

Activated: 06/20/11
Closed:

Version Date: 04/24/2017
Amendment: #8a

CHILDREN'S ONCOLOGY GROUP

AAML1031

A Phase III Randomized Trial for Patients with *de novo* AML using Bortezomib and Sorafenib (IND#114480; NSC# 681239, NSC# 724772) for Patients with High Allelic Ratio FLT3/ITD

IND Sponsor for Bortezomib and Sorafenib: NCI

NCI Supplied Agents: Bortezomib and Sorafenib (IND#114480; NSC# 681239, NSC# 724772)

A Groupwide Phase II/III Study

THIS PROTOCOL IS FOR RESEARCH PURPOSES ONLY, AND SHOULD NOT BE COPIED, REDISTRIBUTED OR USED FOR ANY OTHER PURPOSE. MEDICAL AND SCIENTIFIC INFORMATION CONTAINED WITHIN THIS PROTOCOL IS NOT INCLUDED TO AUTHORIZE OR FACILITATE THE PRACTICE OF MEDICINE BY ANY PERSON OR ENTITY. RESEARCH MEANS A SYSTEMATIC INVESTIGATION, INCLUDING RESEARCH DEVELOPMENT, TESTING AND EVALUATION, DESIGNED TO DEVELOP OR CONTRIBUTE TO GENERALIZABLE KNOWLEDGE. THIS PROTOCOL IS THE RESEARCH PLAN DEVELOPED BY THE CHILDREN'S ONCOLOGY GROUP TO INVESTIGATE A PARTICULAR STUDY QUESTION OR SET OF STUDY QUESTIONS AND SHOULD NOT BE USED TO DIRECT THE PRACTICE OF MEDICINE BY ANY PERSON OR TO PROVIDE INDIVIDUALIZED MEDICAL CARE, TREATMENT, OR ADVICE TO ANY PATIENT OR STUDY SUBJECT. THE PROCEDURES IN THIS PROTOCOL ARE INTENDED ONLY FOR USE BY CLINICAL ONCOLOGISTS IN CAREFULLY STRUCTURED SETTINGS, AND MAY NOT PROVE TO BE MORE EFFECTIVE THAN STANDARD TREATMENT. ANY PERSON WHO REQUIRES MEDICAL CARE IS URGED TO CONSULT WITH HIS OR HER PERSONAL PHYSICIAN OR TREATING PHYSICIAN OR VISIT THE NEAREST LOCAL HOSPITAL OR HEALTHCARE INSTITUTION.

STUDY CHAIR

Richard Aplenc, MD, PhD
Children's Hospital of Philadelphia
Pediatric Oncology/Stem Cell Transplant
4018 CTRB
3501 Civic Center Blvd
Philadelphia, PA 19104-4318
Phone: (267) 426-7252
Fax: (267) 425-0013
E-mail: aplenc@email.chop.edu

TABLE OF CONTENTS

<u>SECTION</u>	<u>PAGE</u>
STUDY COMMITTEE	6
ABSTRACT	9
EXPERIMENTAL DESIGN SCHEMA FOR PATIENTS ENROLLED BEFORE ACCRUAL GOALS FOR ARMS A AND B HAVE BEEN MET	11
EXPERIMENTAL DESIGN SCHEMA FOR PATIENTS ENROLLED AFTER ACCRUAL GOALS FOR ARMS A AND B HAVE BEEN MET	12
1.0 GOALS AND OBJECTIVES	13
1.1 Primary Objectives	13
1.2 Secondary Objectives	13
2.0 BACKGROUND	15
2.1 Rationale for Study Design	15
2.2 Rationale for Bortezomib Feasibility, Definitive Efficacy Testing, and Pharmacokinetic Studies	16
2.3 Rationale for Sorafenib Feasibility Determination	17
2.4 Rationale for Sorafenib Maintenance for HR FLT3/ITD+ AML Patients	19
2.5 Health Related Quality of Life and Parental Stress	21
2.6 Dexrazoxane Use in Pediatric AML	22
2.7 Rationale for Changes in GVHD Prophylaxis and SCT Conditioning Regimen	22
2.8 Biology Correlative Studies	23
3.0 STUDY ENROLLMENT AND PATIENT ELIGIBILITY	33
3.1 Study Enrollment	33
3.2 Patient Eligibility Criteria	35
3.3 Definitions	37
3.4 Pre-Enrollment Baseline Diagnostic Requirements	39
4.0 TREATMENT PLAN	40
4.1 Overview of Treatment Plan	40
4.2 Concomitant Therapy Restrictions	42
4.3 Dose Modifications for CNS Disease	43
4.4 Evaluation of Marrow Status and Extramedullary Disease	44
4.5 End of Induction I	44
4.6 Progression to Subsequent Courses of Therapy	44
4.7 Requirements to Start Each Sorafenib + Chemotherapy Course	45
4.8 Induction I Arm A (ADE 10+3+5)	45
4.9 Induction I Arm B (ADE 10+3+5+ Bortezomib)	48
4.10 Induction I Arm C-Cohort 3 (ADE 10+3+5+ Sorafenib)	52
4.11 Induction I Arm D (ADE 10+3+5)	55
4.12 Induction II Arm A (ADE 8+3+5) – Low Risk Patients	58
4.13 Induction II Arm B (ADE 8+3+5 + Bortezomib) – Low Risk Patients	61
4.14 Induction II Arm A (MA) – High Risk Patients	65
4.15 Induction II Arm B (MA + Bortezomib) – High Risk Patients	68
4.16 Induction II Arm C - Cohort 3 (ADE 8+3+5 + Sorafenib)	71
4.17 Intensification I Arm A (AE)	74
4.18 Intensification I Arm B (AE + Bortezomib)	77
4.19 Intensification I Arm C-Cohort 3 (AE + Sorafenib)	80

4.20	Intensification II Arm A (MA) – Low Risk Patients	83
4.21	Intensification II Arm B (MA + Bortezomib) – Low Risk Patients	86
4.22	Intensification II Arm A & B (HD ARAC/LASP) – High Risk Patients	89
4.23	Intensification II Arm C-Cohort 3 (MA + Sorafenib)	92
4.24	Allogeneic Stem Cell Transplantation	96
4.25	Alternative Donor SCT	101
4.26	Maintenance Arm C (Sorafenib)	103
5.0	DOSE MODIFICATIONS FOR TOXICITIES	106
5.1	Allergy	106
5.2	Cardiac Toxicity	106
5.3	Coagulopathy	108
5.4	Hepatic Toxicity	108
5.5	Neurologic Toxicity	109
5.6	Pancreatitis	110
5.7	Pulmonary Toxicity	110
5.8	Palmar-Plantar Erythrodysesthesia Syndrome, Skin Pain that impacts ADLs or Rash Maculo-Papular	111
5.9	Renal Toxicity	113
5.10	Thrombosis	113
5.11	Dose Reduction for Other Bortezomib-Associated Toxicities	113
5.12	Dose Reductions for Other Sorafenib-Associated Toxicities	114
5.13	Stem Cell Transplant Regimen Agents	116
6.0	DRUG INFORMATION	116
6.1	ASPARAGINASE (E. coli) (07/01/15)	116
6.2	ASPARAGINASE (ERWINIA CHRYSANTHEMI)	118
6.3	BORTEZOMIB	120
6.4	BUSULFAN INJECTION	127
6.5	CYTARABINE – ALL ROUTES	128
6.6	DAUNORUBICIN	131
6.7	ETOPOSIDE INJECTION	132
6.8	FLUDARABINE	134
6.9	LYMPHOCYTE IMMUNE GLOBULIN	137
6.10	METHOTREXATE-IV ONLY	139
6.11	METHYLPREDNISOLONE	140
6.12	MITOXANTRONE	142
6.13	SORAFENIB TOSYLATE	143
6.14	TACROLIMUS	153
7.0	EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED	156
7.1	Required Clinical, Laboratory and Disease Evaluations	156
7.2	Recommended Clinical, Laboratory and Disease Evaluations	157
7.3	Required & Optional Observations for SCT Patients Only	157
7.4	Optional Studies	158
7.5	Follow-up Studies*	159
7.6	Follow-up Reports	159
8.0	CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA	160
8.1	Criteria for Removal from Protocol Therapy	160
8.2	Off Study Criteria	160

9.0	STATISTICAL CONSIDERATIONS	161
9.1	Patient Accrual and Expected Duration of Trial	161
9.2	Statistical Analysis Methods	161
9.3	Interim Monitoring	170
9.4	Gender and Minority Accrual Estimates	172
10.0	EVALUATION CRITERIA	172
10.1	Common Terminology Criteria for Adverse Events (CTCAE)	172
10.2	Response Criteria for Patients with Acute Myeloid Leukemia	172
10.3	Graft Failure following Allogeneic SCT	173
11.0	ADVERSE EVENT REPORTING REQUIREMENTS	174
11.1	Purpose	174
11.2	Determination of reporting requirements	174
11.3	Expedited Reporting Requirements – Serious Adverse Events (SAEs)	174
11.4	Special Situations for Expedited Reporting	175
11.5	Reporting Requirements for Specialized AEs	177
11.6	Exceptions to Expedited Reporting	177
11.7	Reporting Requirements - Investigator Responsibility	178
11.8	General Instructions for Expedited Reporting via CTEP-AERS	178
11.9	Reporting Table for Late Phase 2 and Phase 3 Studies – Table A	179
11.10	Protocol Specific Additional Instructions and Reporting Exceptions	180
11.11	Reporting of Adverse Events for commercial agents – CTEP-AERS abbreviated pathway	180
11.12	Routine Adverse Event Reporting	181
11.13	Definition of Onset and Resolution of Adverse Events	181
12.0	RECORDS AND REPORTING	182
12.1	CDUS	182
12.2	Data and Safety Monitoring Committee	182
12.3	CRADA/CTA	182
13.0	PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS	184
13.1	Immunophenotyping Studies	184
13.2	Cytomorphologic Studies	185
14.0	FLT3 MOLECULAR GENETIC ANALYSIS GUIDELINES AND REQUIREMENTS	187
14.1	NIH-Funded Laboratory for Mutation Analysis at Diagnosis	187
14.2	FLT3 Mutation Analysis	187
14.3	Specimen Requirements	187
14.4	Shipping	187
14.5	Specimens Shipping Options	188
15.0	MRD ANALYSIS GUIDELINES AND REQUIREMENTS	188
15.1	NIH-Funded Laboratory for MRD Testing at Diagnosis	188
15.2	MRD Analysis	188
15.3	Specimen Requirements	189
15.4	Shipping	189
16.0	CYTOGENETIC ANALYSIS GUIDELINES AND REQUIREMENTS	190
16.1	Cytogenetic Analysis Overview	190
16.2	Specimen Collection and Submission	190
17.0	SPECIAL STUDIES SPECIMEN REQUIREMENTS	192

17.1	Pharmacokinetic and Pharmacodynamic Studies	192
17.2	Leukemia Biology Studies	194
18.0	QUALITY OF LIFE AND PARENTAL STRESS ASSESSMENTS	196
18.1	Time points for Assessment	196
18.2	Health Related Quality Of Life (HRQOL) and Parental Stress Questionnaires	197
19.0	RADIATION THERAPY GUIDELINES	198
19.1	Indications for Radiation Therapy	199
19.2	Timing	199
19.3	Emergency Radiation Therapy	199
19.4	Equipment and Methods of Delivery and Verification	199
19.5	Target Volumes	200
19.6	Target Dose	200
19.7	Treatment Technique	201
19.8	Organs at Risk	202
19.9	Dose Calculations and Reporting	202
19.10	Quality Assurance Documentation	203
20.0	HEMATOPOIETIC TRANSPLANT GUIDELINES	205
20.1	Pre-SCT Conditioning	205
20.2	Admission Criteria (see Section 7.3)	205
20.3	Bone Marrow Harvest, Processing, Dose and Infusion	205
20.4	Supportive Care Guidelines	206
20.5	COG Stem Cell Committee Consensus Guidelines for Establishing Organ Stage and Overall Grade of Acute Graft versus Host Disease (GVHD)	206
	APPENDIX I: SUPPORTIVE CARE GUIDELINES	213
	APPENDIX II: SORAFENIB DOSING NOMOGRAM	217
	APPENDIX III: INSTRUCTIONS FOR CHEMOTHERAPY DRUG PREPARATION, ADMINISTRATION AND SAFE HANDLING	218
	APPENDIX IV: LIST CYP3A4 SUBSTRATES, INDUCERS AND INHIBITORS	220
	APPENDIX V: SAMPLE SUBMISSION FOR CYTOGENETIC/FISH STUDIES	222
	APPENDIX VI: BLOOD PRESSURE LEVELS FOR CHILDREN BY AGE AND HEIGHT PERCENTILE	225
	APPENDIX VII: LEUKEMIA BIOLOGY & GENE POLYMORPHISM STUDIES	227
	APPENDIX VIII: LEUKEMIA INITIATING CELL (LIC) STUDY	228
	APPENDIX IX: PROTEIN EXPRESSION (RPPA) AND UNFOLDED PROTEIN RESPONSE (UPR) STUDY	230
	APPENDIX X: SORAFENIB PHARMACOKINETIC (PK) STUDY	232
	APPENDIX XI: SORAFENIB PIA STUDY	235
	APPENDIX XII: YOUTH INFORMATION SHEETS	236
	APPENDIX XIII: BIOMARKERS FOR THE PREDICTION OF GVHD STUDY	238
	APPENDIX XIV: CTEP AND CTSU REGISTRATION PROCEDURES	240
	APPENDIX XV: POSSIBLE DRUG INTERACTIONS	242
	REFERENCES	248

STUDY COMMITTEE

STUDY CHAIR

Richard Aplenc, MD, PhD
Children's Hospital of Philadelphia
Pediatric Oncology/Stem Cell Transplant
4018 CTRB
3501 Civic Center Blvd
Philadelphia, PA 19104-4318
Phone: (267) 426-7252
Fax: (267) 425-0113
E-mail: aplenc@email.chop.edu

STUDY VICE CHAIR

Lillian Sung, MD, PhD
Hematology/Oncology
Hospital for Sick Children
Pediatrics
555 University Avenue
Toronto ON M5G 1X8
Canada
Phone: (416) 813-5287
Fax: (416) 813-5327
E-mail: lillian.sung@sickkids.ca

STUDY VICE CHAIR

Soheil Meshinchi, MD, PhD
Hematology/Oncology
Seattle Children's Hospital
Fred Hutchinson Cancer Research Ctr.
Clinical Research Division
1100 Fairview Avenue North, D5-380
Seattle, WA 98109-1024
Phone: (206) 667-4077
Fax: (206) 667-4310
E-mail: smeshinc@fhcrc.org

STUDY STATISTICIAN

Todd Alonzo, PhD
Statistics
222 E. Huntington Dr, Suite 100
Monrovia, CA 91016
Phone: (626) 241-1522
Fax: (626) 445-4334
E-mail: talonzo@childrensoncologygroup.org

STUDY COMMITTEE MEMBERS

Alan S. Gamis, MD, MPH
Hematology/Oncology
Children's Mercy Hospitals & Clinics
Department Hematology-Oncology
2401 Gillham Road
Kansas City, MO 64108
Phone: (816) 302-6808
Fax: (816) 855-1700
E-mail: agamis@cmh.edu

STUDY COMMITTEE MEMBERS

Michael Joseph Absalon, MD, PhD
Hematology/Oncology
Cincinnati Children's Hospital Medical Center
MLC 7015
3333 Burnet Avenue
Cincinnati, OH 45229
Phone: (513) 636-2061
Fax: (513) 636-3549
E-mail: michael.absalon@cchmc.org

Lamia P. Barakat, PhD
Behavioral Science
Children's Hospital of Philadelphia
Div. of Oncology, Dept. of Pediatrics
3400 Civic Center Blvd.
CHOP North, Room 1483
Philadelphia, PA 19104
Phone: (267) 426-8135
E-mail: barakat@email.chop.edu

Lisa A. Bayer, CPNP
Nursing
Weill Medical College of Cornell University
Division of Pediatric Hematology/Oncology
525 E. 68th Street,
Payson Building #695
New York, NY 10065
Phone: (212) 746-3400
Fax: (212) 746-8609
E-mail: lbayer@med.cornell.edu

Patrick A. Brown, MD
Hematology/Oncology
Johns Hopkins University/Sidney Kimmel Cancer Center
Pediatric Oncology
1650 Orleans St. CRB 2M49
Baltimore, MD 21231
Phone: (410) 614-4915
Fax: (410) 955-8897
E-mail: pbrown2@jhmi.edu

John Kim Choi, MD, PhD
Pathology
St. Jude Children's Research Hospital
Pathology
262 Danny Thomas Place, Mail Stop 250
Memphis, TN 38105
Phone: (901) 595-5347
Fax: (901) 595-3100
E-mail: JohnKim.Choi@stjude.org

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

Samir B. Kahwash, MD
Pathology
Nationwide Children's Hospital
Division of Laboratory Medicine
700 Children's Dr
Columbus, OH 43205
Phone: (614) 722-5450
Fax: (614) 722-5308
E-mail: Samir.Kahwash@nationwidechildrens.org

John E. Levine, MD
Stem Cell Transplantation
Mount Sinai Medical Center
Pediatric Hematology/Oncology/BMT
Blood and Marrow Transplantation Program
1 Gustave L. Levy Place, Box 1410
New York, NY 10029
Phone: (212) 241-3429
Fax: (212) 659-5599
E-mail: jelevine@mssm.edu

Jessica A. Pollard, MD
Hematology/Oncology
Maine Children's Cancer Program
100 Campus Drive
Scarborough, ME 04074
Phone: (207) 396-7565
Email: jpollard@mmc.org

Thomas Merchant, DO, PhD
Radiation Oncology
St. Jude Children's Research Hospital
Radiological Sciences
262 Danny Thomas Place, MS 210
Memphis TN 38105-3678
Phone: (901) 595-3604
Fax: (901) 595-3113
E-mail: thomas.merchant@stjude.org

Rajaram Nagarajan, MD
Hematology/Oncology
Cincinnati Children's Hospital Medical Center
Division of Hematology/Oncology
3333 Burnet Avenue
MLC 7015; TCHRF 2092
Cincinnati, OH 45229
Phone: (513) 636-1248
Fax: (513) 636-3549
E-mail: rajaram.nagarajan@cchmc.org

Susana C. Raimondi, PhD
Cytogenetics
St. Jude Children's Research Hospital
Pathology
262 Danny Thomas Place, MS 250
Memphis, TN 38105-3678
Phone: (901) 595-3537
Fax: (901) 595-3100
E-mail: susana.raimondi@stjude.org

Betsy Hirsch, PhD
Cytogenetics
University of Minnesota/Masonic Cancer Center
Division of Laboratory Medicine
420 Delaware St., SE
MMC Box 609
Minneapolis MN 55455
Phone: (612) 273-4952
Fax: (612) 273-4689
E-mail: hirsc003@umn.edu

Laura Louise Winter, PharmD
Pharmacy
Seattle Children's Hospital
Pediatric Hematology/Oncology
4800 Sand Point Way NE
MB.5.420
Seattle, WA 98105-0371
Phone: (206) 987-2106
Fax: (206) 729-3062
E-mail: laura.winter@seattlechildrens.org

Andrew Christopher Harris, MD
Stem Cell Transplantation
Primary Children's Hospital
Pediatric Hematology/Oncology
100 N. Mario Capecchi Drive, Suite 4100
Salt Lake City, UT 84113
Phone: (801) 662-4700
Fax: (801) 662-4707
Email: andrew.harris@hsc.utah.edu

STUDY COMMITTEE MEMBERS

Brian T. Fisher, DO, MSCE
Epidemiology
Children's Hospital of Philadelphia
Pediatrics, Division of Infectious Diseases
34th Street and Civic Center Boulevard
CHOP North Room 1515
Philadelphia, PA 19104
Phone: (267) 426-7110
Fax: (215) 590-2017
Email: fisherbria@email.chop.edu

Michael G. Douvas, MD
Hematology/Oncology
University of Virginia Cancer Center
Internal Medicine
P.O. BOX 800716
Charlottesville, VA 22908-0716
Phone: 434 982-6399
Fax: 434 243-6086
Email: mgd9a@virginia.edu

Elizabeth Fox, MD
Hematology/Oncology
Children's Hospital of Philadelphia
Pediatrics/Division of Oncology
3501 Civic Center Blvd. CTRB-4016
Philadelphia, PA 19104
Phone: (267) 425-3010
Fax: (267) 425-0113
Email: foxe@email.chop.edu

Gabriela Gheorghe, MD
Pathology
Midwest children's Cancer Center
CHW Department of Pathology
9000 West Wisconsin Avenue
Milwaukee, WI 53226
Phone: (414) 266-2530
Fax: (414) 266-2779
Email: ggheorghe@mcw.edu

STUDY COMMITTEE MEMBERS

Jennifer Truong, MD MPH
Clinical Research Associate
Floating Hospital for Children at Tufts Medical Center
Pediatric Hem/Onc
800 Washington Street, Box 245
Boston, MA 02111
Phone: (617) 636-8885
Fax: (617) 636-8886
Email: jtruong@tuftsmedicalcenter.org

COG PROTOCOL COORDINATOR

Jeannette Cassar
Children's Oncology Group- Operations Center
Study Development Office
222 E. Huntington Dr, Suite 100
Monrovia, CA 91016
Phone: (626) 241-1532
Fax: (626) 445-4334
E-mail: jcassar@childrensoncologygroup.org

COG RESEARCH COORDINATOR

Wendy Lee, LVN
Children's Oncology Group
Statistics and Data Center
222 E. Huntington Dr, Suite 100
Monrovia, CA 91016
Phone : (626) 241-1762
Fax: (626) 445-4334
Email: wlee@childrensoncologygroup.org

AGENT

NSC#

IND#

Bortezomib (supplied by NCI)	NSC# 681239	IND# 114480
Sorafenib (supplied by NCI)	NSC#724772	IND# 114480
Busulfan	NSC#750	Exempt
Cytarabine	NSC#063878	Exempt
Daunorubicin	NSC#82151	Exempt
Etoposide	NSC#141540	Exempt
Fludarabine	NSC#312887	Exempt
L'Asparaginase (E. Coli)	NSC#109229	Exempt
L'Asparaginase (Erwinia)	NSC# 106977	Exempt
Lymphocyte Immune Globulin	NSC#743145	Exempt
Methotrexate	NSC#000740	Exempt
Methylprednisolone	NSC#19987	Exempt
Mitoxantrone	NSC#301739	Exempt
Tacrolimus	NSC#717865	Exempt

IND Sponsor: NCI

SEE SECTIONS [13-16](#) AND [APPENDICES VII-XVII](#) FOR SPECIMEN SHIPPING ADDRESSES.

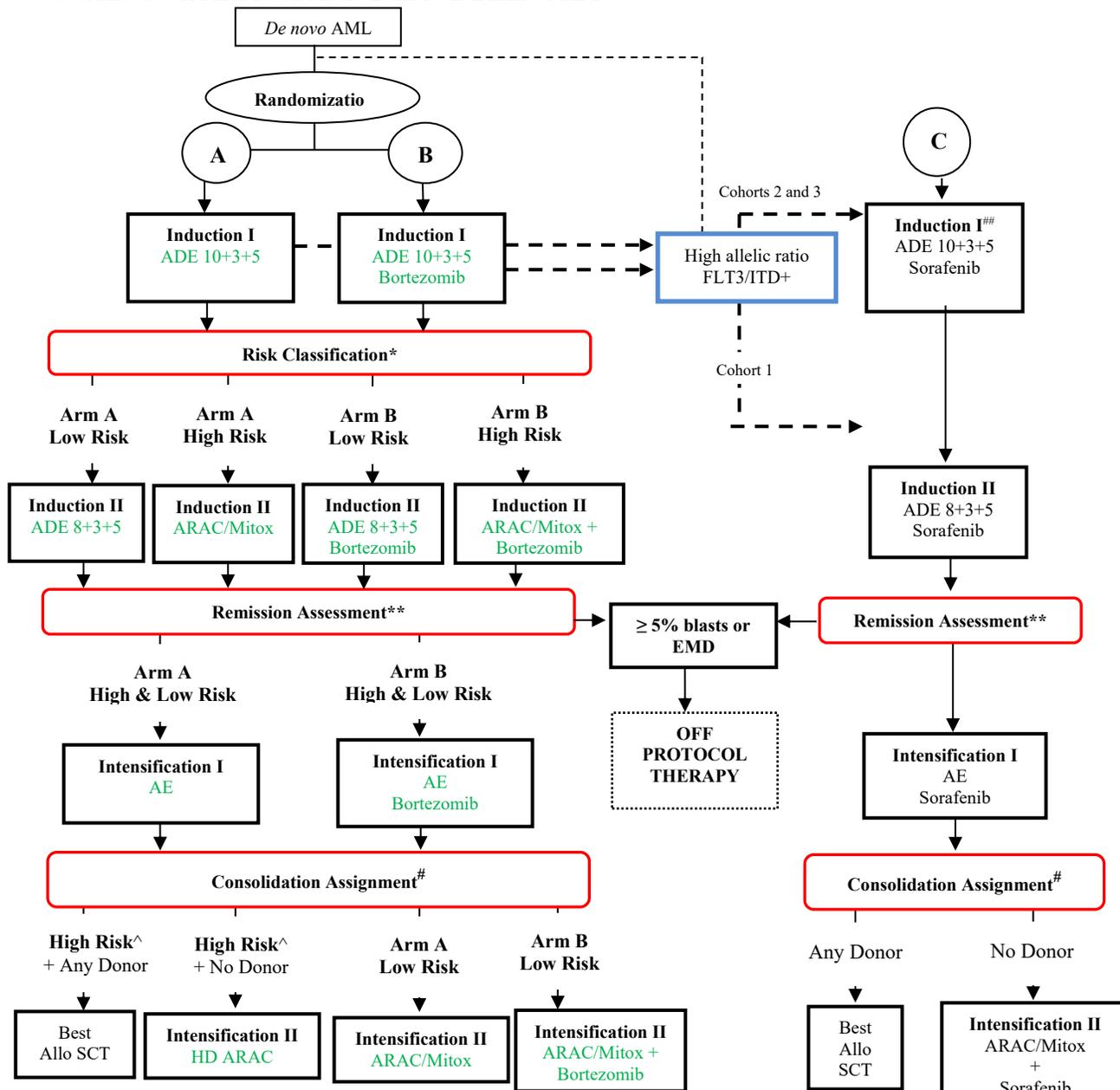
The Children's Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about your subjects collected during the course of our covered 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

Disease recurrence and treatment related toxicities still account for approximately 40% of deaths in pediatric patients with *de novo* Acute Myeloid Leukemia (AML) despite substantial progress made with various treatment modalities. Thus, the incorporation of new approaches to standard AML therapy that will further improve the overall outcome of patients with *de novo* AML by testing clinically relevant hypotheses that enhance better understanding of AML blast biology, more accurate patient risk stratification, and more effective supportive care practices is a high priority. Bortezomib, a proteasome inhibitor has been shown to selectively deplete the leukemic stem cells that are a source of resistance for AML. Despite limited pediatric data, adult and *in vitro* studies demonstrate that bortezomib can be safely combined with standard AML chemotherapy (without high dose cytarabine arabinoside), augmenting their anti-leukemic effects. Fms-like tyrosine kinase 3 (FLT3) which is an AML oncogene that plays a significant role in AML pathogenesis is a target of the multi-target tyrosine kinase inhibitor, sorafenib. Sorafenib has shown encouraging results with its use as a single agent in adults with relapsed FLT3 positive internal tandem duplication (FLT3/ITD+) AML. Despite limited pediatric experience of sorafenib in combination with AML chemotherapy, it has been safely combined with AML chemotherapy in younger adults with AML. This clinical trial seeks to determine the role of bortezomib in upfront therapy for pediatric AML, and to determine the safety of sorafenib in the treatment of patients with high allelic ratio FLT3/ITD+ (HR FLT3/ITD+) AML. In addition, bortezomib and sorafenib pharmacokinetic studies will be performed on a subset of patients. Bortezomib evaluation involved random allocation to treatment with standard pediatric AML therapy only (Arm A), or standard AML therapy with bortezomib (Arm B). With Amendment #7A, Arms A and B are closed to new patient enrollment as accrual goals have been met. Patients enrolled prior to Amendment #7A will continue treatment per their initially assigned arm. Arms A and B of AAML1031 utilize a 3 or 4 course chemotherapy backbone. Patients with low risk disease receive cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/daunorubicin/etoposide (ADE 8+3+5), cytarabine/etoposide (AE), and cytarabine/mitoxantrone (ARAC/Mitox). Patients with high risk disease receive 3 courses of chemotherapy: cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/mitoxantrone (ARAC/Mitox), and cytarabine/etoposide (AE), prior to best allogeneic donor stem cell transplant (SCT). High risk patients without an appropriate allogeneic donor will receive high dose cytarabine/L-asparaginase (HD ARAC/LASP) as a fourth chemotherapy course. Sorafenib evaluation will occur in three cohorts. Cohort 1-Arm C has determined a tolerable sorafenib dose in combination with standard AML chemotherapy in patients with HR FLT3/ITD+ mutations. With Amendment #3A, patients with HR FLT3/ITD+ will be allocated to sorafenib plus standard chemotherapy for additional feasibility and efficacy determination (Cohorts 2 and 3-Arm C) and offered an additional sorafenib maintenance phase. Cohort 2 patients received concomitant sorafenib and conventional chemotherapy whereas with Cohort 3 (Amendment #6A) the dosing schedule of sorafenib during Induction II, Intensification I and Intensification II was changed given concerns of cardiac toxicity. With Amendment #6A, sorafenib was started after completion of conventional chemotherapy. With Amendment #7A, patients with known HR FLT3/ITD+ AML prior to study entry will be enrolled directly onto Arm C and receive three courses of chemotherapy in combination with sorafenib: cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/daunorubicin/etoposide (ADE 8+3+5), cytarabine/etoposide (AE), followed by best allogeneic donor SCT. Patients with HR FLT3/ITD+ who do not have an appropriate allogeneic donor will receive sorafenib with ARAC/Mitoxantrone as a fourth course of chemotherapy. Patients with unknown FLT/ITD

allelic ratio prior to study entry will first enroll onto Arm D (standard pediatric AML Induction chemotherapy) until FLT/ITD results are determined. Patients found to have HR FLT/ITD+ will be treated per Arm C; patients without HR FLT/ITD+ will be removed from study. Risk stratification of patients in AAML1031 will utilize cytogenetics, molecular markers and multidimensional flow cytometry to allocate patients into high risk (HR) and low risk (LR) groups. This study will assess health related quality of life (HRQOL) and parental stress at multiple time points during and after therapy, for longitudinal assessment of QOL and parental stress. AAML1031 will also incorporate correlative studies aimed at contributing to the enhancement of future AML treatment parameters and assessment. These correlative studies include evaluation of the *in vitro* levels of wild type FLT3 in response to sorafenib, analyzing chromosomal abnormalities, complex karyotypes; molecular abnormalities of *WT1*, *RUNX1*, *MLL-PTD* and other novel AML associated genes, leukemic involvement of early progenitor cells, and GVHD biomarkers.

EXPERIMENTAL DESIGN SCHEMA FOR PATIENTS ENROLLED BEFORE ACCRUAL GOALS FOR ARMS A AND B HAVE BEEN MET



***Risk Classification:** Based on cytogenetics, molecular markers and MRD post-Induction I. See Section 3.3 for definitions.

****Remission Assessment:** Patients with $\geq 5\%$ blasts or EMD will be off protocol therapy.

Consolidation Assignment

- High risk patients receive SCT based on availability of appropriate donor.
- Low risk patients receive chemotherapy **only** (based on initial treatment arm randomized to), *without* SCT.

^Includes high-risk patients on both Arm A and Arm B.

High allelic ratio FLT3/ITD+ patients

- If appropriate donor available (MFD or MUD), patients receive best allogeneic donor SCT regardless of cytogenetic risk group followed by maintenance sorafenib.
- If no appropriate donor is available, patients receive a fourth course of chemotherapy followed by maintenance sorafenib, regardless of cytogenetic risk group

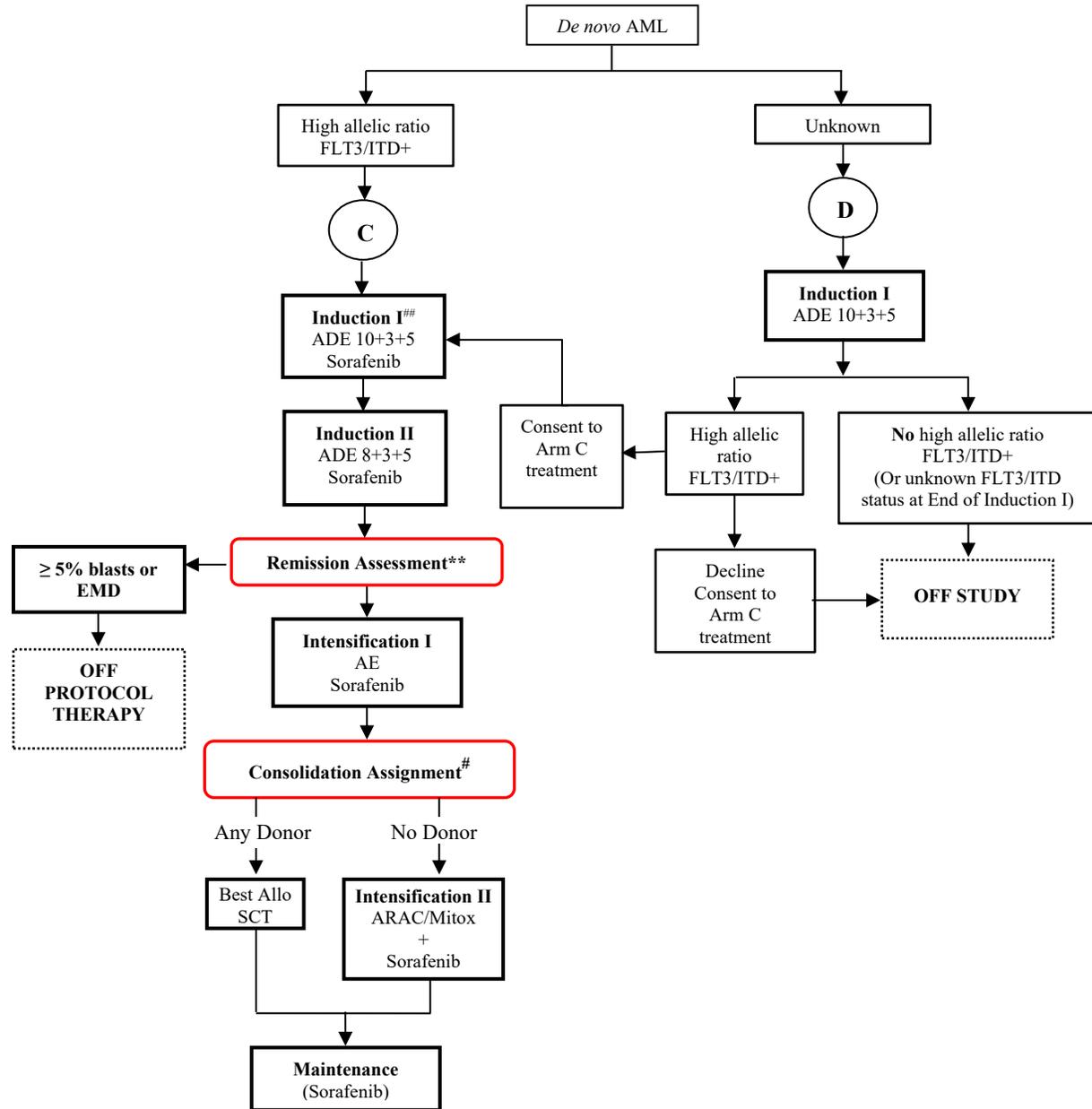
Sorafenib to be given in Induction I (after FLT3/ITD status is known) for Cohorts 2 and 3.

Note: Patients enrolled prior to Amendment #3A activation and currently on therapy may move to Arm C prior to their next treatment course. Patients enrolled during suspension of Arm C may move to Arm C prior to their next treatment course as part of amendment 6a.

See [Section 18.0](#) for optional Quality of Life and Parental Stress Study.

ADE = Cytarabine, Daunorubicin, Etoposide	ITD = Internal tandem duplication
EMD = Extramedullary disease	AE = Cytarabine, Etoposide
MFD = Matched family donor	SCT = Stem Cell Transplant
ARAC/Mitox = Cytarabine, Mitoxantrone	MUD = Matched unrelated donor

**EXPERIMENTAL DESIGN SCHEMA FOR PATIENTS ENROLLED AFTER ACCRUAL
GOALS FOR ARMS A AND B HAVE BEEN MET**



****Remission Assessment:** Patients with $\geq 5\%$ blasts or EMD will be off protocol therapy.

Consolidation Assignment

- High risk patients receive SCT based on availability of appropriate donor.
- Low risk patients receive chemotherapy **only**, *without* SCT.

High allelic ratio FLT3/ITD+ patients

- If appropriate donor available (MFD or MUD), patients receive best allogeneic donor SCT regardless of cytogenetic risk group followed by maintenance sorafenib.
 - If no appropriate donor is available, patients receive a fourth course of chemotherapy followed by maintenance sorafenib, regardless of cytogenetic risk group
- ^{###} Sorafenib to be given in Induction I (as soon as FLT3/ITD status is known).

ADE = Cytarabine, Daunorubicin, Etoposide

ITD = Internal tandem duplication

EMD = Extramedullary disease

AE = Cytarabine, Etoposide

MFD = Matched family donor

SCT = Stem Cell Transplant

ARAC/Mitox = Cytarabine, Mitoxantrone

MUD = Matched unrelated donor.

1.0 GOALS AND OBJECTIVES

With Amendment #7A accrual goals for Arms A and B will have been met and there will be a sufficient number of patients enrolled in order to meet the following protocol objectives:

Primary Objectives: 1.1.1, 1.1.2, 1.1.3

Secondary Objectives: 1.2.2 - 1.2.6, 1.2.14

1.1 Primary Objectives

1.1.1

To compare event free survival (EFS) and overall survival (OS) in patients with *de novo* acute myeloid leukemia (AML) without high allelic ratio FLT3/ITD+ mutations who are randomized to standard therapy versus bortezomib/standard combination therapy.

1.1.2

To determine the feasibility of combining bortezomib with standard chemotherapy in patients with *de novo* AML.

1.1.3

To compare the OS and EFS of high risk patients treated with intensive Induction II with historical controls from AAML03P1 and AAML0531.

1.1.4

To determine the feasibility of administering sorafenib with standard chemotherapy and in a one year maintenance phase in patients with *de novo* high allelic ratio FLT3/ITD+ AML.

1.2 Secondary Objectives

1.2.1

To assess the anti-leukemic activity of sorafenib in patients with *de novo* high allelic ratio FLT3/ITD+ AML.

1.2.2

To compare the percentage of patients converting from positive MRD to negative MRD after Intensive Induction II with historical controls from AAML03P1 and AAML0531.

1.2.3

To compare OS, disease free survival (DFS), cumulative incidence of relapse, and treatment related mortality from end of Intensification I between patients allocated to best allogenic donor stem cell transplant (SCT) and comparable patients on AAML0531 who did not receive allogenic donor SCT.

1.2.4

To compare OS, DFS, cumulative incidence of relapse, treatment related mortality, and severe toxicity between patients allocated to matched family donor SCT on AAML1031 and AAML0531.

1.2.5

To assess the health-related quality of life (HRQOL) of patients treated with chemotherapy and stem cell transplant (SCT) for AML.

1.2.6

To evaluate bortezomib pharmacokinetics (PK) in patients receiving the combination regimen.

1.2.7

To obtain sorafenib and metabolite steady state pharmacokinetics and pharmacokinetic-pharmacodynamic data in subjects with FLT3/ITD receiving sorafenib.

1.2.8

To compare the changes in shortening fraction/ejection fraction over time between patients treated with and without dexrazoxane.

1.2.9

To refine the use of minimal residual disease (MRD) detection with 4-color flow cytometry.

1.2.10

To evaluate the prognostic significance of molecular MRD and its contribution to risk identification with MDF-based MRD in patients with translocations amenable to quantitative RT-PCR (e.g., t (8;21), inv (16), t(9;11), *WT1* expression).

1.2.11

To determine the leukemic involvement of the hematopoietic early progenitor cell and its role in defining response to therapy.

1.2.12

To define the leukemic stem cell population in patients with AML.

1.2.13

To determine the prevalence and prognostic significance of molecular abnormalities of *WT1*, *RUNX1*, *MLL-PTD*, *TET2*, *c-CBL*, *KIT* and other novel AML associated genes in pediatric AML.

1.2.14

Correlate the expression of CD74 antigen as well as *PSMB5* gene expression and mutation with response to bortezomib.

1.2.15

To evaluate the changes in protein expression and unfolded protein response (UPR) in patients with AML.

1.2.16

Determine the expression level of wild type FLT3, and correlate with outcome and *in vitro* sensitivity to FLT3 inhibition.

1.2.17

To collect biology specimens at diagnosis, treatment time points, and relapse for future biology studies.

1.2.18

To create a pediatric-specific algorithm to predict the occurrence of Grade 2-4 acute graft-versus-host disease (GVHD) prior to its clinical manifestations using a combination of pre-transplant clinical variables and serum GVHD biomarker concentrations in the first weeks after SCT.

2.0 BACKGROUND

Despite substantial progress in the treatment of pediatric AML, approximately 40% of patients die from disease recurrence or treatment related toxicities.¹⁰ Further improvements in AML treatment response will require not only new, targeted therapies, but also an improved understanding of AML blast biology, more accurate patient risk stratification, and more effective supportive care practices. The AAML1031 protocol seeks to improve the overall outcome of patients with *de novo* AML by testing clinically relevant hypotheses in all of these areas.

2.1 Rationale for Study Design

This trial (AAML1031) will use a 4 course standard therapy backbone of cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/daunorubicin/etoposide (ADE 8+3+5), cytarabine/etoposide (AE), and cytarabine/mitoxantrone (ARAC/Mitox) for patients with low risk AML. These courses correspond to the first 4 courses of AAML0531. The final course of AAML0531, high dose cytarabine (HD ARAC), has been deleted from the therapy for low risk patients for 2 reasons. First, the available data from the MRC 12 pediatric trial that randomized patients to 4 versus 5 courses of therapy showed no benefit of a fifth course. Specifically, patients randomized to 4 courses had an overall survival (OS) of 81% while patient randomized to 5 courses had an OS of 80%.¹¹ (Personal Communication, Gibson B 2008) Secondly, both the AAML03P1 and AAML0531 trials have had a 10% - 18% withdrawal rate for toxicity after Intensification II (Personal Communication, Alonzo TA 2009). The reported toxicities are primarily delayed count recovery with an attendant increase in infectious complication risk. Currently, patients removed from therapy after Intensification II do not have a decreased event free survival (EFS) or OS on either AAML03P1 or AAML0531 (Personal Communication, Alonzo TA 2009). Thus, the available data indicate that the fifth course (Intensification III) of therapy likely provides no additional benefit but increases toxicity risk.

This trial will use an intensified Induction II regimen of cytarabine/mitoxantrone (ARAC/Mitox) for patients with high risk AML, while low risk patients will receive an Induction II regimen of cytarabine/daunorubicin/etoposide (ADE 10+3+5). For the high risk patients with persistent marrow disease (> 15%) after Induction I, recently released AAML0531 data shows that using ADE 8+3+5 during Induction II resulted in only a 36 - 40% CR rate (using either a flow-based or morphologic CR respectively) (personal communication, A Gamis, MD, 2010). As well, data from AAML03P1 demonstrate that patients with high risk AML have a very poor DFS. Specifically, using the same risk stratification that will be used in AAML1031, DFS at 3 years from Induction I was $68 \pm 9\%$ vs. $20 \pm 16\%$ for the low risk and high risk patients ($p < 0.001$), respectively.¹² While the benefit of intensifying Induction therapy further remains debated, the poor outcome of high risk patients justifies evaluation of alternative chemotherapy strategies. COG's extensive experience with ARAC/Mitox early in the relapsed setting (CCG-2951 and COG AAML00P2) indicates that intensifying Induction with ARAC/Mitox likely will be tolerated and may benefit patients.

Patients with HR FLT3/ITD+ allocated to the sorafenib treatment arm will receive the first 3 courses of low risk therapy backbone followed by best allogeneic donor SCT. These courses are: cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/daunorubicin/etoposide (ADE 8+3+5), and cytarabine/etoposide (AE). Patients with HR FLT3/ITD+ without an appropriate allogeneic donor will receive sorafenib in combination with ARAC/Mitox as a fourth course of chemotherapy. Patients allocated to the sorafenib treatment arm will not receive intensified Induction with ARAC/Mitox as the baseline rate of expected toxicities is not known for the intensified Induction regimen. The absence of baseline toxicity data substantively hinders assessment of sorafenib feasibility, which is the primary aim of the sorafenib treatment arm.

When Arms A and B were open (NOTE: As of Amendment #7A accrual goals for Arms A and B have been met. No additional patients will be enrolled on Arms A or B of AAML1031), this study will use cytogenetics, molecular markers, and multidimensional flow cytometry to risk stratify patients into high risk (HR) and low risk (LR) groups to allocate patients to 1 of 3 treatment groups at diagnosis at the end of Induction I. Prior studies have demonstrated that FLT3 positive internal tandem duplication (FLT3/ITD+), particularly high allelic ratio (HR FLT3/ITD+), is an independent predictor of poor outcome. Likewise, pediatric and adult data have shown core binding factor AML, CEBP α mutations, and nucleophosmin (NPM) mutations as robust markers of good prognosis. Finally, recent work by Dr. Meshinchi has shown that minimal residual disease (MRD) may be used to identify high and low risk groups in patients with standard risk cytogenetic features. (Personal Communication, Meshinchi S 2009) Thus, the risk stratification procedure on this study will incorporate all of these factors. The data supporting MRD use are presented in the Biology Background ([Section 2.7.1](#)) below.

2.2 Rationale for Bortezomib Feasibility, Definitive Efficacy Testing, and Pharmacokinetic Studies

Despite very intensive therapies, patients with AML still fare relatively poorly in comparison to many other pediatric cancers. Given the high intensity of AML therapy, the augmentation of chemotherapy with biologic agents will likely be necessary for further improvements in AML outcome. Bortezomib is a proteasome inhibitor that has single agent efficacy in the treatment of multiple myeloma¹³ and is currently under study in multiple bortezomib/chemotherapy combinations for patients with several malignancies. Bortezomib has been shown to selectively deplete the proposed AML leukemia stem cell population, a proposed source of resistance for AML, and may augment the anti-leukemic effect of standard chemotherapy.¹⁴ Adult trials have demonstrated that bortezomib may be safely combined with AML chemotherapy, although concerns exist about rare cases of acute respiratory distress syndrome (ARDS) in combination with HD AraC.¹⁵⁻¹⁸ Data from the ongoing CALGB10502 Phase I/II trial of bortezomib in older adults provides additional recent data demonstrating that bortezomib can be safely combined with standard AML chemotherapy (Personal Communication, Attar EC 2010). The CALGB trial enrolled 98 patients, of whom 95 received bortezomib and 75 have complete Induction data. The trial defined the maximum tolerated dose (MTD) as 1.3 mg/m², and did not observe idiosyncratic adult respiratory distress syndrome, although patients with pre-existing cardiac and pulmonary dysfunction were excluded from the study. Patients with greater or equal to Grade 2 peripheral neuropathy were also excluded. However, 7 patients experienced Grade 3 or greater peripheral neuropathy with most toxicities resolving over time. In addition, 4 patients experienced autonomic neuropathy with orthostatic hypotension requiring IV fluids. Three of 4 patients have had a resolution of this toxicity, and 1 patient continues with unresolved symptoms. Remission Induction was achieved in 14 of the first 25 patients in Stage 1, and at least 25 of the 70 patients enrolled in Stage 2. Overall and event-free survival data are not yet available. The trial continues for those in complete remission (CR) and combines bortezomib on Days 1, 4, 8, and 11 during 2 courses with high dose cytarabine, 2 gm/m² on Days 1 - 5. Data from this portion of the trial is pending.

As of July 24, 2010, 20 evaluable patients have been treated on the ongoing COG protocol AAML07P1 that combines bortezomib with standard AML chemotherapy. Of these, 14 evaluable patients have enrolled on Arm A of AAML07P1; interim analysis of Arm A efficacy data demonstrated that only 4 of 14 patients attained a CR or complete remission with partial recovery of platelet count (CRp). While an additional 4 patients experienced a complete remission with incomplete blood count recovery (CRi) and 2 a partial response (PR), the study objectives were to assess only CR and CRp, and thus, Arm A has been closed to further accrual (Personal Communication, Alonzo TA 2010). Arm B is nearing completion of dose finding without seeing an episode of ARDS in the patients treated at a bortezomib dose of 1.3 mg/m². Thus, while the available data on bortezomib in pediatric AML is limited, the *in vitro* and adult data demonstrate that bortezomib may be combined safely with AML chemotherapy and that bortezomib has potential for augmenting the efficacy of standard AML therapy.

Although the MTD of bortezomib both as a single agent and in combination with standard ALL induction therapy has been defined,¹⁹⁻²¹ very limited data exist on bortezomib pharmacokinetics. Specifically, of 37 patients enrolled on these three trials, PK data are available on 5 patients treated with single agent bortezomib on the Phase I trial in refractory leukemia.²⁰ Of these, only three patients were treated at the 1.3 mg/m² dose level. Thus, additional data are clearly needed to determine the pharmacokinetic properties of bortezomib.

2.3 Rationale for Sorafenib Feasibility Determination

Sorafenib is a multi-target tyrosine kinase (TKI) inhibitor that targets *FLT3*, *c-KIT*, *PDGF*, *VEGF*, and the RAF/MEK/ERK pathway and is currently approved for the treatment of renal cell carcinoma.²² The current approved adult dose is 400 mg twice daily, and the ADVL0413 pediatric Phase I study has defined the MTD in solid tumor patients at 200 mg/m² twice daily continuous exposure with dose-limiting toxicities (DLT) of hypertension and hand-foot syndrome. Children with refractory leukemias do not tolerate the solid tumor MTD, and therefore the recommended dose is 150 mg/m²/dose for this patient population. (Personal Communication, Widemann B, 2010) In addition, pharmacokinetic data indicate that the half life (T_{1/2}) is > 24 hours and that sorafenib exposure is not dose-proportional above 150 mg/m² dosing.

Sorafenib has shown encouraging results as a single agent in adults with relapsed FLT3/ITD+ AML.²³⁻²⁵ A very recent report from MD Anderson Cancer Center (MDACC) demonstrates that sorafenib can be safely combined with AML chemotherapy in younger adults with AML.²⁶ Moreover, patients with FLT3/ITD+ AML treated with sorafenib had a significantly increased remission induction rate than either patients without FLT3/ITD or with FLT3/ITD+ patients treated with chemotherapy alone. However, no benefit in OS was observed in patients treated with sorafenib.

Pediatric experience with sorafenib in combination with AML chemotherapy in children has been reported in 15 children in addition to the 10 evaluable patients in Arm C, Cohort 1.^{27,28} Data from these reports indicate that the MTD of sorafenib in combination with AML chemotherapy is 150 mg/m² twice daily. Data from Arm C, Cohort 1, indicates that 200 mg/m² once daily may be safely administered with COG standard AML therapy, and this dose will be taken forward for Arm C, Cohorts 2 and 3.

2.3.1 Rationale for Modification of Sorafenib Dosing Schedule (Per Amendment #6A)

As of the 04/2015 interim evaluation, higher than expected rates of cardiac toxicity were observed for Cohort 2 of Arm C. Specifically, 7/38 (18%) patients met criterion for cardiac toxicity that warranted permanent discontinuation of sorafenib treatment. Two patients experienced dose-limiting toxicity at 100 mg/m² dosing and five patients experienced dose limiting toxicity at 200 mg/m² dosing but went off protocol therapy prior to a sorafenib dose reduction. In most children, cardiac toxicity was seen only by study required echocardiograms and the children had no clinical symptoms.

In addition, prospective monitoring of cardiac toxicity on all arms of the study demonstrated that both shortening fraction (SF) and ejection fraction (EF) declined significantly more in patients treated with sorafenib than patients treated with standard chemotherapy. Specifically, the mean SF difference was -1.96% (95% CI -3.02% - 0.9%, p = 0.003) and the mean EF difference was -3.57% (95% CI -5.21% - -1.92%, p < 0.001) when comparing patients treated with standard chemotherapy plus sorafenib and patients treated with standard chemotherapy alone.

Because of concerns for cardiotoxicity, a safety memo was released on 04/27/2015 that mandated a change in the sorafenib dosing schedule for patients during Induction II, Intensification I and, for the limited subset of patients without a SCT donor, Intensification II (as reflected in Amendment #6A).

While the precise mechanisms for sorafenib related cardiotoxicity is not currently known, a number of mechanisms have been postulated based on preclinical models.^{29,30} VEGF is an important regulator of

angiogenesis and VEGF/VEGFR-targeted therapeutics, including sorafenib, block endothelial proliferation and promote apoptosis causing regression of cardiac vasculature. Moreover, inhibition of the VEGF/VEGFR pathway impacts nitric oxide production by endothelial cells, resulting in hypertension.²⁹ Importantly, in patients with poorly controlled hypertension, inhibition of VEGF/VEGFR may have even greater sequelae. In a mouse model of increased pressure load, inhibition of angiogenesis resulted in reduction in myocardial capillary density promoting global contractile dysfunction.^{31,32} A second mechanism for cardiotoxicity may reflect sorafenib's impact on the KIT receptor tyrosine kinase. KIT, the receptor for stem cell factor, is normally expressed on endothelial progenitor cells (EPCs) and proper functioning of this receptor is needed for mobilization of EPCs to sites of injury. As sorafenib inhibits KIT, homing to sites of cardiac injury after a myocardial event may be impaired in the context of sorafenib treatment, a finding supported by a mouse model of sorafenib induced cardiotoxicity.^{4,30} PDGF may also be protective in the context of an ischemic heart and it remains of concern that inhibition of endogenous PDGFR signaling by receptor tyrosine kinases inhibitors, like sorafenib, may be detrimental.^{3,29} Finally, Raf family inhibition by sorafenib may also have deleterious cardiac effects. Raf-1 has an important role in cardioprotection, particularly in the context of pressure overload stress, and deletion of this serine/threonine kinase has been found to result in cardiomyocyte apoptosis and fibrosis.¹

Currently, the optimal schedule of TKIs such as sorafenib administration with standard AML chemotherapy is not known. Both sunitinib and tandutinib have been found to have synergistic effects in combination with both cytarabine and anthracyclines; in the context of tandutinib, this was sequence independent.^{8,9} Importantly however, *in vitro* data of a third TKI, lestaurtinib, demonstrated that co-administration of both lestaurtinib and daunorubicin, both of which are normally protein bound, resulted in competition for protein binding sites and subsequently increased unbound levels of both drugs with resultant cytotoxicity.²

While the initial clinical trials of sorafenib in combination with conventional chemotherapy included concomitant dosing, these treatments were characterized by short duration of sorafenib exposure and/or non-anthracycline regimens.^{6,26,27} Notably, an excess in cardiac toxicity was not observed with such regimens though final analysis of the de novo younger adult AML cohort treated with idarubicin/cytarabine and sorafenib did demonstrate that 6/62 (9.6%) patients experienced Grade 3 or higher cardiac toxicity or hypertension. One patient died of a massive MI during treatment though this was not considered related to sorafenib.⁶ A subsequent elderly AML trial studied sorafenib in a more sequential fashion. In this study by Serve et al, patients received (7 + 3) cytarabine/daunorubicin followed by a 3 day washout and then single agent sorafenib until 3 days before the next chemotherapy course. Consolidation with intermediate dose cytarabine was followed by a similar sorafenib approach. For those who remained on study following Consolidation, an additional period of sorafenib maintenance (up until 1 year from start of treatment) was also possible. With this design, considerable Induction toxicity was observed for patients treated with sorafenib. Though toxicity was largely infectious in nature, Grade 3 or greater Induction cardiac toxicity was observed in 6/104 (5.8%) patients treated with sorafenib.⁵ A more recent abstract published by Rollig et al³³ also utilized a treatment approach in which prolonged sorafenib exposure was delayed until after completion/washout from conventional chemotherapy, the latter of which included anthracyclines (daunorubicin and mitoxantrone). Reported toxicity data is limited to date but does not include reports of significant cardiac effects. Similarly, the Phase I pediatric trial of the alternative TKI quizartinib, in combination with cytarabine/etoposide, utilized a dosing schema in which quizartinib was not initiated until after conventional chemotherapy completion.³⁴ Using this design, there were no significant cardiac toxicities observed (Todd Cooper, personal communication).

Since the precise mechanism of the observed cardiac toxicity is not fully understood, the impact of a sorafenib schedule change on cardiac toxicity risk is unknown. However, given the potential interaction of sorafenib with anthracyclines and limited cardiac toxicity reported by Serve et al and Rollig et al with sequential dosing of anthracyclines/sorafenib despite prolonged TKI exposure, the AAML1031 dosing schema was modified in Amendment #6A to avoid concomitant sorafenib/chemotherapy exposure and

ensure adequate washout between sorafenib and daunorubicin for Induction I and II. Given the low rate of cytarabine/etoposide associated cardiac toxicity, a washout period between cytarabine/etoposide and sorafenib was not included in Intensification I.

2.3.2 Rationale for Extending Study Accrual on Arm C and Creation of Arm D (Amendment #7A)

Extending study accrual to Arm C is necessary to meet the protocol specified specific aims, particularly determining the feasibility of administering sorafenib with standard AML chemotherapy (Aim 1.1.4) and assessing the anti-leukemic activity of sorafenib (Aim 1.2.1). The current enrollment on Arm C is 54 patients (12 patients in Cohort 1, 34 patients in Cohort 2, 5 patients rolling over to Cohort 3 upon reopening with Amendment #6A, and 3 patients starting therapy in Cohort 3) with a target sample size of 80 patients in Cohort 2. The unexpected lower accrual on Arm C is due to multiple periods in which Arm C has been closed, particularly during the approval process for Amendment #4 that added a sorafenib maintenance treatment course. Additional enrollment to Arm C will substantially improve both the feasibility and efficacy data from the AAML1031 trial. In addition, extended accrual on the sequential chemotherapy/sorafenib administration schedule will enable a comparison of the different sorafenib dosing schedules used in the trial and facilitate more granular cardiac toxicity analyses.

As Arms A and B are now closed, Arm D has been created in order to enroll patients on study whose FLT3/ITD allelic ratio is unknown prior to study entry. Patients who enroll on Arm D will receive ADE 10+3+5 Induction therapy, which is the standard Induction regimen for children with *de novo* AML.³⁴

2.4 Rationale for Sorafenib Maintenance for HR FLT3/ITD+ AML Patients

2.4.1 Overview

Single agent sorafenib in the post-SCT period was initially reported in 3 adult AML patients with evidence of FLT3/ITD+ disease relapse during the first year following SCT. Despite lack of standardized dosing (100 - 400 mg/dose) sorafenib was reasonably well tolerated and CR observed in 3/3 patients, including one complete molecular remission. All patients were able to receive dosing for more than 75 days though the drug was held intermittently for marrow suppression.³⁵ The drug was also used in a similar fashion in a 4th reported patient, also with good effect.³⁶ A subsequent abstract provided updated information regarding use of sorafenib post-SCT in 16 patients who recurred post-SCT. Patients received between 200 mg and 800 mg sorafenib daily. In this group, there were 3 partial responses, 2 hematologic responses, 7 bone marrow responses and 4 complete molecular responses. In general, sorafenib was well-tolerated with Grade 3/4 pancytopenia and thrombocytopenia the most significant, but manageable, side effects observed.³⁷ In 2011 Sharma et al described outcomes for n = 16 adult patients with relapsed *FLT3/ITD+* AML following allogeneic SCT. In this series, patients were treated with either sorafenib alone (n = 8) or in combination with cytotoxic chemotherapy (n = 8). While the therapy was well-tolerated overall, there were no patients that survived beyond one year. This finding may reflect the very high risk nature of this study cohort.³⁸ A more recent retrospective review of 65 adult patients with *FLT3/ITD+* AML that were treated with sorafenib demonstrates more compelling results, particularly for the 29 patients treated with sorafenib following SCT. Sixty-three of 65 patients (96%) had relapsed or refractory disease suggesting this was a very high-risk group of patients and yet, overall, 25/65 (38%) achieved morphologic CR, complete morphologic remission with incomplete peripheral blood count recovery (CRi) or complete molecular response (CMR). Rates of CR/CRi/CMR were 11/36 (30%) for patients treated in the absence of SCT versus 14/29 (48%) for patients who received sorafenib following transplantation. Notably, in the post-SCT cohort, 7/29 (24%) patients achieved complete molecular response (CMR), a significantly higher rate than that observed in the setting of conventional chemotherapy (CMR 3/36 = 8%). Moreover, sorafenib induced remissions following HCT lasted, on average, significantly longer than patients treated with sorafenib + conventional chemotherapy. Specifically, 17/36 (47%) of patients without prior SCT developed sorafenib resistance after median treatment duration of 136 days whereas post-SCT patients developed sorafenib resistance less frequently (38%) and significantly later (median 197 days, *P* = .03). Three patients in the

post-SCT cohort have already survived more than 2 years without disease recurrence.³⁹ Together these findings suggest that there is a potentially curative synergism between sorafenib and an allo-immune mediated anti-leukemic effect.³⁹ In this study the median dose of sorafenib was 600 mg daily for the post-SCT cohort (versus 486 mg daily in the chemotherapy group) and the median duration of therapy 74 days (range 1 - 270 days) for this subset of patients. The drug was overall well-tolerated though Grade 3/4 pancytopenia was seen in 40/65 (62%) of the total cohort. Additional toxicities described for the entire study cohort included infection (n = 17), hand-foot syndrome (n = 8), rash (n = 12), mucositis (n = 7), hepatotoxicity (n = 5), hypertension (n = 4), cardiac decompensation (n = 2), GI perforation (n = 1), GI bleeding (n = 1), intracranial bleeding (n = 1), neurotoxicity (n = 1) and non-specific cardiac arrhythmia (n = 1). In 10 instances, such toxicities were fatal (n = 9 infections, n = 1 cardiac decompensation, n = 1 GI perforation, n = 1 intracranial bleed). For the post-HCT cohort, 1 case of severe liver GVHD and 1 case of severe skin GVHD were observed. An additional 5 patients developed mild (< Grade 2) skin GVHD.³⁹

At present there are 2 cooperative adult studies open to study this treatment approach in greater detail. The first study, conducted by the Dana-Farber Cancer Institute, is a Phase I study of sorafenib post-SCT for *FLT3/ITD+* patients age 18 - 75 years. Patients initiate treatment Day 60 - 100 post-SCT and will receive treatment for 1 year. Three dose escalations and 1 de-escalation are planned. As of 9/2012, 7 patients had been enrolled without any DLTs to date and patients continued to be enrolled on the maximum dose planned (400 mg PO BID). (Personal Communication with Study PI, Dr Yi Bin Chen) A second post-SCT multicenter study is being conducted in Germany for *FLT3/ITD+* patients > 18 years. This study is a randomization to +/- sorafenib. For patients on the treatment arm, sorafenib is initiated at Day 60 - 100 post-SCT and is continued for 2 years. The starting dose of sorafenib is 400 mg PO daily (220 mg/m²/day) and assuming this dose is tolerated, the dose is increased over a period of 6 weeks to 800 mg PO daily (440 mg/m²/day). As of 04/2012 a total of 16 patients have been enrolled on this study. The dosing schema has been tolerated to date without significant adverse events and or GVHD observed. Notably, accrual has been significantly limited by the fact that treating physicians are not willing to accept randomization to the observation arm. (Personal Communication with Study PI, Dr Andreas Burchert)

2.4.2 Pediatric Post-SCT Experience

Given the encouraging results described above, sorafenib has been used in a subset of high-risk pediatric AML patients. A retrospective review of unpublished experience at several COG institutions, coupled with 2 published series,^{27,28} suggests that this approach is feasible and has an acceptable toxicity profile. Of 22 pediatric AML patients treated with sorafenib post SCT, the median dosing of drug used was 200 mg/m²/day (range 110-400 mg/m²/day). Overall, the regimen was well tolerated. Specifically, 16/22 (73%) patients had reported toxicity however Grade 3/4 toxicity was relatively low with n = 3 patients experiencing neutropenia, n = 1 rash/skin pain, n = 3 transaminitis, n = 1 hyperbilirubinemia and n=1 sepsis. One additional patient had Grade 4 cardiac toxicity that was possibly related to sorafenib, although additional confounding factors existed. Seven out of 22 (32%) patients who experienced toxicity had their sorafenib dose held and restarted at the same or a lower dose of drug without recurrence of symptoms. An additional 6 patients with toxicity resolved such toxicity without dose modification. Notably, of 5 patients with dose limiting toxicity, 3/5 were treated with exceptionally high doses of therapy (340 - 400 mg/m²/day).

Of particular interest is the potential impact this therapeutic approach had on 15 pediatric *FLT3/ITD+* patients treated with sorafenib following allogeneic SCT. Specifically, of 10 *FLT3/ITD+* patients treated for relapse post-HCT (n = 8 morphologic, n = 2 with MRD), approximately 70% remain disease-free at a median of 12.5 months post start of therapy (range 2.5 - 48 months). Three additional *FLT3/ITD+* patients received the drug prophylactically post-SCT given the high-risk nature of their disease and an additional 2 patient received such treatment given MRD preceding, but not following, SCT. Of this cohort, 4/5 (80%) have remained disease-free for a median of 10 months (range 1 - 16 months). Interestingly, the 1 patient

who recurred had, at time of recurrence, absence of the *FLT3/ITD*+ clone, suggestive of clonal evolution and selection for a minor clone at time of disease recurrence. (Pollard et al. manuscript in preparation)

The Seattle pediatric experience with single agent sorafenib post-SCT in *FLT3/ITD*+ AML suggests that the drug may facilitate clearance of MRD in the post-SCT setting. However, the initial dosing of sorafenib used to treat our patients is not standardized and drug toxicity/intolerance has necessitated doses far lower than that of the defined MTD for relapsed pediatric leukemias.⁴⁰ Given limited data on post-SCT sorafenib use, its impact on pre-existing GVHD remains unclear. Together these findings suggest that a formal study to describe the feasibility of administering sorafenib following SCT is necessary, towards the goal of ultimately improving outcomes for this high-risk patient population.

2.5 Health Related Quality of Life and Parental Stress

Health related quality of life (HRQOL) has become increasingly important in clinical trials of children undergoing cancer therapy and is of particular relevance in childhood AML. AML treatment clearly substantially impacts HRQOL. Short and long term complications following therapy can be quite extensive and are dependent on a variety of factors including chemotherapy regimen, radiation therapy, age, gender, and acute toxicities of therapy. Possible long term sequelae include cardiopulmonary toxicity, second malignancies, infertility, and endocrinopathies.⁴¹ In one study from the Childhood Cancer Survivor Study,⁴² 50% of long term AML survivors had a chronic physical health conditions and 16% had a significant health condition. We lack prospectively collected HRQOL data from large numbers of children with AML receiving homogeneous therapy and in particular, lack good data which examines HRQOL among children treated with stem cell transplant (SCT) and chemotherapy. The measurement of HRQOL is important for several reasons. First, it will help to better understand the expected consequences of therapy and to inform future interventional trials designed to improve HRQOL associated with both SCT and chemotherapy. Second, understanding and quantifying longitudinal changes in HRQOL associated with chemotherapy and SCT treatment will allow us to see how HRQOL changes and when interventions may be most beneficial. Finally, as an exploratory aim, the measurement of HRQOL will further clarify the overall impact of SCT on patients and the overall risk to benefit ratio of SCT.

Despite this clear need, previous work in pediatric AML HRQOL has been very limited. In one of the larger studies by Nicholson et al,⁴³ HRQOL was described in approximately 200 survivors of AML treated on COG trials. Median age at diagnosis was 3 years and age at HRQOL assessment (SF-36) was 19 years. One hundred and twenty four patients were treated with chemotherapy only, 54 with allogenic SCT and 28 with autologous SCT. HRQOL summary scores and sub-scale scores did not differ between those receiving chemotherapy only and those receiving SCT. However, this study was limited by the small number of patients receiving SCT and the cross-sectional nature of the study. Studies have been summarized by Redaelli and colleagues who examined 21 articles that reported on HRQOL in AML patients.^{44,45} Most studies were cross-sectional, post-treatment, and only included adult patients. Clarke et al.⁴⁶ reviewed the literature regarding pediatric SCT and HRQOL; however, studies were small and utilized different assessments in various disease groups. Therefore, there is a lack of information on HRQOL from pediatric AML patients obtained prospectively that compares status during and following chemotherapy or SCT. Furthermore, almost nothing is known about the parenting stress of parents of children treated for pediatric AML. Parenting stress related to caring for a child with an illness can influence the family structure and cohesiveness and impact patient HRQOL.⁴⁷⁻⁴⁹ While this knowledge is less likely to contribute to decision-making, it will help for anticipatory guidance and planning of future intervention trials.

Thus, we believe that both patient HRQOL and parenting stress are important in pediatric AML. This study will provide a unique opportunity to prospectively assess HRQOL in comparable groups of AML patients on a prospective basis in order to definitely describe HRQOL in those receiving SCT and chemotherapy. In addition, longitudinal assessment of HRQOL and determination of predictors from demographic and treatment characteristics and pediatric parenting stress will allow for more accurate identification of

potentially modifiable risk factors for poorer HRQOL and determine how HRQOL changes over time. This in turn will inform future development and evaluation of early, targeted interventions to improve HRQOL and reduce pediatric parenting stress. For children 2 to 18 years of age, measures of HRQOL will be the Pediatric Quality of Life (PedsQL) 4.0 Generic Core Scale,^{50,51} the PedsQL 3.0 Cancer Module,⁵² and the Multidimensional Fatigue Scale.^{52,53} These have been found to be reliable and valid in this population.⁵² For HRQOL assessments, parents will provide proxy assessments for all patients while for patients ≥ 5 years of age, self-report also will be completed by the child (if able and agrees). Parental stress will be assessed using the Pediatric Inventory for Parents (PIP) scale.^{54,55} The PIP was developed to measure parenting stress related to caring for a child with an illness. PIP has been found to be a reliable and valid tool assessing parental stress in the pediatric oncology population.⁵⁵ This instrument will only be completed by parents or guardians.

2.6 Dexrazoxane Use in Pediatric AML

Patients treated with MRC based AML chemotherapy receive high cumulative anthracycline exposures and are at high risk for long term cardiac complications.⁵⁶⁻⁵⁸ Despite this, the available data on the risks and benefits of cardioprotection with dexrazoxane in pediatric AML patients are limited to a single institution case series. Sanchez-Medina et al⁵⁹ reported on 50 patients treated according to the MRC10 protocol and appeared to benefit from the use of dexrazoxane. However, no control group was reported in this study, thus limiting the conclusions of this report. Since dexrazoxane is used by some centers for cardioprotection with AML, this study will prospectively collect data on dexrazoxane use and cardiac outcomes (shortening fraction and ejection fraction) at the end of each course of chemotherapy and in follow-up. These data will enable a comparison of cardiac complications in patients treated with and without dexrazoxane. Dexrazoxane use is per institutional preference and is NOT mandated by the AAML1031 protocol.

2.7 Rationale for Changes in GVHD Prophylaxis and SCT Conditioning Regimen

A survival benefit, presumably due to graft-versus-leukemia effects, following family donor allogeneic SCT compared to chemotherapy only approaches, for children with AML has been shown in sequential randomized cooperative group studies.^{60,61} The benefit of allogeneic SCT for children with AML was confirmed in a recent meta-analysis, which included 1,373 pediatric patients with AML in first complete remission (CR1). In the intermediate-risk group, the estimated disease-free survival (DFS) at 8 years for patients who did not undergo transplantation was $39\% \pm 5\%$, whereas it was $58\% \pm 7\%$ for family donor allogeneic SCT patients.⁶² In all of these analyses, some of the survival benefit was offset by increased treatment related mortality (TRM) in the SCT-treated patients. Transplant-related death is the culmination of a complex pathophysiology that includes conditioning regimen related effects, donor source, and graft-versus-host disease (GVHD). Donor source selection is determined by factors outside investigator control, such as the availability of a family member match or unrelated donor. The 2 areas that can be readily incorporated into the treatment design are GVHD prophylaxis and conditioning regimen.

The GVHD prophylaxis schema recently developed for ASCT0431 represents a consensus approach developed by the COG hematopoietic stem cell transplant (HSCT) committee covering diverse donor sources including matched family member, unrelated adult donor, and cord blood stem cell sources. It is logical, therefore, to include the ASCT0431 standard arm in this study (AAML1031) as the GVHD prophylaxis regimen. While the standard GVHD prophylaxis regimen does not attempt to improve upon TRM, incorporating this regimen in this study has the advantage of creating a standard approach and baseline for future comparisons across the 2 major indications for allogeneic SCT in children. The conditioning regimen for allogeneic SCT in children with AML (myeloablative doses of busulfan and high doses of cyclophosphamide) treated on POG/CCG/COG studies has not been updated in over 20 years, with the exception of the change from oral busulfan to intravenous (IV) busulfan and dosing based on pharmacokinetics in AAML0531. The combination of myeloablative busulfan and high doses of cyclophosphamide (200 mg/kg) has a well-documented track record, but this includes high rates of veno-occlusive disease (VOD) at 20% - 50%,^{63,64} hemorrhagic cystitis (5% - 15%),⁶⁵ and TRM.

Cyclophosphamide metabolism has been directly linked to VOD.⁶⁶ Cyclophosphamide is included in the conditioning regimen primarily for its immunoablative effects. In recent years, fludarabine has been substituted for cyclophosphamide in order to avoid the cyclophosphamide toxicity with excellent results. Falling VOD rates after SCT in recent years can be attributed to the increasing use of fludarabine over cyclophosphamide in the conditioning regimen.⁶³ Fludarabine, a nucleoside analog, has the attractive properties of potent immunosuppression as well as synergy with alkylating agents against leukemia.⁶⁷ In fact, fludarabine, in combination with cytarabine and idarubicin, was used for its anti-AML effects in the predecessor study CCG2961.

Most recently, a combination of fludarabine together with myeloablative doses of busulfan has been shown to be highly effective with rates of TRM ranging from 1% - 7% in adult patients with myeloid leukemias even when alternative donors, such as unrelated donors, were used.^{68,69} The lowest rates of TRM were seen when busulfan was administered as a once-daily IV dose for 4 days (FluBu4) and dose adjusted based on pharmacokinetics (PK). The graft failure rate was approximately 1%. Anti-thymocyte globulin (ATG) was used to facilitate engraftment in recipients of unrelated donors. In unpublished data from the University of Michigan, FluBu4 without ATG resulted in 100% engraftment in 56 patients receiving unrelated donor SCT. In order to compare the FluBu4 experience with BuCy, the MD Anderson group performed a Bayesian analysis of outcomes between nonrandomized patients (148 FluBu4, 67 BuCy). Both groups had comparable pretreatment characteristics, except that FluBu4 patients were older (46 versus 39 years, $p < 0.01$), more often had unrelated donors (47.3% versus 20.9%, $p < 0.0003$), and had shorter median follow-up (39.7 versus 74.6 months). Patients transplanted with FluBu4 in CR1 had a 3 year OS and EFS of 78% and 74%, respectively, whereas CR1 patients younger than age 41 years had a 3 year EFS of 83%. These results compared favorably to 3 year OS and EFS rates of 42% in the BuCy treated patients.⁷⁰

Fludarabine and busulfan conditioning regimens have been tested in pediatric populations. Pulsipher reported 2 year EFS of 40% with TRM of 11% in 47 children with very high risk malignancies who could not undergo a traditional myeloablative conditioning regimen for reasons that included prior myeloablative SCT.⁷¹ Excellent outcomes using fludarabine and busulfan have also been seen in children with both malignant and non-malignant diseases after related, unrelated, and cord blood SCT.⁷²⁻⁷⁵ Unpublished center for international blood and marrow transplant research (CIBMTR) data showed 1 year OS of 73% in 40 children (age < 18 years with AML, sibling donor) conditioned myeloablative doses of fludarabine/busulfan compared to 63% in 200 children conditioned with BuCy (Mary Eapen M.D, personal communication). The data was unadjusted for any other risk factors, but does support the premise that FluBu4 can be considered at least roughly equivalent to BuCy, if not superior. Since 2005, children with AML under the age of 18 years have received myeloablative fludarabine/busulfan for SCT in 8% of cases reported to CIBMTR, making this regimen the third most common regimen after BuCy and total body irradiation (TBI) based conditioning regimens. Given the above data, and a compelling interest in reducing TRM and improving efficacy of SCT, the COG HSCT committee outlined a strategy in 2009 to support a transition from BuCy to FluBu4 to fludarabine/treosulfan in a sequential series of studies for children with AML. Revising the conditioning regimen in this study is in accordance with this aim.

2.8 Biology Correlative Studies

2.8.1 MRD Based Risk Stratification

Utility of MRD in risk identification in childhood AML has been shown in legacy CCG2961.⁷⁶ These findings have further been validated in a prospective study in AAML03P1, where post remission specimens were evaluated for the presence of disease by 4-color multidimensional flow cytometry (MDF), and the presence of disease was correlated with outcome (manuscript in preparation).⁷⁷ End of Induction I specimens were evaluated for evidence of disease by MDF. Disease was detected in 69 of 222 evaluable patients (31%) at various MRD levels, ranging from 0.02% to 43% (median 1.5%). Two patients had MRD

levels < 0.1%, and 6 patients reported to be in morphologic CR had leukemia cells > 5% by MDF. Forty-five of 191 patients (24%) in morphologic CR had a measurable MRD. At the end of Induction I, 27 patients were in PR (5% - 20% blasts, n = 15) or were refractory to initial Induction (> 20% blasts, n = 12). Disease assessment by MDF in these patients revealed that 40% of the patients with PR (5% - ≤ 20% blasts) had no evidence of disease by MRD, whereas, in those with refractory disease, all but one had disease by MDF. Thus, MDF identified residual disease in patients in morphologic CR as well as identified patients with reported morphologic disease who did not have immunophenotypic evidence of disease. Clinical implications of MRD were assessed in patients who had a response to initial chemotherapy (Induction I). Presence of MRD by MDF at the end of Induction I was correlated with relapse risk (RR) and DFS from end of Induction I. Patients with evidence of disease by MDF had a RR at 3 years of $64 \pm 20\%$. In contrast, cumulative RR was $25 \pm 11\%$ in those without MRD ($p < 0.001$). Corresponding DFS at 3 years from initial Induction was $32 \pm 14\%$ for those with MRD compared to $65 \pm 9\%$ to those without MRD ($p < 0.001$). The impact of MRD on patients who were in morphologic CR were evaluated separately. Presence of MRD in patients in CR (n = 191) was associated with a RR of $59 \pm 16\%$ compared to that of $28 \pm 8\%$ in patients without MRD ($p < 0.001$). Corresponding DFS was $31 \pm 15\%$ vs. $65 \pm 9\%$ in those with and without MRD ($p < 0.001$). It was further evaluated whether presence of MRD beyond the Induction I carries clinical significance. One hundred and eighty-five patients had MRD data available at the end of Induction II of whom 36 patients (19.5%) had evidence of disease by MDF ranging from 0.1% to 50% (median 1.0%). Cumulative RR at 3 years from end of Induction II in patients with MRD was $68 \pm 17\%$ compared to $28 \pm 8\%$ in those without MRD ($p < 0.001$) with a corresponding DFS of $28 \pm 17\%$ and $65 \pm 9\%$ respectively ($p < 0.001$). At the end of Intensification III (end of therapy), 7 of 91 patients (8%) were MRD positive. Patients with MRD at the end of therapy had a RR of $86 \pm 26\%$, compared to that of $37 \pm 11\%$ for the MRD negative patients ($p < 0.001$) with a corresponding DFS of $14 \pm 26\%$ and $62 \pm 11\%$, respectively ($p < 0.001$). It is of note that of the 7 patients who were MRD positive at the end of therapy, 3 had a previously documented MRD and in 4 patients, MRD emerged during therapy. We inquired whether clearance of initial MRD correlates with improved outcome. Ninety-one patients had MRD data available at the end of therapy, of which 7 (8%) were MRD positive and the remaining 84 were MRD negative. Of the 84 patients who were MRD-negative at the end of therapy, 22 patients had a previously documented MRD at the end of Induction I or II. The clinical outcome from end of therapy for MRD-negative patients with or without a previously documented MRD was evaluated. Relapse risk at 3 years from end of therapy for MRD positive patients was $86\% \pm 26\%$ vs. $64 \pm 20\%$ for those with a history of MRD and RR of $25 \pm 11\%$ for those without previous MRD ($p < 0.001$).

Prognostic Factors: Prognostic factors were evaluated for RR and DFS from end of Induction I. Cox regression analysis was used to evaluate MRD status, cytogenetic/molecular favorable risk (CBF AML, *NPM* and *CEBPα* mutations), unfavorable risk (-7, -5/del5q, HR FLT3/ITD+), and diagnostic WBC, as predictors of DFS and RR in a univariate model. In the univariate model, the presence of a MRD was a significant prognostic factor for higher relapse risk (HR 2.7, $p < 0.001$) and worse DFS with an HR of 2.47 ($p < 0.001$). In separate univariate models, molecular high-risk patients had a worse DFS with a HR of 1.94 ($p = 0.039$), and those with favorable risk AML had an improved DFS (HR 0.58, $p = 0.02$) compared to those without standard risk AML. In a multivariate model that included the above-mentioned prognostic factors, the presence of MRD remained an independent prognostic factor for higher RR (HR = 2.43, $p < 0.001$) and lower DFS (HR = 2.41, $p < 0.001$).

Implications of MRD in specific risk groups in AML: The clinical implications of MRD in specific risk classes identified by cytogenetic and molecular alterations was assessed. Of 206 patients with CR/PR at the end of Induction I, 195 patients had complete cytogenetic and molecular data available, 91 of which had favorable (CBF AML, *NPM* and *CEBPα* mutations, n=73) or unfavorable (HR FLT3/ITD+, -7, -5 or del5q, n = 18) features. The remaining 104 patients without favorable or unfavorable features were regarded standard risk, of whom 32 (31%) had MRD ranging from 0.02% to 22% (median 2%). Standard risk patients with MRD had a DFS at 3 years from end of Induction I of $34 \pm 18\%$ vs. $65 \pm 13\%$ for the MRD negative

patients ($p = 0.006$). Of the 73 patients with favorable risk features, 12 had MRD (16%) at the end of Induction I. In this favorable risk cohort, DFS at 3 years from CR for those with and without MRD was similar ($p = 0.138$). Eighteen patients were deemed high risk based on their cytogenetic or molecular features, of which 8 patients had MRD (44%).

Implications of pre-transplant MRD: Hematopoietic cell transplantation (HCT) is utilized in the treatment of a subset of children with AML. Above it was demonstrated that the presence of MRD is associated with a high relapse rate in children with AML, however, the clinical implications of MRD prior to HCT have not previously been defined. We explored the clinical outcomes associated with the presence of morphologic or sub-morphologic disease prior to HCT.⁷⁸ In the period of 1995 - 2007, 89 patients age 1 - 18 years received myeloablative conditioning and allogeneic HCT from related ($n = 36$) or unrelated ($n = 53$) donors at Fred Hutchinson Cancer Research Center. At the time of HCT, 63 patients received transplants in CR and 26 had morphologic disease. Among those in morphologic CR, 43 were in CR1, 17 in second complete remission (CR2) and 3 were in second complete remission (CR3). All patients had bone marrow evaluation for MRD by MDF 2-4 weeks prior to HCT. MDF testing showed that 10 of 63 patients (16%) who received transplants in morphologic CR had MRD levels ranging from 0.3% to 12% (median 3.7%) while the remaining 53 patients had no detectable MRD. The median follow up for the 38 survivors is 6 years (range 1 – 12 years). Actuarial relapse rate for those with MRD was 65% vs. 17% for those without MRD ($p < 0.001$). Corresponding OS for patients who received transplants in morphologic CR with MRD was 15% vs. 67% for those without MRD, $p = 0.001$. This data suggests that MRD data at the end of Intensification I would provide significant insight into the post-transplant outcome of patients who are to receive stem cell transplant.

While MRD has prognostic significance for risk stratification at the end of Induction I, standard morphology criteria will be used to determine remission induction and relapse rate. Standard morphological remission criteria will be used for several reasons. First, morphology remains the standard measurement of disease response in all pediatric and adult cooperative groups. Thus, use of standard morphological criteria will facilitate comparisons of AAML1031 outcomes with other cooperative group trials. Second, AAML1031 standard therapy has omitted the fifth course of chemotherapy. Evaluation of the efficacy of the AAML1031 chemotherapy will require comparison with prior trials that have used morphologic criteria. Finally, additional work is needed to determine the reproducibility of centrally performed 4-color flow cytometry in other clinical laboratories.

2.8.2 Evaluation of the prognostic significance of molecular MRD and its contribution to risk identification with MDF based MRD in patients with translocations amenable to quantitative RT-PCR (e.g., $t(8;21)$, $inv(16)$, $t(9;11)$, *WT1* expression.

Molecular markers have been considered candidates for post remission disease status assessment to be utilized for outcome prediction. Specific translocations including $t(8;21)$, $inv(16)$ and $t(9;11)$ are common in childhood AML and provide for excellent tools for MRD assessment in a large cohort of patients. In addition, expression levels of *WT1* gene have been associated with relapse risk in AML. Ribonucleic acid (RNA) extracted from remission specimens will be evaluated for quantitative expression of specific fusion transcripts as well as for *WT1* expression levels. Molecular MRD will be correlated with MRD by MDF to determine whether molecular MRD provides any additional information to that obtained from MDF.

2.8.3 Determination of the leukemic involvement of hematopoietic early progenitor

We hypothesize that the extent of leukemic involvement of the early hematopoietic progenitors may be associated with response to chemotherapy, where those with leukemia limited to more differentiated progenitors would have improved outcome. In contrast, those with leukemic involvement of more primitive stem/progenitor population would be more resistant to conventional chemotherapy and may require more stem-cell targeted therapy (e.g., bortezomib) or stem cell transplantation. It has been demonstrated that early progenitor expression of the FLT3/ITD was associated with significantly worse outcome,⁷⁹ compared

to those where disease was limited to more differentiated cells. We will use early progenitor isolation and ex-vivo functional analysis, including stromal based culture, combined with molecular genotyping or clonal analysis based on expression of polymorphic X-linked genes in heterozygous females to determine early progenitor involvement and correlate it to response to therapy.

2.8.4 Definition of the Leukemia Stem Cell Population in Patients with AML

The stem cell model proposes that AML arises from a biologically distinct subpopulation of cells referred to as leukemia initiating cells (LIC) with a capacity to self-renew, differentiate, and give rise to non-self-renewing progeny. Additional data supports a key role for LIC in disease relapse. Murine models of AML have largely confirmed the observations made in primary human samples. While similar in some characteristics to normal hematopoietic stem cells (HSC), LIC have been shown to possess traits that are distinct from normal HSC including antigen expression.^{80,81} These differences allow them to be physically separated from normal HSC and may allow novel therapies to target LIC while sparing their normal HSC counterpart. Efforts to design therapies based on this model include drugs that target antigens on the surface of the LSC as well as agents that selectively deplete LSC, while sparing normal HSC.⁸²⁻⁸⁴ The underlying premise to the above studies is that the stem cell model is relevant to a majority of patients with AML in all stages of disease but there is little data that supports this assumption. The ability to identify and isolate the LIC populations from primary human AML samples has allowed the identification of several key molecular features of this population of leukemic cells. Dr. Becker and Dr. Jordan's labs have identified LIC specific pathways using a genome wide approach.⁸⁵⁻⁸⁷ Expression profiles of highly enriched leukemic CD123CD34+CD38- cells and enriched normal HSC were analyzed and the expression profiles of leukemic and normal stem cell populations compared. This approach identified signaling pathways that are aberrantly regulated in LIC. Dr. Jordan's lab demonstrated the aberrant activation of the NF- κ B pathway in LIC and altered LIC response to changes in oxidative state.⁸⁵ This data suggested that LIC may be more sensitive to bortezomib than HSC or the bulk leukemia population. Dr. Jordan's lab demonstrated that AML stem cells are selectively sensitive to proteasome inhibition and that proteasome inhibitors are synergistic with cytotoxic AML agents such as idarubicin.⁸⁸ Dr. Becker and his colleagues identified additional aberrantly regulated pathways in LIC, including the MAP kinase pathway, Wnt signaling, ribosomal proteins, the actin cytoskeleton and tight junction pathways.⁸⁷ Several of the pathways identified by our analysis had not been previously implicated in the regulation of LIC function. These differences between normal and malignant stem cell compartments of the bone marrow represent potential targets to improve AML therapy for this deadly disease. However, it is critical to determine the frequency at which these pathways are disrupted in a cohort of patients with AML as well as determine if these findings are stable during treatment. To accomplish the task of studying LIC throughout the course of disease, Dr. Becker and Dr. Jordan's labs have collaborated on the development of a flow cytometry platform that will permit the separation of potential LIC populations from the normal cell population at time of diagnosis, in remission and at relapse. The putative leukemia initiating cell-leukemia aberrant immunophenotype (LIC-LAIP) will be identified using a 10-color flow sorting strategy. It is not possible to enumerate LIC using 4-color flow cytometry. The published phenotype of CD123+CD45^{dim}CD34+CD38- (LIC) has been shown to be sufficient to isolate a population of cells with LIC properties from many patients at time of high disease burden.^{81,89} In contrast to the study of de novo and relapsed samples, the above phenotype has been shown to be insufficient for the study of LIC in remission. Schuurhuis et al reported that by using a panel of antigens such as CLL-1, CD5, CD7, CD19 and CD56, he was able to identify leukemic (CD45^{dim}CD34+CD38-) cells in the remission samples of patients undergoing treatment for AML.^{89,90} While an aberrant population of CD45^{dim}CD34+CD38- cells was identified in the majority of patients, there was significant inter-patient heterogeneity in informative cell surface markers. Building upon this effort, we have characterized the surface antigen profile of over 20 different antigens on normal and leukemic CD123+CD45^{dim}CD34+CD38- cells. These antigens were chosen based on prior published studies as well as data from expression profiling of LIC. AML samples used for this effort were obtained from patients at time of high disease burden to decrease the potential for residual normal marrow elements contamination. We found significant variability between patients with respect to surface antigen expression on the leukemic

CD123+CD45^{dim}CD34+CD38- population. In contrast the expression profile for normal CD123+CD45^{dim}CD34+CD38- cells was conserved. Two antigens, BB9 and CD62L, expressed on normal CD123+CD45^{dim}CD34+CD38- cells, were absent on same population for the majority of patients with AML. In addition to BB9 and CD62L, for many of the AML samples examined, aberrant expression of at least 1 additional antigen was identified. Using the gating strategy employed, we were able to identify a leukemia specific expression profile that may be employed to identify the LIC population in remission.

2.8.5 Prevalence and Prognostic Significance of Molecular Abnormalities of *WT1*, *RUNX1*, *MLL-PTD*, *TET2*, *c-CBL*, *KIT* and other novel AML Associated Genes in Pediatric AML

As part of our continued efforts to identify molecular alteration in AML we will evaluate the prognostic significance of genes implicated in AML pathogenesis. Presence of these mutations will be correlated with clinical characteristics and outcome. We have documented the prevalence of numerous molecular alterations in genes involved in hematopoietic development. The list of genes and their overall prevalence is shown below. These genes are associated with regulation of normal hematopoiesis and the altered protein product as a result of genomic alteration may contribute to malignant transformation. Presence of some of these mutations may be associated with clinical outcome, and others may be potential targets for directed therapies. For example, N-RAS mutations are identified with a prevalence of 10% - 15% in childhood AML in an age dependent manner. Patients with N-RAS mutations have a constitutively activated RAS/RAF/MAP/ERK pathway, and although presence of N-RAS does not have prognostic significance, its presence may identify a pathway that may be amenable for targeting with specific small molecule inhibitors. Comprehensive mutation profiling in AML is essential for identification of patient populations with biologically distinct disease with altered disease response. As part of the integrated aims of this study we will create a complete genomic profile that will be used to correlate the information with specific patient demographics, disease characteristics and clinical outcome. Such a comprehensive genotypic profiling will allow more complete correlation of molecular alterations with clinical outcome. As part of complete profiling of specimens from AAML1031, we will screen all diagnostic specimens for disease-associated mutations that may impact disease initiation and progression and may act as targets for directed therapy. These include *KIT* mutations in *CBF AML*, *WT1* mutations and *WT1* single nucleotide polymorphisms (SNP), *IDH1* mutations and *IDH1* SNP, *NRAS* mutations, *TET2* mutations and *TET2* SNP, *RUNX1* mutations as well as novel mutations identified as disease associated alteration in AML. In addition to disease associated mutations, new data is emerging on the prognostic significance of common germline polymorphisms (whether synonymous or not) in disease outcome.

***N-RAS* mutations (*NRAS*^{mut}):** *NRAS*^{mut} occur in 10% - 20% of adult and pediatric patients with AML, however the prognostic significance remains disputed. We report on the incidence and prognostic significance of *NRAS*^{mut} in 1,241 pediatric AML patients treated on CCG2961 and AAML03P1. Of 825 patients who underwent *NRAS* mutational analysis, 86 (10%) were positive. Gender, age, race, FAB subtype and cytogenetics were comparable between patients with and without *NRAS*^{mut}. *FLT3/ITD* were less common (2% vs. 9%, $p = 0.03$), while *NPM* mutations were more common in those with *NRAS*^{mut} (13% vs. 5%, $p = 0.02$), resulting an overall significant correlation with low risk disease ($p = 0.04$). *NRAS*^{mut} and non-mutated patients had identical CR rates of 79% following 2 courses of Induction and a similar rate of relapse. Five year EFS and OS were also comparable however *NRAS*^{mut} patients demonstrated a marked increase in treatment related mortality (TRM) (21% vs. 14%, $p = 0.03$). This increased TRM occurred exclusively for patients treated on CCG2961, which correlated with decreased OS and DFS. *NRAS*^{mut} did not contribute to increased disease recurrence in pediatric AML, but further study may identify a novel downstream target for directed therapy.

***KIT* mutations:** *KIT* receptor tyrosine kinase mutations are implicated as a prognostic factor in adults with core binding factor (CBF) AML. However, their prevalence and prognostic significance in pediatric CBF AML is not well established. We performed *KIT* mutational analysis (exon 8 and exon 17) on diagnostic specimens from 203 pediatric patients with CBF AML enrolled on 4 pediatric AML protocols.⁹¹ *KIT*

mutations were detected in 38 (19%) of 203 (95% CI, 14% - 25%) patient samples of which 20 (52.5%) of 38 (95% CI, 36% - 69%) involved exon 8, 17 (45%) of 38 (95% CI, 29% - 62%) involved exon 17, and 1 (2.5%; 95% CI, 0% - 14%) involved both locations. Patients with *KIT* mutations had a 5-year EFS of 55% (\pm 17%) compared with 59% (\pm 9%) for patients with wild-type *KIT* ($p = 0.86$). Rates of CR, OS, DFS, or relapse were not significantly different for patients with or without *KIT* mutations. Location of the *KIT* mutation and analysis by cytogenetic subtype [t(8;21) vs inv(16)] also lacked prognostic significance. Our study shows that *KIT* mutations lack prognostic significance in a large series of pediatric patients with CBF AML.

***WT1* mutations (*WT1*^{mut}):** Recent studies of *WT1* mutations in AML report conflicting associations with clinical outcome. We screened 842 patients treated on 3 consecutive pediatric AML trials for *WT1* zinc-finger mutations.⁹² Eighty-five mutations were detected in 70 of 842 patients (8.3%). Mutations occurred predominantly in exon 7 ($n = 74$), but were also found in exons 8 ($n = 5$) and 9 ($n = 6$). Normal karyotype was observed in 35.3% of *WT1*^{mut} patients, while 27.5% *WT1*^{mut} patients harbored favorable-risk cytogenetics. Patients with or without mutations had similar rates of CR after 1 course of Induction chemotherapy. Overall survival for patients with *WT1* mutations was 41% vs. 54% for those without mutations ($p = 0.016$). Corresponding EFS was also significantly worse for those with *WT1* mutations (28% vs. 42%, $p = 0.01$). FLT3/ITD was present in 36% of the *WT1*^{mut} cohort. *WT1*^{mut} patients without FLT3/ITD had similar OS (56% vs. 56% respectively, $p = 0.8$) and EFS (35% and 44% respectively, $p = 0.34$) to patients who were wild-type for both mutations. In current risk-stratification schemes incorporating cytogenetics and FLT3/ITD status, the presence of *WT1* mutations has no independent prognostic significance in predicting outcome in pediatric AML.

***WT1* Single Nucleotide Polymorphism (SNP):** A recent study in adult AML reported that the presence of the minor allele of the *WT1* SNP rs16754 correlated significantly with improved survival outcomes. To determine the prevalence of the minor allele of SNP rs16754 in pediatric AML, we sequenced exon 7 of the *WT1* gene in diagnostic marrow specimens from 562 children with *de novo* AML treated on the trial CCG2961. Presence of at least 1 copy of the minor allele was detected in 157 of 562 (28%) patients. Patients with or without the minor SNP allele did not differ significantly in terms of FAB class, diagnostic white blood cell count (WBC), or the concomitant presence CBF translocations or FLT3/ITD, *CEBPA*, or *NPM* mutations. Sixty percent of minor-allele positive patients were male, and 4 out of 5 patients with monosomy 5 harbored the minor allele. Asian patients accounted for 6.1% of minor allele positive patients, vs. 1.9% of those without the minor allele ($p = 0.020$). Minor-allele positive patients were younger (median age 9.2 years vs. 10.8 years, $p = 0.021$) than those with only the major allele. Rates of CR after 1 course of Induction therapy were similar between the 2 groups. However, minor allele positive patients had a significantly higher 5 year OS from study entry (62% vs. 44%, $p = 0.004$) than those with only the major allele. In addition, minor allele positive patients had a significantly higher OS from relapse (36% vs. 19%, $p = 0.021$) suggesting a higher rate of salvage success. The biologic mechanism explaining why the presence of a synonymous SNP correlates with favorable outcome remains to be determined. However, the high prevalence of this SNP in pediatric AML, and the strongly significant association with improved OS, identifies the minor allele of *WT1* SNP rs16754 as an important molecular marker of prognosis that can be incorporated into the risk stratification schemes.

***WT1* expression:** Expression level of *WT1* gene has been implicated in disease pathogenesis and is suggested as a prognostic marker in AML,⁹³ although comprehensive evaluation of *WT1* expression in childhood AML is lacking.⁹⁴ As part of our evaluation of the role of *WT1* gene in AML pathogenesis, we have assessed *WT1* expression by quantitative RT-PCR in patients treated on AAML03P1. In the initial 100 specimens studied, *WT1* expression varied over 4 log fold and there was no correlation with cytogenetic class. Those with highest *WT1* expression had elevated blast percentage and had a higher incidence of FLT3/ITD ($p = 0.042$). We are investigating the correlation of *WT1* expression level with *WT1* mutation and SNP and clinical outcome. We are also evaluating the *WT1* expression level in post remission

specimens and its correlation with flow based MRD as well as clinical outcome. In the context of AAML1031 we will evaluate the *WT1* expression level and attempt to identify patient population in which *WT1* expression level can be utilized for risk allocation

***IDH1* mutation and *IDH1* SNP:** Recent whole-genome sequencing efforts led to the identification of *IDH1*(R132) mutations in acute myeloid leukemia (AML) patients. We studied the prevalence and clinical implications of *IDH1* genomic alterations in pediatric and adult AML.⁹⁵ Diagnostic deoxy ribonucleic acid (DNA) from 531 AML patients treated on AAML03P1 (n = 257), and Southwest Oncology Group trials SWOG-9031, SWOG-9333 and SWOG-9500 (n = 274), were tested for *IDH1* mutations. Codon R132 mutations were absent in the pediatric cohort, but were found in 12 of 274 adult patients (4.4%, 95% CI 2.3 - 7.5). *IDH1*(R132) mutations occurred most commonly in patients with normal karyotype, and those with *FLT3*/ITD and *NPM* mutations. Patients with *IDH1*(R132) mutations trended toward higher median diagnostic WBC (59.2 x 10⁹ vs 29.1 x 10⁹) per liter, p = 0.19) than those without mutations, but the 2 groups did not differ significantly in age, bone marrow blast percentage, OS or relapse-free survival. Eleven patients (2.1%) harbored a novel V71I sequence alteration, which was found to be a germ-line polymorphism. In addition to the abovementioned mutations, 58 patients (12%) had silent G105G polymorphisms, 11 of which also harbored an additional, novel V71I sequence alteration. Recent studies have demonstrated that the presence of these synonymous SNP are associated with clinical outcome.⁹⁶ As a result we inquired whether presence of SNP rs11554137 is associated with clinical outcome in pediatric and adult AML. Early studies from AAML03P1 demonstrated that presence of V71I was associated with improved outcome, where those with this polymorphism did not relapse. This data is being validated in a larger cohort of pediatric AML as well as adult patients treated on SWOG trials.

***RUNX1* mutations:** The *RUNX1* gene (previously *AML1*) encodes the alpha subunit of core binding factor (CBF α), which is implicated in normal and malignant hematopoiesis. Translocations involving *RUNX1* have been associated with favorable prognosis in acute leukemias (e.g., t(8;21) in AML and t(12;21) in ALL). Point mutations of *RUNX1* have been identified in exon 3 and exon 8 of *RUNX1* gene in a subset of AML patients and are most closely associated with myelodysplastic syndrome (MDS) associated AML and AML subtype M0. We evaluated the prevalence and prognostic significance of *RUNX1* mutations in pediatric patients with *de novo* AML treated on pediatric AML trials CCG2941 and CCG2961. Initial evaluation of exon 3 and exon 8 of *RUNX1* gene was conducted on a cohort of 100 randomly selected patient specimens. In this initial analysis, we identified 4 missense mutations of exon 3 that resulted in sequence alteration. No exon 8 mutations were identified. Subsequent molecular genotyping of the remaining 484 patient specimens were limited to exon 3. Of the 584 diagnostic specimens tested, missense mutations of exon 3 were detected in 19 patients (3.3%). All detected mutations were missense mutations at nucleotide 167 (T to C) causing a leucine to serine conversion at amino acid 56 (L56S). Two patients had silent mutations (G to A at base pair 182, G182G). Demographic, laboratory characteristics and clinical outcome was compared between those with and without *RUNX1* mutations. There was no significant difference with regards to age, gender or race between these groups. Those with *RUNX1* mutations had a significantly lower prevalence of organomegaly (11% vs. 32%, p < 0.05) and significantly higher rate of extramedullary disease (chloroma 26% vs. 10%, p < 0.05). There was no significant difference in association with other known cytogenetic abnormalities or risk groupings (standard, low, or high risk grouping on these studies). t(8;21) translocations were detected in 22% of those with *RUNX1* mutations compared to 16% of those without mutation (p=0.6) and there was no *RUNX1* mutation detected in patients with inv(16). Of the 85 patients with *FLT3*/ITD, *NPM1* or *CEBP α* mutations, only 3 patients had the concomitant *RUNX1* mutation (2 with *FLT3*/ITD and 1 with *NPM1* mutation). Remission induction rate was compared between patients with and without *RUNX1* mutations. Those with *RUNX1* mutations had a similar CR rates to those without mutations (89% vs. 79%, p = 0.4). Overall survival from remission for patients with and without *RUNX1* mutation was 38% and 60% respectively (p = 0.1) with a corresponding DFS of 34% vs. 49%. *RUNX1* mutations may represent biologically distinct groups that are present in 3.3% of pediatric AML patients across different morphologic and cytogenetic populations. Alterations of *RUNX1*

are implicated in signal transduction pathway and may be exploited in defining a population for directed and risk based therapy.

***TET2* mutations:** Deletions and rearrangements involving chromosome 4q24 in patients with myelodysplastic syndrome lead to the discovery that the *TET2* gene found in this genomic region may be important to myeloid cancers. The function of *TET2* is unknown although it is likely a tumor suppressor gene since full deletions of the gene have been demonstrated in some MDS patients. Further study has demonstrated that *TET2* mutations are found in acute myeloid leukemia. Abdel-Wahab, et al.⁹⁷ reported a prevalence of 12% (11 of 91 patients) in adults with primary AML. We tested a cohort of 172 pediatric patients treated on the study CCG2961. DNA was isolated from diagnostic bone marrow samples. All 11 exons of the *TET2* gene were amplified by PCR then sequenced for mutational analysis. Excluding synonymous mutations, mutations found in the single nucleotide polymorphism database (dbSNP) and mutations described as SNP by Abdel-Wahab, et al.⁹⁷, there were 9 heterozygous missense mutations (K423R, N767D, R814H, E1010D, S1039L, A1443V, V1718L, H1817N and E1973K), 2 heterozygous nonsense mutations (Q958X and E1323X) and one heterozygous single base insertion (ins1870-1871) which caused a frame shift beginning with T624N and causing an early termination codon (E637X). Each mutation was found in a single patient sample except for 1 missense mutation (V1718L) which was found in 2 samples. Also, 1 patient sample had 2 missense mutations (E1010D and E1973K) and another patient sample contained both of the nonsense mutations. Therefore a total of 11 out of 172 samples (6.4%) had a *TET2* mutation.

***c-CBL* mutations:** *c-CBL* encodes an ubiquitin ligase that targets multiple activated tyrosine kinases for degradation. Analysis of several series of patients with myeloid malignancies demonstrated mutations in the RING finger domain and linker domains (encoded by exons 8 and 9 of the *c-CBL* gene). Loh, et al.⁹⁸ analyzed 157 samples from pediatric patients with juvenile myelomonocytic leukemia (JMML) for mutations in exons 8 and 9 of the *c-CBL* gene. They found *c-CBL* mutations in 27 of the 157 samples (17% prevalence). We sought to determine the prevalence of mutations in *c-CBL* exons 8 and 9 in pediatric patients with *de novo* AML. We analyzed 290 samples from pediatric patients treated on the study AAML03P1. DNA was isolated from diagnostic bone marrow samples. Exons 8 and 9 of the *TET2* gene were amplified by PCR then sequenced for mutational analysis. One patient sample had a heterozygous splice site mutation (1,096 -1G > T). One patient sample had a homozygous missense mutation (C384S). Two samples each had a synonymous heterozygous mutation (P433P and I429I). We also found that 3 samples had heterozygous insertions and deletions that included part of these exons. One sample had a 121 base pair deletion that included 66 base pairs at the 3' end of exon 8 and the first 55 base pairs of intron 8. Another sample had complex insertion/deletions with deletion of 73 base pairs including 31 base pairs in intron 7 and 42 base pairs at the 5' end of exon 8 along with an insertion of 26 base pairs at the site of the deletion. A third sample had a 3 base pair duplication in exon 9 (1,382 - 1,383 ins TGA) with TGA repeated 7 rather than 6 times. Excluding the synonymous mutations, there were a total of 5 samples out of 290 (1.7%) with *c-CBL* exon 8 or 9 mutations. Therefore *c-CBL* mutations in the RING finger and linker domains are rare in pediatric *de novo* AML compared to the previously reported prevalence in JMML.

2.8.6 Correlate the expression of CD74 antigen as well as *PSMB5* gene expression and mutation with response to bortezomib

CD74 expression determination: CD74 is a major histocompatibility complex molecule which signals thru NF- κ B. In a study by Attar et al.¹⁵ it was shown that CD74 expression level correlated with response to bortezomib. In this study CD74 was expressed at a 6-fold increase in patients who achieved a CR compared with patients who failed Induction (p = 0.06). As part of our diagnostic evaluations of marrow specimens by flow cytometry, we will evaluate CD74 expression level in all patients enrolled on AAML1031. CD74 antibody can be included with ease into our diagnostic panel and expression level of CD74 on blast cells determined. CD74 expression will be subsequently correlated with CR and relapse rates in patients with

and without exposure to bortezomib to determine whether CD74 exposure correlates with response to bortezomib.

***PSMB5* mutations and expression:** The ubiquitin-proteasome pathway plays an essential role in the degradation of cellular proteins. The C-terminal glycine residues of poly ubiquitin chains conjugate to specific lysine moieties on the protein targeted for degradation. The proteasome is a 26S enzyme complex that comprised of a core 20S catalytic complex and a 19S regulatory complex. Within the 20S core, proteins are degraded to small peptides. The 20S proteasome core has chymotrypsin- like, trypsin-like, and peptidyl glutamyl-like activities that are associated with three distinct units: b5, b2, b1, respectively. Chymotrypsin-like activity at proteasome b5 subunit (PSMB5) is associated with the rate-limiting step of proteolysis. Recent data suggest that genomic alterations, SNPs and expression variations of *PSMB5* gene may mediate response to bortezomib.^{99,100} Investigators created high levels of acquired resistance to bortezomib in THP1 cells by exposure to increasing concentrations of bortezomib and demonstrated an Ala49Thr mutation residing in the bortezomib-binding pocket in the PSMB5 protein.

2.8.7 Evaluation of Protein Expression and Unfolded Protein Response in Patients with AML

Understanding the changes in protein expression that occur in “bulk” leukemia cells and in leukemia initiating cells (LIC) in response to conventional and targeted chemotherapy is crucial to determining how LIC evade chemotherapy. We have recently developed the means to study protein expression and activation in small numbers of cells using reverse-phase protein lysate array (RPPA) technology. Using this technique we can globally assess the effects of targeted anti-cancer agents on the proteome by studying protein expression and phosphorylation of over 200 proteins using as few as 200,000 myeloblasts. We plan to use RPPA to identify protein pathways that correlate with bulk leukemia and LIC response to therapy. While previous work in adult AML has demonstrated that functional proteomic profiling can predict treatment response and survival,¹⁰¹ no functional proteomic profiling or unfolded protein response data are available in the pediatric AML population. We hypothesize that functional proteomic profiling and analysis of the unfolded protein response (UPR) will allow us to 1) further characterize the protein pathways associated with LIC and bulk leukemia cell response to bortezomib and sorafenib treatment, and 2) help to identify biomarkers of either clinical response or therapy resistance. Induction of the endoplasmic-reticulum (ER) - mediated UPR and the triggering of autophagy may be important to targeting LIC. Properties that distinguish LIC from their normal counterparts have been characterized^{14,102} and these properties have been used to identify anti-LIC targets.^{101,103} Among these are the NF- κ B pathway and pathways controlled by the ubiquitin-proteasome pathway which regulate how rapidly AML cells multiply, die or mature.¹⁸ Bortezomib is known to block NF- κ B activation, to trigger the UPR¹⁰⁴ and to induce autophagy in many cell types¹⁰⁵⁻¹⁰⁷ suggesting that this may be a means of selectively enhance LIC targeting. Sorafenib also targets the LIC through its effects on FLT3 inhibition.¹⁰¹

2.8.8 Expression Levels of Wild Type FLT3 and its *In Vitro* Response to FLT3 Inhibitor Sorafenib.

We have demonstrated that expression level of wild type FLT3 correlates with clinical outcome and those with high wild type FLT3 (WT-FLT3) expression are exquisitely sensitive to pro-apoptotic effects of FLT3 inhibitors. We will prospectively evaluate the expression of FLT3 by flow cytometry (included in diagnostic flow platform) as well as by RT-PCR and define its impact on outcome. This approach may identify additional high risk patients who may benefit from therapy with FLT3 inhibitors. In addition, in vitro cytotoxicity will be performed on specimens identified to have high versus low FLT3 expression in order to demonstrate correlation of in vitro response to FLT3 expression levels.

2.8.9 Sorafenib Pharmacokinetics and Pharmacodynamics

The sorafenib dosing needed to achieve adequate mutant FLT3/ITD inhibition is poorly understood because of the very few patients treated with sorafenib/chemotherapy combinations and very limited data on the role of the sorafenib N-oxide metabolite. Thus, this trial will use a limited sampling approach to determine sorafenib and N-oxide metabolite exposure in plasma. Steady state PK sampling with each course of

Induction and Intensification and at various time points during the maintenance phase will provide information on systemic exposure to sorafenib and its N-oxide metabolite. These data can be compared to pharmacokinetic data obtained during ADVL0413 Phase I study of sorafenib in children and extension study in FLT3/ITD leukemia. In addition, the pharmacokinetic-pharmacodynamic relationship of FLT3/ITD inhibition will be studied using a plasma inhibitory assay (PIA) and trough plasma sorafenib and N-oxide metabolite concentrations. While standard PK assays accurately measure the concentration of total drug in plasma, they do not measure the amount of free drug, which varies widely depending upon the affinity of a drug for the various plasma proteins (e.g., albumin, alpha-1-acid glycoprotein (AGP) and the concentration of these proteins in an individual patient's plasma. Since only the free, unbound fractions of sorafenib and the N-oxide metabolite are available to inhibit FLT3 in target cells, and since these compounds are known to be highly bound to plasma proteins, PK values cannot necessarily be used as a valid surrogate for biologic activity. The PIA assay is a pharmacodynamic (PD) assay can serve as a powerful surrogate for *in vivo* FLT3 inhibition in patients receiving sorafenib by measuring the amount of FLT3 inhibitory activity in plasma taken from patients just before and at various trough time points during sorafenib therapy. These data will be correlated with PK measurements from the same time points to determine the PK-PD relationship for sorafenib and its N-oxide metabolite.

2.8.10 Serum Biomarkers for the Prediction of Graft-Versus-Host Disease in Children

Approximately 45% of the children receiving allogeneic bone marrow transplants will develop GVHD of the skin, GI tract, or liver, of sufficient severity to warrant additional treatment despite advances in HLA-matching techniques and GVHD prophylaxis strategies.¹⁰⁸ Children experiencing moderate to severe GVHD (extensive rash, diarrhea, or jaundice) are twice as likely to die as those who experience no or mild GVHD [no more than a limited skin rash; relative risk = 1.96].¹⁰⁸ Given the danger associated with GVHD and its treatment, there has been considerable interest in identifying patients at highest risk for GVHD. While clinical factors such as donor type (related, unrelated, cord) and HLA-match permit some calculation of GVHD risk,¹⁰⁹ the choice of donor or HLA-match is typically constrained by limited availability. Recent research at the University of Michigan has identified several proteins in the bloodstream (biomarkers) that are associated with GVHD.¹¹⁰⁻¹¹² Increased concentrations of one biomarker, TNFR1, measured 7 days post-SCT, correlated with GVHD risk in both pediatric (n = 82) and adult (n = 356) patients.^{113,114} These observations were extended to create a day 7 biomarker panel in primarily adult patients that included 4 biomarkers: TNFR1, IL-2R α , REG3 α (a lower gastrointestinal-specific GVHD biomarker) and elafin (a skin-specific GVHD biomarker). The 4 protein panel identified GVHD after unrelated donor SCT with 67% sensitivity and 50% specificity.^{115,116} We now have preliminary data that shows the combination of pre-SCT clinical factors (e.g. degree of HLA-match) and the 4 biomarker panel effectively predicts for GVHD following related donor SCT. Patients identified as high risk were twice as likely to develop GVHD (38% vs. 20%, $p < .001$), which translated into significantly higher risk for non-relapse mortality (12% vs. 3%, $p = .001$).

The data from related and unrelated patients was mostly derived from adult patients. Therefore, the goal of this aim is to develop a pediatric specific algorithm using a similar strategy. There are several reasons why a pediatric algorithm will be different from adults. First, preliminary data showed that the absolute values of GVHD biomarker concentrations in 96 children were half the value of those in 456 adults. Predictive algorithms assign a specific weight to each clinical factor and biomarker concentration, thus biomarkers will be weighted differently in a pediatric algorithm. Furthermore, differences in clinical factors (e.g. more common use of cord blood as stem cell source) exist between the pediatric and adult population, and these differences will influence the predictive algorithm. A first step to developing a GVHD prediction algorithm in children is to study a fairly homogeneous population as is available on this protocol which involves patients with one underlying disease (AML), a preferred conditioning regimen and specified GVHD prophylaxis regimen. These results will form a baseline for future studies involving more heterogeneous populations.

2.8.11 Biology Specimen Collection at Diagnosis, Treatment Time Points, and Relapse

The banking of biologic specimens is a requirement for innovative and potentially therapeutic biologic studies. Thus, this trial will strongly encourage banking of specimens at diagnosis, scheduled bone marrow evaluations, and relapse.

3.0 STUDY ENROLLMENT 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 OPEN (Oncology Patient Enrollment Network) 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 newly diagnosed patient needs to be offered participation in ACCRN07, *Protocol for the Enrollment on the Official COG Registry*, *The Childhood Cancer Research Network (CCRN)* or *APEC14B1, Project:EveryChild*.

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.

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.ctsuo.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.ctsuo.org in the members' section) under the Regulatory Submission Portal, faxed (1-215-569-0206), E-mailed (CTSURegulatory@ctsuo.coccg.org) or mailed to the CTSU Regulatory office.

When a site has a pending patient enrollment within the next 24 hours, this is considered a "Time of Need" registration. For Time of Need registrations, in addition to marking your submissions as 'URGENT' and faxing the regulatory documents, call the CTSU Regulatory Helpdesk at: 1(866) 651-CTSUS. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1(888) 823-5923.

3.1.3 Health Related Quality-of-Life and Parental Stress Assessments

These studies are closed to new patient accrual as of 05-15-2015.

Participation in the HRQOL and parental stress assessments is optional but strongly encouraged for patients between the ages of 2 years old and 18 years old (≥ 2 years and ≤ 18 years) with a parent/guardian who is able to speak and read English. Those under the age of 2 years and those 19 years old and over will not be eligible. For those who agree to the HRQOL and parental stress study, parents or alternative caregivers must provide assessments while children ≥ 5 years of age who can understand English will be given the opportunity to concurrently participate and provide self-report HRQOL data. The institutional CRA will be

responsible for ensuring the quality of life and parental stress assessments are completed at the appropriate time points, and entry of responses into the COG eRDE system. See [Section 18](#) for further details.

3.1.4 Study Enrollment

Patients may be enrolled on the study once all eligibility requirements for the study have been met. Study enrollment is accomplished by going to the Enrollment application in the RDE system. If you have problems with enrollment, refer to online help in the Applications area of the COG website.

3.1.5 Timing

Patients must be enrolled before treatment begins. The date protocol therapy is projected to start must be no later than five (5) calendar days after the date of study enrollment. Administration of IT chemotherapy is permitted before enrollment when administered as part of an initial diagnostic lumbar puncture.

3.1.5.1 Cytogenetics

Specimens for cytogenetics analysis are required, must be obtained prior to therapy initiation, and it is strongly recommended that they be sent to a COG-approved institutional cytogenetics laboratory (see [Section 16.2](#)). A listing of these laboratories may be found on the protocol web page as well as methods of attaining COG approval for local cytogenetics laboratories without COG approval.

Because the chromosome analysis results should be finalized by the end of Induction I, and will be used for subsequent risk stratification and in order to guide therapy by Day 28 of a patient's enrollment, **the reports should be sent to the appropriate reviewer by Day 14 (see [Appendix V](#)).** **With Amendment #7A, only those patients with HR FLT3/ITD (AR > 0.4) who consent to Arm C should have cytogenetic data sent for Central Review.**

Results of cytogenetics are not required to be completed prior to enrollment, but samples must be collected prior to therapy initiation and submitted to a cytogenetics laboratory.

3.1.5.2 FLT3, NPM and CEBP α Mutation Analysis

Specimens for FLT3 (with ITD allelic ratio testing) mutation analysis will be used for risk assignment, must be obtained prior to therapy initiation, and must be performed at the Molecular Oncology Laboratory at the Seattle Cancer Care Alliance (SCCA). Testing is free of charge to the patient.

With Amendment #7A, NPM1 and CEBP α testing will no longer be done as part of the clinical protocol but remain part of best clinical practice.

Patients enrolled prior to Amendment #7A that do not have adequate specimens submitted will receive a risk assignment based upon other criteria. For patients enrolled after Amendment #7A, adequate specimen must be submitted in order to determine FLT3/ITD allelic ratio. Per Amendment #7A, patients with unknown FLT3/ITD status at End of Induction I on Arm D will be removed from study with no further data submission. See [Section 14.0](#) for specimen collection and shipping details.

Results of FLT3 testing are not required to be completed prior to enrollment, but samples must be collected prior to therapy initiation and submitted to the SCCA.

3.1.5.3 MRD Analysis

Specimens for MRD analysis at the end of Induction I are required, and must be obtained prior to start of Induction II. Samples must be sent to the central laboratory for MRD determination (Hematologics Inc.). The result of the MRD analysis will be used for risk assignment. All patients that do not have adequate specimens submitted will receive a risk assignment based upon other criteria. See [Section 15.0](#) for specimen collection and shipping details.

3.1.6 Clarification of Consent for Diagnostic Bone Marrow Samples

It is recommended that patients initially provide consent for drawing and shipping extra bone marrow/peripheral blood. This may spare the child from having a second procedure. Investigators may use the sample consent for bone marrow sampling provided with this protocol or may use their own institutional consent for diagnostic bone marrow sampling. If an institutional consent is used, it should include within it that extra samples of bone marrow will be obtained and shipped for potential research studies should the diagnosis of AML be made.

Once the diagnosis of acute leukemia is confirmed and AML is diagnosed, a separate consent must be completed prior to enrollment onto AAML1031. Permission to use the extra bone marrow samples for the performance of research studies will be obtained within this therapeutic study consent. The consent that pertains to the bone marrow sampling should permit the immediate shipment of the samples to the AML reference laboratory. This will maximize the viability of the samples. The samples will only be kept if the patient enrolls on AAML1031 and only if the patient's family has approved their use as identified in the therapeutic study consent. In other words, the samples for those who do not enroll on AAML1031 for any reason will be destroyed. If, at any time, in the future, the patient wants banked tissue destroyed, the COG tissue bank must be notified immediately by the local institution and the RDE system will be updated and forwarded to the appropriate Reference Laboratory, who will destroy the tissue. Patients must be informed that their tissue can only be prospectively destroyed; that is, any research studies that have already been conducted prior to the date that they decided they wanted their tissue destroyed cannot be amended.

3.1.7 Inclusion of Women and Minorities

Men, women and children of all races and ethnic groups are eligible for this study. To allow non-English speaking patients to participate in the study, bilingual health care services will be provided in the appropriate language.

3.1.8 Randomization

As of Amendment #7A accrual goals for Arms A and B have been met and as such, there will be no randomization on AAML1031. Rather, patients enrolled after Amendment #7A with unknown FLT3 status at time of study enrollment will be offered treatment with standard induction chemotherapy treatment arm (Arm D, see [Section 4.11.1](#)) while FLT3 testing is being performed.

Patients later determined to have HR FLT3/ITD+ AML, will be offered re-allocation from Arm D to the sorafenib treatment arm (Arm C, see Section 4.10.1), whereas patients determined not to have HR FLT3/ITD+ AML will be removed from study with no further data submission. Patients whose FLT3/ITD status is unknown at End of Arm D Induction I will be removed from study with no further data submission, as will HR FLT3/ITD+ patients who decline Arm C treatment.

Patients with known HR FLT3/ITD+ AML prior to study enrollment will be offered to enroll directly onto the sorafenib treatment arm (Arm C).

3.2 Patient Eligibility Criteria

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived (per COG policy 7.12). 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. BMA/biopsy must be obtained within 2 weeks prior to start of protocol therapy (repeat the BMA/biopsy if necessary).

INCLUSION CRITERIA

3.2.1 Age

Patients must be less than 30 years of age at the time of study enrollment.

3.2.2 Diagnosis

Patients must be newly diagnosed with *de novo* acute myelogenous leukemia.

3.2.2.1

Patients with previously untreated primary AML who meet the customary criteria for AML with $\geq 20\%$ bone marrow blasts as set out in the 2008 WHO Myeloid Neoplasm Classification are eligible.¹¹⁷

Attempts to obtain bone marrow either by aspirate or biopsy must be made unless clinically prohibitive. In cases where it is clinically prohibitive, peripheral blood with an excess of 20% blasts and in which adequate flow cytometric and cytogenetics/FISH testing is feasible can be substituted for the marrow exam at diagnosis.

3.2.2.2

Patients with $< 20\%$ bone marrow blasts are eligible if they have:

- A karyotypic abnormality characteristic of *de novo* AML (*t(8;21)(q22;q22)*, *inv(16)(p13q22)* or *t(16;16)(p13;q22)* or *11q23* abnormalities,
- The unequivocal presence of megakaryoblasts, or
- Biopsy proven isolated myeloid sarcoma (myeloblastoma; chloroma, including leukemia cutis).

3.2.3 Performance Level

Patients with any performance status are eligible for enrollment.

3.2.4 Prior Therapy

Prior therapy with hydroxyurea, all-trans retinoic acid (ATRA), corticosteroids (any route), and IT cytarabine given at diagnosis is allowed. Hydroxyurea and ATRA must be discontinued prior to initiation of protocol therapy. Patients who have previously received any other chemotherapy, radiation therapy or any other antileukemic therapy are not eligible for this protocol.

EXCLUSION CRITERIA

3.2.5 Excluded Constitutional Conditions

Patients with any of the following constitutional conditions are not eligible:

- Fanconi anemia
- Shwachman syndrome
- Any other known bone marrow failure syndrome
- Patients with constitutional trisomy 21 or with constitutional mosaicism of trisomy 21

Note: Enrollment may occur pending results of clinically indicated studies to exclude these conditions.

3.2.6 Other Excluded Conditions

Patients with any of the following oncologic diagnoses are not eligible:

- Any concurrent malignancy
- Juvenile myelomonocytic leukemia (JMML)
- Philadelphia chromosome positive AML
- Biphenotypic or bilineal acute leukemia
- Acute promyelocytic leukemia

- Acute myeloid leukemia arising from myelodysplasia
- Therapy-related myeloid neoplasms

Note: Enrollment may occur pending results of clinically indicated studies to exclude these conditions.

3.2.7 Pregnancy and Breast Feeding:

3.2.7.1

Female patients who are pregnant are ineligible since fetal toxicities and teratogenic effects have been noted for several of the study drugs.

3.2.7.2

Lactating females are not eligible unless they have agreed not to breastfeed their infants.

3.2.7.3

Female patients of childbearing potential are not eligible unless a negative pregnancy test result has been obtained.

3.2.7.4

Sexually active patients of reproductive potential are not eligible unless they have agreed to use an effective contraceptive method for the duration of their study participation and for 30 days after the last dose of sorafenib.

REGULATORY

3.2.8

All patients and/or their parents or legal guardians must sign a written informed consent.

3.2.9

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.

3.3.2

INITIAL PLATELET COUNT: The first platelet count at the treating COG institution, or the count before transfusion of platelets if transfused prior to arrival.

3.3.3

INITIAL HEMOGLOBIN: The first hemoglobin at the treating COG institution, or the hemoglobin prior to intravenous fluid or red cell transfusions, whichever occurred first.

3.3.4

CNS LEUKEMIA AT DIAGNOSIS:

3.3.4.1

CNS disease at diagnosis is defined as:

Any number of blasts on a cytospin prep in an atraumatic (< 100 RBCs) lumbar puncture.

Blasts in a traumatic tap in which the WBC/RBC ratio in the CSF is twice that in the peripheral blood.

Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome). Extra-ocular orbital masses are not considered CNS leukemia.

Radiographic evidence of an intracranial, intradural mass consistent with a chloroma.

3.3.4.2

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 blasts, the following algorithm should be used to diagnose CNS disease:

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

A patient with CSF blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis. Example: CSF WBC = 60/ μ L; CSF RBC = 1,500/ μ L; blood WBC = 46,000/ μ L; blood RBC = 3 X 10⁶/ μ L:

$$\frac{60}{1,500} = \mathbf{0.04} > \mathbf{2X} \quad \frac{46,000}{3 \times 10^6} = \mathbf{0.015}$$

3.3.5 RISK CATEGORY STRATIFICATION

AAML1031 is utilizing stratification for relapse risk to guide both therapy and analyses. The section below outlines and defines the 2 risk categories – Low and High Risk. These classifications should be made no later than Day 1 of Induction II.

HIGH RISK:

- 1) FLT3/ITD+ with high allelic ratio > 0.4 (HR FLT3/ITD+) regardless of low risk features.
- 2) Presence of monosomy 7, monosomy 5, or del5q, without inv(16)/t(16;16) or t(8;21) cytogenetics or NPM or CEBP α mutations.
- 3) AML without inv(16)/t(16;16), t(8;21), NPM, CEBP α mutations, monosomy 7, monosomy 5, del5q, or HR FLT3/ITD+, but with evidence of residual AML (MRD \geq 0.1%) at end of Induction I.
- 4) AML without inv(16)/t(16;16), t(8;21), NPM, CEBP α mutations, monosomy 7, monosomy 5, del5q, or HR FLT3/ITD+, but with clinical or radiographic evidence of progressive extramedullary AML at end of Induction I.

All patients in this category will receive SCT, if possible, after Intensification I. Those for whom no suitable alternative donor can be found should continue with their assigned chemotherapy regimen.

LOW RISK:

- 1) Presence of inv(16)/t(16;16) or t(8;21) cytogenetic features or NPM or CEBP α mutation regardless of monosomy 7, monosomy 5, or del5q and regardless of MRD at end of Induction I.
- 2) Negative MRD (< 0.1%) at end of Induction I and no high risk disease features.

These patients do NOT receive SCT in first remission, regardless of whether a matched related donor is available.

Note: Patients who do not have MRD data and have non-informative molecular studies (NPM, CEBP α , and cytogenetics) will be classified as having Low Risk disease. Patients with multiple MRD measurements will be risk stratified based on the most recent MRD measurement.

RISK ASSIGNMENT:	Low Risk		High Risk		
	LR Group 1	LR Group 2	HR Group 1	HR Group 2	HR Group 3
FLT3 ITD allelic ratio	Low/Negative	Low/Negative	High	Low/Negative	Low/Negative
Good risk molecular markers*	Present	Absent	Any	Absent	Absent
Poor risk cytogenetic markers**	Any	Absent	Any	Present	Absent
Minimal residual disease	Any	Negative	Any	Any	Positive
⁺ Groups are based upon combinations of risk factors which may be found in any individual patient *NPM1, CEBP α , t(8;21), inv(16) **Monosomy 7, Monosomy 5, del(5q) Bold indicates the overriding risk factor in risk group assignment					

3.4 Pre-Enrollment Baseline Diagnostic Requirements

See [Section 7.0](#) for all required and recommended baseline pre-enrollment evaluations.

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 per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

4.1 Overview of Treatment Plan

With Amendment #7A, Arms A and B are closed to new patient enrollment as accrual goals have been met. Patients enrolled on AAML1031 prior to Amendment #7A who were randomized to either Arm A or Arm B received either a 3 or 4 course chemotherapy backbone. Patients with low risk AML will receive cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/daunorubicin/etoposide (ADE 8+3+5), cytarabine/etoposide (AE), and cytarabine/mitoxantrone (ARAC/Mitox). Patients with high risk AML received 3 courses of chemotherapy followed by best allogenic donor SCT if available. These high risk AML patients received cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/mitoxantrone (ARAC/Mitox), and cytarabine/etoposide (AE). Patients with high risk AML who did not have an appropriate allogenic donor received high dose cytarabine/L-Asparaginase (HD ARAC/LASP) as a fourth course.

With Amendment #7A, patients with HR FLT3/ITD+ AML enrolled on the sorafenib treatment arm (Arm C) will receive 3 courses of chemotherapy in combination with sorafenib: cytarabine/daunorubicin/etoposide (ADE 10+3+5), cytarabine/daunorubicin/etoposide (ADE 8+3+5), cytarabine/etoposide (AE), followed by best allogenic donor SCT. Patients with HR FLT3/ITD+ who do not have an appropriate allogenic donor will receive sorafenib with ARAC/Mitox as a fourth course of chemotherapy. Finally, all patients allocated to the sorafenib treatment arm will be eligible to receive a one year sorafenib maintenance course.

4.1.1 Treatment Assignments

Effective with Amendment #7A, Arms A and B are closed to new patient enrollment as accrual goals have been met; therefore, there will be no randomization on AAML1031 for patients enrolling after Amendment #7A. Patients enrolled prior to Amendment #7A will continue treatment per their initially assigned arm.

Bortezomib safety analysis will be performed in an ongoing manner while patients are accrued. Formal interim bortezomib safety analysis will be performed ([Section 9.3](#)) in the first 100 patients without HR FLT3/ITD+ randomized to receive Arm B. Study suspension is not planned, but may be undertaken if needed, for this interim analysis.

Patients with known HR FLT3/ITD+ status (from the SCCA central laboratory) at time of study entry will be enrolled onto the sorafenib treatment arm (Arm C). Should the patient decline treatment with sorafenib, the patient will not be enrolled on study.

With Amendment #7A, patients with unknown FLT3/ITD allelic ratio prior to study entry will be offered enrollment on Arm D (standard pediatric AML Induction chemotherapy with ADE) while FLT3/ITD results are pending. Patients later found to have HR FLT3/ITD+ (prior to End of Arm D Induction I) will be offered treatment on the sorafenib treatment arm (Arm C). Patients with HR FLT3/ITD+ who decline participation in Arm C, patients without HR FLT3/ITD+, and patients with unknown FLT3/ITD status at End of Arm D Induction I will be removed from study with no further data submission. Patients enrolled prior to Amendment #7A, currently on therapy, and now found to have HR FLT3/ITD+ after initial randomization, will be offered participation in the sorafenib treatment arm (Arm C). Patients initially randomized to Arm B will stop bortezomib as soon as consent is obtained for participation in the sorafenib Arm C.

The sorafenib treatment arm entails 3 cohorts. Cohort 1 has been completed. Patients in Cohort 1 received sorafenib starting in Induction II and in the remaining chemotherapy courses. Cohort 1, which accrued and evaluated a total of 12 patients, determined that sorafenib at a dose of 200 mg/m² daily given Day 1 to Day 28 is tolerable in combination with standard AML chemotherapy. Cohort 2, which accrued 34 patients, opened with Amendment #3a and later closed with Amendment #6A. Cohort 2 received 200 mg/m² sorafenib daily at the time of known HR FLT3/ITD+ (including in Induction I and concurrently with chemotherapy) until a safety memo was released on 04/27/2015 that resulted in subsequent sorafenib dosing being delayed until after conventional chemotherapy for a given course was complete. Cohort 3 opened with Amendment #6A and patients enrolled in Cohort 3 of Arm C receive sorafenib 200 mg/m² daily. Per Amendment #6A, Cohort 3 sorafenib therapy starts on Day 11 of Induction I and continues until Day 28; Induction II and Intensification I, sorafenib starts on Days 9 and 6, respectively and is administered for a total of 28 days. While most Arm C patients proceed to hematopoietic stem cell transplant following Intensification I, should Intensification II treatment be administered, sorafenib would start on Day 7 and continue for 28 days.

Any HR FLT3/ITD+ patient treated on Arm C will also be eligible for sorafenib maintenance. For Arm C patients who undergo SCT, sorafenib maintenance therapy must be started between Day 40 - 100 post-SCT and patients must meet baseline clinical criteria to initiate therapy (see [Section 4.26](#)). For Arm C patients who do not undergo SCT, sorafenib maintenance therapy must be started between Day 40 - 100 following Intensification II and patients must meet baseline clinical criteria to initiate therapy (see [Section 4.26](#)).

4.1.2 Stem Cell Transplant Assignments

SCT allocation for patients will be based on risk stratification, to be determined no later than Day 1 of Induction II. Patients with low risk status will receive chemotherapy only and patients with high risk status will receive best allogeneic donor SCT or alternative donor SCT depending on availability. All high risk patients without a suitable allogeneic or alternative donor will proceed to a final course of high dose cytarabine/L-Asparaginase (HD ARAC/LASP).

All HR FLT3/ITD+ patients will receive best allogeneic donor SCT or alternative donor SCT depending on availability. Patients without a suitable allogeneic or alternative donor will proceed to a final course of cytarabine/mitoxantrone with sorafenib followed by 1 year of sorafenib maintenance. Enrollment on AAML05P1 will be permitted for patients with an available unrelated donor.

4.1.3 HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Participation in the HRQOL and parental stress assessments is optional but strongly encouraged for patients between the ages of 2 years old and 18 years old (≥ 2 years and ≤ 18 years) with a parent/guardian who is able to speak and read English. Those under the age of 2 years and those 19 years old and over will not be eligible. For those who agree to the HRQOL and parental stress study, parents or alternative caregivers must provide assessments while children ≥ 5 years of age who can understand English will be given the opportunity to concurrently participate and provide self-report HRQOL data.

All eligible patients will be evaluated near diagnosis (within 14 days of Induction initiation), on or after Day 21 of Induction II but prior to start of Intensification I, and on or after Day 21 of Intensification I but prior to start of Intensification II. Patients assigned to chemotherapy in Intensification II will have evaluations 1 month (± 7 days) and 4 months (± 1 month) from the start of Intensification II, while patients assigned to SCT will have evaluations at 1 month (± 7 days) and 4 months (± 1 month) from the start of the preparative regimen. Evaluations 6, 7 and 8 occur at 12 (± 1 month), 24 (± 3 months) and 36 (± 3 months)

months from diagnosis, respectively. Please see [Section 18.0](#) for instructions on timing and specific test to be administered.

4.1.4 Standard Chemotherapy Dosing Guide

Cytotoxic chemotherapy doses will be modified only for patients with a BSA < 0.6 m². **For patients with BSA < 0.6 m², chemotherapy will be dosed in mg/kg, as specified in the treatment plan.**

Bortezomib and sorafenib will be dosed in mg/m² for all patients.

No chemotherapy dose modification will be made for obese patients except in stem cell transplant. These dosing guidelines are described in Section 4.24.

Age and weight adjusted busulfan dosing guidelines are found in Section 4.24.3.

4.2 Concomitant Therapy Restrictions

4.2.1 Restrictions for all patients

Myeloid growth factor support can be administered to patients in the following clinical scenarios: (1) prolonged neutropenia that is clinically significant; (2) clinical or culture proven bacteremia during neutropenia, or with invasive fungal infection. Growth factors should not be initiated before notification of the Study Chair.

4.2.2 Restrictions during Induction I, II and Intensification I (all treatment arms)

Elimination of etoposide is partially mediated through cytochrome P450 3A4 (CYP3A4), CYP1A2 and 2E1. The clinical outcome and significance of CYP450 interactions with etoposide is not clear. It is recommended to avoid concomitant administration with strong CYP3A4 inducers (refer to [Appendix IV](#) for a list of strong inducers for this protocol) when possible, given theoretical concerns for reduced clinical efficacy. Similarly, strong CYP3A4 inhibitors (refer to [Appendix IV](#) for a list of strong inhibitors for this protocol) should be avoided if possible. If concomitant administration is necessary, close monitoring for toxicity is recommended. Please consult frequently updated medical references for additional inducers or inhibitors of CYP450 isoenzymes.

4.2.3 Restrictions for patients receiving bortezomib

Bortezomib is metabolized by multiple cytochrome P450 (CYP) enzymes, with CYP3A4 being the primary contributor, and with only minor contributions of CYPs 2C19, 1A2, 2C9 and 2D6. It is a weak inhibitor of CYP2C19 and does not inhibit CYP1A2, 2C9, 2D6, and 3A4 at clinically relevant concentrations (IC₅₀ > 30 μM). Co-administration of strong CYP3A4 inducers should be avoided throughout therapy with bortezomib (refer to [Appendix IV](#) for a list of strong inducers for this protocol). Co-administration of strong and clinically relevant moderate CYP3A4 inhibitors should be avoided until 72 hours beyond administration of Day 8 bortezomib for each course (exception: administration of prophylactic fluconazole may begin ≥ 24 hours after the Day 8 bortezomib dose). Similarly, patients receiving strong and clinically relevant moderate inhibitors should discontinue use of these agents 72 hours prior to start of Day 1 bortezomib in all courses (refer to [Appendix IV](#) for a list of strong and clinically relevant moderate inhibitors for this protocol). Please consult frequently updated medical references for additional inducers or inhibitors of CYP450 enzymes.

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.^{118,119} A more recent study has concluded that there is no interaction when plasma concentrations are commensurate with dietary oral intake.¹²⁰ 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 recommended daily allowance (RDA) for age (i.e., normally balanced diets are acceptable).

4.2.4 Restrictions for patients receiving sorafenib

Sorafenib is primarily metabolized by cytochrome CYP3A4. While CYP3A4 inducers/inhibitors do not seem to substantially change sorafenib pharmacokinetics, levels of the N-oxide sorafenib metabolite are substantially altered by CYP3A4 inducers/inhibitors. Since the N-oxide metabolite has substantial activity against FLT3, concomitant administration of strong CYP3A4 inducers and inhibitors (including clinically relevant moderate inhibitors) is prohibited with the exception of voriconazole, fluconazole, and posaconazole in the sorafenib maintenance phase. Please refer to [Appendix IV](#) for a list of strong inducers and inhibitors for this protocol. Please consult frequently updated medical references for additional inducers or inhibitors of CYP450 enzymes.

Sorafenib may increase systemic exposure to CYP2B6 or CYP2C8 substrates; caution is advised when substrates of CYP2B6 or CYP2C8 are used concomitantly with sorafenib. However, no CYP2B6 or CYP2C8 substrates are excluded.

Sorafenib does not appear to affect the metabolism of warfarin (a CYP2C9 substrate) *in vivo*; however, infrequent bleeding events or elevations in the international normalized ratio (INR) have been reported in some patients receiving concomitant therapy with warfarin. Patients taking concomitant warfarin should be monitored regularly for changes in prothrombin time, INR or clinical bleeding episodes.

4.3 **Dose Modifications for CNS Disease**

4.3.1 Intrathecal Chemotherapy for CNS Disease

CNS disease at diagnosis: Patients with CSF involvement will receive IT cytarabine twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement.

Patients with CNS chloromatous disease at diagnosis will receive 6 intrathecal treatments regardless of CSF blast count at diagnosis.

CNS disease at the start of Induction II: If CSF involvement is present at start of Induction II but was not present at Induction I, patients will receive IT cytarabine twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement.

Patients with refractory CNS leukemia at the end of Induction I or Induction II will be taken Off Protocol Therapy for failure to attain remission. Patients with CNS disease at any time point after completion of Induction II will be taken Off Protocol Therapy for CNS relapse. See [Section 10.2.2](#) and [10.2.3](#).

4.3.2 IT Chemotherapy Administration Timing

The administration of subsequent IT chemotherapy in all courses of therapy after Induction I occurs on Day 1 of each course (except for the Intensification II course for high risk patients). It is permissible, to avoid additional sedation procedures, to administer this Day 1 dose of IT chemotherapy for Induction II, Intensification I, and Intensification II courses with the end of course bone marrow aspirations obtained for remission assessment at the end of the preceding course of therapy.

4.4 Evaluation of Marrow Status and Extramedullary Disease

A bone marrow aspirate to assess remission status should be performed approximately 18 – 25 days after the last day of chemotherapy on each course. Bone marrow aspirate may be delayed beyond 25 days if adequate peripheral count recovery has not yet occurred. If the marrow is hypoplastic and/or there is little or no evidence of normal hematopoiesis, a repeat marrow should be performed after a further 7 – 21 days (based upon the peripheral blood count recovery and the clinician's judgment) and remission status assessed at this later time point. If the bone marrow remains hypoplastic, then marrow studies should be repeated every 1 - 3 weeks (based upon the peripheral blood count recovery and the clinician's judgment) until an accurate bone marrow status is ascertained.

Differentiation between recovery bone marrow and AML blasts can be very difficult. Thus, repeat bone marrow examination in 7 - 10 days is strongly recommended when bone marrow blast percentage is $\geq 5\%$ after each course.

If clinically feasible, tissue biopsy should be used to determine extramedullary disease response. If tissue biopsy is not clinically appropriate, then radiographic studies should be used to determine extramedullary disease response. The differentiation between residual disease and residual scar tissue on radiographic studies may be difficult and the final determination of radiographic disease status should be made by the treating clinician in consultation with the local radiologist.

Progression to the next course of therapy should await marrow and extramedullary disease response determination. Refer to [Section 10.2](#) for the specific definitions of remission, persistent disease, refractory disease, and relapse.

4.5 End of Induction I

All patients will proceed to Induction II regardless of remission status after Induction I. It is suggested, but not required, that patients have an ANC $> 1,000/\mu\text{L}$ and a platelet count $> 75,000/\mu\text{L}$, after chemotherapy-induced count nadir, before proceeding with therapy.

4.6 Progression to Subsequent Courses of Therapy

Patients should progress to the next course of therapy as soon as clinically acceptable to the treating institution. It is recommended but not required that the ANC $> 1,000/\mu\text{L}$ and a platelet count $> 75,000/\mu\text{L}$ be achieved before proceeding with therapy. Patients should have time for full or partial recovery (judged by the treating institution) from any toxicities experienced in the prior course(s) of therapy before proceeding to the next course. Disease status evaluations with bone marrow examination should be completed, where required, prior to proceeding to the next course of therapy. Administration of the next course's intrathecal therapy at the time of these bone marrow evaluations is permitted to avoid additional sedation procedures.

4.7 Requirements to Start Each Sorafenib + Chemotherapy Course

Patients must meet the following parameters before starting sorafenib in any sorafenib + chemotherapy course. Patients may start sorafenib as soon as these criteria are met:

- Shortening fraction $\geq 28\%$, ejection fraction $\geq 55\%$
- BP less than the 95th percentile for age, height and gender (antihypertensive therapy is allowed)
- No concomitant treatment with CYP3A4 inducers/inhibitors listed in [Section 4.2.4](#)
- No limitations in activities of daily living if a maculo-papular rash is present
- Less than Grade 2 palmar-plantar erythrodysesthesia syndrome.

Patients who experienced any of the following while on study may not start sorafenib in any subsequent courses of therapy (please refer to [Sections 5.2](#) and [5.8](#) for additional details regarding the following toxicities as well as [Section 5.12](#) for guidance regarding dose modifications):

- Grade 4 hypertension
- Any maculo-papular rash or palmar-plantar erythrodysesthesia syndrome that warranted permanent discontinuation of sorafenib in an earlier phase of treatment
- Reduction in cardiac function to SF $< 28\%$ and/or EF $< 55\%$ on sorafenib 100 mg/m²/day (see [Section 5.2](#))

4.8 Induction I Arm A (ADE 10+3+5)

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Induction I therapy guidelines are for patients who were randomized to Arm A of the study prior to Amendment #7A. Induction I lasts 28 days or longer.

Note: Patients determined to be HR FLT3/ITD+ will be eligible to participate in sorafenib Arm C. During Arm C – Cohort 3: consenting patients begin sorafenib treatment in Induction I (see [Section 4.10](#)).

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.

Intrathecal Cytarabine (IT ARAC): IT

Given at time of diagnostic lumbar puncture or Day 1.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For CNS positive patients: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See

[Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

If IT cytarabine is given prior to diagnosis, a separate institutional consent must be obtained.

Cytarabine (IV ARAC): IV over 1 - 30 minutes

Days: 1 through 10.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or
3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes

Days: 1, 3 and 5.

Dose: 50 mg/m²/dose or
1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose or
3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the first evaluation time point can occur anytime within 14 days of the start of Induction I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction I (Arm A) is on the next page.

Following Induction I Arm A, Induction II Arm A (Section 4.12 or 4.14) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later). This should not precede the end of course marrow exam.

For patients without HR FLT3/ITD+:

Continue treatment on Induction II Arm A therapy, see Section 4.12 or 4.14.

For patients with HR FLT3/ITD+ disease (and who consent to participate in Arm C):

Continue treatment on sorafenib Arm C-Cohort 3 **starting in Induction I**, see [Section 4.10](#).

Patients who decline participation on Arm C continue treatment on Induction II Arm A (high risk) therapy, see Section 4.14.

4.8.1 Induction I Arm A (ADE 10+3+5) This therapy is for patients randomized to receive standard therapy only.	Patient name or initials _____	DOB _____
---	--------------------------------	-----------

Induction I lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1 - 4.6](#) and [4.8](#).

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age ≥ 3 years)	Given at time of Diagnostic LP or Day 1	If given prior to diagnosis, a separate institutional consent must be obtained.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total) b. BMA/clot section or biopsy, MRD c. HRQOL and Parental Stress Studies ¹ d. Optional biology studies (see Section 7.4).
<i>Note: For CNS positive patients see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.</i>					
Cytarabine (IV ARAC)	IV over 1 - 30 minutes	100 mg/m ² /dose, every 12 hours or 3.3 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 10	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6 m ²	See Section 7.1 for complete list of observations.
DAUNOrubicin (DAUN)	IV over 1 - 15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5		¹ For patients enrolled on this study, evaluation can be obtained anytime within 14 days of starting Induction I (see Section 18.0 for details).
Etoposide (ETOP)	IV over 60 - 120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5		OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	ARAC IT mg	ARAC IV mg	DAUN mg	ETOP mg	Studies	Comments (Include any held doses, or dose modifications)	
Enter calculated dose above and actual dose administered below										
		1	1	mg [#]	mg	mg	mg	a, c, d		
			2		mg	mg	mg			
			3		mg	mg	mg			
			4	mg*	mg	mg	mg			
			5		mg	mg	mg			
			6		mg	mg				
			7		mg	mg				
		2	8	mg*	mg	mg				
			9		mg	mg				
			10		mg	mg				
			11	mg*						
		3	15	mg*						
			18	mg*						
		4	28					b, d		
			29	Start next course (Induction II Arm A, Section 4.12 or 4.14) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later). This should not precede the end of course marrow exam.						

[#] If IT ARAC is given at diagnosis (instead of Day 1), record the date of administration: _____

* Note: additional IT ARAC doses for CNS+ patients only.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.9 Induction I Arm B (ADE 10+3+5+ Bortezomib)

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Induction I therapy guidelines are for patients who were randomized to Arm B of the study prior to Amendment #7A. Patients will receive bortezomib in addition to ADE therapy. Induction I lasts 28 days or longer.

Note: Patients determined to be HR FLT3/ITD+ will be eligible to participate in sorafenib Arm C. During Arm C–Cohort 3: consenting patients stop receiving bortezomib and continue treatment on Arm C–Cohort 3, starting on Day 11 of Induction I (see [Section 4.10](#)).

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://members.childrononcologygroup.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.

Intrathecal Cytarabine (IT ARAC): IT

Given at time of diagnostic lumbar puncture or Day 1.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For CNS positive patients: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

If IT cytarabine is given prior to diagnosis, a separate institutional consent must be obtained.

Cytarabine (IV ARAC): IV over 1 - 30 minutes

Days: 1 through 10.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or
3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes

Days: 1, 3 and 5.

Dose: 50 mg/m²/dose, or
1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose, or

3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

Bortezomib (BORTEZ): IV bolus over 3 - 5 seconds

Days: 1, 4 and 8.

Dose: 1.3 mg/m²/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.” Additional wording may be considered at the institution’s discretion.

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. 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.

On Days 1 and 4: administer after the end of the etoposide infusion.

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

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

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the first evaluation time point can occur anytime within 14 days of the start of Induction I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction I (Arm B) is on the next page.

Following completion of Induction I Arm B, Induction II Arm B (Section 4.13 or 4.15) starts on Day 29 or when blood count parameters and clinical conditions are acceptable (whichever occurs later). This should not precede the end of course marrow exam.

For patients without HR FLT3/ITD+:

Continue treatment on Induction II Arm B therapy, see Section 4.13 or 4.15.

For patients with HR FLT3/ITD+ disease (and who consent to participate in Arm C):

Continue treatment on sorafenib Arm C-Cohort 3 **starting in Induction I**, see [Section 4.10](#).

Patients who decline participation on Arm C continue treatment on Induction II Arm B (high risk) therapy, see Section 4.15.

4.9.1 Induction I Arm B (ADE 10+3+5 + Bortezomib) This therapy is for patients receiving standard therapy and bortezomib.	_____ Patient name or initials	_____ DOB
--	-----------------------------------	--------------

Induction I lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1 - 4.6](#) and [4.9](#).

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age ≥ 3 years)	Given at time of Diagnostic LP <u>or</u> Day 1	If given prior to diagnosis, a separate institutional consent must be obtained.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), neurologic exam b. BMA/clot section or biopsy, MRD c. HRQOL and Parental Stress Studies ¹ d. Optional biology studies (see Section 7.4) e. Pulse oximetry (prior to bortez)
Note: For CNS positive patients see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.					
Cytarabine (IV ARAC)	IV over 1 - 30 minutes	100 mg/m ² /dose, every 12 hours or 3.3 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 10	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6m ²	See Section 7.1 for complete list of observations.
DAUNOrubicin (DAUN)	IV over 1 - 15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5		¹ For patients enrolled on this study, evaluation can be obtained anytime within 14 days of starting Induction I (see Section 18.0 for details).
Etoposide (ETOP)	IV over 60 - 120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5		
Bortezomib (BORTEZ) IND# 114480	IV over 3 - 5 seconds	1.3 mg/m ² /dose	Days 1, 4, 8	Consecutive doses must be at least 72 hours apart. On Days 1 and 4: administer after the end of the etoposide infusion.	

OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	Ht	cm	Wt	kg	BSA	m ²	ARAC IT mg	ARAC IV mg	mg	DAUN mg	ETOP mg	BORTEZ mg	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below																	
		1	1	mg [#]		mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	a, c, d, e	
			2			mg	mg				mg	mg					
			3			mg	mg				mg	mg					
			4	mg*		mg	mg				mg	mg			mg	e	
			5			mg	mg				mg	mg					
			6			mg	mg				mg	mg					
			7			mg	mg				mg	mg					
		2	8	mg*		mg	mg				mg	mg			mg	e	
			9			mg	mg				mg	mg					
			10			mg	mg				mg	mg					
			11	mg*													
		3	15	mg*													
			18	mg*													
		4	28													b, d	
			29	Start next course (Induction II Arm B, Section 4.12 or 4.14) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later). This should not precede the end of course marrow exam.													

If IT ARAC is given at diagnosis (instead of Day 1), record the date of administration: _____

* Note: additional IT ARAC doses for CNS+ patients only.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.10 Induction I Arm C-Cohort 3 (ADE 10+3+5+ Sorafenib)

The following Induction I therapy guidelines are for patients with HR FLT3/ITD+ disease during the sorafenib feasibility part of the study (Cohort 3). Patients on Arm C-Cohort 3 will receive standard ADE therapy and sorafenib. Induction I lasts 28 days or longer.

See [Section 4.7](#) for requirements to start each sorafenib course.

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.

Intrathecal Cytarabine (IT ARAC): IT

Given at time of diagnostic lumbar puncture or Day 1.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For CNS positive patients: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

If IT cytarabine is given prior to diagnosis, a separate institutional consent must be obtained.

Cytarabine (IV ARAC): IV over 1 - 30 minutes

Days: 1 through 10.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or
3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes.

Days: 1, 3 and 5.

Dose: 50 mg/m²/dose, or
1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose if BSA, or
3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

Sorafenib (SORAF): PO

Days: 11 through 28.

Dose: 200 mg/m²/dose daily, rounded to accommodate tablet size. The maximum dose will be 400 mg.
Dose to be administered per dosing nomogram in [Appendix II](#).

Note: Sorafenib tablets should be taken at least 1 hour before or 2 hours after food. Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 years and 4 to 8 ounces for ≥ 12 years). If taken with food, sorafenib should be taken with a moderate to low fat meal. Tablets should not be crushed, but should be swallowed whole. However, sorafenib tablets can be dispersed in water to facilitate the administration to subjects that cannot swallow tablets (see drug monograph in [Section 6.12](#)). Grapefruit and its juice should be avoided for the duration of treatment with sorafenib.

Sorafenib is supplied by Bayer HealthCare Pharmaceuticals, and distributed by the NCI DTCD. Do not use commercially available drug.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the first evaluation time point can occur anytime within 14 days of the start of Induction I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction I Arm C- Cohort 3 is on the next page.

Following completion of Induction I, Induction II Arm C- Cohort 3 (Section 4.16) starts on Day 29 or when blood count parameters and clinical conditions are acceptable (whichever occurs later). This should not precede the end of course marrow exam.

4.10.1 Induction I - Arm C-Cohort 3 (ADE 10+3+5 + Sorafenib)
 This therapy is for HR FLT3/ITD+ patients receiving standard therapy and sorafenib during sorafenib feasibility assessment. Patient name or initials _____ DOB _____

Induction I lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Section 4.1-4.7](#) and [4.10](#).

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age ≥ 3 years)	Given at time of Diagnostic LP <u>or</u> Day 1	If given prior to diagnosis, a separate institutional consent must be obtained.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), BP, urinalysis b. BMA/clot section or biopsy, MRD c. HRQOL and Parental Stress Studies ¹ d. Optional biology studies (see Section 7.4) e. Clinical cardiac examination See Section 7.1 for complete list of observations.
<i>Note: For CNS positive patients see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.</i>					
Cytarabine (IV ARAC)	IV over 1 - 30 minutes	100 mg/m ² /dose, every 12 hrs or 3.3 mg/kg/dose, every 12 hrs if BSA < 0.6 m ²	Days 1 - 10	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6 m ²	¹ For patients enrolled on this study, evaluation can be obtained anytime within 14 days of starting Induction I (see Section 18.0 for details). OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE
DAUNOrubicin (DAUN)	IV over 1 - 15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5		
Etoposide (ETOP)	IV over 60 - 120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5		
Sorafenib (SORAF) IND# 114480	PO	200 mg/m ² /dose daily	Days 11 - 28	See Section 4.10 for administration guidelines. See Section 5.0 for dose modification plan. The maximum dose is 400 mg.	

Date Due	Date Given	Week	Day	ARAC IT mg	ARAC IV mg	DAUN mg	ETOP mg	SORAF mg	BSA m ²	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below											
		1	1	mg [#]	mg	mg	mg	mg		a, c, d, , e	
			2		mg	mg		mg			
			3		mg	mg	mg	mg			
			4	mg*	mg	mg		mg			
			5		mg	mg	mg	mg			
			6		mg	mg					
			7		mg	mg					
		2	8	mg*	mg	mg				e	
			9		mg	mg					
			10	mg*	mg	mg					
			11					mg			
			12					↓ mg			
			13								
			14							d	
		3	15	mg*						e	
			16								
			17								
			18	mg*							
			21							d	
		4	22							e	
			28							b, d	

Start next course (Induction II Arm C, Section 4.16) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later). This should not precede the end of course marrow exam.

[#] If IT ARAC is given at diagnosis (instead of Day 1), record the date of administration: _____ * Note: additional IT ARAC doses for CNS+ patients only.
 SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX 1](#) FOR SUPPORTIVE CARE GUIDELINES.

4.11 Induction I Arm D (ADE 10+3+5)

The following Induction I therapy guidelines are for patients enrolled on study after implementation of Amendment #7A and whose FLT3/ITD status is **unknown** prior to study enrollment. Induction I lasts 28 days or longer.

Note: Patients determined to be HR FLT3/ITD+ will be eligible to participate in sorafenib Arm C. During Arm C–Cohort 3: consenting patients begin sorafenib treatment in Induction I (see [Section 4.10](#)).

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.

Intrathecal Cytarabine (IT ARAC): IT

Given at time of diagnostic lumbar puncture or Day 1.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For CNS positive patients: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

If IT cytarabine is given prior to diagnosis, a separate institutional consent must be obtained.

Cytarabine (IV ARAC): IV over 1 - 30 minutes

Days: 1 through 10.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or 3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes

Days: 1, 3 and 5.

Dose: 50 mg/m²/dose or 1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose or
3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction I (Arm D) is on the next page.

For patients without HR FLT3/ITD+:

Remove from study at the time of FLT3/ITD test results with no further data submission. See Section 7.1 for required End of Therapy evaluations

For patients with HR FLT3/ITD+ disease:

Patients who consent to participate in Arm C will switch to the sorafenib treatment arm (Arm C-Cohort 3) **starting with Induction I** (see [Section 4.10](#)) at the time of FLT3/ITD test results.

Patients who decline participation on Arm C are removed from study with no further data submission. See Section 7.1 for required End of Therapy evaluations

For patients with unknown FLT3/ITD at End of Induction I:

Remove from study with no further data submission. See Section 7.1 for required End of Therapy evaluations

4.11.1 Induction I Arm D (ADE 10+3+5) This therapy is for patients enrolled on study with unknown FLT3/ITD status only enrolled after Amendment #7A.	_____	_____
	Patient name or initials	DOB

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age ≥ 3 years)	Given at time of Diagnostic LP <u>or</u> Day 1	If given prior to diagnosis, a separate institutional consent must be obtained.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total) b. BMA/clot section or biopsy, MRD c. Optional biology studies (see Section 7.4).
<i>Note: For CNS positive patients see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.</i>					
Cytarabine (IV ARAC)	IV over 1 - 30 minutes	100 mg/m ² /dose, every 12 hours or 3.3 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 10	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6 m ²	See Section 7.1 for complete list of observations.
DAUNOrubicin (DAUN)	IV over 1 - 15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5		OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE
Etoposide (ETOP)	IV over 60 - 120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5		

Date Due	Date Given	Week	Day	ARAC IT mg	ARAC IV mg	Ht cm	Wt kg	DAUN mg	ETOP mg	BSA m ²	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below												
		1	1	mg [#]	mg	mg		mg	mg		a, c	
			2		mg	mg			mg			
			3		mg	mg		mg	mg			
			4	mg*	mg	mg			mg			
			5		mg	mg		mg	mg			
			6		mg	mg						
			7		mg	mg						
		2	8	mg*	mg	mg						
			9		mg	mg						
			10		mg	mg						
			11	mg*								
		3	15	mg*								
			18	mg*								
		4	28								b, c	
			29	Switch patients with HR FLT3/ITD+ to sorafenib treatment arm (Arm C-cohort 3, Section 4.10) after consenting them to Arm C treatment. Patients without HR FLT3/ITD+, with unknown FLT3/ITD status at End of Induction I, or who do not consent to Arm C are Off Study. See Section 7.1 for required end of therapy evaluations								

If IT ARAC is given at diagnosis (instead of Day 1), record the date of administration: _____

* Note: additional IT ARAC doses for CNS+ patients only.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.12 Induction II Arm A (ADE 8+3+5) – Low Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Induction II therapy guidelines are for low risk patients randomized to Arm A of the study. Arm A consists of the standard therapy. Induction II lasts 28 days or longer.

All patients will proceed to Induction II regardless of bone marrow remission status after Induction I. Progression to Induction II should await disease response determination (MRD and extramedullary disease) in order to correctly assign risk stratum. Patients with high or low risk cytogenetic/molecular features may proceed on to Induction II therapy before disease response results are available. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. By the beginning of Induction II, patients should have been classified as Low or High risk.

Patients who were CSF positive at start of Induction I and are CSF positive at start of Induction II will be taken off protocol therapy ([Section 8.1](#)).

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Induction II or with the bone marrow evaluation at the end of Induction I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For newly detected CNS positive patients who were CNS negative at diagnosis: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

Cytarabine (ARAC IV): IV over 1 - 30 minutes

Days: 1 through 8.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or
3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes

Days: 1, 3, and 5.

Dose: 50 mg/m²/dose, or
1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose or
3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the second evaluation time is on or after Day 21 of Induction II, but prior to the start of Intensification I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction II (Arm A, Low Risk) is on the next page.

End of Induction II: Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I. Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study.

Following Induction II Arm A, the next course (Intensification I - Arm A, [Section 4.17](#)) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.12.1 Induction II Arm A (ADE 8+3+5) – Low Risk Patients

This therapy is for low risk patients randomized to receive standard therapy.

Patient name or initials

DOB

Induction II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Section 4.1-4.6](#) and [4.12](#). **All patients will proceed to Induction II regardless of remission status after Induction I.** Progression to Induction II should await disease response determination so as to properly assign risk strata. It is suggested, but not required, that patients have an ANC > 1000/ μ L and a platelet count > 75 000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine [#] (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	[#] May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), Echo or MUGA and ECG b. BMA/clot section or biopsy c. Optional biology studies (see Section 7.4) d. HRQOL and Parental Stress Studies ¹
Note: For CNS positive patients (initially CNS negative at diagnosis) see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.					
Cytarabine (IV ARAC)	IV over 1 - 30 minutes	100 mg/m ² /dose, every 12 hours or 3.3 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 8	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6 m ²	See Section 7.1 for complete list of observations.
DAUNOrubicin (DAUN)	IV over 1 - 15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5		¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Induction II, but prior to Intensification I (see Section 18.0 for details).
Etoposide (ETOP)	IV over 60 - 120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5		OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	Ht cm		Wt kg		BSA m ²	Studies	Comments (Include any held doses, or dose modifications)	
				ARAC IT mg	ARAC IV mg	DAUN mg	ETOP mg				
Enter calculated dose above and actual dose administered below											
		1	1	mg [#]	mg	mg	mg	mg	a		
			2		mg	mg		mg			
			3		mg	mg	mg	mg			
			4	mg*	mg	mg		mg			
			5		mg	mg	mg	mg			
			6		mg	mg					
			7		mg	mg					
		2	8	mg*	mg	mg					
			11	mg*							
		3	15	mg*							
			18	mg*							
			21						d		
		4	28						b, c		
			29	Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in Section 10.2.1 will proceed to Intensification I (AE). Patients with refractory disease as defined in Section 10.2.2 are Off Protocol Therapy but not Off Study. Start next course (Intensification I Arm A, Section 4.17) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).							

[#] May be performed with BM done at end of previous course.

*Note: additional IT ARAC doses for CNS+ patients only; those with **no** CNS disease at dx, but CNS disease present at start of Induction II (see [Section 4.3](#)).

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.13 Induction II Arm B (ADE 8+3+5 + Bortezomib) – Low Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Induction II therapy guidelines are for low risk patients randomized to Arm B of the study. Patients will receive bortezomib in addition to ADE therapy. Induction II lasts 28 days or longer.

All patients will proceed to Induction II regardless of bone marrow remission status after Induction I. Progression to Induction II should await disease response determination (MRD and extramedullary disease) in order to correctly assign risk stratum. Patients with high or low risk cytogenetic/molecular features may proceed on to Induction II therapy before disease response results are available. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. By the beginning of Induction II, patients should have been classified as Low or High risk.

Patients who were CSF positive at start of Induction I and are CSF positive at start of Induction II will be taken off protocol therapy ([Section 8.1](#)).

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Induction II or with the bone marrow evaluation at the end of Induction I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For newly detected CNS positive patients who were CNS negative at diagnosis: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

Cytarabine (IV ARAC): IV over 1 - 30 minutes

Days: 1 through 8.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or
3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes

Days: 1, 3, and 5.

Dose: 50 mg/m²/dose, or
1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose, or

3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

Bortezomib (BORTEZ): IV push over 3 - 5 seconds

Days: 1, 4 and 8.

Dose: 1.3 mg/m²/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." Additional wording may be considered at the institution's discretion.

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. 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.

On Days 1 and 4: administer after the end of the etoposide infusion.

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

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the second evaluation time is on or after Day 21 of Induction II, but prior to the start of Intensification I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction II (Arm B, Low Risk) is on the next page.

End of Induction II: Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I. Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study.

Following Induction II Arm B, the next course (Intensification I - Arm B, Section 4.18) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.13.1 Induction II Arm B (ADE 8+3+5+ Bortezomib) – Low Risk Patients This therapy is for low risk patients receiving standard therapy and bortezomib.	_____ Patient name or initials	_____ DOB
--	-----------------------------------	--------------

Induction II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1-4.6](#) and [4.13](#). *All patients will proceed to Induction II regardless of remission status after Induction I.* Progression to Induction II should await disease response determination so as to properly assign risk strata. It is suggested, but not required, that patients have an ANC > 1000/ μ L and a platelet count > 75 000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS	
Cytarabine [#] (IT ARAC)	IT	20 mg (age 0-0.99 years) 30 mg (age 1-1.99 years) 50 mg (age 2-2.99 years) 70 mg (age \geq 3 years)	Day 1	[#] May be administered with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), neurologic exam, Echo or MUGA and ECG b. BMA/clot section or biopsy c. Optional biology studies (see Section 7.4) d. HRQOL and Parental Stress Studies ¹ e. Pulse oximetry (prior to bortez) f. PK studies (see Appendices XII-XV , closed to new patients as of 6/25/13) See Section 7.1 for complete list of observations. ¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Induction II, but prior to Intensification I (see Section 18.0 for details). OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE	
<i>Note: For CNS positive patients (initially CNS negative at diagnosis) see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.</i>						
Cytarabine (IV ARAC)	IV over 1-30 minutes	100 mg/m ² /dose, every 12 hours or 3.3 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1-8	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6 m ²		
DAUNorubicin (DAUN)	IV over 1-15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5			
Etoposide (ETOP)	IV over 60-120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1-5			
Bortezomib (BORTEZ) IND# 114480	IV over 3-5 seconds	1.3 mg/m ² /dose	Days 1, 4, 8	Consecutive doses must be at least 72 hours apart. On Days 1 and 4: administer after the end of the etoposide infusion		

Date Due	Date Given	Week	Day	ARAC IT mg	ARAC IV mg	DAUN mg	ETOP mg	BORTEZ mg	Studies	Comments (Include any held doses, or dose modifications)	
Enter calculated dose above and actual dose administered below											
		1	1	mg [#]	mg mg	mg	mg	mg	a, e		
			2		mg mg		mg				
			3		mg mg	mg	mg				
			4	mg*	mg mg		mg	mg	e		
			5		mg mg	mg	mg				
			6		mg mg						
			7		mg mg						
		2	8	mg*	mg mg			mg	e, f		
			11	mg*							
		3	15	mg*							
			18	mg*							
			21						d		
		4	28						b, c		
			29	Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in Section 10.2.1 will proceed to Intensification Course I (AE). Patients with refractory disease as defined in Section 10.2.2 are Off Protocol Therapy but not Off Study. Start next course (Intensification I – Arm B, Section 4.18) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).							

[#] May be performed with BM done at end of previous course.

* Note: add¹ IT ARAC doses for CNS+ pts only; those with **no** CNS disease at dx, but CNS disease present at start of Induction II (see [Sect. 4.3](#)).

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.14 Induction II Arm A (MA) – High Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Induction II therapy guidelines are for high risk patients randomized to Arm A of the study. Induction II lasts 28 days or longer.

All patients will proceed to Induction II regardless of bone marrow remission status after Induction I. Progression to Induction II should await disease response determination (MRD and extramedullary disease) in order to correctly assign risk stratum. Patients with high or low risk cytogenetic/molecular features may proceed on to Induction II therapy before disease response results are available. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. By the beginning of Induction II, patients should have been classified as Low or High risk. **If a patient is classified as High risk at the end of Induction I and no family HLA match is found, initiation of an unrelated donor search should occur.**

Patients who were CSF positive at start of Induction I and are CSF positive at start of Induction II will be taken off protocol therapy ([Section 8.1](#)).

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Induction II or with the bone marrow evaluation at the end of Induction I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For newly detected CNS positive patients who were CNS negative at diagnosis: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 4.

Dose: 1000 mg/m²/dose, every 12 hours (i.e., 2000 mg/m²/day, divided BID) or 33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

MitoXANTRONE (MITOX): IV over 15 - 30 minutes

Days: 3 through 6.

Dose: 12 mg/m²/dose or
0.4 mg/kg/dose if BSA < 0.6 m².

Note: Administer through the tubing of a rapidly infusing solution of D₅W or 0.9% NaCl. Avoid extravasation; the use of a central line is suggested. On the Days 3 and 4, mitoXANTRONE should be given 8 hours after the 5th and 7th high dose cytarabine infusions are complete.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the second evaluation time is on or after Day 21 of Induction II, but prior to the start of Intensification I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction II (Arm A, High Risk) is on the next page.

End of Induction II: Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I. Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study.

Following Induction II Arm A, the next course (Intensification I - Arm A, [Section 4.17](#)) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.14.1 Induction II Arm A (MA) – High Risk Patients

This therapy is for high risk patients randomized to receive standard therapy.

Patient name or initials

DOB

Induction II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Section 4.1-4.6](#) and [4.14](#). **All patients will proceed to Induction II regardless of remission status after Induction I.** Progression to Induction II should await disease response determination so as to properly assign risk strata. It is suggested, but not required, that patients have an ANC > 1000/ μ L and a platelet count > 75 000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine [#] (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	[#] May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), Echo or MUGA and ECG b. CrCl c. BMA/clot section or biopsy d. Optional biology studies (see Section 7.4) e. HRQOL and Parental Stress Studies ¹ See Section 7.1 for complete list of observations.
Note: For CNS positive patients (initially CNS negative at diagnosis) see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.					
Cytarabine (HD ARAC)	IV over 1 - 3 hours	1000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 4	Total dose: 2000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops as described in Section 4.1.	¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Induction II, but prior to Intensification I (see Section 18.0 for details).
MitoXANTRONE (MITOX)	IV over 15 - 30 minutes	12 mg/m ² /dose or 0.4 mg/kg/dose if BSA < 0.6 m ²	Days 3 - 6	See Section 4.14 for administration guidelines. On Days 3 and 4, administer 8 hours after the 5 th and 7 th cytarabine infusions are completed.	OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	ARAC IT mg	Ht cm HD ARAC mg	Wt kg MITOX mg	BSA m ² Studies	Comments (Include any held doses, or dose modifications)	
Enter calculated dose above and actual dose administered below									
		1	1	mg [#]	mg	mg		a, b ⁺	
			2		mg	mg			
			3		mg	mg	mg		
			4	mg*	mg	mg	mg		
			5				mg		
			6				mg		
			7						
		2	8	mg*					
			11	mg*					
			15	mg*					
			18	mg*					
			21					e	
			28					c, d	
			29	Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in Section 10.2.1 will proceed to Intensification I (AE). Patients with refractory disease as defined in Section 10.2.2 are Off Protocol Therapy but not Off Study. Start next course (Intensification I Arm A, Section 4.17) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).					

[#] May be performed with BM done at end of previous course.

* Note: add¹ IT ARAC doses for CNS+ pts only; those with no CNS disease at dx, but CNS+ at start of Ind II ([Sect. 4.3](#)). ⁺ For pts in whom serum Cr is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.15 Induction II Arm B (MA + Bortezomib) – High Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Induction II therapy guidelines are for high risk patients randomized to Arm B of the study. Patients will receive bortezomib in addition to MA therapy. Induction II lasts 28 days or longer.

All patients will proceed to Induction II regardless of bone marrow remission status after Induction I. Progression to Induction II should await disease response determination (MRD and extramedullary disease) in order to correctly assign risk stratum. Patients with high or low risk cytogenetic/molecular features may proceed on to Induction II therapy before disease response results are available. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. By the beginning of Induction II, patients should have been classified as Low or High risk. **If a patient is classified as High risk at the end of Induction I and no family HLA match is found, initiation of an unrelated donor search should occur.**

Patients who were CSF positive at start of Induction I and are CSF positive at start of Induction II will be taken off protocol therapy ([Section 8.1](#)).

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Induction II or with the bone marrow evaluation at the end of Induction I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For newly detected CNS positive patients who were CNS negative at diagnosis: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 4.

Dose: 1000 mg/m²/dose, every 12 hours (i.e., 2000 mg/m²/day, divided BID) or
33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL

or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

MitoXANTRONE (MITOX): IV over 15 - 30 minutes

Days: 3 through 6.

Dose: 12 mg/m²/dose or

0.4 mg/kg/dose if BSA < 0.6 m²

Note: Administer through the tubing of a rapidly infusing solution of D₅W or 0.9% NaCl. Avoid extravasation; the use of a central line is suggested. On Days 3 and 4, mitoXANTRONE should be given 8 hours after the 5th and 7th high dose cytarabine infusions are complete.

Bortezomib (BORTEZ): IV over 3 - 5 seconds

Days: 1, 4 and 8.

Dose: 1.3 mg/m²/dose.

On Day 4: Administer after the end of the mitoXANTRONE infusion.

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.” Additional wording may be considered at the institution’s discretion.

Note: at least 72 hours must have elapsed between doses. Grapefruit and its juice should be avoided for the duration of treatment with bortezomib. 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.

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

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the second evaluation time is on or after Day 21 of Induction II, but prior to the start of Intensification I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction II (Arm B, High Risk) is on the next page.

End of Induction II: Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I. Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study.

Following Induction II Arm B, the next course (Intensification I - Arm B, [Section 4.18](#)) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.15.1 Induction II Arm B (MA+ Bortezomib) – High Risk Patients

This therapy is for high risk patients receiving standard therapy and bortezomib.

Patient name or initials

DOB

Induction II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1-4.6](#) and [4.15](#). **All patients will proceed to Induction II regardless of remission status after Induction I.** Progression to Induction II should await disease response determination so as to properly assign risk strata. It is suggested, but not required, that patients have an ANC > 1000/ μ L and a platelet count > 75 000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine [#] (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	[#] May be administered with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct & total), neurologic exam, Echo or MUGA & ECG b. CrCl c. BMA/clot section or biopsy d. Optional biology studies (see Section 7.4) e. HRQOL and Parental Stress Studies ¹ f. Pulse oximetry (prior to bortez) g. PK studies (see Appendices XII-XV , closed to new patients as of 6/25/13)
<i>Note: For CNS positive patients (initially CNS negative at diagnosis) see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.</i>					
High dose Cytarabine (HD ARAC)	IV over 1 - 3 hours	1000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 4	Total dose: 2000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² Use eye drops as described in Section 4.15 .	See Section 7.1 for complete list of observations. ¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Induction II, but prior to Intensification I (see Section 18.0). OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE
MitoXANTRONE (MITOX)	IV over 15 - 30 minutes	12 mg/m ² /dose or 0.4 mg/kg/dose if BSA < 0.6 m ²	Days 3 - 6	See Section 4.15 for administration guidelines. On Days 3 and 4, administer 8 hours after the 5 th and 7 th cytarabine infusions are completed.	
Bortezomib (BORTEZ) IND# 114480	IV over 3 - 5 seconds	1.3 mg/m ² /dose	Days 1, 4, 8	Consecutive doses must be at least 72 hours apart. On Day 4, administer <u>after</u> the end of the mitoXANTRONE infusion.	

Date Due	Date Given	Week	Day	ARAC IT mg	Ht cm HD ARAC mg mg	Wt kg MITOX mg	BSA m ² BORTEZ mg	Studies	Comments (Include any held doses, or dose modifications)	
Enter calculated dose above and actual dose administered below										
		1	1	mg [#]	mg mg		mg	a, b [†] , f		
			2		mg mg					
			3		mg mg	mg				
			4	mg*	mg mg	mg	mg	f		
			5			mg				
			6			mg				
			7							
		2	8	mg*			mg	f, g		
			11	mg*						
		3	15	mg*						
			18	mg*						
			21					e		
		4	28					c, d		
			29	Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in Section 10.2.1 will proceed to Intensification Course I (AE). Patients with refractory disease as defined in Section 10.2.2 are Off Protocol Therapy but not Off Study. Start next course (Intensification I – Arm B, Section 4.18) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).						

[#]May be performed with BM done at end of previous course. ^{*}Note: add[†] IT ARAC doses for CNS+ pts only; those with **no** CNS disease at dx, but CNS+ at start of Ind II ([Sect. 4.3](#)). [†] For pts in whom serum Cr is > 2 mg/dL or > 2 x normal for age. SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX 1](#) FOR SUPPORTIVE CARE GUIDELINES.

4.16 Induction II Arm C - Cohort 3 (ADE 8+3+5 + Sorafenib)

The following Induction II therapy guidelines are for patients with HR FLT3/ITD+ disease during the sorafenib feasibility part of the study (Cohort 3). Patients on Arm C will receive standard ADE therapy and sorafenib. Induction II lasts 36 days or longer.

All patients will proceed to Induction II regardless of bone marrow remission status after Induction I. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. **All patients with HR FLT3/ITD+ should have evaluation for a family HLA matched donor. If no family HLA match is found, initiation of an unrelated donor search should occur.**

Patients who were CSF positive at start of Induction I and are CSF positive at start of Induction II will be taken off protocol therapy ([Section 8.1](#)).

See [Section 4.7](#) for requirements to start each sorafenib course.

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Induction II or with the bone marrow evaluation at the end of Induction I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

For newly detected CNS positive patients who were CNS negative at diagnosis: IT cytarabine is to be administered twice weekly until the CSF is clear. A minimum of 4 intrathecal treatments must be given and a maximum of 6 intrathecal treatments may be given. Patients whose CSF is clear at the second spinal tap will receive 3 intrathecal treatments once the CSF is clear and all other patients will receive 2 intrathecal treatments once the CSF is clear. See [Section 3.3.4](#) for definitions of CSF involvement. Patients with refractory CNS leukemia following 6 doses of therapy will be taken off protocol therapy.

Cytarabine (IV ARAC): IV over 1 - 30 minutes

Days: 1 through 8.

Dose: 100 mg/m²/dose, every 12 hours (i.e., 200 mg/m²/day, divided BID), or
3.3 mg/kg/dose, every 12 hours (i.e., 6.6 mg/kg/day, divided BID) if BSA < 0.6 m².

DAUNOrubicin (DAUN): IV over 1 - 15 minutes

Days: 1, 3 and 5.

Dose: 50 mg/m²/dose, or
1.7 mg/kg/dose if BSA < 0.6 m².

Note: 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 D₅W 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.

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 100 mg/m²/dose, or
3.3 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested.

Sorafenib (SORAF): PO

Days: 9 through 36.

Dose: 200 mg/m²/dose daily, rounded to accommodate tablet size. The maximum dose will be 400 mg.
Dose to be administered per the dosing nomogram in [Appendix II](#).

Note: Sorafenib tablets should be taken at least 1 hour before or 2 hours after food. Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 years and 4 to 8 ounces for ≥ 12 years). If taken with food, sorafenib should be taken with a moderate to low fat meal. Tablets should not be crushed, but should be swallowed whole. However, sorafenib tablets can be dispersed in water to facilitate the administration to subjects that cannot swallow tablets (see drug monograph in [Section 6.12](#)). Grapefruit and its juice should be avoided for the duration of treatment with sorafenib.

Sorafenib is supplied by Bayer HealthCare Pharmaceuticals, and distributed by the NCI DTCD. Do not use commercially available drug.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the second evaluation time is on or after Day 21 of Induction II, but prior to the start of Intensification I. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Induction II Arm C-Cohort 2 is on the next page.

End of Induction II: Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I. Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study.

Following Induction II Arm C - Cohort 3, the next course (Intensification I Arm C - Cohort 3, [Section 4.19](#)) starts on Day 37 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.16.1 Induction II Arm C-Cohort 3 (ADE 8+3+5 Sorafenib) This therapy is for HR FLT3/ITD+ patients receiving standard therapy and sorafenib during sorafenib feasibility assessment.	Patient name or initials _____ DOB _____
--	---

Induction II lasts 36 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Section 4.1-4.7](#) and [4.16](#). **All patients will proceed to Induction II regardless of remission status after Induction I.** It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine [#] (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	[#] May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), Echo or MUGA and ECG, BP, urinalysis b. BMA/clot section or biopsy c. Optional biology studies (see Sect. 7.4) d. HRQOL and Parental Stress Studies ¹ e. Clinical cardiac examination See Section 7.1 for complete list of observations.
Note: For CNS positive patients (initially CNS negative at diagnosis) see Section 4.3.1 for details of additional doses of intrathecal ARAC needed.					
Cytarabine (IV ARAC)	IV over 1 - 30 minutes	100 mg/m ² /dose, every 12 hours or 3.3 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 8	Total dose: 200 mg/m ² /day, divided BID or 6.6 mg/kg/day, divided BID if BSA < 0.6 m ²	¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Induction II, but prior to Intensification I (see Sect. 18.0 for details) OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE
DAUNOrubicin (DAUN)	IV over 1 - 15 minutes	50 mg/m ² /dose or 1.7 mg/kg/dose if BSA < 0.6 m ²	Days 1, 3, 5		
Etoposide (ETOP)	IV over 60 - 120 minutes	100 mg/m ² /dose or 3.3 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5		
Sorafenib (SORAF) IND# 114480	PO	200 mg/m ² /dose once daily	Days 9 - 36	See Section 4.16 for administration guidelines. See Section 5.0 for dose modification plan. The maximum dose will be 400 mg.	

Date Due	Date Given	Week	Day	ARAC IT mg	ARAC IV mg	DAUN mg	ETOP mg	SORAF mg	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below										
		1	1	mg [#]	mg	mg	mg	mg	a, c, e	
			2		mg	mg	mg			
			3		mg	mg	mg			
			4	mg*	mg	mg	mg			
			5		mg	mg	mg			
			6		mg	mg				
			7		mg	mg				
		2	8	mg*	mg	mg			e	
			9					mg		
			11	mg*						
			14						c	
		3	15	mg*					e	
			18	mg*						
			21						c, d	
		4	22						e	
			28						c	
		5	29						e	
		6	36						b, c, e	

Remission status at the end of Induction II will determine whether patients receive further protocol therapy. All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I Arm C-Cohort 3 (AE). Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study.
Start next course (Intensification I Arm C-Cohort , [Section 4.19](#)) on Day 37 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

[#] May be performed with BM done at end of previous course.

* Note: additional IT ARAC doses for CNS+ patients only; those with **no** CNS disease at dx, but CNS+ at start of Induction II (see [Section 4.3](#)).

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.17 Intensification I Arm A (AE)

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Intensification I therapy guidelines are for patients randomized to Arm A of the study. Arm A consists of the standard therapy. Intensification I lasts 28 days or longer.

All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I (AE). Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with Intensification I therapy.

Patients with high risk status will proceed to best allogeneic donor SCT (see [Section 4.24](#)) or alternative donor SCT (see [Section 4.25](#)) following Intensification I. All high risk patients without a suitable donor will proceed to a final course of high dose cytarabine/L-Asparaginase ([Section 4.22](#)). Patients with low risk status will proceed to a final course of cytarabine/mitoxantrone ([Section 4.20](#)) regardless of the availability of a MFD (i.e., no SCT for these patients). Enrollment on AAML05P1 will be permitted for patients with an available unrelated donor.

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Intensification I or with the bone marrow evaluation at the end of Induction II.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 5.

Dose: 1000 mg/m²/dose, every 12 hours, (i.e., 2000 mg/m²/day, divided BID), or 33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

Etoposide (ETOP): IV over 60 - 120 minutes

Days: 1 through 5.

Dose: 150 mg/m²/dose, or

5 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested. Each dose of etoposide should immediately follow the 1st, 3rd, 5th, 7th and 9th doses of cytarabine.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the third evaluation time point is on or after Day 21 of Intensification I, but prior to Intensification II. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification I (Arm A) is on the next page.

End of Intensification I: Remission status at the end of Intensification I will determine whether patients receive further protocol therapy. Bone marrow exam after Intensification I must show continued complete remission. Patients not in remission as defined in [Section 10.2.1](#) will be Off Protocol Therapy but not Off Study. All patients in remission will proceed on to Intensification II or SCT.

Following Intensification I – Arm A, the next course (Intensification II – Arm A [[Section 4.20](#) or [4.22](#)], or SCT [[Section 4.24](#) or [4.25](#) depending on availability of a suitable donor]) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.17.1 Intensification I Arm A (AE) This therapy is for patients randomized to receive standard therapy.				Patient name or initials _____	DOB _____
Intensification I lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive details on treatment assignment are provided in Sections 4.1-4.6 and 4.17 . It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.					
DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine* (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	* May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), Echo or MUGA and ECG b. CrCl c. BMA/clot section or biopsy. (For SCT patients: the end of therapy BMA/Biopsy is to be done within 1 month of ANC recovery.) d. Optional biology studies (see Section 7.4) e. HRQOL and Parental Stress Studies ¹
High dose Cytarabine (HD ARAC)	IV over 1 - 3 hours	1000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 5	Total dose: 2000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops as described in Section 4.17 .	
Etoposide (ETOP)	IV over 60 - 120 minutes	150 mg/m ² /dose or 5 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5	Each dose of etoposide should immediately follow the 1 st , 3 rd , 5 th , 7 th and 9 th doses of cytarabine.	See Section 7.1 for complete list of observations. ¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Intensification I, but prior to Intensification II (see Section 18.0 for details). OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	ARAC IT mg	HD ARAC mg	ETOP mg	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below								
		1	1	mg*	mg	mg	a, b [#]	
			2		mg	mg		
			3		mg	mg		
			4		mg	mg		
			5		mg	mg		

		3	21				e	

		4	28				c, d	
			29	Remission status at the end of Intensification I will determine whether patients receive further protocol therapy. Patients not in remission as defined in Section 10.2.1 will be Off Protocol Therapy but not Off Study. All patients in remission will proceed on to Intensification II or SCT. Start next course (Intensification II – Arm A, Section 4.20 or 4.22) or SCT (Section 4.24 or 4.25) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).				

* May be performed with BM done at end of previous course.

For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.18 Intensification I Arm B (AE + Bortezomib)

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Intensification I therapy guidelines are for patients randomized to Arm B of the study. Patients on Arm B receive bortezomib in addition to AE therapy. Intensification I lasts 28 days or longer.

All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I (AE). Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

Patients with high risk status will proceed to best allogenic donor SCT (see [Section 4.24](#)) or alternative donor SCT (see [Section 4.25](#)) following Intensification I. All high risk patients without a suitable donor will proceed to a final course of high dose cytarabine/L-Asparaginase ([Section 4.22](#)). Patients with low risk status will proceed to a final course of cytarabine/mitoxantrone ([Section 4.21](#)) regardless of the availability of a MFD (i.e., no SCT for these patients). Enrollment on AAML05P1 will be permitted for patients with an available unrelated donor.

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Intensification I or with the bone marrow evaluation at the end of Induction II.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 5.

Dose: 1,000 mg/m²/dose, every 12 hours (i.e., 2,000 mg/m²/day, divided BID), or
33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 150 mg/m²/dose or
5 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested. Each dose of etoposide should immediately follow the 1st, 3rd, 5th, 7th and 9th doses of cytarabine.

Bortezomib (BORTEZ): IV over 3 - 5 seconds

Days: 1, 4 and 8.

Dose: 1.3 mg/m²/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.” Additional wording may be considered at the institution’s discretion.

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. 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.

On Days 1 and 4: Administer after the end of the etoposide infusion.

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

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the third evaluation time point is on or after Day 21 of Intensification I, but prior to Intensification II. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification I - Arm B is on the next page.

End of Intensification I: Remission status at the end of Intensification I will determine whether patients receive further protocol therapy. Bone marrow exam after Intensification I must show continued complete remission. Patients not in remission as defined in [Section 10.2.1](#) will be Off Protocol Therapy but not Off Study. All patients in remission will proceed on to Intensification II or SCT accordingly:

Following Intensification I – Arm B, the next course (Intensification II – Arm B [[Section 4.21](#) or [4.22](#)] or SCT [see [Section 4.24](#) or [4.25](#) depending on availability of a suitable donor]) starts on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.18.1 Intensification I Arm B (AE + Bortezomib) This therapy is for patients randomized to receive bortezomib and standard therapy.	Patient name or initials	DOB
--	--------------------------	-----

Intensification I lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive details on treatment assignment are provided in [Sections 4.1-4.6](#) and [4.18](#). It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine* (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	* May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), neurologic exam, Echo or MUGA and ECG b. CrCl c. BMA/clot section or biopsy. (For SCT patients: the end of therapy BMA/Biopsy is to be done within 1 month of ANC recovery.) d. Optional biology studies (see Section 7.4) e. HRQOL and Parental Stress Studies ¹ f. Pulse oximetry (prior to bortez)
High dose Cytarabine (HD ARAC)	IV over 1 - 3 hours	1,000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 5	Total dose: 2,000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops as described in Section 4.18 .	See Section 7.1 for complete list of observations. ¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Intensification I, but prior to Intensification II (see Section 18.0 for details). OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE
Etoposide (ETOP)	IV over 60 - 120 minutes	150 mg/m ² /dose or 5 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5	Each dose of etoposide should immediately follow the 1 st , 3 rd , 5 th , 7 th and 9 th doses of cytarabine.	
Bortezomib (BORTEZ) IND# 114480	IV over 3 - 5 seconds	1.3 mg/m ² /dose	Days 1, 4, 8	Consecutive doses must be at least 72 hours apart. On Days 1 and 4: administer after the end of the etoposide infusion.	

Date Due	Date Given	Week	Day	ARAC IT mg	HD ARAC mg	ETOP mg	BORTEZ mg	Studies	Comments (Include any held doses, or dose modifications)	
				Enter calculated dose above and actual dose administered below						
		1	1	mg*	mg	mg	mg	a, b [#] , f		
			2		mg	mg	mg			
			3		mg	mg	mg			
			4		mg	mg	mg	f		
			5		mg	mg	mg			
		2	8				mg	f		

		3	21					e		

		4	28					c, d		
			29	Remission status at the end of Intensification I will determine whether patients receive further protocol therapy. Patients not in remission as defined in Section 10.2.1 will be Off Protocol Therapy but not Off Study. All patients in remission will proceed on to Intensification II or SCT. Start next course (Intensification II – Arm B, Section 4.21 or 4.22) or SCT (Section 4.24 or 4.25) on Day 29 or when blood count parameters and clinical condition are acceptable (whichever occurs later).						

* May be performed with BM done at end of previous course.

For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.19 Intensification I Arm C-Cohort 3 (AE + Sorafenib)

The following Intensification I therapy guidelines are for patients with HR FLT3/ITD+ disease during the sorafenib feasibility part of the study (Cohort 3). Intensification I lasts 33 days or longer.

All patients in CR as defined in [Section 10.2.1](#) will proceed to Intensification I (AE). Patients with refractory disease as defined in [Section 10.2.2](#) are Off Protocol Therapy but not Off Study. It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

All HR FLT3/ITD+ patients will receive best allogeneic donor SCT (see [Section 4.24](#)) or alternative donor SCT (see [Section 4.25](#)) following Intensification I. Patients without a suitable donor will proceed to a final course of cytarabine/mitoxantrone with sorafenib. Enrollment on AAML05P1 will be permitted for patients with an available unrelated donor.

See [Section 4.7](#) for requirements to start each sorafenib course.

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Intensification I or with the bone marrow evaluation at the end of Induction II.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 5.

Dose: 1,000 mg/m²/dose, every 12 hours (i.e., 2,000 mg/m²/day, divided BID) or 33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

Etoposide (ETOP): IV over at least 60 - 120 minutes

Days: 1 through 5.

Dose: 150 mg/m²/dose or 5 mg/kg/dose if BSA < 0.6 m².

Note: Slow rate of administration if hypotension occurs. The use of an in-line filter during the infusion is suggested. Each dose of etoposide should immediately follow the 1st, 3rd, 5th, 7th and 9th doses of cytarabine.

Sorafenib (SORAF): PO

Days: 6 through 33.

Dose: 200 mg/m²/dose daily, rounded to accommodate tablet size. The maximum dose will be 400 mg.

Dose to be administered per dosing nomogram in [Appendix II](#).

Note: Sorafenib tablets should be taken at least 1 hour before or 2 hours after food. Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 years and 4 to 8 ounces for ≥ 12 years). If taken with food, sorafenib should be taken with a moderate to low fat meal. Tablets should not be crushed, but should be swallowed whole. However, sorafenib tablets can be dispersed in water to facilitate the administration to subjects that cannot swallow tablets (see drug monograph in [Section 6.12](#)). Grapefruit and its juice should be avoided for the duration of treatment with sorafenib.

Sorafenib is supplied by Bayer HealthCare Pharmaceuticals, and distributed by the NCI DTCD. Do not use commercially available drug.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: For patients who consent and are enrolled on the HRQOL and parental stress studies, the third evaluation time point is on or after Day 21 of Intensification I, but prior to Intensification II. See [Section 18.0](#) for details of evaluation schedule.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification I Arm C-Cohort 3 is on the next page.

End of Intensification I: Remission status at the end of Intensification I will determine whether patients receive further protocol therapy. Bone marrow exam after Intensification I must show continued complete remission. Patients not in remission as defined in [Section 10.2.1](#) will be Off Protocol Therapy but not Off Study. All patients in remission will proceed on to Intensification II or SCT accordingly:

Following Intensification I Arm C - Cohort 3, the next course (Intensification II Arm C - Cohort 3 [[Section 4.23](#)] or SCT [see [Section 4.24](#) or [4.25](#) depending on availability of a suitable donor]) starts on Day 34 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

4.19.1 Intensification I Arm C-Cohort 3 (AE + Sorafenib)	_____
This therapy is for HR FLT3/ITD+ patients receiving standard therapy and sorafenib during sorafenib feasibility assessment.	Patient name or initials _____ DOB _____

Intensification I lasts 33 days or longer and this Therapy Delivery Map is on 1 page. Extensive details on treatment assignment are provided in [Sections 4.1-4.7](#) and [4.18](#). It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine* (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	* May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct & total), BP, Echo or MUGA & ECG, urinalysis b. CrCl c. BMA/clot section or biopsy. (For SCT patients: the end of therapy BMA/Biopsy is to be done within 1 month of ANC recovery.)
High dose Cytarabine (HD ARAC)	IV over 1 - 3 hours	1,000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 5	Total dose: 2,000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops as described in Section 4.19 .	d. Optional biology studies (see Section 7.4)
Etoposide (ETOP)	IV over 60 - 120 minutes	150 mg/m ² /dose or 5 mg/kg/dose if BSA < 0.6 m ²	Days 1 - 5	Each dose of etoposide should immediately follow the 1 st , 3 rd , 5 th , 7 th and 9 th doses of cytarabine.	e. HRQOL and Parental Stress Studies ¹ f. Clinical cardiac examination See Sect. 7.1 for complete list of observations.
Sorafenib (SORAF) IND# 114480	PO	200 mg/m ² /dose once daily	Days 6 - 33	See Section 4.19 for administration guidelines. See Section 5.0 for dose modification plan. The maximum dose will be 400 mg.	¹ For patients enrolled on this study, evaluation can be obtained on or after Day 21 of Intensification I, but prior to Intensification II (see Section 18.0 for details). OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	ARAC IT mg	HD ARAC mg	ETOP mg	SORAF mg	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below									
		1	1	_____ mg*	_____ mg	_____ mg	_____ mg	a, b [#] , f	
			2		_____ mg	_____ mg	_____ mg		
			3		_____ mg	_____ mg	_____ mg		
			4		_____ mg	_____ mg	_____ mg		
			5		_____ mg	_____ mg	_____ mg		
			6				_____ mg		

		2	8					f	
			14					d	
		3	15					f	
			--						
			21					d, e	
		4	22					f	
			28					d	
		5	29					f	
			33					c, d	

Remission status at the end of Intensification I will determine whether patients receive further protocol therapy. Patients not in remission as defined in [Section 10.2.1](#) will be Off Protocol Therapy but not Off Study. All patients in remission will proceed on to Intensification II or SCT.
Start next course (Intensification II Arm C-Cohort 3, [Sect. 4.23](#)) or SCT ([Sect. 4.24](#) or [4.25](#)) on Day 34 or when blood count parameters and clinical condition are acceptable (whichever occurs later).

* May be performed with BM done at end of previous course.
For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.
SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.20 Intensification II Arm A (MA) – Low Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Intensification II therapy guidelines are for patients with low risk disease randomized to Arm A of the study. Arm A consists of the standard therapy. Intensification II lasts 28 days or longer.

It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Intensification II or with the bone marrow evaluation at the end of Intensification I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 4.

Dose: 1,000 mg/m²/dose, every 12 hours (i.e., 2,000 mg/m²/day, divided BID) or 33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

MitoXANTRONE (MITOX): IV over 15 - 30 minutes

Days: 3 through 6.

Dose: 12 mg/m²/dose or 0.4 mg/kg/dose if BSA < 0.6 m².

Note: Administer through the tubing of a rapidly infusing solution of D₅W or 0.9% NaCl. Avoid extravasation; the use of a central line is suggested. On the Days 3 and 4, mitoXANTRONE should be given 8 hours after the 5th and 7th high dose cytarabine infusions are complete.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: Patients assigned to chemotherapy- For patients who consent and are enrolled on the HRQOL and parental stress studies, the fourth and fifth evaluation time points occur at 1 month (\pm 7 days) and 4 months (\pm 1 month) respectively, from the start of Intensification II. Evaluations 6, 7 and 8 occur at 12

(\pm 1 month), 24 (\pm 3 months) and 36 (\pm 3 months) months from diagnosis, respectively. See [Section 18.0](#) for complete details of evaluation schedules.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification II (Arm A, Low Risk) is on the next page.

<p>4.20.1 Intensification II Arm A (MA) – Low Risk Patients This therapy is for low risk patients randomized to receive standard therapy.</p>	<p>_____ Patient name or initials</p> <p>_____ DOB</p>
---	--

Intensification II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1-4.6](#) and [4.20](#). It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. BM exam after Intensification I must show continued complete remission.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine (IT ARAC)	IT	<u>Age-based dosing:</u> 20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	* May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), Echo or MUGA and ECG b. CrCl c. HRQOL and Parental Stress Studies ¹
Cytarabine (HD ARAC)	IV over 1 - 3 hours	1,000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 – 4	Total dose: 2,000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops as described in Section 4.20 .	See Section 7.1 for complete list of observations
MitoXANTRONE (MITOX)	IV over 15 - 30 minutes	12 mg/m ² /dose or 0.4 mg/kg/dose if BSA < 0.6 m ²	Days 3 - 6	See Section 4.20 for administration guidelines. On Days 3 and 4, administer 8 hours after the 5 th and 7 th cytarabine infusions are completed.	¹ For patients enrolled on this study, see Section 18.0 for details on evaluation time points 4, 5, 6, 7 and 8. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	ARAC IT mg	HD ARAC mg	MITOX mg	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below								
		1	1	mg*	mg mg		a, b [#]	
			2		mg mg			
			3		mg mg	mg		
			4		mg mg	mg		
			5			mg		
			6			mg		

		4	28				c	
See Section 7.1 for required end of therapy evaluations								

* May be performed with BM done at end of previous course

For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.21 Intensification II Arm B (MA + Bortezomib) – Low Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Intensification II therapy guidelines are for patients with low risk disease randomized to Arm B of the study. Patients on Arm B receive bortezomib in addition to MA therapy. Intensification II lasts 28 days or longer.

It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Intensification II or with the bone marrow evaluation at the end of Intensification I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

High Dose Cytarabine (HD ARAC): IV over 1 - 3 hours

Days: 1 through 4.

Dose: 1,000 mg/m²/dose, every 12 hours (i.e., 2,000 mg/m²/day, divided BID) or 33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

MitoXANTRONE (MITOX): IV over 15 - 30 minutes

Days: 3 through 6.

Dose: 12 mg/m²/dose or 0.4 mg/kg/dose if BSA < 0.6 m²

Note: Administer through the tubing of a rapidly infusing solution of D₅W or 0.9% NaCl. Avoid extravasation; the use of a central line is suggested. On Days 3 and 4, mitoXANTRONE should be given 8 hours after the 5th and 7th high dose cytarabine infusions are complete.

Bortezomib (BORTEZ): IV over 3 - 5 seconds

Days: 1, 4 and 8.

Dose: 1.3 mg/m²/dose.

On Day 4: Administer after the end of the mitoXANTRONE infusion.

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.” Additional wording may be considered at the institution’s discretion.

Note: at least 72 hours must have elapsed between doses. Grapefruit and its juice should be avoided for the duration of treatment with bortezomib. 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.

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

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: Patients assigned to chemotherapy- For patients who consent and are enrolled on the HRQOL and parental stress studies, the fourth and fifth evaluation time points occur at 1 month (\pm 7 days) and 4 months (\pm 1 month) respectively, from the start of Intensification II. Evaluations 6, 7 and 8 occur at 12 (\pm 1 month), 24 (\pm 3 months) and 36 (\pm 3 months) months from diagnosis, respectively. See [Section 18.0](#) for complete details of evaluation schedules.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification II (Arm B, Low Risk) is on the next page.

4.21.1 Intensification II Arm B (MA + Bortezomib) – Low Risk Patients This therapy is for low risk patients randomized to receive bortezomib and standard therapy.	_____ Patient name or initials	_____ DOB
---	-----------------------------------	--------------

Intensification II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1-4.6](#) and [4.21](#). It is suggested, but not required, that patients have an ANC > 1000/ μ L and a platelet count > 75 000/ μ L before proceeding with therapy. BM exam after Intensification I must show continued complete remission.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine* (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	*May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), neurologic exam, Echo or MUGA and ECG b. CrCl
High dose Cytarabine (HD ARAC)	IV over 1 - 3 hours	1000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 4	Total dose: 2000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² Use eye drops as described in Section 4.21 .	c. Pulse oximetry (prior to bortez) d. HRQOL and Parental Stress Studies ¹ See Section 7.1 for complete list of observations.
MitoXANTRONE (MITOX)	IV over 15 - 30 minutes	12 mg/m ² /dose or 0.4 mg/kg/dose if BSA < 0.6 m ²	Days 3 - 6	See Section 4.21 for administration guidelines. On Days 3 and 4, administer 8 hours after the 5 th and 7 th cytarabine infusions are completed.	¹ For patients enrolled on this study, see Section 18.0 for details on evaluation time points 4, 5, 6, 7 and 8.
Bortezomib (BORTEZ) IND# 114480	IV over 3 - 5 seconds	1.3 mg/m ² /dose	Days 1, 4, 8	Consecutive doses must be at least 72 hours apart. On Day 4, administer <u>after</u> the end of the mitoXANTRONE infusion.	OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	Ht cm	Wt kg	BSA m ²	ARAC IT mg	HD ARAC mg mg	MITOX mg	BORTEZ mg	Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below												
		1	1				mg*	mg mg		mg	a, b#, c	
			2					mg mg				
			3					mg mg	mg			
			4					mg mg	mg	mg	c	
			5						mg			
			6						mg			
			7									
		2	8							mg	c	

		4	28								d	

See [Section 7.1](#) for Required end of therapy observations.

* May be performed with BM done at end of previous course.

For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.22 Intensification II Arm A & B (HD ARAC/LASP) – High Risk Patients

NOTE: AS OF AMENDMENT #7A ACCRUAL FOR ARMS A AND B HAVE BEEN MET. NO ADDITIONAL PATIENTS WILL BE ENROLLED ON ARMS A OR B OF AAML1031.

The following Intensification II therapy guidelines are for patients with high risk disease, randomized to Arm A or Arm B of the study, who do not have an appropriate donor for SCT. Intensification II lasts 28 days or longer.

It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

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.

High Dose Cytarabine (HD ARAC): IV over 3 hours

Days: 1, 2 and 8, 9

Dose: 3,000 mg/m²/dose, every 12 hours (i.e., 6,000 mg/m²/day, divided BID), or
100 mg/kg/dose, every 12 hours (i.e., 200 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Days 1 and 8) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

E. coli L-Asparaginase (LASP): IM

Days: 2 and 9. To be given 6 hours after the start of the 4th and 8th dose of cytarabine (Hour 42).

Dose: 6,000 International Units/m²/dose (200 International Units/kg/dose if BSA < 0.6 m²).

If *E. coli* L-asparaginase (i.e., Elspar or Kidrolase) is not available, please use Erwinia asparaginase (Erwinaze) as follows:

Erwinia L-asparaginase (Erwinaze): IM (may be given IV over 1 hour)

Days: 2, 9 (one dose per day, 2 doses total for the entire course)

Dose: 25,000 IU/m² (830 IU/kg/dose if BSA < 0.6 m²)

Note: Erwinia asparaginase should be given 6 hours after the start of cytarabine doses 4 and 8.

If Erwinia asparaginase is not available, pegasparaginase should not be given. Rather, asparaginase should be omitted.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: Patients assigned to chemotherapy- For patients who consent and are enrolled on the HRQOL and parental stress studies, the fourth and fifth evaluation time points occur at 1 month (\pm 7 days) and 4 months (\pm 1 month) respectively, from the start of Intensification II. Evaluations 6, 7 and 8 occur at 12 (\pm 1 month), 24 (\pm 3 months) and 36 (\pm 3 months) months from diagnosis, respectively. See [Section 18.0](#) for complete details of evaluation schedules.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification II (Arm A & B, High Risk) is on the next page.

<p>4.22.1 Intensification II Arm A & B (HD ARAC/LASP) – High Risk Patients This therapy is for high risk patients who do not have an appropriate donor for SCT.</p>	<p>_____</p> <p>Patient name or initials _____ DOB _____</p>
--	--

Intensification II lasts 28 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1-4.6](#) and [4.22](#). It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. BM exam after Intensification I must show continued complete remission.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine (HD ARAC)	IV over 3 hours	3,000 mg/m ² /dose, every 12 hours or 100 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1, 2 and 8, 9	Total dose: 6,000 mg/m ² /day, divided BID or 200 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops as described in Section 4.22 . Check serum Cr.	a. CBC, BUN/Cr, AST/ALT, bili (direct and total), Echo or MUGA and ECG b. CrCl c. HRQOL and Parental Stress Studies ¹
<i>E. coli</i> L-Asparaginase (LASP)	IM	6,000 International Units/m ² /dose (200 International Units/kg/dose if BSA < 0.6 m ²)	Day 2 and Day 9. To be given 6 hours after the start of the 4th and 8th dose of cytarabine (Hour 42).	If E. Coli version is not available use: <i>Erwinia L-asparaginase (Erwinaze)</i> : 25,000 IU/m ² (830 IU/kg/dose if BSA < 0.6 m ²) on Days 2 and 9 (2 doses total for the entire course). IM (may be given IV over 1 hour). See Section 4.22 .	See Section 7.1 for complete list of observations ¹ For patients enrolled on this study, see Section 18.0 for details on evaluation time points 4, 5, 6, 7 and 8. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	Ht cm HD ARAC mg	Wt kg LASP units	BSA m ² Studies	Comments (Include any held doses, or dose modifications)
Enter calculated dose above and actual dose administered below							
		1	1	mg mg		a, b*	
			2	mg mg	units		

			8	mg mg		b*	
			9	mg mg	units		

		4	28			c	

See [Section 7.1](#) for required end of therapy evaluations.

* For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.23 Intensification II Arm C-Cohort 3 (MA + Sorafenib)

The following Intensification II therapy guidelines are for patients with HR FLT3/ITD+ disease without a suitable donor for SCT, during the sorafenib feasibility part of the study (Cohort 3). Intensification II lasts 34 days or longer.

It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy.

See [Section 4.7](#) for requirements to start each sorafenib course.

See the Parenteral Chemotherapy Administration Guidelines (CAGs) on the COG website at: https://members.childrononcologygroup.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.

Intrathecal Cytarabine (IT ARAC): IT

IT ARAC may be administered on Day 1 of Intensification II or with the bone marrow evaluation at the end of Intensification I.

Age-based dosing:

<u>Age (yrs)</u>	<u>Dose</u>
0 - 0.99	20 mg
1 - 1.99	30 mg
2 - 2.99	50 mg
≥ 3	70 mg

High Dose Cytarabine (HD ARAC): IV over 1-3 hours

Days: 1 through 4.

Dose: 1,000 mg/m²/dose, every 12 hours (i.e., 2,000 mg/m²/day, divided BID) or 33 mg/kg/dose, every 12 hours (i.e., 66 mg/kg/day, divided BID) if BSA < 0.6 m².

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. Patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age, must have a CrCl performed (Day 1) and doses of cytarabine adjusted as outlined in [Section 5.9.1](#).

MitoXANTRONE (MITOX): IV over 15 - 30 minutes

Days: 3 through 6.

Dose: 12 mg/m²/dose or 0.4 mg/kg/dose if BSA < 0.6 m²

Note: Administer through the tubing of a rapidly infusing solution of D₅W or 0.9% NaCl. Avoid extravasation; the use of a central line is suggested. On the Days 3 and 4, mitoXANTRONE should be given 8 hours after the 5th and 7th high dose cytarabine infusions are complete.

Sorafenib (SORAF): PO

Days: 7 through 34.

Dose: 200 mg/m²/dose daily, rounded to accommodate tablet size. The maximum dose will be 400 mg.

Dose to be administered per dosing nomogram in [Appendix II](#).

Note: Sorafenib tablets should be taken at least 1 hour before or 2 hours after food. Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 years and 4 to 8 ounces for ≥ 12 years). If taken with food, sorafenib should be taken with a moderate to low fat meal. Tablets should not be crushed, but should be swallowed whole. However, sorafenib tablets can be dispersed in water to facilitate the administration to subjects that cannot swallow tablets (see drug monograph in [Section 6.12](#)). Grapefruit and its juice should be avoided for the duration of treatment with sorafenib.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note:

Patients assigned to chemotherapy- For patients who consent and are enrolled on the HRQOL and parental stress studies, the fourth and fifth evaluation time points occur at 1 month (± 7 days) and 4 months (± 1 month) respectively, from the start of Intensification II. Evaluations 6, 7 and 8 occur at 12 (± 1 month), 24 (± 3 months) and 36 months (± 3 months) from diagnosis, respectively. See [Section 18.0](#) for complete details of evaluation schedules.

Sorafenib is supplied by Bayer HealthCare Pharmaceuticals, and distributed by the NCI DTCD. Do not use commercially available drug.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Intensification II Arm C-Cohort 3 is on the next page.

End of Intensification II:

Arm C patients will complete protocol therapy after Intensification II if:

1. They were unable to tolerate the sorafenib 100 mg/m² dose level during any therapy phase to date,
or
2. They experienced Grade 4 hypertension.

All other patients may proceed to Maintenance therapy (see [Section 4.26](#)). Maintenance therapy will begin between 40 - 100 days following Intensification II.

4.23.1 Intensification II Arm C – Cohort 3 (MA + Sorafenib) This therapy is for patients with HR FLT3/ITD+ disease without an appropriate SCT donor receiving standard therapy and sorafenib during sorafenib feasibility assessment.	_____ Patient name or initials	_____ DOB
---	-----------------------------------	--------------

Intensification II lasts 34 days or longer and this Therapy Delivery Map is on 1 page. Extensive treatment details are provided in [Sections 4.1-4.7](#) and [4.23](#). It is suggested, but not required, that patients have an ANC > 1,000/ μ L and a platelet count > 75,000/ μ L before proceeding with therapy. BM exam after Intensification I must show continued complete remission.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Cytarabine* (IT ARAC)	IT	20 mg (age 0 - 0.99 years) 30 mg (age 1 - 1.99 years) 50 mg (age 2 - 2.99 years) 70 mg (age \geq 3 years)	Day 1	* May be performed with BM done at end of previous course.	a. CBC, BUN/Creat, AST/ALT, bili (direct and total), Echo or MUGA and ECG, BP, urinalysis b. CrCl
High dose Cytarabine (HD ARAC)	IV over 1 - 3 hours	1,000 mg/m ² /dose, every 12 hours or 33 mg/kg/dose, every 12 hours if BSA < 0.6 m ²	Days 1 - 4	Total dose: 2,000 mg/m ² /day, divided BID or 66 mg/kg/day, divided BID if BSA < 0.6 m ² . Use eye drops described in Section 4.23 .	c. Optional biology studies (see Sect. 7.4) d. HRQOL and Parental Stress Studies ¹ e. Clinical cardiac examination
MitoXANTRONE (MITOX)	IV over 15 - 30 minutes	12 mg/m ² /dose or 0.4 mg/kg/dose if BSA < 0.6 m ²	Days 3 - 6	See Section 4.23 for administration guidelines. On Days 3 and 4, administer 8 hours after the 5 th and 7 th cytarabine infusions are completed.	See Sect. 7.1 for complete list of observations. ¹ For patients enrolled on this study, see Sect. 18.0 for details on evaluation time point 4.
Sorafenib (SORAF) IND# 114480	PO	200 mg/m ² /dose once daily	Days 7 - 34	See Section 4.23 for administration guidelines. See Section 5.0 for dose modification plan. The maximum dose will be 400 mg.	OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE

Date Due	Date Given	Week	Day	Ht cm		Wt kg		BSA m ²		Studies	Comments (Include any held doses, or dose modifications)
				ARAC mg	HD ARAC mg mg	MITOX mg	SORAF mg				
Enter calculated dose above and actual dose administered below											
		1	1	mg*	mg mg					a, b [#] , c, e	
			2		mg mg						
			3		mg mg	mg					
			4		mg mg	mg					
			5			mg					
			6			mg					
			7								
		2	8					mg			
			14							e	
		3	15							c	
			21							e	
			21							c	
		4	22							e	
			28							c, d	
		5	29							e	
			34								
			35								

See [Section 7.1](#) for required evaluations. Arm C patients will complete protocol therapy after Intensification II if (1) they were unable to tolerate the sorafenib 100 mg/m² dose level during any therapy phase to date, or (2) they experienced Grade 4 hypertension or Grade 3 maculo-papular rash during any therapy phase to date. All other patients may proceed to maintenance therapy (see [Section 4.26](#)). Maintenance therapy will begin between 40 - 100 days following Intensification II.

* May be performed with BM done at end of previous course.

For patients in whom serum creatinine is > 2 mg/dL or > 2 x normal for age.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

4.24 Allogeneic Stem Cell Transplantation

Stem cell transplantation in this study will be utilized in those patients with high risk leukemia (see [Section 3.3.5](#) for risk categorizations). All patients in whom low risk cytogenetics are present will **NOT** proceed to SCT. All high risk patients with a family match (see below) will proceed with a MFD SCT after Intensification I.

All high risk patients in whom no MFD is available will proceed with an alternative donor SCT (see [Section 4.25](#)) after Intensification I. If the donor is not available after Intensification I, and the delay in the availability of the donor will exceed 8 weeks after count recovery, the patient should proceed with the chemotherapy according to the arm to which they were originally assigned. If the donor becomes available at a later date, the institution may proceed with the SCT. For those in whom donors later become available and in whom the subsequent Intensification course of therapy has been administered, the treating clinicians may choose to defer SCT altogether during CR. See [Section 3.3.5](#) for further guidelines for high risk patients without a MFD.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: Patients assigned to stem cell transplant therapy- Patients who consent and are enrolled on the HRQOL and parental stress studies, will have their fourth and fifth evaluations at 1 month (\pm 7 days) and 4 months (\pm 1 month) respectively, from the start of the preparative regimen. Evaluations 6, 7 and 8 occur at 12 (\pm 1 month), 24 (\pm 3 months) and 36 (\pm 3 months) months from diagnosis respectively. See [Section 18.0](#) for complete details of evaluation schedules.

4.24.1

Bone marrow SCT **must** be performed at a COG certified SCT institution. Patients should begin their SCT preparative regimen following Intensification I (cytarabine and etoposide).

4.24.2 MFD Donor Selection:

- 1) HLA, A, B, C, DRB1, identical, or 1 antigen or allele mismatched family donor.
- 2) HLA typing must be performed using molecular high resolution technique.
- 3) All available first degree family members (parents and siblings) must be HLA typed.
- 4) Use of syngeneic donors will NOT be permitted in this study

4.24.3 MFD SCT Conditioning Regimen

Dose adjustments will be made for obesity, defined as > 125% of IBW:

Adjusted weight = $1.25 \times \text{IBW}$

Day -6	Begin anti-seizure prophylaxis (lorazepam [phenytoin or levetiracetam can be used as alternatives]: see Section 4.24.4)
Day -5 to -2	Fludarabine 40 mg/m ² /dose daily IV or 1.3 mg/kg/dose daily IV if < 10 kg
Day -5 to -2	Busulfan daily IV* <ul style="list-style-type: none"> • With age-based dosing (see Section 4.24.6.1) • With 1st dose pharmacokinetics (see Section 17.1.3)
Day -2	Begin Tacrolimus
Day -1	Rest

Day 0	Bone Marrow Infusion
Day 1	Methotrexate 5 mg/m ² /dose IV
Day 3	Methotrexate 5 mg/m ² /dose IV
Day 6	Methotrexate 5 mg/m ² /dose IV
Day 11	Methotrexate 5 mg/m ² /dose IV (mismatched family donors only, see Section 4.24.8.2). Matched sibling and UCB SCTs do not receive Day 11 MTX.

* Divided dosing (4 times daily is permitted per institutional standard)

4.24.4 Anti-seizure prophylaxis (Day -6)

4.24.4.1

Seizure prophylaxis is mandatory. The preferred regimen is lorazepam (0.02 - 0.05 mg/kg/dose, maximum dose: 2 mg) given 30 minutes prior to each busulfan dose and then continuing for at least 24 hours after last busulfan dose. Alternative acceptable regimens include phenytoin or levetiracetam beginning 12 hours prior to busulfan and continue at least 24 hours after completion of busulfan.

4.24.4.2

The dose of phenytoin is 2.5 mg/kg/dose PO BID (maximum dose: 150 mg).

The dose of levetiracetam is 10 mg/kg/dose PO BID (maximum dose: 1,000 mg).

4.24.4.3

Loading dose and therapeutic monitoring are not necessary. For patients with a known seizure disorder, consider IV dosing with loading and therapeutic monitoring.

4.24.4.4

Phenytoin interacts with busulfan causing reduced plasma levels, whereas lorazepam and levetiracetam do not affect busulfan levels. Adjusting the busulfan dose based on 1st day pharmacokinetics is recommended.

4.24.4.5

Acetaminophen should be held for 72 hours before and during busulfan administration, but may be given per individual institutional standard policies if clinically necessary.

4.24.5 Fludarabine (Day -5 to -2)

4.24.5.1

≥ 10 kg: Fludarabine 40 mg/m² IV daily in 0.9% NaCl or D₅W over 30 minutes on Days -5, -4, -3, -2 for a total of 4 doses.

< 10 kg: Fludarabine 1.33 mg/kg IV daily in 0.9% NaCl or D₅W over 30 minutes on Days -5, -4, -3, -2 for a total of 4 doses.

4.24.6 Busulfan (Day -5 to -2)

4.24.6.1

**Dosing of intravenous busulfan (Busulfex™):
Daily Dosing Schedule (Preferred Dosing Schedule)**

< 10 kg: 3.2 mg/kg/dose IV daily x 4 doses

≥ 10 kg but ≤ 4 years old: 4 mg/kg/dose IV daily x 4 doses
> 4 years: 3.2 mg/kg/dose IV daily x 4 doses

Q6 Hour Dosing Schedule:

< 10 kg: 0.8 mg/kg/dose as a starting dose q 6 hours x 16 doses
≥ 10 kg but ≤ 4 years old: 1 mg/kg/dose as a starting dose q 6 hours x 16 doses
> 4 years: 0.8 mg/kg/dose as a starting dose q 6 hours x 16 doses

Note: DOSING WILL BE ADJUSTED BASED ON 1ST DOSE PHARMACOKINETICS (see [Section 17.1.3](#)).

4.24.6.2 Administration

The preferred schedule is busulfan administered intravenously via a central venous catheter as a **3 hour** infusion once daily for 4 consecutive days for a total of 4 doses. Institutional practice (divided dosing) is permitted. In this case, busulfan should be administered as a **2 hour** infusion every 6 hours for 16 consecutive doses. Please note that the timing of busulfan PK sample collection ([Section 17.1.3](#)) is based on whether a 2 hour or 3 hour infusion is used. Refrain from using polycarbonate syringes or filter needles during busulfan preparation.

4.24.7 Marrow Infusion (Day 0)

Stem cells for allogeneic SCT is infused within 36 to 48 hours after the last dose of busulfan. A cell dose of at least 2×10^8 nucleated cells per kilogram should be given. Higher cell doses can be administered if donor size allows. For MFD SCT, the use of peripheral blood stem cells (PBSC) is not permitted on this study.

4.24.8 GVHD Prophylaxis

4.24.8.1 Tacrolimus Administration, Monitoring and Dose Adjustments

Tacrolimus should be administered by continuous IV infusion until patients are able to take PO. Levels should be drawn at least twice per week while hospitalized, then weekly or monthly thereafter unless a change in medication (e.g. use of itraconazole) or renal function might result in an acute change in level. At that point, levels will be measured as clinically indicated. Levels sent when dosing by continuous infusion are not true trough concentrations, however, the same target range of drug levels will be used for both continuous IV and bolus PO routes of administration.

The target serum trough level for tacrolimus is 5 - 12 ng/mL (8 - 12 ng/mL for mismatched or unrelated donors). Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. For levels < 5 ng/mL, it is suggested that the dose of tacrolimus be increased by approximately 25% increments every 1 - 2 days, rounded to the nearest 0.5 milligram (when dosing is oral) until the target range is achieved (dose adjustments may vary from the suggested 25% as needed to achieve the therapeutic range based upon the measured level and the clinical status of the patient). Conversely, for levels > 12 ng/mL, it is suggested that the dose of tacrolimus be decreased by approximately 25% every 1 - 2 days until the target level is achieved. For very high levels (> 20 ng/mL), tacrolimus may need to be held entirely for a period of time. This may be done as long as serum levels are monitored daily and the drug is restarted at an appropriate dosage when the level returns to the therapeutic range.

4.24.8.2 Methotrexate Administration, Monitoring and Dose Adjustments

“Mini-dose” methotrexate should be given at a dose of 5 mg/m^2 on Days +1, 3, and 6 after transplant for matched sibling and cord blood donor sources and an additional dose at Day +11 after transplant for unrelated or “other” related marrow/PBSC donors (non-genotypically matched or partially mismatched

related donors). All doses of methotrexate should be administered as scheduled if possible, but centers may modify or hold methotrexate for significant toxicity (see [Section 5.12.1](#) for methotrexate dose modification guidelines).

4.24.8.3 Leucovorin Administration

Leucovorin may be given at the physician’s discretion for patients at risk for methotrexate toxicity. Patients at risk for methotrexate toxicity include those with extravascular fluid collections (ascites, pleural effusions) or with decreased renal function (see [Section 5.12.1](#)).

4.24.8.4 GVHD Prophylaxis Administration (the tacrolimus dose listed is a starting dose; doses should be adjusted to achieve target trough levels).

Drug	Route	Dose	Days	Important Notes
Tacrolimus	IV, PO when able	0.02 mg/kg/day continuous infusion	Begins Day -2	Target levels 5 - 12 ng/mL (8 - 12 ng/mL for mismatched or unrelated donors). Taper as per Section 4.23.9.3
Methotrexate	IV	5 mg/m ² /dose	Days +1, +3, +6 (matched sibling/cord blood donors) (Days +1, +3, +6 and +11 (other related/unrelated donors)	Modify/hold for toxicity as per Section 5.12.1

4.24.9 Tapering Immune Suppressive Medications

4.24.9.1 Tapering immune suppression in patients with GVHD

Patients who develop either acute or chronic GVHD will be treated according to institutional practice.

4.24.9.2 Tapering immune suppression in patients without GVHD

Tapering of Tacrolimus for Patients without GVHD

Drug	Stem cell source	Taper Schedule
Tacrolimus	Matched Sibling	Start Day +42 and taper over 8 weeks, off by Day +98.
Tacrolimus	“Other” Related, Unrelated, or Cord Blood	Start Day +100, off by Day +180.

4.24.9.3 Tacrolimus taper

- **Tacrolimus taper for patients receiving matched sibling donor allografts:**
In the absence of GVHD, patients receiving matched sibling donor allografts will taper tacrolimus starting on Day +42 over 8 weeks, tapering every 1 - 3 weeks depending on capsule sizes, and off by Day +98.
- **Tacrolimus taper for patients receiving “other” related or unrelated allografts (matched, mismatched, and cord blood):**
Patients receiving either “other” (non-genotypically matched) related or unrelated allografts or cord blood will start tapering tacrolimus at Day +100, tapering every 1 - 3 weeks depending on capsule sizes, and off by Day +180.

4.24.10

Patients who develop \geq Grade 2 GVHD may be treated with steroids, antithymocyte globulin (ATG) or other agents according to local protocols. Tacrolimus therapy will continue during GVHD treatment and can be weaned per institutional guidelines.

4.24.11

Arm A & B Patients: Refer to End of Therapy evaluation requirements in [Section 7.0](#). Patients enrolled on Arm C who are not eligible for Sorafenib Maintenance ([Section 4.25](#)) should also proceed to [Section 7.0](#) for end of therapy requirements.

For patients enrolled on Arm C who are eligible for Sorafenib Maintenance, refer to [Section 4.25](#) for additional monitoring in the context of sorafenib and [Section 7.3](#) for additional post-SCT monitoring.

4.25 Alternative Donor SCT

For patients with high risk AML (see [Section 3.3.5](#) for high risk definition), SCT should be pursued after the completion of Intensification I. If a MFD is available, this donor should be utilized and the guidelines in [Section 4.24](#) followed. For patients without an MFD, efforts should be made to identify an alternative donor for SCT. All high risk AML patients for whom a suitable alternative donor cannot be identified should continue with their assigned chemotherapy arm.

HRQOL and Parental Stress Studies

These studies are closed to new patient accrual as of 05-15-2015.

Please note: Patients assigned to stem cell transplant therapy- Patients who consent and are enrolled on the HRQOL and parental stress studies, will have their fourth and fifth evaluations at 1 month (± 7 days) and 4 months (± 1 month) respectively, from the start of the preparative regimen. Evaluations 6, 7 and 8 occur at 12 (± 1 month), 24 (± 3 months) and 36 (± 3 months) months from diagnosis respectively. See [Section 18.0](#) for complete details of evaluation schedules.

4.25.1 Timing of Alternative Donor SCT

Bone marrow transplantation **must** be performed at a COG certified SCT institution. All patients with high risk disease should begin their transplant preparative regimen following Intensification I. If a suitable donor is unavailable at this time, they should continue with their assigned chemotherapy arm until such time a donor is identified and blood counts have recovered from their most recent course of chemotherapy. If no suitable alternative donor is found, they may continue to receive their assigned chemotherapy regimen without proceeding to SCT until such time that a donor becomes available.

4.25.2 HLA Matching in Alternative Donor SCT

It is strongly recommended that all children with AML are HLA-typed at high resolution at the time of study enrollment so that rapid donor search can be performed if the patient is categorized as high risk. Donor search should be performed as quickly as possible using the institutional criteria for optimal donor selection. Minimal criteria for donor selection are described below:

- 1) HLA, A, B, C, DRB1, identical or 1 antigen or allele mismatched unrelated donor.
- 2) HLA A, B, DRB1 4 of 6 antigen matched unrelated donor cord blood unit with an adequate cell dose. "Adequate" will be judged by institutional criteria, but is commonly considered to be a nucleated cell dose $> 3.7 \times 10^7/\text{kg}$ or CD34+ cell dose $> 2 \times 10^5/\text{kg}$.
- 3) Mismatched family member donor with at least one haplotype match, or 5 of 6 antigen phenotypic match.

4.25.3 Graft Source for Alternative Donors

Bone marrow and umbilical cord blood are the recommended stem cell sources for alternative donor SCT. Use of peripheral blood stem cells is discouraged but allowed.

4.25.4 Transplant Conditioning Regimen

The following conditioning regimen, GVHD prophylaxis, and supportive care plan, are based on the COG package and the COG's broad experience with family donors. Although these guidelines are recommended for AAML1031 patients allocated to alternative donor SCT, use of institutional treatment standards for alternative donor SCT are acceptable.

**Dose adjustments will be made for obesity, defined as > 125% of IBW:
Adjusted weight = 1.25*IBW**

Day -6	Begin anti-seizure prophylaxis (lorazepam, [phenytoin or levetiracetam can be used as alternatives]: see Section 4.24.4)
Day -5 to -2	Fludarabine 40 mg/m ² /dose IV daily or 1.3mg/kg/dose daily IV if < 10 kg
Day -5 to Day -2	Busulfan daily IV* <ul style="list-style-type: none"> • With age-based dosing (see Section 4.24.6.1) • With 1st dose pharmacokinetics (see Section 17.1.3)
Day -3 to -1	ATGAM (Equine ATG) 30 mg/kg/dose IV daily
Day -2	Begin Tacrolimus
Day 0	Bone Marrow Infusion
Day 1	Methotrexate 5 mg/m ² /dose IV
Day 3	Methotrexate 5 mg/m ² /dose IV
Day 6	Methotrexate 5 mg/m ² /dose IV
Day 11	Methotrexate 5 mg/m ² /dose IV. UCB SCTs do not receive Day 11 MTX.

*Divided dosing (4 times daily is permitted per institutional standard)

4.25.5

See [Sections 4.24.4](#) through [4.24.10](#) with the following additions or exceptions.

4.25.6 Equine Lymphocyte Immune Globulin

Lymphocyte immune globulin equine (ATGAM®) is utilized in addition to fludarabine and busulfan in patients with alternative donors as follows:

ATGAM 30 mg/kg/dose IV over 6-8 hours on Days -3, -2, -1. Premedication will be given prior to infusion with diphenhydramine 1 mg/kg/dose, max 50 mg (or comparable anti-histamine) acetaminophen 10 - 15 mg/kg (max 1,000 mg), and methylprednisone 1 mg/kg/dose. Premedications may be repeated or increased as needed to control allergic reactions, chills or fever. Patients who are unable to tolerate the equine product may receive rabbit antithymocyte globulin (thymoglobulin ®) 2.5 mg/kg/day.

4.25.7 Tacrolimus

Follow the guidelines in [Section 4.24.8.1](#).

4.25.8

Arm A & B Patients: Refer to End of Therapy evaluation requirements in [Section 7.0](#). Patients enrolled on Arm C who are not eligible for Sorafenib Maintenance ([Section 4.26](#)) should also proceed to [Section 7.0](#) for end of therapy requirements.

For patients enrolled on Arm C who are eligible for Sorafenib Maintenance, refer to [Section 4.26](#) for additional monitoring in the context of sorafenib and [Section 7.3](#) for additional post SCT monitoring.

4.26 Maintenance Arm C (Sorafenib)

The following Maintenance therapy guidelines are for patients with HR FLT3/ITD+ disease who did not experience dose limiting toxicity on the Sorafenib 100 mg/m² dose level during previous therapy.

Maintenance therapy will begin between Days 40 - 100 post completion of Intensification II or stem cell transplant and continue for 1 year.

Baseline Criteria for Initiation of Maintenance Therapy

The following criteria must be met to initiate sorafenib maintenance therapy:

- Adequate hematopoietic recovery (ANC > 1,000/μL and a platelet count > 75,000/μL)
- No evidence of acute GVHD on physical examination
- No evidence of relapse on baseline bone marrow examination post Intensification II or SCT
- Shortening fraction ≥ 28% and ejection fraction ≥ 55%
- Blood pressure less than the 95th percentile for age, height, and gender (anti-hypertensive therapy is allowed)
- No history of Grade 4 hypertension
- No history of maculo-papular rash or palmar plantar erythrodysesthesia syndrome that warranted permanent discontinuation of sorafenib in previous therapy cycles.

Sorafenib (SORAF): PO

Days: 1 through 364.

Starting Dose: 100 mg/m²/dose daily, rounded to accommodate tablet size. Dose to be administered per dosing nomogram in [Appendix II](#). See table below for guidelines for dose adjustment during maintenance. Refer to [Appendix II](#) for max dosing.

Note: Sorafenib tablets should be taken at least 1 hour before or 2 hours after food. Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 years and 4 to 8 ounces for ≥ 12 years). If taken with food, sorafenib should be taken with a moderate to low fat meal. Tablets should not be crushed, but should be swallowed whole. However, sorafenib tablets can be dispersed in water to facilitate the administration to subjects that cannot swallow tablets (see drug monograph in [Section 6.12](#)). Grapefruit and its juice should be avoided for the duration of treatment with sorafenib.

Maintenance Sorafenib Dose Adjustment:

Patient doses may change as described in Table 1 below over the course of maintenance with the exception that sorafenib doses may not be re-escalated in patients experiencing any grade of cardiac toxicities regardless of attribution or Grade 4 non-cardiac toxicities thought to be possibly, probably or definitely related to sorafenib by the treating physician.

Table 1: Maintenance Sorafenib Dose Adjustment Guidelines

Dose	Toxicity*	Timing of Dose Adjustment	Dose Adjustment
100 mg/m ² /dose, once daily	Dose limiting	Immediate	Hold dose and refer to dose modification guidelines. If further dose reduction mandated for toxicity then sorafenib should be discontinued permanently.
100 mg/m ² /dose, once daily	No toxicity, or toxicities limited to ≤ Grade 2 that improve to ≤ Grade 1 within one week	After 28 days	Increase dose to 200 mg/m ² once daily
200 mg/m ² /dose, once daily	Dose limiting	Immediate	Hold dose and adjust per dose modification guidelines. If dose reduction warranted, decrease dose to 100 mg/m ² /dose, once daily
200 mg/m ² /dose, once daily	No toxicity, or toxicities limited to ≤ Grade 2 that improve to ≤ Grade 1 within one week	After 28 days	Increase dose to 150 mg/m ² /dose twice daily
150 mg/m ² /dose, TWICE daily	Dose limiting	Immediate	Hold dose and adjust per dose modification guidelines. If dose reduction warranted, decrease dose to 200 mg/m ² /dose, once daily
150 mg/m ² /dose, TWICE daily	No toxicity, or toxicities limited to ≤ Grade 2 that improve to ≤ Grade 1 within one week	NA	Maintain dose

* Toxicity possibly, probably or definitely related to sorafenib.

Sorafenib is supplied by Bayer HealthCare Pharmaceuticals, and distributed by the NCI DTCD. Do not use commercially available drug.

SEE [SECTION 5.0](#) FOR DOSE MODIFICATIONS BASED ON TOXICITIES. SEE [APPENDIX I](#) FOR SUPPORTIVE CARE GUIDELINES.

The therapy delivery map (TDM) for Maintenance Arm C is on the next page.

5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 Allergy

5.1.1 Allergy to Asparaginase

5.1.1.1 Local Reactions (Inflammation at Injection Site, Swelling):

Continue asparaginase (*E.coli* or *Erwinia*) administration in the presence of Grade 1 allergic reaction (transient flushing or rash; drug fever < 38°C). 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 associated with the presence of asparaginase neutralizing antibodies, which render asparaginase therapy ineffective.

5.1.1.2 Anaphylaxis/Systemic Allergic Reactions:

Discontinue asparaginase (*E.coli* or *Erwinia*) if the patient develops a systemic allergic reaction (urticaria, wheezing, laryngospasm, hypotension, etc). Should an allergy be diagnosed after the first dose given on Day 2 during Intensification II (for high risk patients), then the dose due on Day 9 should not be administered.

5.1.2 Allergy to Etoposide

Etoposide allergic reactions may be managed with pre-medications such as diphenhydramine 1 mg/kg IV (maximum dose 50 mg) or an equivalent H-1 receptor antagonist, ranitidine 1 mg/kg IV (maximum dose 50 mg) or an equivalent H-2 receptor antagonist, hydrocortisone 1 - 4 mg/kg IV, and by slowing the rate of the infusion OR etoposide phosphate may be substituted in the same dose and at the same rate. Premedication for etoposide phosphate is recommended.

5.2 Cardiac Toxicity

Note: Patients may be on ACE inhibitors at time of echocardiogram reassessment and still meet criteria for sorafenib re-initiation described below. However, patients would need to be off all inotropic support, including but not limited to oral digoxin, for a minimum of 7 days, before meeting echocardiogram/MUGA criteria for restarting sorafenib therapy described below.

5.2.1 Left Ventricular Systolic Dysfunction

Daunorubicin, mitoxantrone, and sorafenib will be held if there is significant evidence of cardiac disease by echocardiogram or MUGA (shortening fraction < 28% or EF < 55%). Cardiac examination with echocardiogram (or MUGA) is required prior to the start of all chemotherapy courses, at the end of protocol therapy, and in follow up. Echocardiogram and cardiac biomarker evaluation (i.e. troponin) are also strongly recommended, particularly in clinical scenarios with potential or suspected cardiac dysfunction. Please see [Section 7.5](#) for long-term follow-up monitoring.

5.2.1.1 Daunorubicin and Mitoxantrone:

Do not re-start anthracyclines if held for left ventricular shortening dysfunction that is not associated with a microbiologically proven bacteremia or sepsis. If the left ventricular shortening dysfunction occurred in the setting of microbiologically proven bacteremia or sepsis, then anthracyclines may be reinstated at the treating clinician's discretion once the shortening fraction has returned to $\geq 28\%$ or $EF \geq 55\%$.

5.2.1.2 Sorafenib in Combination with Chemotherapy:

Sorafenib should be held for a shortening fraction of < 28% or $EF < 55\%$ regardless of attribution. Sorafenib should be restarted at a reduced dose (100 mg/m²) when the shortening fraction has returned to $\geq 28\%$ or $EF \geq 55\%$. If SF decreases below 28% or EF decreases below 55% on 100 mg/m², then sorafenib should be discontinued permanently. Sorafenib dose may not be re-escalated in combination with chemotherapy.

5.2.1.3 Sorafenib in Maintenance

Sorafenib should be held for a shortening fraction of <28% or EF < 55% regardless of attribution. Sorafenib should be restarted at 1 dose level lower when the shortening fraction has returned to ≥ 28% or EF ≥ 55%. Sorafenib may not be re-escalated during maintenance in patients experiencing a shortening fraction of <28% or EF < 55% regardless of attribution. Patients experiencing a SF < 28% or EF < 55% at the 100 mg/m² dose level will permanently discontinue sorafenib.

5.2.2 Hypertension

The table below will be used to manage hypertension possibly, probably or definitely related to sorafenib. Management of hypertension not related to sorafenib is at the treating clinician's discretion. Should initiation of anti-hypertensive therapy be required, single agent therapy (such as amlodipine or nifedipine) should be started (see [Appendix I](#)) and the blood pressure should be monitored at least twice weekly until BP is within the 95th percentile for age, height, and gender per [Appendix VI](#). For patients greater than age 18, rely on CTCAE v.4.0 grading of hypertension for consideration of dose adjustment.

Grade (CTCAE v4.0)	Antihypertensive Therapy	Blood Pressure Monitoring	Sorafenib Dose
Grade 1 (or Grade 2 lasting ≤ 72 hours)	None	Routine	No change
Grade 2 lasting > 72 hours	Initiate monotherapy	Increase frequency and monitor (by health professional) at least twice/week until BP is within the 95th percentile for age, height, and gender.	No change
Grade 3	Add additional agent(s) per institutional standards.	Monitor every 2 days* until BP is within the 95th percentile for age, height, and gender; continue every other day monitoring after sorafenib restarted until BP is within the 95th percentile for age, height, and gender.	<ol style="list-style-type: none"> 1. Hold sorafenib until diastolic BP in range for age, height, and gender. 2. If BP within the 95th percentile for age, height, and gender within 14 days: <ul style="list-style-type: none"> • For patients receiving sorafenib + chemotherapy restart sorafenib at reduced dose (100 mg/m²/day). • For patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level. 3. If BP is not within the 95th percentile for age, height, weight within 14 days, or if hypertension occurred at 100 mg/m² dose level discontinue sorafenib.
Grade 4			Discontinue sorafenib permanently.

* May monitor q3 days over weekend, i.e., evaluation on Friday and Monday

5.3 Coagulopathy

If symptomatic, omit Day 9 asparaginase and consider factor replacement (FFP, cryoprecipitate, factor VIIa). Do not hold asparaginase (*E.coli* or *Erwinia*) for abnormal laboratory findings without clinical symptoms.

5.4 Hepatic Toxicity

5.4.1 Transaminases

If the ALT or AST are > 10 x ULN, attempts should be made to identify the cause and notify the Study Chair. In most cases, the therapy will proceed without modification.

5.4.2 Hyperbilirubinemia

If the direct bilirubin is > 3 mg/dL, notify the Study Chair. In some cases it may be necessary to proceed if the bilirubin elevation is a result of the leukemia itself. If the elevated direct bilirubin is not a result of the leukemia, modify the doses of daunorubicin, etoposide, mitoxantrone and bortezomib as follows below. For all cases in which the direct bilirubin is elevated at the point in time that the next course is to begin, consider delaying the course for 1 week to determine whether the direct bilirubin falls to an acceptable level.

Asparaginase

L-asparaginase has been associated with hepatic toxicity but dosing guidelines for hepatic toxicity are not available. Thus, asparaginase administration in the setting of hepatic toxicity is at the clinician's discretion.

Bortezomib

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. Consider dose escalation to 1 mg/m ² if transaminitis resolves and is not possibly, probably or definitely related to bortezomib.
Severe	> 3x upper limit of normal	

* No adjustment necessary for elevated SGOT (ALT).

Daunorubicin, Etoposide and Mitoxantrone

In severe liver dysfunction, the half-life of mitoxantrone is prolonged and the AUC may be more than 3-fold that of patients with normal hepatic function. However, there are no available dose adjustment guidelines in the literature. Therefore, similar dose reductions of mitoxantrone as outlined below for daunorubicin are utilized on this protocol.

Direct Bilirubin	Daunorubicin	Etoposide	Mitoxantrone
≥ 2 and < 3 mg/dL	50% of the calculated dose	50% of the calculated dose	50% of the calculated dose
≥ 3 and < 5 mg/dL	25% of the calculated dose	25% of the calculated dose	25% of the calculated dose
≥ 5 mg/dL	Hold dose and notify study chair	Hold dose and notify study chair	Hold dose and notify study chair

Full dose of daunorubicin, etoposide and mitoxantrone may resume when the direct bilirubin has fallen to < 1.2 mg/dL.

5.5 Neurologic Toxicity

5.5.1 Cytarabine

Patients with \geq Grade 3 CTCAE v 4 nervous system disorders from high dose cytarabine should not receive further high dose cytarabine. The most common nervous system disorder is an acute cerebellar syndrome that may manifest itself as ataxia, nystagmus, dysarthria, or dysmetria. However, seizures and encephalopathy have also occurred following therapy with high dose cytarabine. **If nervous system disorders \geq Grade 3 due to high dose cytarabine occurs during protocol therapy, the patient will go Off Protocol Therapy for toxicity.**

5.5.2 Bortezomib-Related Peripheral Neuropathy

Patients with bortezomib-related neurotoxicity will remain on protocol therapy but have bortezomib dose de-escalated or held as specified below. Peripheral neuropathy will be closely monitored during each course of treatment and toxicities graded using CTCAE v4.0. A complete neurological exam should be documented prior to the start of each course of therapy.

Peripheral sensory neuropathy grading should be based on the maximum toxicity occurring during the previous course. All dose modifications should be based on the worst preceding toxicity. Bortezomib dose will be decreased for sensory peripheral neuropathy as follows:

Severity of peripheral sensory neuropathy	Bortezomib modification
Grades 1 - 2 with no pain	Same dose as previous.
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 have resolved to \leq Grade 1. Do not make up missed doses. When toxicity resolves, re-initiate bortezomib at 1 mg/m ² /dose
Grade 4	Discontinue bortezomib

5.5.2.1

If Grade 2 peripheral sensory neuropathy with pain or Grade 3 peripheral sensory neuropathy persists for > 2 weeks, bortezomib should be discontinued. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

5.5.2.2

Patients experiencing Grade 4 peripheral sensory neuropathy will discontinue bortezomib. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

5.5.2.3

If Grade 2 or 3 peripheral sensory neuropathy with pain recurs despite bortezomib dose reduction to 1 mg/m², the patient should discontinue bortezomib in Induction I. See [Section 5.5.2.4](#) if peripheral neuropathy resolves before the start of Induction II. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

5.5.2.4

Patients whose peripheral sensory neuropathy resolves to \leq Grade 1 or baseline within 2 weeks will receive bortezomib in Induction II at 1 mg/m²/dose.

5.6 Pancreatitis

Discontinue asparaginase (*E.coli* or *Erwinia*) in the presence of hemorrhagic pancreatitis or severe pancreatitis (abdominal pain > 72 hours and > Grade 3 amylase elevation (> 2.0 x ULN). In the case of mild pancreatitis after Day 2, Day 9 asparaginase may be given only if symptoms and signs subside, and amylase levels return to normal. Severe pancreatitis is a contraindication to additional asparaginase administration.

5.7 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 [Section 5.7.3](#) for management of new-onset dyspnea, hypoxia or chest infiltrates not explained by infection or other known cause.

Bortezomib should be held in patients with ARDS that may be bortezomib related.

5.7.1 Monitoring

Pulmonary function will be monitored by pulse oximetry. Measurement of pulse oximetry should be documented within 12 hours prior to each dose of bortezomib. A chest radiograph should be performed with the development of respiratory symptoms. In the absence of identifiable causes of respiratory stress, all patients should demonstrate oxygen saturation on room air of $\geq 94\%$ at sea level ($> 90\%$ at high altitude) prior to each bortezomib dose. Pulmonary toxicity due to bortezomib is frequently delayed and can occur up to 3 weeks after the final dose of bortezomib is administered.

Pulmonary symptoms such as increased respiratory rate may indicate toxicity related to bortezomib and pulse oximetry should be checked for persistent, unexplained hyperpnea. Respiratory symptoms such as cough, hypoxia and dyspnea should be aggressively evaluated. See [Section 5.7.3](#) for management of new-onset dyspnea, hypoxia or chest infiltrates not explained by infection.

5.7.2 Dose Reductions and Discontinuation of Bortezomib for Pulmonary Toxicity

Bortezomib will be dose reduced to 1 mg/m^2 for any resolving Grade 3 pulmonary toxicity (excluding hiccups or voice changes/laryngitis), including hypoxia 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 hiccups and voice changes/laryngitis) will discontinue bortezomib. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

Dose reduction within a course:

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 \leq Grade 1, bortezomib can be restarted at a decreased dose of 1 mg/m^2 for **subsequent** doses. Missed doses of bortezomib in a course should not be made up. Patients who have a Grade 3 pulmonary toxicity that worsens (i.e., > Grade 3) by the next scheduled dose of bortezomib should discontinue bortezomib. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

Dose reduction between courses:

The bortezomib dose will be decreased to 1 mg/m^2 for qualifying Grade 3 or 4 toxicities (see above) that resolve to \leq Grade 1 prior to the beginning of the next course. Doses reduced for an adverse event will not be increased, even if there is minimal or no toxicity at the reduced dose.

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

5.7.3 Management of Drug-induced Pulmonary Toxicity

Both cytarabine and bortezomib have been associated with ARDS however, if a patient experiences dyspnea, hypoxia (oxygen saturation < 92%), or bilateral infiltrates on a CXR, other causes must be ruled out. Additional testing for infectious complications and congestive heart failure should be obtained. A CT scan is strongly recommended to evaluate for infectious complications. A repeat ECHO is advised since anthracyclines can be associated with sudden decreases in left ventricular function. In addition, formal consultation from a pediatric pulmonologist is strongly recommended. Bronchoscopy is encouraged if clinically indicated. In the absence of evidence of an infectious etiology or congestive heart failure, the following steroid therapy is recommended for patients with Grades 1 - 3 ARDS that may be related to bortezomib:

Methylprednisolone 1 mg/kg IV daily.

For patients with Grade 4 ARDS, the following steroid regimens may also be considered:

- Methylprednisolone 1 gram/m² IV, followed by 500 mg/m² IV daily, or
- Dexamethasone 6 mg/m² IV q8 hrs, or
- Prednisone 40 mg/m² PO daily divided BID.

Other similar steroid regimens suitable for treatment of non-infectious pneumonitis may be used. It is recommended that steroids be rapidly tapered after 4 days based on the patient’s clinical status. Prolonged use of steroids is strongly discouraged to prevent an increased incidence in invasive fungal infections.

5.8 Palmar-Plantar Erythrodysesthesia Syndrome, Skin Pain that impacts ADLs or Rash Maculo-Papular

The following tables detail sorafenib modifications for palmar-plantar erythrodysesthesia syndrome, skin pain that impacts ADLs, or rash maculo-papular.

Grading for rash maculo-papular is performed solely on the basis of ADL limitation and not percentage of body surface area involved.

Table 2: Palmar-Plantar Erythrodysesthesia Syndrome or Pain (in absence of rash) with impact on ADLs

Grade	Occurrence	Dose Modification
Grade 1	Any	Continue sorafenib. Topical therapy and supportive care measures. *
Grade 2	First	1. Hold sorafenib. Institute topical therapy and supportive care measures. * 2. If toxicity improves to < Grade 2 within 14 days, restart at same dose. 3. If toxicity does not improve to < Grade 2 within 14 days, hold until toxicity resolved. <ul style="list-style-type: none"> • For patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. • For patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level. • Discontinue sorafenib if toxicity occurred at 100 mg/m² dose
	Second	1. Hold sorafenib. Institute topical therapy and supportive care measures* 2. If toxicity improves to < Grade 2 within 14 days: <ul style="list-style-type: none"> • patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day

		<ul style="list-style-type: none"> patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level Discontinue sorafenib if toxicity occurred at 100 mg/m² dose. <p>3. Discontinue sorafenib if toxicity does not improve to < Grade 2 within 14 days.</p>
	Third	Institute topical therapy and supportive care measures.* Discontinue sorafenib permanently.
Grade 3	First	<p>1. Hold sorafenib. Institute topical therapy and supportive care measures. *</p> <p>2. If toxicity improves to < Grade 2 within 14 days:</p> <ul style="list-style-type: none"> patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level Discontinue sorafenib if toxicity occurred at 100 mg/m² dose. <p>3. Discontinue sorafenib if toxicity does not improve to < Grade 2 within 14 days.</p>
	Second	Institute topical therapy and supportive care measures. * Discontinue sorafenib permanently.

* see [Appendix I](#)

Table 3: Rash, Maculo-Papular- Grade only for ADL Limitation

Grade	Occurrence	Dose Modification
Grade 1	Any	Continue sorafenib. Topical therapy and supportive care measures. *
Grade 2	First	<p>1. Hold sorafenib. Institute topical therapy and supportive care measures. *</p> <p>2. If toxicity improves to < Grade 2 within 14 days, restart at same dose.</p> <p>3. If toxicity does not improve to < Grade 2 within 14 days, hold until toxicity resolved.</p> <ul style="list-style-type: none"> For patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. For patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level. Discontinue sorafenib if toxicity occurred at 100 mg/m² dose
	Second	<p>1. Hold sorafenib. Institute topical therapy and supportive care measures. *</p> <p>2. If toxicity improves to < Grade 2 within 14 days:</p> <ul style="list-style-type: none"> patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level. Discontinue sorafenib if toxicity occurred at 100 mg/m² dose. <p>3. Discontinue sorafenib if toxicity does not improve to < Grade 2 within 14 days.</p>
	Third	Institute topical therapy and supportive care measures.* Discontinue sorafenib permanently.
Grade 3	First	<p>1. Hold sorafenib. Institute topical therapy and supportive care measures. *</p> <p>2. If toxicity improves to < Grade 2 within 14 days:</p> <ul style="list-style-type: none"> patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level. Discontinue sorafenib if toxicity occurred at 100 mg/m² dose. <p>3. Discontinue sorafenib if toxicity does not improve to < Grade 2 within 14 days.</p>
	Second	Institute topical therapy and supportive care measures. * Discontinue sorafenib permanently.

* see [Appendix I](#)

5.9 Renal Toxicity

5.9.1 Cytarabine

Patients with nephrotoxicity secondary to antibiotics, or antifungals, may have prolonged excretion of cytarabine leading to more severe marrow and extramedullary toxicity. Patients with a serum creatinine > 2 mg/dL or > 2 x normal for age should be hydrated orally or intravenously. Following hydration, the patient must have a creatinine clearance ≥ 60 mL/min/1.73m² as measured preferably by a nuclear GFR Scan or timed urine collection for creatinine clearance before proceeding with high dose cytarabine therapy (doses of 1,000 mg/m² or greater). If the CrCl is abnormal (< 60 mL/min/1.73m²) then high dose cytarabine should be reduced from twice daily to once daily dosing, at the same previously prescribed doses (e.g., 50% daily dose reduction). With this approach, previous research has shown the prevention of subsequent neurotoxicity in recipients of high dose cytarabine in the face of renal insufficiency.¹²¹

5.9.2 Etoposide

In patients with impaired renal function, the following **initial** dose modification of etoposide should be considered based on measured creatinine clearance: for CrCl > 60 mL/min give full dose, for CrCl of 15 – 60 mL/min give 75% of the dose (25% dose reduction). For CrCl < 15 mL/min notify the Study Chair. (Please note: For dose adjustment in renal dysfunction for children, use the “corrected” value for creatinine clearance measured in mL/min/1.73 m², which ‘corrects’ that value for the standardized values of CrCl in adults, measured in mL/min. The “corrected” value is loosely interpreted as being equivalent to creatinine clearance measured in an adult patient). **Subsequent doses** should be based on patient tolerance and clinical effect.

5.10 Thrombosis

Discontinue asparaginase and treat with appropriate antithrombotic therapy, as indicated. Do not hold asparaginase for abnormal laboratory findings without clinical sequelae. For significant thrombosis, not line related, consider evaluation for inherited predisposition to thrombosis.

5.11 Dose Reduction for Other Bortezomib-Associated Toxicities

5.11.1 Dose Reduction for Non-Hematological Toxicity (other than described above)

Bortezomib will be dose reduced to 1 mg/m² for non-hematological severe toxicities (other than those described above) that are possibly, probably or definitely related to bortezomib. A severe toxicity is defined as any Grade 3 or 4 toxicity (with the exception of fever, infection, hyperfibrinogenemia, or metabolic/laboratory abnormalities). Follow dose reduction guidelines detailed in [Section 5.7.2](#).

Bortezomib doses may not be re-escalated in patients with dose limiting toxicity.

If a specific bortezomib-related toxicity recurs despite one dose reduction, patient will discontinue bortezomib. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

5.11.2 Dose Reduction for Hematologic Toxicity

Bortezomib will be dose reduced to 1 mg/m² for patients experiencing persistent myelosuppression following chemotherapy. Persistent myelosuppression is defined as neutropenia (ANC $< 500/\mu\text{L}$) or thrombocytopenia (platelet count $< 50,000/\mu\text{L}$) lasting longer than 40 days in Induction I, Induction II, and Intensification I or longer than 75 days in Intensification II. Patients that have a recurrence of persistent neutropenia or thrombocytopenia despite 1 bortezomib dose reduction will discontinue bortezomib.

Bortezomib doses may not be re-escalated in patients with dose limiting toxicity. Patients who discontinue bortezomib will continue with the standard chemotherapy regimen and will remain on protocol therapy.

5.12 Dose Reductions for Other Sorafenib-Associated Toxicities

Dose reductions for sorafenib-associated toxicities other than left ventricular shortening dysfunction ([Section 5.2](#)), hypertension ([Section 5.2.2](#)), and rash (maculo-papular or palmar plantar erythrodysesthesia syndrome, [Section 5.8](#)) or skin pain without rash that limits ADLs should be made according to the table below. There is a dose modification table for both the sorafenib/chemotherapy and sorafenib maintenance phases of the protocol.

Sorafenib dose may not be re-escalated after de-escalation for dose limiting toxicity in the sorafenib/chemotherapy phase of the protocol. Patients should receive 100 mg/m²/day until maintenance, at which time dose-escalation may proceed according to Table 1 in [Section 4.26](#).

Sorafenib dose may be re-escalated after de-escalation for Grade 2 or 3 dose limiting toxicity in the sorafenib maintenance phase per [Section 4.26](#).

Dose re-escalation may not occur if the toxicity was Grade 4 and possibly, probably or definitely related to sorafenib, or for any grade of cardiac toxicity regardless of attribution.

Non-Hematologic Toxicities (Possibly, Probably or Definitely) Related to Sorafenib:

Grade	Dose Modification
Grade 1	Continue same dose.
Grade 2	Continue same dose unless toxicity intolerable to the patient. If toxicity intolerant to the patient, dose adjust per Grade 3 dose adjustment guidelines.
Grade 3	Hold sorafenib until toxicity is Grade < 2. If toxicity improves to Grade < 2 within 14 days, resume at the same dose. If patient experiences a second Grade 3 toxicity or if resolution of the first takes > 14 days, hold sorafenib until toxicity is Grade < 2, then: <ul style="list-style-type: none"> For patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. Discontinue sorafenib if toxicity occurred at 100 mg/m²/dose.
Grade 4	Hold sorafenib until toxicity is Grade < 2, then: <ul style="list-style-type: none"> For patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. For patients receiving sorafenib maintenance, refer to Table 1 in Section 4.26 and dose reduce 1 dose level. Discontinue sorafenib if toxicity occurred at 100 mg/m²/dose. If clinically indicated, sorafenib at 200 mg/m ² or 300 mg/m ² (150 mg/m ² BID) dose levels may be held indefinitely.

Hematologic Toxicities (Possibly, Probably or Definitely) Related to Sorafenib + conventional chemotherapy phases (prior to maintenance therapy):

Grade	Dose Modification
Grade 1	Continue same dose.
Grade 2	Continue same dose.
Grade 3	Hold sorafenib until toxicity is Grade < 2. If toxicity improves to < Grade 2 within 14 days, resume at the same dose.

	<p>If patient experiences a second Grade 3 toxicity or if resolution of the first takes > 14 days, hold sorafenib until toxicity is Grade < 2, then:</p> <ul style="list-style-type: none"> • For patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. • Discontinue sorafenib if toxicity occurred at 100 mg/m² dose
Grade 4	<p>Hold sorafenib until toxicity is Grade < 2, then:</p> <ul style="list-style-type: none"> • For patients receiving sorafenib (200 mg/m²/day) + chemotherapy, restart at 100 mg/m²/day. • Discontinue sorafenib if toxicity occurred at 100 mg/m²/dose. <p>If clinically indicated, sorafenib at 200 mg/m² or 300 mg/m² (150 mg/m² BID) dose levels may be held indefinitely.</p>

Hematologic Toxicities (Possibly, Probably or Definitely) Related to Sorafenib Maintenance therapy:

Grade	Dose Modification
Grade 1	Continue same dose.
Grade 2	Continue same dose.
Grade 3	<p>Hold sorafenib until toxicity is Grade < 2. If toxicity improves to < Grade 2 within 14 days, resume at the same dose. If patient experiences a second Grade 3 toxicity or if resolution of the first takes > 14 days, hold sorafenib until toxicity is Grade < 2, then:</p> <ul style="list-style-type: none"> • Refer to Table 1 in Section 4.26 and dose reduce 1 dose level. <p>Discontinue sorafenib if toxicity occurred at 100 mg/m² dose</p>
Grade 4	<p>Hold sorafenib until toxicity is Grade < 2, then:</p> <ul style="list-style-type: none"> • Refer to Table 1 in Section 4.26 and dose reduce 1 dose level. • Discontinue sorafenib if toxicity occurred at 100 mg/m²/dose. If clinically indicated, sorafenib at 200 mg/m² or 300 mg/m² (150 mg/m² BID) dose levels may be held indefinitely.

5.13 Stem Cell Transplant Regimen Agents

5.13.1 Methotrexate Dose Adjustments

Every attempt possible should be made to administer methotrexate as prescribed since dose reduction and omission have been associated with an increased incidence of acute GVHD. Not every impairment in renal function and not every increase in third space fluid are contraindications to the use of methotrexate. It may be advantageous to give methotrexate, possibly at a reduced dose (75%-50%), and offer rescue with leucovorin. Several studies have shown that this will reduce toxicity without increasing the risk of GVHD. Any questionable situation must be discussed with the Transplant Study Committee Representative and serum methotrexate levels determined. If methotrexate concentration is assessed and is greater than 0.1 μM at 24 hours, then leucovorin 10 mg/m^2 IV q 6 hours should be initiated until resolution of elevated methotrexate levels.

5.13.2 Tacrolimus

Tacrolimus commonly causes mild/moderate hypertension and alopecia and less commonly kidney or liver dysfunction, transplant associated microangiopathy (TAM), and neurological changes associated with significant hypertension. When trough levels are kept in the therapeutic range and patients receive adequate hydration and magnesium replacement, most of these side effects can be minimized. Hypertension should be managed with single or combination antihypertensive therapy per institutional standards. Tacrolimus should be held for severe toxicities thought to be related to its administration (significant neurological changes/malignant hypertension, TAM, kidney failure, etc). Other immune suppressive medications may be substituted if tacrolimus is not tolerated (MMF, cyclosporine, etc).

6.0 DRUG INFORMATION

Agents are listed alphabetically.

6.1 ASPARAGINASE (*E. coli*)

(07/01/15)

(*E. coli* L-asparaginase, EC 3.5.2.2, colaspase, L-asnase, Elspar®, Kidrolase®, Crasnitin®, Leunase®)
NSC#109229

Source and Pharmacology:

L-asparagine is a nonessential amino acid synthesized by the transamination of L-aspartic acid by a reaction catalyzed by the enzyme L-asparagine synthetase. The ability to synthesize asparagine is notably lacking in malignancies of lymphoid origin. Asparaginase contains the enzyme L-asparagine amidohydrolase type EC-2, which is derived from *Escherichia coli* and depletes L-asparagine from leukemic cells (especially lymphoblasts) by catalyzing the conversion of L-asparagine to aspartic acid and ammonia. L-asparaginase is administered either intravenously or intramuscularly. The intramuscular route produces peak blood levels 50% lower than the intravenous route, but the former may be less immunogenic and is more commonly used. L-asparaginase concentration in plasma is proportional to a total dose up to 200,000 IU/ m^2 and falls with a primary half-life of 14 to 22 hours after administration. Blood levels of the *E. coli* enzyme are detectable for 1 to 2 weeks after a single dose, and concentrations of L-asparagine fall below 1 mmol within minutes of enzyme injection and remain low for 7 to 10 days after completion of therapy. The half-life is independent of the dose administered, disease status, renal or hepatic function, age, or gender. Plasma $t_{1/2}$ varies from 8 to 30 hours after IV administration and 39 to 49 hours after IM administration. 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), pain at injection site	Rash	Anaphylaxis, periorbital edema, chills, fever, myalgia, mild nausea & vomiting, abdominal pain, 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), hemorrhage (L), DIC, thrombosis, anorexia, CNS ischemic attacks, edema, azotemia and decreased renal function, mild leukopenia and thrombocytopenia, coma and stupor, hypertriglyceridemia, hyperlipidemia, Parkinson-like syndrome with tremor and increase in muscular tone, CNS changes including irritability, depression, confusion, EEG changes, hallucinations
Delayed: Any time later during therapy, excluding the above conditions			Renal failure
Unknown Frequency and Timing:	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:

Asparaginase is a white lyophilized plug or powder supplied in a sterile 10 mL vial containing 10,000 IU of asparaginase and 80 mg mannitol, an inactive ingredient. The specific activity of asparaginase is at least 225 IU per mg of protein. Store at 2°-8°C (36°-46°F). Asparaginase powder for injection is stable for at least 48 hours at room temperature.

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

For intramuscular use (IM): asparaginase is reconstituted by adding 1 or 2 mL of NS to the vial containing 10,000 IU of asparaginase. Rotate the vial to dissolve (shaking will cause excessive foaming making withdrawal of the entire contents difficult). It is suggested that no more than 2 mL should be given at any one injection site.

For intravenous use (IV): Reconstitute a 10,000 units vial with 5mL of SWFI or with NS and used for direct intravenous infusion within 8 hours. Asparaginase may be further diluted in 50-250mL of D5W or NS and infuse within 8 hours, only if clear.

Asparaginase does not contain a preservative. Unused, reconstituted solution should be discarded after eight hours or sooner if it becomes cloudy.

Occasionally a very small number of gelatinous, fiber-like particles may develop in asparaginase solutions on standing. These particles may be removed without loss of potency by filtration through a 5-micron filter. Some loss of potency has occurred with the use of a 0.2-micron filter.

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: US formulation of *E.Coli* Asparaginase, Elspar®, has been discontinued.

CANADIAN SITES: *E.Coli* Asparaginase is commercially available in Canada. See Canadian package insert for further information.

6.2 ASPARAGINASE (ERWINIA CHRYSANTHEMI) (*Erwinia chrysanthemi*, Erwinase®, Erwinaze™, Crisantaspase) (10/10/2016)

Source and Pharmacology:

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

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

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

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

Effective asparaginase levels have been defined as activity of ≥ 0.1 International Units per mL. Clinical trials with asparaginase *Erwinia chrysanthemi* demonstrated that 100% of patients achieved effective asparaginase levels at 48 and 72 hours (n=35 and n=13, respectively) following the third total dose when given on a Monday, Wednesday, Friday schedule using the IM route of administration. In a multicenter study characterizing the pharmacokinetic profile of 25,000 International Units/m² Erwinaze® given intravenously over one hour on the same dosing schedule of Monday, Wednesday, Friday for 2 consecutive

weeks, 83% (20/24) and 43% (9/21) of evaluable patients achieved an asparaginase activity level of ≥ 0.1 International Units/mL at 48 post-dose 5 and 72 hours post-dose 6, respectively.. No formal drug interaction studies have been performed with asparaginase *Erwinia chrysanthemi*.

Toxicity:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug		Allergic reactions, anaphylaxis, urticaria	Local injection site reactions, fever
Prompt: Within 2-3 weeks, prior to the next course			Pancreatitis, glucose intolerance, thrombosis, hemorrhage, transient ischemic attack, disseminated intravascular coagulation, hyperbilirubinemia, alanine aminotransferase increased, aspartate aminotransferase increased, hyperglycemia, hyperammonemia, vomiting, nausea, abdominal pain, headache, diarrhea, seizure
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of L-asparaginase have been noted in animals. It is unknown whether the drug is excreted in breast milk. Adequate, well-controlled studies of asparaginase <i>Erwinia chrysanthemi</i> have NOT been conducted. It is not known whether asparaginase <i>Erwinia chrysanthemi</i> will cause fetal harm or affect the ability to reproduce. It is not known if asparaginase <i>Erwinia chrysanthemi</i> is excreted into breast milk. The use of asparaginase <i>Erwinia chrysanthemi</i> should be avoided in pregnant or lactating patients.		

(L) Toxicity may also occur later.

Formulation and Stability:

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

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

Erwinia asparaginase can be administered by intramuscular injection or by intravenous infusion. Use appropriate precautions for preparation of a hazardous agent. Visually inspect the powder in vial for foreign particles or discoloration prior to reconstitution.

For intramuscular administration, the contents of each vial should be reconstituted by slowly adding 1 mL or 2 mL of sterile, preservative-free NS to the inner vial wall. The final concentration is 10,000 International Units per mL when using 1 mL for reconstitution or 5,000 International Units per mL when using 2 mL for reconstitution. Gently mix or swirl the contents to dissolve the contents of the vial. Do not shake or invert the vial. The resulting solution should be clear and colorless. Discard if any particulate matter or protein aggregates are visible. **Withdraw the appropriate dosing volume into a polypropylene syringe within 15 minutes of reconstitution.** Polycarbonate luer-lok syringes from B-D (1 mL) are also acceptable (personal communication, EUSA Pharma). Discard any unused drug; do not save or use any unused drug remaining the in the vial. No more than 2 mL should be given at any one injection site. Doses larger than 2 mL should be divided and given in separate administration sites.

For intravenous use, slowly inject the appropriate volume of reconstituted solution into a Normal Saline 100 mL infusion bag; do not shake or squeeze the bag. Infuse *Erwinia* asparaginase over 1-2 hours. Do

not infuse other intravenous drugs through the same intravenous line while infusing *Erwinia* asparaginase.

Administer the dose within a 4 hour time period from reconstitution. If the dose is not used within this time period, discard the dose. Do not freeze or refrigerate the reconstituted solution.

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.

Drug Ordering:

In the United States, asparaginase *Erwinia chrysanthemi* (Erwinaze®) is distributed by McKesson Plasma and Biologics. Verify your institution has a contract with McKesson Plasma and Biologics before ordering. If not, contact McKesson at 877-625-2566 for assistance setting up an account.

Orders may be placed online or via phone, fax, or email.

1. Orders may be placed online via <http://Connect.McKesson.com>
2. Orders may be submitted via fax to 888-752-7626
3. Orders may be submitted via email or MPBOrders@McKesson.com
4. Email all other information requests to MPB@McKesson.com
5. Regular order hours: M-F 9:00 am – 7:30 pm EST;
6. Emergency order after hours services (24/7/365): 877-625-2566

Orders placed by 7:30 pm EST will ship the next day.

CANADIAN SITES

Asparaginase *Erwinia chrysanthemi* is commercially available in Canada. Canadian sites may purchase the Canadian commercial supply from EUSA via CGF Pharmatech, Montreal, Quebec, a subsidiary of EUSA (order desk phone: 1-514-343-0344 or 1-866-343-0344, fax: 1-514-343-0340). CGF requests that a site fax a Purchase Order number. There is no special fax order form. Shipments are sent Monday to Wednesday only and usually arrive at the site within 48-72 hours.

6.3 **BORTEZOMIB**

(Velcade, N-Pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid, PS-341, MLN341, LDP-341)
NSC# 681239, IND# 114480 (08/04/14)

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 which 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/m² dose and 76 to 108 hours after the 1.3 mg/m² dose. *In vitro* studies with human liver microsomes and human cDNA-expressed cytochrome P450 isozymes indicate that bortezomib is

primarily oxidatively metabolized via cytochrome P450 enzymes 3A4, 2C19, and 1A2. Bortezomib metabolism by CYP 2D6 and 2C9 enzymes is minor. The major metabolic pathway is deboronation to form 2 deboronated metabolites that subsequently undergo hydroxylation to several metabolites. Deboronated bortezomib metabolites are inactive as 26S proteasome inhibitors.

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

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

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

Toxicity:

Comprehensive Adverse Events and Potential Risks list (CAEPR) for PS-341 (Bortezomib, Velcade, NSC 681239)

The Comprehensive Adverse Events 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 PS-341 (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 2.5, June 30, 2014¹⁰

Adverse Events with Possible Relationship to PS-341 (Bortezomib, Velcade) (CTCAE 4.0 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			<i>Anemia (Gr 3)</i>
CARDIAC DISORDERS			
		Heart failure	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		<i>Abdominal pain (Gr 3)</i>
Constipation			<i>Constipation (Gr 3)</i>
Diarrhea			<i>Diarrhea (Gr 3)</i>
	Dyspepsia		<i>Dyspepsia (Gr 2)</i>
	Gastrointestinal hemorrhage ¹²²		
		Gastrointestinal perforation ¹¹	
	Ileus		<i>Ileus (Gr 3)</i>
Nausea			<i>Nausea (Gr 3)</i>
Vomiting			<i>Vomiting (Gr 3)</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		<i>Chills (Gr 2)</i>
	Edema limbs		<i>Edema limbs (Gr 3)</i>
Fatigue			<i>Fatigue (Gr 3)</i>
Fever			<i>Fever (Gr 3)</i>
INFECTIONS AND INFESTATIONS			
Infection ¹²³			<i>Infection⁴ (Gr 3)</i>
INVESTIGATIONS			
	Neutrophil count decreased		<i>Neutrophil count decreased (Gr 4)</i>
Platelet count decreased			<i>Platelet count decreased (Gr 4)</i>
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
Anorexia			<i>Anorexia (Gr 3)</i>
	Dehydration		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		<i>Arthralgia (Gr 2)</i>
	Back pain		<i>Back pain (Gr 2)</i>
	Bone pain		<i>Bone pain (Gr 2)</i>
	Musculoskeletal and connective tissue disorder - Other (muscle spasms)		
	Myalgia		<i>Myalgia (Gr 3)</i>
	Pain in extremity		<i>Pain in extremity (Gr 2)</i>
NERVOUS SYSTEM DISORDERS			
	Dizziness		<i>Dizziness (Gr 3)</i>

Adverse Events with Possible Relationship to PS-341 (Bortezomib, Velcade) (CTCAE 4.0 Term) [n= 2084]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
	Headache		<i>Headache (Gr 2)</i>
		Leukoencephalopathy	
	Neuralgia		<i>Neuralgia (Gr 3)</i>
	Paresthesia		
Peripheral motor neuropathy			<i>Peripheral motor neuropathy (Gr 3)</i>
Peripheral sensory neuropathy			<i>Peripheral sensory neuropathy (Gr 3)</i>
		Reversible posterior leukoencephalopathy syndrome	
PSYCHIATRIC DISORDERS			
	Anxiety		
	Insomnia		<i>Insomnia (Gr 2)</i>
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome	
	Cough		<i>Cough (Gr 2)</i>
	Dyspnea		<i>Dyspnea (Gr 3)</i>
	Pharyngeal mucositis		<i>Pharyngeal mucositis (Gr 2)</i>
		Pulmonary hypertension	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		<i>Rash maculo-papular (Gr 3)</i>
VASCULAR DISORDERS			
	Hypotension		<i>Hypotension (Gr 3)</i>

¹This 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.

²Gastrointestinal 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.

³Gastrointestinal 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.

⁴Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on PS-341 (bortezomib, Velcade) trials but with the relationship to PS-341 (bortezomib, Velcade) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (hematocrit low); Blood and lymphatic system disorders - Other (lymphadenopathy); Disseminated intravascular coagulation; Febrile neutropenia; Hemolytic uremic syndrome; Leukocytosis

CARDIAC DISORDERS - Acute coronary syndrome; 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 - External ear inflammation; Hearing impaired; Tinnitus

ENDOCRINE DISORDERS - Hypothyroidism

EYE DISORDERS - Blurred vision; Conjunctivitis; 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; Watery eyes

GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; 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 (eructation); 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

HEPATOBIILIARY DISORDERS - Hepatic failure; Hepatobiliary disorders - Other (hepatitis); Hepatobiliary disorders - Other (portal vein thrombosis); Hepatobiliary disorders - Other (VOD)

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; Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; 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; GGT increased; INR increased; Investigations - Other (albumin); Investigations - Other (BUN); Investigations - Other (low chloride); Investigations - Other (pancytopenia); 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); Tumor lysis syndrome

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; Musculoskeletal and connective tissue disorder - Other (cramping); 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); Nervous system disorders - Other (Bell's palsy); 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 (spinal cord compression); Nervous system disorders - Other (tongue paralysis); Presyncope; Seizure; Somnolence; 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 (calculus renal); Renal and urinary disorders - Other (glomerular nephritis proliferative); 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 (pleurisy); 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: PS-341 (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.

PLEASE NOTE: The potential risks listed in the CAEPR whose relationship to bortezomib is still undetermined are not required by CTEP to be described in the ICD; however, they may be communicated to patients according to local IRB requirements.

Other Potential Risks:

The CAEPR above lists “leukoencephalopathy” and “Reversible posterior leukoencephalopathy syndrome” as “Rare but Serious” adverse events with potential association to bortezomib. The prescribing information for bortezomib was updated in 2012 to include Progressive Multifocal Leukoencephalopathy (PML) as a specific adverse event noted in post-marketing reports. Institutions may use their discretion with adding this toxicity to the Informed Consent document.

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/m²) 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 25°C (77°F); excursions permitted from 15°C to 30°C (59°F to 86°F). 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.jsp> >. 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.

6.4 BUSULFAN INJECTION

(Busulfex®)

NSC #750

(07/01/15)

Source and Pharmacology:

Busulfan is a non-cell cycle specific bifunctional alkylating agent. In aqueous media, busulfan hydrolyzes to release methanesulfonate groups. This produces reactive carbonium ions that interact with cellular thiol groups and nucleic acids to form DNA cross-links. Busulfan injection is 100% bioavailable by definition of intravenous administration. The elimination of busulfan appears to be independent of renal function, presumably reflecting the extensive metabolism of the drug in the liver, since less than 2% of the administered dose is excreted in the urine unchanged within 24 hours. The drug is metabolized by enzymatic activity to at least 12 metabolites, among which tetrahydrothiophene, tetrahydrothiophene 12-oxide, sulfolane, and 3-hydroxysulfolane were identified. These metabolites do not have cytotoxic activity. Irreversible binding to plasma proteins (primarily albumin) is approximately 32.4%. Busulfan has a plasma terminal elimination half-life ($t_{1/2}$) of about 2.6 hours and demonstrates linear kinetics. It is rapidly distributed into tissue and crosses the blood-brain and the placental barriers. CSF concentrations are approximately equal to those in plasma. Itraconazole reduced busulfan clearance by up to 25% in patients receiving itraconazole compared to patients who did not receive itraconazole. Higher busulfan exposure due to concomitant itraconazole could lead to toxic plasma levels in some patients. Fluconazole had no effect on the clearance of busulfan.

Toxicities:

	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, electrolyte changes (hypokalemia, hypomagnesemia, hypocalcemia, hypophosphatemia, and hyponatremia), hyperglycemia, dizziness, rash, pruritus, urticaria, injection site pain and inflammation, back pain, tachycardia, chest pain, edema, insomnia, anxiety, depression, headache, abdominal pain, diarrhea (L) or constipation, anorexia, rectal discomfort, dyspnea, epistaxis	Weight gain, confusion	Seizures (rare with phenytoin prophylaxis), hematemesis, hyperuricemia, arrhythmias other than tachycardia, pleural effusion, alveolar hemorrhage
Prompt: Within 2-3 weeks	Myelosuppression, asthenia, immunosuppression (L), mucositis, hyperbilirubinemia	Hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD) (L), mild alopecia (L), arthralgia, myalgia, hemorrhagic cystitis, hyperpigmentation (L), elevated creatinine and BUN	Reduced adrenal function (L), esophagitis, radiation recall reactions
Late: Any time after completion of treatment	Infertility, testicular atrophy and azoospermia, amenorrhea, ovarian failure		Secondary malignancy, breast enlargement, cataracts, idiopathic pulmonary syndrome (cough, dyspnea, pleural effusion, infiltrates, and hypoxemia), bronchopulmonary dysplasia with interstitial pulmonary fibrosis and pneumonitis, myocardial fibrosis, osteonecrosis

Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of busulfan and its solvent have been noted in animals. Toxicities include: multiple anomalies and low birth weight. It is unknown whether the drug or its solvent is excreted in breast milk.
--------------------------------------	---

(L)Toxicity may also occur later

Formulation and Stability:

Each ampoule or vial of busulfan injection contains 60 mg (6 mg/mL) of busulfan, N,N-dimethylacetamide (DMA) 33% vol/vol and Polyethylene Glycol 400, 67% vol/vol. Store refrigerated at 2°-8°C, (36°-46°F).

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

Dilute busulfan injection to a final concentration of approximately 0.5 mg/mL with NS or D5W. The drug should not be infused with any other drug or IV solution other than NS or D5W. Always add the busulfan to the diluent, not the diluent to the busulfan injection. Mix thoroughly by inverting several times. **Do not use polycarbonate syringes or filter needles with busulfan injection.** Busulfan injection diluted in NS or D5W is stable at room temperature (25°C) for up to 8 hours but the infusion must be completed within that time. Busulfan injection diluted in NS is stable at refrigerated conditions 2°-8°C (36°-46°F) for up to 12 hours but the infusion must be completed within that time.

Busulfan injection contains N,N-dimethylacetamide, which is incompatible with many closed-system transfer devices (CSTDs); the plastic components of CSTDs may dissolve and result in subsequent leakage and potential infusion of dissolved plastic into the patient.

Busulfan injection should be administered by IV infusion through a central venous catheter. Patients receiving busulfan in a conditioning regimen for bone marrow transplant must receive seizure prophylaxis. If phenytoin is used, it should be given 12 hours prior to the start of busulfan, then daily during busulfan administration and for 48 hours after completion of busulfan. In dose-finding studies of busulfan where patients received concomitant busulfan and phenytoin, phenytoin reduced busulfan plasma AUC by approximately 15%. Use of other anticonvulsants may result in higher busulfan plasma AUCs, and an increased risk of sinusoidal obstruction syndrome, (SOS, formerly VOD) or seizures. After an initial dose of busulfan injection, blood levels are monitored with bone marrow transplant patients in order to achieve a target area-under-the-curve (AUC) plasma concentration.

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

6.5 CYTARABINE – ALL ROUTES

(Cytosine arabinoside, Ara-C, Cytosar®)

NSC #63878

(07/13/15)

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 $t_{1/2}$ of about 10 minutes, with a secondary elimination phase $t_{1/2}$ 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 $t_{1/2}$ 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 <i>With High Dose:</i> 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) <i>With High Dose:</i> 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 <i>With High Dose:</i> 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 $> 200 \text{ mg/m}^2$. 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 ($\leq 200 \text{ mg/m}^2/\text{dose}$): For administration by IV push, reconstitute to a concentration of 20-100 mg/mL.

High Dose ($\geq 1000 \text{ mg/m}^2/\text{dose}$): 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.

Intrathecal:

For intrathecal administration, dilute with 5-10 mL (or volume per institutional practice) preservative free 0.9% sodium chloride injection, lactated Ringer's injection, Elliot's B solution. The volume of CSF removed should be equal to at least $\frac{1}{2}$ the volume delivered.

Patient Age (years)	Recommended volume	10% CSF volume	CSF Volume *
1 – 1.99	5–10 mL	5 mL	50 \pm 10 mL (babies)
2 – 2.99	5-10 mL	8 mL	80 \pm 20 mL (younger children)
3 – 8.99	5-10 mL	10 mL	100 \pm 20 mL (older children)
9 or greater	5-10 mL	13 mL	130 \pm 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.

Stability: Intrathecal cytarabine mixed in NS, lactated Ringer's injection, or Elliot's B solution is stable for 24 hours at 25°C 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.

6.6 DAUNORUBICIN

(Daunomycin, rubidomycin, Cerubidine®)

NSC #82151

(05/09/11)

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 cytotoxic 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 aldo-ketoreductases 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_{1/2} is approximately 45 minutes followed by a terminal t_{1/2} 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/m ² , 400 mg/m ² with mediastinal radiation, 300 mg/m ² in children, or 10 mg/kg in children < 2 yrs or 0.5 m ²) (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 powder¹ for injection in 20 mg single dose vials and a preservative free 5 mg/mL solution² 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 unreconstituted vial 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 Modifications 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.

6.7 ETOPOSIDE INJECTION

(Toposar®, VePesid®, Etopophos®, VP-16) NSC #141540 (11/15/2016)

Source and Pharmacology:

A semisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G₂ phase of the cell cycle. The initial t_{1/2} is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m² or about 35% of the total body clearance over a dose range of 80 to 600 mg/m². Etoposide, therefore, is cleared by both renal and non renal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non renal clearance of etoposide.

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

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

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 1/20 th 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 0.4mg/mL in NS. To avoid leaching, prepare the etoposide solution as close as possible, preferably within 4 hours, to the time of administration or alternatively as per institutional policy; glass or polyethylene-lined (non-PVC) containers and polyethylene-lined tubing may be used to minimize exposure to DEHP.

Etoposide Phosphate:

Reconstitute the 100 mg vial with 5 or 10 mL of Sterile Water for Injection, D5W, NS, Bacteriostatic Water for Injection with Benzyl Alcohol, or Bacteriostatic Sodium Chloride for Injection with Benzyl Alcohol for a concentration equivalent to 20 mg/mL or 10 mg/mL etoposide equivalent (22.7 mg/mL or 11.4 mg/mL etoposide phosphate), respectively. **Use diluents without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol.**

When reconstituted as directed, etoposide phosphate solutions can be stored in glass or plastic containers under refrigeration for 7 days. When reconstituted with a diluent containing a bacteriostat, store at controlled room temperature for up to 48 hours. Following reconstitution with SWFI, D5W, or NS store at controlled room temperature for up to 24 hours.

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

Supplier:

Commercially available 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 FLUDARABINE

(Fludara®, fludarabine phosphate, 2-fluoro-ara-AMP) NSC# 312887

(05/09/11)

Source and Pharmacology:

Fludarabine phosphate is a synthetic purine nucleoside. It differs from the physiologic nucleosides, adenosine, in that the sugar moiety is arabinose instead of ribose, and by the addition of a fluorine atom to the purine base adenine. Fludarabine is also a fluorinated nucleotide analog the antiviral agent vidarabine, (ara-A). The addition of fluorine results in increased aqueous solubility and resistance to enzymatic degradation by adenosine deaminase. Fludarabine (2-fluoro-ara-A) is commercially available as the monophosphate salt (2-fluoro-ara-AMP). The monophosphorylation increases the drug's aqueous solubility while maintaining pharmacologic activity. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono β-D-arabino-furanosyl) (2-fluoro-ara-AMP) and the molecular weight is 365.2.

Fludarabine is a purine antagonist antimetabolite. *In vivo*, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then it is phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Phase I studies in humans have demonstrated that within several minutes after intravenous infusion, fludarabine phosphate is converted to the active metabolite, 2-fluoro-ara-A and becomes undetectable. Therefore, pharmacokinetics studies have focused on 2-fluoro-ara-A. Fludarabine phosphate 25 mg/m² infused intravenously over 30 minutes to adult cancer patients, showed a moderate accumulation of 2-fluoro-ara-A. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2.

Fludarabine is widely distributed. The volume of distribution at steady state (V_{ss}) reported after daily administration of 25mg/m² for 5 days to adults averaged at 96-98 L/m². Tissue distribution studies in animals indicate that the highest concentrations of the drug are in liver, kidney, and spleen. Although the extent to which fludarabine and/or its metabolites distribute into the CNS in humans has not been determined to date, severe neurologic toxicity (e.g., blindness, coma) has been reported in patients receiving the drug, particularly in high dosages. There is evidence from animal studies that fludarabine distributes into the CNS and that a toxic metabolite (2-fluoroadenine, possibly formed by bacteria in the GI tract), can be absorbed systematically via enterohepatic circulation and distributed into CSF. According to *in vitro* data, about 19-29% of fludarabine is bound to plasma proteins.

Following IV administration, fludarabine phosphate is dephosphorylated rapidly to fludarabine. Plasma concentrations of fludarabine decline in a linear, dose-independent manner. The elimination profile of fludarabine also has been reported to be either biphasic or triphasic; however, reported terminal elimination half-lives have been similar. In adult cancer patients receiving fludarabine 25 mg/m² as a 30-minute IV infusion daily for 5 days, a terminal half-life of about 20 hours was reported. In a limited number of pediatric patients, the plasma concentration profile of fludarabine exhibited both monoexponential and biexponential decay, with a mean t_{1/2} of 10.5 hours in patients with monoexponential elimination and a t_{1/2} of 1.2-1.4 and 12.4-19 hours, respectively, in patients with biexponential elimination.

Renal clearance accounts for about 40% of the total body clearance of fludarabine. Renal elimination appears to become more important at high dosages of the drug. The dose of fludarabine needs to be adjusted in patients with moderate renal impairment.

The use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

Toxicity:

	Common Happens to 21-100 subjects out of every 100	Occasional Happens to 5-20 subjects out of every 100	Rare Happens to < 5 subjects out of every 100
Immediate: Within 1-2 days of receiving drug	Fever, fatigue, weakness, pain, nausea, vomiting, anorexia, cough, dyspnea	Edema including peripheral edema, chills, rash, diarrhea, rhinitis, diaphoresis, malaise, abdominal pain, headache, back pain, myalgia, stomatitis, flu-like syndrome	Anaphylaxis, tumor lysis syndrome, dehydration*
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression (anemia, neutropenia, thrombocytopenia), infection (urinary tract infection, herpes simplex infection, pneumonia, upper respiratory)	Weight loss, gastrointestinal bleeding, hemoptysis, paresthesia, allergic pneumonitis, bronchitis, pharyngitis, visual disturbance, hearing loss, hyperglycemia	Sinusitis, dysuria, opportunistic infections and reactivation of latent viral infections like Epstein-Barr virus (EBV), herpes zoster and John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML]) ^L , EBV associated lymphoproliferative disorder, pancytopenia (can be prolonged),

	Common Happens to 21-100 subjects out of every 100	Occasional Happens to 5-20 subjects out of every 100	Rare Happens to < 5 subjects out of every 100
			pulmonary hypersensitivity ^a (dyspnea, cough, hypoxia, interstitial pulmonary infiltrate), pulmonary toxicity (acute respiratory distress syndrome [ARDS], pulmonary fibrosis, pulmonary hemorrhage, respiratory distress, respiratory failure), pericardial effusion, skin toxicity (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigus), liver failure, renal failure, hemorrhage, transfusion-associated graft-versus-host disease has occurred following transfusion of nonirradiated blood products, phlebitis*, sleep disorder*, cerebellar syndrome*, depression*, mentation impaired*, alopecia*, pruritus*, seborrhea*, esophagitis*, constipation*, mucositis*, dysphagia*, hesitancy*, cholelithiasis*, abnormal liver function tests *, osteoporosis*, arthralgia*, abnormal renal function test*, proteinuria*, epistaxis*, hemorrhagic cystitis*, eosinophilia*
Delayed: Any time later during therapy, excluding the above conditions			Neurotoxicity (increased with high doses): seizures, agitation, confusion, weakness, visual disturbances, optic neuritis, optic neuropathy, photophobia, blindness, paralysis, coma, death, peripheral neuropathy ^a); autoimmune phenomena: thrombocytopenia/thrombocytopenic purpura (ITP), Evans syndrome, hemolytic anemia, acquired hemophilia
Late: Any time after completion of treatment			Myelodysplastic syndrome/acute myeloid leukemia (mainly associated with prior or concomitant or subsequent treatment with other anticancer treatments), skin cancer (new onset or exacerbation)
Unknown Frequency and Timing:	Pregnancy Category D Based on its mechanism of action, fludarabine phosphate can cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was embryolethal and teratogenic in both rats and rabbits.		

(L) Toxicity may also occur later.

* Reported in ≤ 3% of subjects. Since these are not considered life threatening they are not included in the consent.

^a These effects were not reported in children.

Formulation and Stability:

Fludarabine phosphate injection is available as sterile lyophilized powder and in solution. Each single-dose vial of powder contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and

sodium hydroxide to adjust the pH to 7.7. After reconstitution, the pH range for the final product is 7.2-8.2. The single-dose solution vial contains 25 mg/mL, 2 mL of fludarabine phosphate. It may contain mannitol and is preservative-free.

Fludarabine phosphate vials should be stored refrigerated at 2-8°C (36-46°F).

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

Prior to administration fludarabine phosphate powder should be reconstituted with Sterile Water for Injection and further diluted in D5W or NS to a concentration of 10 – 25 mg/mL. Fludarabine 25 mg/mL solution should be further diluted in the same manner.

Fludarabine phosphate powder should be reconstituted with 2 mL of Sterile Water for Injection. The solid cake should fully dissolve in 15 seconds or less. The resulting concentration is 25 mg/mL. When reconstituted to a final concentration of 25 mg/mL, the drug is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the solution be used within 8 hours after reconstitution.

Prior to administration, fludarabine 25 mg/mL solution or the reconstituted 25 mg/mL solution should be further diluted in 100 mL or 125 mL of D5W or NS. When diluted to a final concentration of 1 mg/mL, fludarabine is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the diluted solution be used within 8 hours after preparation. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

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

6.9 LYMPHOCYTE IMMUNE GLOBULIN

(Antithymocyte globulin (equine), ATG, Atgam®) NSC# 743145

(05/09/11)

Source and Pharmacology:

Lymphocyte immune globulin is a purified concentrated sterile gamma globulin (primarily monomeric IgG) from hyper immune serum of horses immunized with human thymus lymphocytes. It has a molecular weight of between 150,000 to 160,000 Daltons. Lymphocyte immune globulin is a lymphocyte-selective immunosuppressant, demonstrated by its ability to reduce the number of circulating thymus-dependent lymphocytes that form rosettes with sheep erythrocytes. This anti-lymphocytic effect is believed to alter the function of lymphocytes which are responsible in part for cell mediated immunity and are involved in humoral immunity. When given in combination with other immunosuppressive therapy (antimetabolites and corticosteroids) the patient's antibody response to horse IgG is minimal. Lymphocyte Immune Globulin administered with other immunosuppressive therapy and measured as horse IgG has a serum half-life of 5.7 ±3 days. Peak plasma levels are reached after 5 days of infusions. These levels can vary depending on the individual's ability to metabolize foreign IgG. Distribution is poor into lymphoid tissues. Lymphocyte Immune Globulin binds to circulating lymphocytes, granulocytes, platelets, and bone marrow cells. Approximately 1% of the dose is excreted in the urine.

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	Fever, rash, dyspnea	Chills, apnea, pain (chest, back or flank), diarrhea, nausea, vomiting, pruritus, urticaria	Anaphylaxis, laryngospasm, hypertension, hypotension, syncope, dizziness, tachycardia, pulmonary edema, diaphoresis, swelling, redness or phlebitis at the infusion site (peripheral veins), extravasation (rare) but if occurs = local ulceration headache, malaise, seizures, epigastric pain
Prompt:	Thrombocytopenia (transient)	Leukopenia, infection, abnormal renal function tests	Hemolysis (rarely clinically significant), hyperglycemia, sore mouth or throat, thrombosis, HSV or other viral infection,

Within 2-3 weeks, prior to next course			edema, eosinophilia, abnormal liver function test, CHF, vasculitis, arthralgia, myalgia, paresthesias
Delayed: Any time later during therapy, excluding the above conditions		Serum sickness (L) which can have all or some of the following symptoms: glomerulonephritis, fever, myalgia, arthralgia and periorbital edema – incidence reduced with use with corticosteroid premedication	GI bleeding or perforation, acute renal failure
Unknown Frequency and Timing:	Animal reproduction studies have not been conducted with lymphocyte immune globulin. It is not known whether lymphocyte immune globulin can cause fetal harm when administered to a pregnant woman or affect reproductive capacity. It is unknown whether the drug is excreted in breast milk.		

(L) Toxicity may also occur later.

Formulation and Stability: Each milliliter of Lymphocyte Immune Globulin contains 50 mg of horse gamma globulin stabilized in 0.3 molar glycine to a pH of approximately 6.8 (available in 5 mL vials). It may appear colorless to faintly pink or brown and is nearly odorless. It may develop a slight granular or flaky deposit during storage. Store in a refrigerator at 2°-8°C (36°-46°F). DO NOT FREEZE.

Guidelines for Administration:

Lymphocyte Immune Globulin (diluted or undiluted) should not be shaken because excessive foaming and/or denaturation of the protein may occur. Lymphocyte Immune Globulin may be added to NS or dextrose/saline containing solutions. Do not add to dextrose alone as low salt concentrations may result in precipitation. Add Lymphocyte Immune Globulin to an inverted bottle of sterile vehicle so the undiluted Lymphocyte Immune Globulin does not contact the air inside. The concentration should not exceed 4 mg/mL. The diluted solution should be gently rotated or swirled to effect thorough mixing. Store diluted solution in the refrigerator until use. The diluted solution should be allowed to reach room temperature before infusion. Once diluted, Lymphocyte Immune Globulin has been shown to be physically and chemically stable for up to 24 hours at concentrations of up to 4 mg/mL. Lymphocyte Immune Globulin is appropriately administered into a vascular shunt, arterial venous fistula, or a high-flow central vein through an in-line filter with a pore size of 0.2 to 1.0 micron. The in-line filter should be used with all infusions to prevent the administration of any insoluble material that may develop in the product during storage. The use of high-flow veins will minimize the occurrence of phlebitis and thrombosis. Do not infuse a dose in less than 4 hours.

Pretreat patient with an antipyretic, antihistamine, and/or corticosteroid to prevent chills, fever, itching, and erythema. Always keep appropriate resuscitation equipment at the patient’s bedside while Lymphocyte Immune Globulin is being administered. Observe the patient continuously for possible allergic reactions throughout the infusions.

Skin Testing

Before the first infusion of Lymphocyte Immune Globulin, Pharmacia & Upjohn Company strongly recommend that patients be tested with an intradermal injection of 0.1 mL of a 1:1,000 dilution (5 mcg horse IgG) of Lymphocyte Immune Globulin in NS and a contralateral NS injection control. Use only freshly diluted Lymphocyte Immune Globulin for skin testing. The patient, and specifically the skin test, should be observed every 15 to 20 minutes over the first hour after intradermal injection. A local reaction of 10 mm or greater with a wheal or erythema, or both, with or without pseudopod formation and itching or a marked local swelling should be considered a positive test. Note: The predictive value of this test has not been proved clinically. Allergic reactions such as anaphylaxis have occurred in patients whose skin test is negative. In the presence of a locally positive skin test, the risk to benefit ratio must be carefully weighed.

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

6.10 METHOTREXATE-IV ONLY

(MTX, amethopterin) NSC #000740

(02/29/12)

Source and Pharmacology:

A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 μmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half life of 8-15 hours. About 50% is bound to protein. MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

Toxicity:

	Common Happens to 21-100 children out of every 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, pruritis, 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.

Formulation and Stability:

Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43, 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative.

Sterile methotrexate powder or solution is stable at 20°-25°C (68°-77°F); excursions permitted to 15°-30°C (59°- 86 F°). Protect from light.

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

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

Do not use the preserved solution for high dose methotrexate administration due to risk of benzyl alcohol toxicity. Methotrexate dilutions are chemically stable for at least 7 days at room temperature but contain no preservative and should be used within 24 hours. Diluted solutions especially those containing bicarbonate exposed to direct sunlight for periods exceeding 4 hours should be protected from light.

High dose Methotrexate requires alkalinization of the urine, adequate hydration and leucovorin rescue. Avoid probenecid, penicillins, cephalosporins, aspirin, proton pump inhibitors, and NSAIDS as renal excretion of MTX is inhibited by these agents.

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

6.11 **METHYLPREDNISOLONE**(Solu-Medrol®, A-Methapred®, Medrol®) NSC #19987
(05/09/11)

Source and Pharmacology:

Methylprednisolone is a corticosteroid and the methyl derivative of prednisolone. 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. At the cellular level, corticosteroids appear to act by controlling the rate of protein synthesis. The half-life of methylprednisolone is approximately 2.5 hours; however, the metabolic effects at the tissue level persist for up to 20-30 hours. It is primarily metabolized in the liver and excreted by the kidneys. Methylprednisolone 4 mg has equivalent potency to prednisone 5mg.

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	Anaphylaxis, cardiac Arrhythmias, urticaria 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), electrolyte imbalance (Na retention, hypokalemia, hypocalcemia)(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, pseudo tumor cerebri (increased intracranial pressure with papilledema, headache), aseptic necrosis of the femoral and humeral heads (L)
Late: Any time after completion of treatment		Cataracts (which may be reversible on discontinuation in children)	
Unknown Frequency and Timing:	Fetal and teratogenic toxicities: Corticosteroids cross 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. Other studies have found no anomalies and neonatal adrenal insufficiency appears to be rare. Methylprednisolone is excreted into breast milk in humans; however, several studies suggest that amounts excreted in breast milk are negligible with methylprednisolone doses of < 8 mg/day.		

(L) Toxicity may also occur later.

Formulation and Stability:

Methylprednisolone is available for oral use in 2, 4, 8, 16, and 32 mg tablets. Inactive ingredients vary depending on manufacturer but tablet formulations may include: calcium stearate, corn starch, erythrosine sodium, lactose, mineral oil, sorbic acid, sucrose, croscarmellose sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polacrillin potassium, sodium starch glycolate, and stearic acid.

Lyophilized Powder for Injection as methylprednisolone sodium succinate equivalent to methylprednisolone is available as: **Dual chamber vials** of 40 mg (1 mL), 125 mg (2 mL), 500 mg (4 mL), 1000 mg (8 mL) also containing mono, dibasic sodium phosphate and benzyl alcohol (diluent); Vials of 500 mg, 1 g, 2 g also containing monobasic and dibasic sodium phosphate with or without separate vials of diluent containing benzyl alcohol. Protect from light.

Store unconstituted product at controlled room temperature 20°- 25°C (68°- 77°F).

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

Only the sodium succinate formulation may be given intravenously. The acetate salt should NOT be given intravenously.

Dilute lyophilized powder vials for injection with sufficient diluent to obtain a concentration of 62.5 mg/mL. The manufacturer recommends the use of Bacteriostatic sterile water for injection containing benzyl alcohol. **Use sterile water for injection without benzyl alcohol for neonates and infants < 2 years of age or patients with hypersensitivity to benzyl alcohol.**

Mix dual chamber vials as directed. Store solution at controlled room temperature 20°-25°C (68°-77°F). Use solution within 48 hours after mixing.

May be further diluted in Saline or Dextrose containing solutions for intravenous administration.

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

6.12 MITOXANTRONE

(Novantrone®, CL 232315, DAD, DHAD, Mitozantrone) NSC #301739

(07/14/15)

Source and Pharmacology:

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

Toxicity

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, diarrhea, fever, anorexia, green blue discoloration of the urine and/or sclera	Abdominal pain, back pain, headache, phlebitis, constipation	Anaphylaxis, angioedema, cardiac arrhythmias ¹ (bradycardia), seizures, extravasation reactions rare but if occur can lead to: (erythema, swelling, pain, burning and/or blue discoloration of the skin and rarely tissue necrosis), tumor lysis
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (L), mucositis /stomatitis, immunosuppression, alopecia, fatigue	Transient elevation of LFTs, pruritus with desquamation of the skin due to progressive dryness	Rash, conjunctivitis, (GI) hemorrhage, interstitial pneumonitis
Delayed: Any time later during therapy	Amenorrhea, menstrual disorders, temporary reduction in sperm count	Cardiotoxicity (decreased LVEF) ² (L)	CHF, hepatotoxicity
Late: Any time after completion of treatment			Secondary malignancy

Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of mitoxantrone have been noted in animals. Toxicities include: low birth weight and prematurity. Mitoxantrone is excreted in human milk and significant concentrations (18 ng/mL) have been reported for 28 days after the last administration.
--------------------------------------	---

¹ Rarely clinically significant.

² Risk increases with chest radiation and prior anthracycline dosage

(L) Toxicity may also occur later.

Formulation and Stability:

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

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

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

Supplier: Commercially available. See package insert for more detailed information.

Special precautions: Incompatible with heparin; precipitation may form

6.13 SORAFENIB TOSYLATE

(BAY 43-9006 tosylate, BAY 54-9085 Nexavar®) NSC# 724772, IND#114480 (03/05/2017)

Source and Pharmacology: Sorafenib tosylate has the chemical name:

4-(4-{3-[4-Chloro-3-(trifluoromethyl)phenyl]ureido}phenoxy)N2-methylpyridine-2-carboxamide 4-methylbenzenesulfonate. Sorafenib is a kinase inhibitor that decreases tumor cell proliferation *in vitro*. Sorafenib was shown to inhibit multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT-3, RET, VEGFR-1, VEGFR-2, VEGFR-3, and PDGFR- β). Several of these kinases are thought to be involved in tumor cell signaling, angiogenesis, and apoptosis. Sorafenib inhibited tumor growth and angiogenesis of human hepatocellular carcinoma, human renal cell carcinoma, and several other human tumor xenografts in immunocompromised mice.

In previously conducted clinical trials, sorafenib was predominantly administered as sorafenib tosylate (BAY 54-9085) tablets, however the dosage of sorafenib is expressed as free base sorafenib (BAY 43-9006). The conversion factor between the free base sorafenib and the tosylate salt is 1.3705. No conversion should be used when calculating the dose in this clinical trial.

The mean relative bioavailability of sorafenib administered as an oral tablet was 38–49% when compared to an oral solution. Pharmacokinetics studies in adults who received oral sorafenib showed that sorafenib reaches peak plasma levels in approximately 3 hours. When given with a moderate-fat meal (30% fat; 700 calories), the bioavailability was similar to that in the fasted state, however, with a high-fat meal (50% fat; 900 calories), sorafenib bioavailability was reduced by 29% compared to administration in the fasted state. Therefore, it is recommended that sorafenib be administered without food.

The mean maximum plasma concentration (C_{max}) and area under the concentration curve (AUC) increased less than proportionally when doses greater than 400 mg are given orally twice daily. *In vitro* binding of sorafenib to human plasma proteins is 99.5%.

Sorafenib is metabolized mainly in the liver through oxidation by cytochrome P-450 (CYP) isoenzyme 3A4, and glucuronidation by uridine diphosphate-glucuronosyltransferase (UGT) 1A9. Sorafenib accounted for approximately 70–85% of the circulating analytes in plasma at steady state. Eight metabolites of sorafenib have been identified. The primary metabolite, a pyridine *N*-oxide derivative, is pharmacologically active, shows *in vitro* potency similar to that of sorafenib, and accounts for approximately 9-16% of total plasma concentrations of the drug. Approximately 77% of an oral dose of sorafenib is excreted in feces; 19% is eliminated in urine as glucuronidated metabolites. Unchanged sorafenib is recovered in feces and accounts for 51% of a single dose. No unchanged sorafenib is recovered in the urine.

The mean elimination half-life of sorafenib is approximately 25 to 48 hours. Multiple dosing of sorafenib for 7 days resulted in a 2.5 to 7-fold accumulation compared to single dose administration. Steady-state plasma sorafenib concentrations are achieved within 7 days, with a peak-to-trough ratio of mean concentrations of less than 2.

A study of the pharmacokinetics of sorafenib indicated that the mean AUC of sorafenib in Asians (N=78) was 30% lower than the mean AUC in Caucasians (N=40). In adult patients, gender and age do not appear to have a clinically meaningful effect on the pharmacokinetics of sorafenib.

In children with refractory solid tumors or leukemias, single agent sorafenib pharmacokinetics were similar to adults. Significant interpatient variability was observed both on Day 1 and at steady state. Drug exposure increased only marginally with dose escalation from 150 to 200 mg/m² for day 1 pharmacokinetics. The terminal half-life in children appeared to be prolonged (≥ 24 hours) but could not be estimated. Sorafenib accumulated after multiple doses and steady state appeared to be achieved after 5 to 7 days. Steady-state sorafenib concentrations ($AUC_{0-12h}/12$) were 4.1 ± 2.1 µg/mL at the 150 mg/m² and 5.4 ± 1.8 µg/mL at 200 mg/m² dose levels, respectively. The median apparent sorafenib clearance at the MTD was 56.0 mL/min/m². The apparent sorafenib clearance increased with patient age. The observed single agent sorafenib MTD in pediatric patients was 200 mg/m²/dose twice daily for solid tumors and 150 mg/m²/dose twice daily for leukemias.

The pharmacokinetics of sorafenib in patients with varying degrees of renal and hepatic impairment have been studied. No dose adjustment is necessary in patients with renal impairment or with mild to moderate hepatic impairment.

Repeat dosing of sorafenib to young and growing dogs resulted in irregular thickening of the femoral growth plate at daily doses ≥ 600 mg/m² (approximately 0.3 times the AUC at the recommended human dose), hypocellularity of the bone marrow adjoining the growth plate at 200 mg/m²/day (approximately 0.1 times the AUC at the recommended human dose), and alterations of the dentin composition at 600 mg/m²/day. Similar effects were not observed in adult dogs when dosed for 4 weeks or less.

Potential Drug Interactions: Sorafenib is metabolized primarily by CYP3A4 during phase I metabolism (oxidation) and primarily by UGT1A9 in phase II (conjugation). Co-administration of rifampin resulted in sorafenib AUC reduction of approximately 37%. Therefore, use caution when co-administering with strong CYP3A4 inducers, such as St. John's wort, phenytoin, carbamazepine, phenobarbital and dexamethasone as they can reduce sorafenib exposure. CYP3A4 inhibitors are not expected to cause clinically relevant changes to sorafenib exposure.

In vitro, sorafenib is a moderate inhibitor CYP2C19, 2D6, and 3A4 and a strong inhibitor of CYP2B6, 2C8, and 2C9. It also inhibits pathway enzymes UGT1A1 and UGT1A9 in phase II conjugation. Clinical data suggests sorafenib does not increase exposure of other drugs metabolized by CYP pathways and therefore does not appear to be clinically relevant. Use caution when co-administered with sensitive substrates of UGT1A1.

While sorafenib solubility is pH dependent, co-administration with omeprazole did not result in clinically relevant change in sorafenib exposure.

Sorafenib may prolong the QT/QTc interval. Avoid concomitant drugs that may induce the QTc prolongation.

Sorafenib also inhibited P-glycoprotein *in vitro* and could increase the concentrations of concomitantly administered drugs that are P-glycoprotein substrates.

Concomitant use of docetaxel or doxorubicin with continuous sorafenib administration resulted in an increase in the AUC of both docetaxel and doxorubicin. With fluorouracil, both increases and decreases in AUC were seen. Therefore, caution is recommended when administering sorafenib with these chemotherapeutic drugs.

Sorafenib does not appear to affect the metabolism of warfarin (a CYP2C9 substrate) *in vivo*, however, infrequent bleeding events or elevations of the International Normalized Ratio (INR) have been reported in some patients receiving concomitant therapy with warfarin. Patients taking concomitant warfarin should be monitored regularly for changes in prothrombin time, INR or clinical bleeding episodes.

Patient Care Implications: Hand-foot skin rash can be treated with topical emollients, high-potency topical steroids, or keratolytic cream (urea/salicylic acid).

Females of childbearing potential should avoid becoming pregnant while they or their male partners are taking sorafenib and should use effective contraception during and for at least 30 days after completion of therapy.

Toxicity:

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Sorafenib (BAY 43-9006; Nexavar, NSC 724772)

The Comprehensive Adverse Events 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 2571 patients. Below is the CAEPR for Sorafenib (BAY 43-9006; Nexavar).

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, November 16, 2015¹

Adverse Events with Possible Relationship to Sorafenib (BAY 43-9006; Nexavar) (CTCAE 4.0 Term) [n= 2571]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			<i>Anemia (Gr 3)</i>
CARDIAC DISORDERS			
		Acute coronary syndrome	
	Chest pain - cardiac		
		Heart failure	
		Left ventricular systolic dysfunction	
		Myocardial infarction	
GASTROINTESTINAL DISORDERS			
Abdominal pain			<i>Abdominal pain (Gr 3)</i>
	Ascites		
	Constipation		<i>Constipation (Gr 2)</i>
Diarrhea			<i>Diarrhea (Gr 3)</i>
	Gastrointestinal hemorrhage ²		<i>Gastrointestinal hemorrhage² (Gr 3)</i>
		Gastrointestinal perforation ³	
	Mucositis oral		
Nausea			<i>Nausea (Gr 3)</i>
	Vomiting		<i>Vomiting (Gr 3)</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			<i>Fatigue (Gr 3)</i>
	Fever		<i>Fever (Gr 2)</i>
HEPATOBIILIARY DISORDERS			
		Hepatic failure	
IMMUNE SYSTEM DISORDERS			
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection ⁴		
INVESTIGATIONS			
	Activated partial thromboplastin time prolonged		<i>Activated partial thromboplastin time prolonged (Gr 2)</i>
Alanine aminotransferase increased			<i>Alanine aminotransferase increased (Gr 3)</i>
Alkaline phosphatase increased			<i>Alkaline phosphatase increased (Gr 3)</i>
Aspartate aminotransferase increased			<i>Aspartate aminotransferase increased (Gr 3)</i>
Blood bilirubin increased			<i>Blood bilirubin increased (Gr 3)</i>

Adverse Events with Possible Relationship to Sorafenib (BAY 43-9006; Nexavar) (CTCAE 4.0 Term) [n= 2571]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
Creatinine increased			<i>Creatinine increased (Gr 3)</i>
		Electrocardiogram QT corrected interval prolonged	
	GGT increased		
INR increased			<i>INR increased (Gr 3)</i>
	Investigations - Other (bicarbonate-serum low)		
Lipase increased			<i>Lipase increased (Gr 3)</i>
Lymphocyte count decreased			<i>Lymphocyte count decreased (Gr 3)</i>
	Neutrophil count decreased		<i>Neutrophil count decreased (Gr 4)</i>
Platelet count decreased			<i>Platelet count decreased (Gr 4)</i>
Serum amylase increased			<i>Serum amylase increased (Gr 3)</i>
Weight loss			<i>Weight loss (Gr 2)</i>
White blood cell decreased			<i>White blood cell decreased (Gr 4)</i>
METABOLISM AND NUTRITION DISORDERS			
Anorexia			<i>Anorexia (Gr 3)</i>
	Hypercalcemia		
Hyperglycemia			<i>Hyperglycemia (Gr 3)</i>
	Hyperkalemia		<i>Hyperkalemia (Gr 3)</i>
	Hypernatremia		
Hypoalbuminemia			<i>Hypoalbuminemia (Gr 3)</i>
Hypocalcemia			<i>Hypocalcemia (Gr 3)</i>
	Hypoglycemia		<i>Hypoglycemia (Gr 2)</i>
	Hypokalemia		<i>Hypokalemia (Gr 3)</i>
Hyponatremia			<i>Hyponatremia (Gr 3)</i>
Hypophosphatemia			<i>Hypophosphatemia (Gr 3)</i>
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		<i>Arthralgia (Gr 3)</i>
	Back pain		<i>Back pain (Gr 3)</i>
	Bone pain		
	Musculoskeletal and connective tissue disorder - Other (muscle spasm)		
	Myalgia		
	Pain in extremity		<i>Pain in extremity (Gr 3)</i>
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Treatment related secondary malignancy		
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		<i>Headache (Gr 3)</i>
		Intracranial hemorrhage	
		Reversible posterior leukoencephalopathy syndrome	
PSYCHIATRIC DISORDERS			
	Insomnia		

Adverse Events with Possible Relationship to Sorafenib (BAY 43-9006; Nexavar) (CTCAE 4.0 Term) [n= 2571]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
RENAL AND URINARY DISORDERS			
	Acute kidney injury		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		<i>Cough (Gr 2)</i>
	Dyspnea		<i>Dyspnea (Gr 3)</i>
	Respiratory hemorrhage ⁵		
	Voice alteration		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
Alopecia			<i>Alopecia (Gr 2)</i>
	Dry skin		<i>Dry skin (Gr 2)</i>
		Erythema multiforme	
Palmar-plantar erythrodysesthesia syndrome			<i>Palmar-plantar erythrodysesthesia syndrome (Gr 3)</i>
	Pruritus		<i>Pruritus (Gr 3)</i>
Rash maculo-papular			<i>Rash maculo-papular (Gr 3)</i>
		Stevens-Johnson syndrome	
		Toxic epidermal necrolysis	
VASCULAR DISORDERS			
	Hypertension		<i>Hypertension (Gr 3)</i>
		Thromboembolic event	

¹This 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.

²Gastrointestinal hemorrhage may include 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.

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

⁴Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

⁵Respiratory hemorrhage may include bronchopulmonary hemorrhage, epistaxis, laryngeal hemorrhage, mediastinal hemorrhage, pharyngeal hemorrhage, and pleural hemorrhage under the RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS SOC.

⁶Febrile neutropenia is seen mostly in combination with other agents.

Adverse events reported on sorafenib (BAY 43-9006; Nexavar) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that sorafenib (BAY 43-9006; Nexavar) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (Thrombotic microangiopathy [e.g., TTP or HUS]); Febrile neutropenia⁶

CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Cardiac arrest; Palpitations; Pericardial effusion; Pericarditis; Right ventricular dysfunction; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia; Ventricular arrhythmia; Ventricular tachycardia

EAR AND LABYRINTH DISORDERS - Hearing impaired; Tinnitus

ENDOCRINE DISORDERS - Adrenal insufficiency; Hyperthyroidism; Hypothyroidism

EYE DISORDERS - Blurred vision; Cataract; Dry eye; Extraocular muscle paresis; Eye disorders - Other (color vision deficits); Eye disorders - Other (light to dark adaptation); Eye disorders - Other (retinal vein occlusion); Eye disorders - Other (retinal hemorrhage); Eye disorders - Other (visual field distortion); Flashing lights; Keratitis; Photophobia; Retinal detachment

GASTROINTESTINAL DISORDERS - Abdominal distension; Anal fistula; Anal mucositis; Anal pain; Anal ulcer; Cheilitis; Colitis; Colonic obstruction; Colonic ulcer; Dry mouth; Duodenal ulcer; Dyspepsia; Dysphagia; Enterocolitis; Esophageal pain; Esophagitis; Flatulence; Gastric ulcer; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (small bowel NOS fistula); Gastrointestinal fistula; Hemorrhoids; Ileal fistula; Ileus; Oral pain; Pancreatitis; Proctitis; Rectal fistula; Rectal mucositis; Rectal obstruction; Rectal pain; Small intestinal obstruction; Stomach pain

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Facial pain; Flu like symptoms; Localized edema; Multi-organ failure; Non-cardiac chest pain; Pain

HEPATOBIILIARY DISORDERS - Cholecystitis; Hepatic hemorrhage; Hepatobiliary disorders - Other (biliary obstruction secondary to multiple biliary stones)

IMMUNE SYSTEM DISORDERS - Allergic reaction; Cytokine release syndrome; Immune system disorders - Other (systemic inflammatory response syndrome)

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Fall; Fracture; Hip fracture; Vascular access complication; Wound complication; Wound dehiscence

INVESTIGATIONS - CPK increased; Cardiac troponin I increased; Cardiac troponin T increased; Cholesterol high; Ejection fraction decreased; Fibrinogen decreased; Investigations - Other (blood urea nitrogen high)

METABOLISM AND NUTRITION DISORDERS - Acidosis; Alkalosis; Dehydration; Hypermagnesemia; Hypertriglyceridemia; Hyperuricemia; Hypomagnesemia; Tumor lysis syndrome

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Chest wall pain; Generalized muscle weakness; Joint range of motion decreased; Muscle weakness left-sided; Muscle weakness lower limb; Muscle weakness right-sided; Muscle weakness upper limb; Musculoskeletal and connective tissue disorders - Other (cramping); Myositis; Neck pain

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Leukemia secondary to oncology chemotherapy; Myelodysplastic syndrome; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (tumor hemorrhage); Tumor pain

NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Depressed level of consciousness; Dysgeusia; Dysphasia; Encephalopathy; Extrapyrmidal disorder; Hydrocephalus; Ischemia cerebrovascular; Lethargy; Leukoencephalopathy; Memory impairment; Neuralgia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Stroke; Syncope; Tremor; Vasovagal reaction

PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Libido decreased; Personality change; Psychosis

RENAL AND URINARY DISORDERS - Chronic kidney disease; Hematuria; Proteinuria; Renal and urinary disorders - Other (focal segmental glomerulosclerosis); Renal and urinary disorders - Other (nephrotic syndrome); Renal and urinary disorders - Other (right ureter rupture); Renal calculi; Renal hemorrhage; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urine discoloration

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Erectile dysfunction; Gynecomastia; Hematosalpinx; Menorrhagia; Ovarian hemorrhage; Prostatic hemorrhage; Spermatic cord hemorrhage; Testicular hemorrhage; Uterine hemorrhage; Vaginal fistula; Vaginal hemorrhage

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Allergic rhinitis; Bronchospasm; Hiccups; Hoarseness; Hypoxia; Laryngeal mucositis; Pharyngeal mucositis; Pharyngolaryngeal pain; Pleural effusion; Pneumonitis; Pneumothorax; Pulmonary edema; Pulmonary fibrosis; Respiratory, thoracic and mediastinal disorders - Other (nasal septal perforation); Tracheal mucositis

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Erythroderma; Hyperhidrosis; Nail loss; Pain of skin; Purpura; Rash acneiform; Scalp pain; Skin and subcutaneous tissue disorders - Other (folliculitis); Skin and

subcutaneous tissue disorders - Other (non-life threatening squamous cell carcinoma of skin: keratoacanthoma type); Skin hyperpigmentation; Skin hypopigmentation; Skin ulceration; Urticaria
VASCULAR DISORDERS - Flushing; Hematoma; Hot flashes; Hypotension; Phlebitis; Vascular disorders - Other (ruptured aortic aneurysm); Vascular disorders - Other (visceral arterial ischemia); Vasculitis

Note: Sorafenib (BAY 43-9006; Nexavar) 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.

The potential risks listed in the CAEPR whose relationship to sorafenib is still undetermined are not required by CTEP to be described in the ICD; however, they may be communicated to patients according to local IRB requirements. The terminology for CTEP's suggested lay terms may change periodically.

Pregnancy and lactation:

Pregnancy Category D. Sorafenib may cause fetal harm when administered to a pregnant woman. In rats and rabbits, sorafenib has been shown to be teratogenic and to induce embryo-fetal toxicity including increased post-implantation loss, resorptions, skeletal retardations, and retarded fetal weight. The effects occurred at doses considerably below the recommended human dose of 400 mg twice daily. Adverse intrauterine development effects were seen at doses ≥ 1.2 mg/m²/day in rats and 3.6 mg/m²/day in rabbits (approximately 0.008 times the AUC seen in cancer patients at the recommended human dose). There are no adequate and well-controlled studies in pregnant women using sorafenib. Women of childbearing potential should be advised to avoid becoming pregnant while on sorafenib. Men and women of childbearing potential should be instructed to use an effective method of birth control during treatment with sorafenib and for at least 2 weeks after stopping treatment.

It is not known whether sorafenib is excreted in human milk. Following administration of radiolabeled sorafenib to lactating Wistar rats, approximately 27% of the radioactivity was secreted into the milk. The milk to plasma AUC ratio was approximately 5:1.

Cardiac Toxicities in Arm C (per Amendment #6a)

Per amendment #6a, The AAML1031 Study Committee has observed a higher than expected rate of cardiac toxicities in Cohort 2 of Arm C of the AAML1031 trial. The schedule of sorafenib administration for subjects enrolled on Arm C of this study has changed in response to these findings, as detailed in [Section 2.3.1](#).

Formulation and Stability:

The tablets are available as 200 mg and 50 mg immediate release tablets. The inactive ingredients for both tablets are microcrystalline cellulose, croscarmellose sodium, hydroxypropylmethyl cellulose, magnesium stearate, sodium lauryl sulfate, and a film-coat with hydroxypropylmethylcellulose, polyethylene glycol, titanium dioxide and red iron oxide.

- The commercially labeled 200 mg tablets are round, biconvex, red film-coated tablets, debossed with the "Bayer cross" on one side and "200" on the other side and packaged in HDPE bottles of 120 tablets.
- The 50 mg tablets for pediatric studies are yellow orange color, 6 mm round shape weighing about 87 mg and packaged in HDPE bottles containing 100 tablets.

NOTE: Sorafenib tablets may be repackaged in HDPE pharmacy dispensing bottle other than the original container with expiration date not to exceed 30 days.

Storage: Store intact bottles at controlled room temperature, not to exceed 25°C.

If a storage temperature excursion is identified, promptly return sorafenib to controlled room temperature (not to exceed 25°C) and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability studies are ongoing for investigationally-labeled supplies. Refer to package labeling for shelf life of commercially-labeled supplies.

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

Sorafenib tablets should be taken on an empty stomach (at least one hour before or two hours after food) or with a moderate fat meal. Ideally, tablets should be swallowed whole and should not be crushed. However, dispersion in water is allowed to facilitate administration to subjects that cannot swallow tablets (see below). Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 and 4 to 8 ounces for ≥ 12 years). If sorafenib tablets must be taken with food, they should be taken with a moderate to low fat meal. Grapefruit or its juice is not allowed for the duration of treatment with sorafenib.

A suspension of sorafenib tosylate can be prepared by dispersing 50 mg and 200 mg tablets in drinking water. The volume of water depends on the dose and is presented in the table.

Sorafenib dose range*	Volume of drinking water	Volume of rinsing water (total volume)
50 mg to 199 mg	30 mL (1 oz)	90 mL (3 oz)
200 mg to 400 mg	60 mL (2 oz)	180 mL (6 oz)

**This represents each INDIVIDUAL dose. Doses should not be prepared in advance.*

Patient/caregiver instructions for preparation of the dispersed tablet may be found in [Appendix III](#).

Preparation instructions:

1. First, choose a quiet working space away from food, windows, fans or heat ducts.
2. Clean the working space with damp paper towels, and place a pad or paper towel on the clean working space.
3. Next, place all needed items and your SORAFENIB tablets on the pad or paper towel.
4. Then, wash your hands with soap and water before preparing this drug. The use of gloves and a mask for preparation of this drug is recommended.
5. Fill a drinking glass with the volume of water appropriate for the dose as indicated in the table above.
6. Place the number of tablets required for the dose into the specified amount of water. Record the time.
7. Let the tablet(s) sit in the water for approximately 5 minutes, then begin stirring. Stir the contents until all tablets are completely disintegrated (broken into tiny pieces). You may see a thin film forming on the top of the water. This does not change the accuracy of your dose. The preparation (suspension) should be ready for administration after 10 minutes.
8. Place dirty gloves, other dirty utensils (disposable spoon and cup), and the used pad or paper towel in the provided waste container or bag and seal.
9. Wash your hands thoroughly.
10. Administer the suspension immediately or within one (1) hour after preparation according to the steps that follow.

Administration of the suspension

1. Administer the suspension within one (1) hour of preparation.
2. Swirl the suspension before taking it by mouth.
3. Sorafenib should be taken **once or twice a day** on an empty stomach, at least one (1) hour before or two (2) hours after food.
4. Refer to the table above to determine the appropriate amount of rinsing water per dose. Rinse the glass with this additional water and drink this rinse water to capture all the remaining drugs.
 - a. **Do not** drink grapefruit juice or eat grapefruit with this drug.
 - b. If dose is skipped or missed, **do not** replace it. Wait for the next dose schedule to take the drug.

Supplier: Supplied by Bayer HealthCare AG and distributed by PMB, CTEP, DCTD NCI. **Do not use commercially available drug.**

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.jsp> >. 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 receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

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.

Investigator Brochure Availability

The current version(s) of the IB(s) for the agent will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status and a “current” password. Questions about IB access may be directed to the PMB IB coordinator via email.

Useful Links and Contacts

- CTEP Forms, Templates, Documents: <http://ctep.cancer.gov/forms/>
- NCI CTEP Investigator Registration: PMBRegPend@ctep.nci.nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: <https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jsp>
- CTEP Identity and Access Management (IAM) account: <https://eapps-ctep.nci.nih.gov/iam/>
- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

6.14 TACROLIMUS

(FK-506, Prograf®) NSC # 717865

(05/10/11)

Source and Pharmacology:

Tacrolimus is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. Tacrolimus is a potent immunosuppressive agent which prolongs the survival of the host and transplanted grafts in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (immunosuppression). Additionally, tacrolimus may inhibit cellular activities such as nitric oxide synthetase activation and apoptosis, and may potentiate the action of corticosteroids in these processes. Tacrolimus activity is primarily due to the parent drug. The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The $t_{1/2}$ in adult patients ranges from 11-19 hours.

The pharmacokinetics of tacrolimus have been studied in pediatric liver transplant patients (0.7 to 13.2 years of age). Following the IV administration of a 0.037 mg/kg/day dose to 12 pediatric patients, mean terminal half-life, volume of distribution and clearance were 11.5 ± 3.8 hours, 2.6 ± 2.1 L/kg and 0.138 ± 0.071 L/hr/kg, respectively. Following oral administration to 9 pediatric patients, the absolute bioavailability was $31 \pm 21\%$. Whole blood trough concentrations from 31 patients less than 12 years old showed that pediatric patients needed higher doses than adults to achieve similar tacrolimus trough concentrations. Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A) in the liver and to a lesser extent in the intestinal mucosa. The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. The main route of elimination is via the biliary tract and excretion in faeces. The mean clearance in renal dysfunction and mild hepatic dysfunction is the same as normal volunteers. Severe hepatic dysfunction (Pugh score > 10) led to a substantially decreased clearance. A retrospective comparison of Black and Caucasian kidney transplant patients indicated that Black patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences. The absorption of tacrolimus from the gastrointestinal tract is incomplete and variable exhibiting large intra- and inter-patient variability. Administration with food significantly decreases the rate and extent of absorption. Drugs that stimulate or inhibit hepatic p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.

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	Headache (L), hypertension (L), nausea, vomiting, anorexia, immunosuppression (L), diarrhea, constipation, fever	Chest pain	Anaphylaxis with the injection, allergic reaction, hypotension, asthma, dyspnea, increased cough, flu like syndrome, pleural effusion, seizure (L), tachycardia, angina
Prompt: Within 2-3 weeks, prior to the next course	Tremor (L), , renal dysfunction (acute with decrease in GFR, impaired urinary concentrating ability, and sodium retention), elevated creatinine/BUN, anemia, insomnia, asthenia, pain (abdominal, back, pain), hyperglycemia, hypomagnesemia (L), hyper/hypokalemia (L), hypophosphatemia, paresthesia	Alopecia, dizziness, elevated LFTs, UTI, peripheral edema, rash, pruritus, hyperlipidemia, hypercholesteremia	Dyspepsia, dysphagia, gastritis, esophagitis, flatulence, CNS abnormalities (confusion (L), somnolence (L), depression (L), anxiety, anxiousness, abnormal dreams, emotional lability, hallucinations, psychosis, hypertonia, incoordination, neuropathy, nervousness encephalopathy), coagulation disorder, leukopenia (L), thrombocytopenia, polycythemia, anemia, leukocytosis, infections (bacterial, fungal, viral –sepsis, cellulites, fungal dermatitis, herpes simplex, sinusitis, pharyngitis, abscess, pneumonia, bronchitis, peritonitis), hyperbilirubinemia (L), thrombosis, phlebitis, arthralgia, myalgia, electrolyte abnormalities
Delayed: Any time later during therapy, excluding the above conditions			Acne, exfoliative dermatitis, skin discoloration, photosensitivity reaction, skin ulcer, delayed wound healing, hirsutism (hypertrichosis) (L), gingival hyperplasia, abnormal vision, amblyopia, ear pain, otitis,

			tinnitus, GI hemorrhage, GI perforation, cholelithiasis, cholestatic jaundice, chronic renal dysfunction, renal failure, post-transplant diabetes mellitus (L), myocardial hypertrophy, elevated liver function tests, liver damage, ascites
Late: Any time after completion of treatment			Lymphoproliferative disorders, skin malignancies
Unknown Frequency and Timing:	Fetal toxic effects of tacrolimus have been noted in animals. Tacrolimus is transported across the placenta and its use during pregnancy has been associated with neonatal hyperkalemia and renal dysfunction. Tacrolimus is excreted in human milk, nursing should be avoided.		

(L) Toxicity may also occur later.

Formulation and Stability:

Injection:

Tacrolimus is available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus per 1 mL. Each mL also contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, *USP*, 80% v/v. Store between 5°C and 25°C (41°F and 77°F).

Oral:

Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).

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

Injection:

Tacrolimus Injection must be diluted with NS or D5W before use to a concentration between 0.004 mg/mL and 0.02 mg/mL. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The polyoxyethylated castor oil contained in the concentrate for intravenous infusion can cause phthalate stripping from PVC. **It is strongly recommended that glass bottles and non-PVC tubing be used to minimize patient exposure to DEHP.** Due to the chemical instability of tacrolimus in alkaline media, tacrolimus injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir).

Monitor closely for an acute allergic reaction for the first 30 minutes and at frequent intervals thereafter.

Oral: Administer at a consistent time of day and at consistent intervals with regard to meals. Tacrolimus may be given with food as long as it is given the same way each time; however, administration with food significantly decreases the rate and extent of absorption. Grapefruit juice should be avoided during the entire course of tacrolimus administration.

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

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).

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.

7.1 Required Clinical, Laboratory and Disease Evaluations

Obtain prior to start of phase unless otherwise indicated.

	Baseline	Each course of chemotherapy	Arm C Maintenance*	End of therapy	Relapse/Refractory
History	X		Q month		
Physical Exam (Ht, Wt, BSA, & VS)	X	X ¹	Q month ¹		
CBC	X	X	Q month	X	X
BUN and Creatinine	X	X ²	Q month		
AST, ALT, and bili (direct and total)	X	X	Q month		
Calcium, phosphorus, albumin			Q month		
Either Echo or MUGA, and ECG	X	Before Induction II Before Intensification I Before Intensification II	Q 4 months	X	
High Resolution HLA/DNA typing: patient, parents, siblings ³	X				
Biopsy and CT or MRI of chloroma ⁴	X				X
Blood Pressure ⁵	X	X	Q month	X	
Pulse Oximetry (O ₂ saturation) ⁶	X	X ⁶			
Neurologic Examination ⁷	X	X ⁷			
Urinalysis (Arm C only)	X	X	Q month		
BMA/clot section (biopsy if aspirate unsuccessful) ⁸	X	After Induction I After Induction II After Intensification I	Before Maintenance	X ⁹	X
BMA cytogenetics and FISH ¹⁰	X ¹⁰				X ¹⁰
LP-CSF for cell count, cytospin ¹¹	X				X
BMA for MRD ¹²	X	After Induction I			
BMA for FLT3 testing	X ¹³				
Pregnancy Test, if applicable	X				

¹ Patients on Arm C should have weekly clinical cardiac examinations performed while receiving sorafenib with chemotherapy. Patients on Arm C Maintenance must have at least monthly clinical cardiac examinations. A clinical cardiac examination should include a review of systems, vital sign assessment, pulse oximetry, and cardiac auscultation to identify signs and symptoms of cardiac dysfunction.

² Prior to HD AraC, obtain CrCl for patients with serum creatinine > 2 mg/dL or > 2 x normal for age. See [Section 4](#) TDMs for timing.

³ All AML patients will have HLA-typing at diagnosis. If an HLA-identical sibling is not identified at presentation, then high resolution typing of the patient should be performed at HLA-A, B, C and DRB1. If HLA typing is not able to be performed at diagnosis, then this should be pursued as soon as possible upon recovery from Induction I.

⁴ For patients with granulocytic sarcomas (chloroma) only. Biopsy only required if other AML diagnostic methods are unsuccessful.

⁵ Only for patients receiving sorafenib containing therapy. Blood pressure (BP) will be measured with an appropriate sized cuff at rest. Guidelines for the grading and management of hypertension are in [Section 5.2.2](#), [Appendix I](#).

⁶ Only for patients randomized to receive bortezomib containing therapy. Prior to each bortezomib dose.

⁷ Only for patients randomized to receive bortezomib containing therapy. Prior to each course of therapy.

⁸ If it is inadvisable clinically to perform a BM at diagnosis or relapse, the use of peripheral blood may be substituted in cases where there are adequate numbers of blasts to conclusively make the diagnosis of AML. In these cases, peripheral blood should also be sent for all studies requested at diagnosis or relapse. Clot section is only required at diagnosis.

⁹ For all patients. For Arm A or B SCT patients, the end of therapy BMA/Biopsy is to be done within 1 month of ANC recovery after SCT. For Arm C SCT patients, the end of therapy evaluation is within one month of completing sorafenib maintenance.

¹⁰ See Cytogenetics [Section 16.0](#) for submission guidelines. Submit sample to local cytogenetics lab in green top tube.

¹¹ May be deferred if clinically indicated for patient safety.

¹² Extra marrow (or peripheral blood) is to be collected in a green top tube at time of disease status evaluation. See [Section 15.0](#).

¹³ See [Section 14.0](#) for instructions regarding submission of FLT3 samples. Submit sample in purple top tube.

* Patients receiving Maintenance post-SCT are referred to [Section 7.3](#) for additional evaluations required/recommended post-SCT.

This table only includes evaluations necessary to answer the primary and secondary aims. Obtain other studies as indicated for good clinical care.

7.2 Recommended Clinical, Laboratory and Disease Evaluations

	Baseline	Each course of chemotherapy	End of therapy*	Relapse
History and Physical	per 7.1	Weekly	X	X
Urinalysis	X	X	X	X
Electrolytes, Ca, Phos	X	X	X	X
BUN/Cr	per 7.1	per 7.1	X	X
LDH	X			X
Uric Acid	X			X
Quant. IgG	X	X (monthly)		
AST/ALT, bili (direct & total)	per 7.1	per 7.1	X	X
CMV serology	X			
Varicella serology	X			
HSV serology	X			
CXR	X			
Echo/EKG or MUGA	per 7.1	per 7.1	per 7.1	X
PT/PTT, fib, FDP or d-dimers	X			
Cr clearance or GFR	X	Prior to Intensification I		

*End of therapy evaluation for patients on Arm C who receive Sorafenib Maintenance will occur within 1 month of completion of Sorafenib Maintenance.

7.3 Required & Optional Observations for SCT Patients Only

Required:	Pre-SCT	Post- SCT	Minimum Criteria to Proceed to SCT
Pulmonary Function Tests (if able to perform; O ₂ saturation if unable to perform)	X		DLCO > 50%
Echo/ECG	X		Shortening fraction \geq 28% or EF \geq 55%
Electrolytes	X		Within normal institutional limits
Liver Function Tests	X		Transaminases < 2.5 x normal; Total bilirubin < 2 mg/dL
Creatinine Clearance or GFR	X		\geq 60 mL/minute/1.73m ²
BMA/clot section (biopsy if aspirate unsuccessful)	X	X**	Documented remission
LP-CSF for cell count, cytospin	X		No leukemic infiltrate
HSV and CMV titers	X		Required Observations
Hep B by surface antigen/ Hep C by serology	X		Required Observations
HIV by serology	X		Negative
1 st Dose Busulfan Pharmacokinetics*	X		To achieve an AUC for busulfan of 900 to 1500 (micromole/liter)*minute.
Optional:***			
GVHD status		X	At onset of GVHD, Day +56 and Day +100

- * See [Section 17.1.3](#). Note that laboratories may report busulfan pharmacokinetics as AUC or C_{ss} values. The target refers to results expressed as AUC.
 - ** BMA is required within 1 month after ANC recovery (> 500/ μ L). If patient is Arm A or B: also collect End of Therapy optional biology bone marrow sample if patients has consented to participate in optional biology studies. If patient is on Arm C: End of Therapy optional biology will be collected within one month of completing Sorafenib Maintenance.
 - *** For patients that have consented to the optional GVHD predictor study.
- Note:** Arm C patients: please also refer to [Section 7.1](#) for Sorafenib Maintenance phase.
- 7.3.1 Required Busulfan Pharmacokinetic Studies* (See [Section 17.1.3](#)).

For Single IV Dosing

Time Following Start of 1st Dose
3 hours
3 hours 15 minutes
4 hours
5 hours
6 hours
8 hours

- * To be done on 1st dose only.

For Q6 hour IV Dosing

Time Following Start of 1st Dose
2 hours
2 hours 15 minutes
2 hours 30 minutes
3 hours
4 hours
5 hours
6 hours

- * To be done on 1st dose only.

7.3.2 Recommended Clinical, Laboratory and Disease Evaluations During and Following SCT

	During Hospitalization	Day 100 post SCT	6 months post SCT	9 months post SCT	1 year post SCT
Physical Examination	Daily during hospitalization	X	X	X	X
CBC	Daily until APC is rising	X	X	X	X
Electrolytes	Weekly	X	X	X	X
AST/ALT, bilirubin	Weekly	X	X	X	X
Donor Chimerism		X	X		

Note: Arm C patients: please also refer to [Section 7.1](#) for specific monitoring post-SCT during Sorafenib Maintenance phase.

7.4 **Optional Studies**

The following table lists samples to be obtained from patients who have provided consent for optional correlative studies.

	Baseline	Each course of therapy	End of therapy	Relapse/Refractory	Special Tubes
BMA for Leukemia Biology Studies #	X#	After Induction I # After Induction II # After Intensification I #	X#	X#	3 - 10 mL/sample purple/lavender top
BMA for Optional MRD @		After Induction II @ After Intensification I @	X@	X@	2 - 4 mL/sample green top
Peripheral blood for Gene Polymorphism studies ^	X^	After Induction I only ^			5 mL purple/lavender top
Peripheral blood for UPR/RPPA § studies		During Induction I only §These studies have been completed per memo 2-17-2017			3 mL/sample CellSave tube 2mL/sample heparin tube
Peripheral blood for Sorafenib PK studies +		During Induction I During Induction II During Intensification I During Intensification II During Maintenance			3 mL/sample green top
Peripheral blood for Sorafenib PIA studies &		Per Amendment #6A, there are only pre-course and post-chemotherapy samples. See Appendix XI			3 mL/sample green top
Bone Marrow for LIC studies *		After Induction I After Induction II *			5 mL/sample green top
Peripheral blood for GVHD predictor study (for pts receiving SCT only) †		Post-SCT on Days +7, +14, +28 !These studies have been completed per memo 2-24-2017			3 - 6 mL/sample red top
HRQOL Studies (Closed to new patient accrual as of 05-15-15. COG memo posted on 05-08-15.)	See Section 18.0 for specific evaluation time points				

- # Extra marrow (or peripheral blood) is to be collected in purple/lavender top tube (green top preservative-free heparin tube if blood) at time of disease status evaluation. See [Appendix VII](#) for details on collection time points and send out guidelines.
- @ Extra marrow (or peripheral blood) is to be collected in green top tube (preservative-free heparin) at time of disease status evaluation. See [Section 15.0](#) for send out guidelines.
- ^ See [Appendix VII](#) for details on collection time points and send out guidelines.
- § These studies have been completed per memo 2-17-2017. See [Appendix IX](#).
- + For patients on sorafenib Arm C only. See [Appendix X](#) for details on collection time points and send out guidelines.
- & For patients on sorafenib Arm C only. See [Appendix XI](#) for details on collection time points and send out guidelines.
- * See [Appendix VIII](#) for details on collection and send out guidelines. Submit Induction II sample only if patient is MRD+ at the end of Induction I.
- † These studies have been completed per memo 2-24-2017. See [Appendix XIII](#).

7.5 Follow-up Studies*

	First 6 months	Second 6 months	Second Year	Third Year	Annually Thereafter
PE & CBC	Monthly	Q other month	Q 4 months	Q 6 months	Q year
Echo (or MUGA)/ECG		Once	Yearly	Yearly	Q year

* For patients who complete chemotherapy. Patients who go on to SCT will need, in addition to these items, further follow-up as required by their SCT institution. More frequent or additional studies should be pursued as clinically warranted.

These studies are required until the patient is off study as defined in [Section 8.2](#) and reported as defined in [Section 7.6](#).

7.6 Follow-up Reports

Follow-up data are expected to be submitted per the Case Report Forms (CRF) schedule.

See COG Late Effects Guidelines for recommended post treatment follow-up:
<http://www.childrensoncologygroup.org/disc/LE/default.htm>.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

- a) Failure to attain or maintain remission following Induction II (\geq 5% malignant blasts or extramedullary disease).
- b) Relapse after Induction II of any site following remission.
- c) Refractory CNS leukemia following 6 doses of IT cytarabine therapy in Induction I or Induction II.
- d) CNS leukemia at start of Induction II after treatment for CNS leukemia in Induction I.
- e) Grade 3 or greater neurotoxicity from HD cytarabine or,
- f) Other intolerable or unacceptable toxicity secondary to the standard chemotherapy backbone.
- g) Refusal of further protocol therapy by patient/parent/guardian.
- h) Completion of planned therapy.
- i) Physician determines it is in patient's best interest.
- j) Development of a second malignancy.
- k) Graft failure
- l) For patients enrolled on Arm C: Failure to meet required criterion for start of maintenance therapy.

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).*
- d) Withdrawal of consent for any further data submission.
- e) Tenth anniversary of study entry.
- f) Excluded constitutional condition or oncologic diagnosis is indicated following enrollment (see [Section 3.2.5](#) and [Section 3.2.6](#)).
- g) For patients enrolled after implementation of Amendment #7A: absence of FLT3/ITD or FLT3/ITD allelic ratio \leq 0.4.
- h) For patients enrolled after implementation of Amendment #7A: FLT3/ITD status is unknown at End of Induction I.
- i) For patients enrolled after implementation of Amendment #7A: HR FLT3/ITD (AR > 0.4) and decline transition to Arm C chemotherapy.

* For subsequent studies with a significant interval between enrollment and beginning of therapy (i.e., studies involving stem cell transplant where there will be an attempt to identify an adequate donor), the patient will come off AAML1031 at the time when therapy is initiated on the subsequent study (and not at time of enrollment onto that study).

9.0 STATISTICAL CONSIDERATIONS

9.1 Patient Accrual and Expected Duration of Trial

Since approximately 8% of eligible patients are expected to be HR FLT3/ITD+, enrollment of an additional 500 patients post-amendment #7A should result in the enrollment of approximately 40 additional patients with HR FLT3/ITD+. Based on the current accrual estimate of 290 patients per year, it is estimated that it will take approximately 1.7 years to accrue 500 patients post-amendment #7A which would result in total enrollment of up to 1,750 patients. Of these 1,750 patients, a total of approximately 100 patients are expected in Arm C (60 patients prior to Amendment 7A and 40 additional patients post-Amendment 7A).

Extension of accrual to Arm C with Amendment 7A occurred for two reasons. First, accrual to Arm C during the main randomization substantially lagged behind expected accrual. Initial Arm C accrual estimates placed the total number of Arm C patients at approximately 80 patients. However, the extended closure of Arm C for Amendment 3 substantially decreased the time available for patient enrollment on Arm C. Second, Amendment 6, which changed the timing of sorafenib initiation to follow chemotherapy completion, has enrolled 14 patients. Since Cohort 3 patients are treated with a different sorafenib schedule, accrual of additional patients would enable more precise estimates of both toxicity, particularly left ventricular systolic dysfunction, and response.

9.2 Statistical Analysis Methods

9.2.1 Study Endpoints

The primary outcome for Aim 1.1.1 is EFS, defined as the time on study to Induction failure, relapse or death. Secondary endpoints include OS, remission rate after 1 and 2 courses of therapy, the proportion of patients dying in each course of therapy, course duration, length of hospitalization, time to count recovery, relapse rate and treatment-related mortality, and frequency of toxicities, including infectious and cardiac complications. The safety of the combination of bortezomib and chemotherapy will be assessed by evaluating the proportion of patients experiencing Grade 3 or higher non-hematologic toxicities, infections, time to count recovery, and length of hospitalization (Aim 1.1.2).

The feasibility of combining sorafenib with AML chemotherapy and in maintenance will be carried out as described in the analysis plan (Aim 1.1.4). Preliminary efficacy data will be obtained by comparing end of Induction I MRD clearance between HR FLT3/ITD patients who did and did not receive sorafenib in Induction I (Aim 1.1.4). In addition, OS and EFS from study entry will be estimated for patients who receive sorafenib in Induction I. OS and EFS will also be described separately for Arm C Cohort 1 patients, Cohort 2 patients, and Cohort 3 patients. Given the small size of these patient groups, the OS and EFS analyses will be descriptive.

The endpoints for the HRQOL aim will be the instruments as described in [Section 18.0](#) and the PIP ([Aim 1.2.5](#)).

9.2.2 Analysis Plan

Aim 1.1.1

Patients determined to have HR FLT3/ITD+ will not contribute to or be included in analysis for Aim 1. The Kaplan-Meier method will be used to estimate OS, EFS, and DFS.¹²⁶ The log-rank test will be used to compare survival between treatment groups. Comparison of EFS will occur when the total event horizon is reached. Comparison of OS will be performed only if a significant difference is found on EFS analysis. Cumulative incidence estimates that account for competing events will be used to estimate relapse rate, treatment-related mortality, and time to count recovery. The occurrence of a clinically important statistical interaction effect on outcomes for the main treatment comparisons is thought to be unlikely. Hence, power calculations are based on the assumption that the stratified analysis of either factor across levels of the other

factor is valid. However, analysis of the study data will examine the validity of this assumption using a Cox regression model. If needed, separate analyses within strata of the factors will be performed but this will have limited power due to limited sample size. If the proportional hazards assumption is violated, alternative statistical methods will be employed.

Aim 1.1.2

Patients determined to have HR FLT3/ITD+ who are randomized to receive Bortezomib and elect to not participate in Arm C will be included in analysis for Aim 2. The proportion and corresponding confidence intervals of patients experiencing Grade 3 or higher non-hematologic toxicities and infections will be estimated. Descriptive statistics will be used to summarize length of hospitalization time. Cumulative incidence estimates that account for competing events will be used to estimate count recovery. Stopping rules for unacceptable bortezomib related toxicity are described in [Section 9.3.1](#).

Aim 1.1.3

The Kaplan-Meier method will be used to estimate OS and EFS.¹²⁶ We will assume the null hypothesis that the EFS experience from the end of Induction I for high risk children without HR FLT3/ITD+ treated on AAML03P1 and AAML0531 follows an exponential mixture cure model. Since AAML0531 data collection is still ongoing, the cure model parameters to be used in the one-sample log-rank test will be estimated at the time of the first interim analysis. We will assume the null hypothesis that the EFS experience on this study follows the exponential cure model. Cumulative incidence estimates that account for competing events will be used to estimate relapse rate, treatment-related mortality, and time to count recovery.

Aim 1.1.4

The proportion of patients experiencing Grade 3 or higher non-hematologic toxicities and infections will be estimated. Descriptive statistics will be used to summarize length of hospitalization time. Cumulative incidence estimates that account for competing events will be used to estimate count recovery. The Kaplan-Meier method will be used to estimate OS and EFS for patients treated with sorafenib.

Aim 1.2.2

The proportion of high risk children without HR FLT3/ITD+ converting from positive MRD at end of Induction I to negative MRD at the end of Induction II will be estimated as well as the corresponding confidence interval. In addition, this proportion will be compared with the proportion for high risk children without HR FLT3/ITD+ treated on AAML03P1 and AAML0531.

Aim 1.2.5

The primary analysis will be the description of parent-report Generic Core Scale, Acute Cancer Module, and Multidimensional Fatigue Module summary and dimension scores of HRQOL and PIP at 4 months following start of SCT or Intensification II of chemotherapy; we will present mean values and their 95% CI.

A secondary analysis will be the description of HRQOL and PIP outcomes over time in recipients of SCT or chemotherapy. This analysis will begin at the baseline assessment and we will describe summary measures at each time point as well as the overall trajectory over time. We also will perform a mixed linear regression model with repeated measures incorporating covariates as appropriate. Another secondary analysis will be the examination of concordance of parent and child measures using the intraclass correlation coefficient at each time point.

As an exploratory analysis, we also will compare HRQOL and PIP between SCT and chemotherapy over time using a mixed linear regression model which includes an indicator variable for treatment and other covariates as appropriate.

Aim 1.2.6

Bortezomib Pharmacokinetics Plasma concentration-time profiles on Day 8 of Induction II will be analyzed using descriptive statistics and will be graphically displayed by age group. PK data will be analyzed using methods such as nonlinear mixed effects modeling to estimate bortezomib clearance (Cl) and volume of distribution (Vd) with associated 95% confidence intervals in each age group (2-11 years and 12-16 years of age). To enable these analyses, data from this study will be combined with PK data collected on AALL07P1. Potential relationships between measures of bortezomib plasma exposure (eg, model-estimated AUC_{0-last} values) in individual patients and efficacy and/or safety endpoints may be examined graphically to guide exploratory assessment of exposure-response relationships. Up to 60 evaluable patients will be studied for bortezomib pharmacokinetic endpoints. Amendment #3A Update: This study was closed to new patient participation on June 25, 2013, as accrual goals were met.

Aim 1.2.7

Steady state pharmacokinetics of sorafenib and N-Oxide metabolite will be obtained in patients who receive sorafenib and provide consent/assent. Descriptive statistics will be used to summarize the systemic exposure of sorafenib and N-oxide metabolite for each course of induction and intensification (CL, Vd, T_{max}/2, AUC). If sufficient data is available, comparison to steady state pharmacokinetic data from ADVL0413 will be performed using Wilcoxon Sign Rank Test. For PIA-PK correlations, a random effects linear regression model will be used to describe the relationship between PIA and PK levels for each of the trough plasma samples collected for each patient. Random effects are included to account for the potential correlation between observations from the same patient.

Aim 1.2.8

The proportion of patients treated with and without dexrazoxane will be estimated for each course of anthracycline containing chemotherapy. Shortening fraction/ejection fraction percentages and change over time will be analyzed by repeated measures ANOVA accounting for dexrazoxane exposure and other clinically relevant covariates, including age, gender, body mass index, risk group, and treatment arm.

Given the ecologic study design, definitive conclusions about the impact of dexrazoxane on cardiac toxicity and relapse risk are not possible. Thus, the proposed analyses will serve as preliminary data to inform discussion of a randomized trial to evaluate the utility of dexrazoxane in pediatric AML therapy.

Aim 1.2.18

Serum concentrations of GVHD biomarker will be measured on samples collected on post-SCT Days 7, 14 and 28. Samples will be excluded from analysis if collected after the onset of Grade 2-4 GVHD. The biomarker concentrations will be combined with the following two clinical variables: donor type (related versus unrelated versus cord blood donor) and HLA-match (matched versus mismatch). Logistic regression modeling will be used to assign individual weights to each individual biomarker and clinical variable in order to maximize sensitivity and specificity of a mathematical algorithm for the prediction of Grade 2-4 GVHD occurring on or before Day 56 post-SCT. We will test whether algorithms using the Day 7 biomarker panel, the Day 14 biomarker panel, the Day 28 biomarker panel, or a combination of the biomarker panels at these different time points are most useful for the prediction of GVHD. We will compare the different algorithms on the basis of maximal specificity and sensitivity using the Akaike Information Criterion, which measures the tradeoff of accuracy for complexity.¹²⁷

A secondary analysis will explore the correlation of high and low risk profiles identified by the GVHD predictive algorithm with non-relapse mortality, relapse incidence and overall survival.

9.2.3 Power Calculations

9.2.3.1 Power Calculations Aim 1.1.1: EFS

Power calculations are performed using a cure model assuming proportional hazards, the failure distribution contains a plateau corresponding to the proportion of patients who are cured, and those not cured have an exponential failure distribution.¹²⁸ Based on this model with 1-sided testing and 2.5% type I error rate, there will be 80% power to detect a 9% difference in EFS plateaus (52% vs. 61%, hazard ratio = 0.78) between patients randomized to standard therapy versus bortezomib/standard combination therapy if the EFS at 1 year is assumed to 69% for the group with inferior EFS. AML has a long term EFS of 52% with 90% of events observed by 1 year of follow-up. Thus enrollment of 1050 eligible patients without HR FLT3/ITD+ would be expected under the alternative hypothesis to generate 407 events by 1 year of follow-up.

9.2.3.2 Power Calculations Aim 1.1.2: Feasibility of Combining Bortezomib with Standard Therapy

Assuming 100 evaluable patients without HR FLT3/ITD+ are randomized to receive bortezomib, this study will provide a worst case precision of $\pm 9.8\%$ at a prevalence of 50% of patients experiencing Grade 3 or higher non-hematologic toxicities. This precision will improve as the prevalence moves away from 50%, with a $\pm 7.8\%$ precision at a prevalence of 10%.

9.2.3.3 Power Calculations Aim 1.1.4: Feasibility of Combining Sorafenib with Standard Therapy

The table below provides the probability of determining a dose de-escalation is needed (i.e. DLT rate of 30% or more at any time after the enrollment of the first 2 and up to 10 patients enrolled) if the true probability a patient experiences a DLT varies from 0.05 to 0.4. Any patient who experiences a severe toxicity at any time during protocol therapy after receiving at least 1 dose of study drug is considered evaluable for toxicity. Patients without a severe toxicity who receive at least 85% of the prescribed dose per protocol guidelines are also considered evaluable for toxicity. In the dose finding phase, patients who are not evaluable for hematologic toxicity because of progressive disease will not be replaced.

Probability of DLT	Probability of determining dose de-escalation is needed	Mean # of patients needed to make determination
0.05	0.16	8.8
0.1	0.30	7.8
0.15	0.43	6.9
0.2	0.55	6.0
0.25	0.67	5.3
0.3	0.76	4.6
0.35	0.82	4.1
0.4	0.88	3.6

Given the concerns for cardiac toxicity in patients receiving sorafenib with standard chemotherapy, the addition of approximately 40 patients to Arm C, cohort 3 will provide improved precision in the estimates of mean change in shortening and ejection fraction between the end of Induction I and end of therapy. The table below provides the decrease in half-width for 95% CI for the change in shorten fraction/ejection fraction for standard deviations of change ranging from 5 to 8.

# of Arm C patients	# evaluable for SF/EF	95% CI half width SD = 5	95% CI half width SD = 6	95% CI half width SD = 7	95% CI half width SD = 8
14	9	3.8	4.6	5.4	6.1
54	36	1.7	2.0	2.4	2.7

9.2.3.4 Power Calculation for Aim 1.1.3: Comparison of High Risk Outcome with Historical Controls

Assuming that 25% of the children without HR FLT3/ITD+ will be high risk, this study will have 80% power (1-sided testing at the 5% level of statistical significance) to detect a relative risk of failure of 0.78 (roughly a 9% improvement in EFS).

9.2.3.5 Power Calculations Aim 1.2.1: Assessment of Anti-leukemic Activity of Sorafenib in HR FLT3/ITD+

Several analyses will be performed to assess the efficacy of sorafenib. First, minimal residual disease levels at the end of Induction I will be compared, using a test of proportions, between patients who receive sorafenib starting in Induction I (sorafenib cohorts 2 and 3) with those patients who receive sorafenib starting in Induction II (first sorafenib cohort). Since only 10 patients are expected to be enrolled in the first sorafenib cohort, this analysis may be exploratory. Second, prevalence of minimal residual disease at the end of Induction I and II will be compared, using tests of proportions, between patients treated with sorafenib in Cohorts 2 and 3 on AAML1031 with patients with HR FLT3/ITD+ enrolled on AAML0531 (there are 64 patients with HR FLT3/ITD+ enrolled on AAML0531 as of September 27, 2010. (Todd Alonzo personal communication, 2010). Given the sample numbers of patients expected in Cohorts 2 and 3, these analyses will be exploratory. Finally, OS and EFS from study entry will be compared, using log-rank tests, for patients treated with sorafenib in the second and third cohorts on AAML1031 with patients with HR FLT3/ITD+ enrolled on AAML0531. These analyses will be exploratory.

The table below summarizes the decrease in estimated half-width of the 95% CI with an additional 40 patients enrolled on Arm C, Cohort 3 for MRD positivity at the end of Induction assuming MRD positivity of 50% (the MRD positivity with the greatest variability).

# of Arm C patients	# evaluable for MRD	95% CI half-width
14	13	28%
54	50	15%

9.2.3.6 Power Calculations for Aim 1.2.2: Comparison of MRD Conversion with Historical Controls

Assuming that 25% of the children without HR FLT3/ITD+ will be high risk and 83% of them will be MRD+ at the end of Induction I, this study will have 80% power (2-sided testing at the 5% level of statistical significance) to detect between a 7% and 9% difference in MRD conversion rates.

9.2.3.7 Power Calculations Aim 1.2.3: Comparison of SCT Outcome with Historical Controls

Outcomes for high risk patients on AAML1031 who are assigned alternative SCT will be compared to comparable patients on AAML0531 who were not assigned alternative SCT. Specifically, OS and DFS from the end of Intensification I will be compared using the log-rank test and cumulative incidence of treatment related mortality (TRM) and relapse will be compared using Gray's test. Given the small sample size, this analysis will be exploratory.

9.2.3.8 Power Calculations Aim 1.2.4: Comparison of SCT Outcome with Historical Controls

OS and DFS from the end of Intensification I will be compared, using the log-rank test, between AAML1031 patients assigned MFD SCT and AAML0531 patients assigned MFD SCT. In addition, the cumulative incidence of TRM, relapse, and severe toxicity will be compared using Gray's test. As of September 27, 2010, approximately 100 AAML0531 patients have been assigned MFD SCT (Todd Alonzo personal communication, 2010). If we assume 77% of the non-HR FLT3/ITD+ patients will still be on protocol therapy at end of Intensification I (this is consistent with AML0531) and 25% of these are high risk, then there will be 202 high risk patients still on protocol therapy at the end of Intensification I. If 25% of these have a MFD, then about 50 will receive MFD SCT on protocol therapy. There will be approximately 80% power with 2-sided 5% type I error rate to detect a 23% difference in DFS plateaus

from the end of Intensification I (75% vs 52%, 0.44 hazard ratio) between patients assigned MFD SCT on AAML1031 and patients assigned MFD SCT on AAML0531

9.2.3.9 Power Calculations Aim 1.2.5: HRQOL

We have powered the study to ensure sufficient precision in HRQOL estimates at 4 months following start of SCT or Intensification II of chemotherapy separately. If we assume that there are 1050 non-HR FLT3/ITD+ subjects available, we estimate that 77% will be between 2 and 18 years of age (derived from AAML0531), leaving 809 patients in the appropriate age range. If we assume that 70% of these patients would meet other eligibility criteria and consent to participate, this leaves 566 subjects that will be enrolled onto the HRQOL aim of the study. If we assume 70% of these patients will still be on study and event free at 4 months following start of SCT or Intensification II (consistent with AAML0531) and 25% of these are high risk, then there will be 99 high risk and thus assigned to SCT and 297 low risk patients and thus assigned to chemotherapy subjects still on protocol therapy at 4 months following SCT or Intensification II. Assuming a standard deviation of 20 points, the half-width of the 95% confidence interval for HRQOL will be 3.99 points and 2.28 points for patients assigned SCT and chemotherapy, respectively. In total, this approach would include 435 patients in the analysis for the time point at 4 months following start of SCT or Intensification II. This precision is sufficient for our purposes. Given the limited number of HR FLT3/ITD+ patients expected to be enrolled onto the HRQOL aim of the study, analysis of HRQOL for HR FLT3/ITD+ patients will be exploratory.

9.2.3.10 Power Calculations for Aim 1.2.8: Changes in Cardiac Function with/without Dexrazoxane

Since the prevalence of dexrazoxane use is not known, it is not possible to estimate the available study power for Aim 1.2.5. If the prevalence of dexrazoxane use is low, then the comparison of changes in shortening fraction over time between patients treated with and without dexrazoxane will be exploratory.

9.2.3.11 Power Calculations Aim 1.2.9: Refinement of MRD Based Risk Stratification

Outcomes for non HR FLT3/ITD+ subjects in morphologic remission at the end of Induction I will be compared for those with and without detectable leukemia by flow cytometry. OS from the end of the first course of therapy will be calculated as well as DFS and cumulative incidence of relapse. The Kaplan-Meier method will be used to calculate estimates of OS and DFS. Comparisons of outcomes will be accomplished using the log-rank test, Grey's test, and multivariate Cox regressions that adjust for treatment arm, and clinical, demographic, and laboratory features, such as diagnostic WBC, cytogenetic class, and race. In addition, clinical outcome of the patients with minimal residual disease will be compared using varying levels of minimal residual disease and at various time points.

If 85% of non HR FLT3/ITD+ subjects are in morphologic remission at the end of Induction I and 20% of these have detectable leukemia by flow cytometry, then this study will have 80% power to detect a 11% difference in plateaus for OS from end of Induction I (62% vs 73%, 1.5 hazard ratio) for those with and without detectable leukemia by flow cytometry.

9.2.3.12 Power Calculations Aim 1.2.10: Prognostic significance of molecular MRD and its contribution to risk identification with MDF based MRD

Molecular MRD and morphologic MRD will be assessed in non HR FLT3/ITD+ subjects with t(8;21), inv(16), and t(9;11). The proportions of these subjects in which each type of MRD is detected will be estimated along with corresponding confidence intervals. Assuming MRD results are available for 367 (35%) non HR FLT3/ITD+ subjects with t(8;21), inv(16), and t(9;11), this study will provide a precision of $\pm 4.1\%$ at a prevalence of 20% of patients with MRD.

9.2.3.13 Power Calculations Aim 1.2.11: Determine leukemic involvement of the hematopoietic early progenitor cell

Outcomes, e.g. DFS and time to relapse, will be compared for patients who achieve a MRD level of less than 1 in 10,000 versus those who fail to achieve a MRD level less than 1 in 10,000. The morphologic remission (< 5% myeloblasts) rates after induction I will also be compared. The Kaplan-Meier method will be used to calculate estimates of DFS while methods that account for competing events will be used to estimate cumulative incidence of relapse. Comparisons of outcomes will be accomplished using the log-rank test, Grey's test, and multivariate Cox regressions that adjust for treatment arm, and clinical, demographic, and laboratory features, such as diagnostic WBC, cytogenetic class, age, and race. Assuming MRD and morphology results are available for 200 patients (100 ADE and 100 ADE + Bortezomib) and 40% of patients have MRD, then there will be 80% power to detect a 20% difference in DFS plateaus (46% vs 66%, 1.9 hazard ratio) between those with and without MRD collapsed across treatment arms. Analysis for a particular treatment arm will be 80% power to detect a 28% difference in DFS plateaus (41% vs 69%, 2.4 hazard ratio) between those with and without MRD. This study will have 80% power to detect an 18% difference in CR rates after Induction I collapsed across treatment arms.

9.2.3.14 Power Calculations Aim 1.2.12: Definition of the Leukemia Stem cell Population in Patients with AML

Outcomes, e.g. DFS and time to relapse, will be compared for subjects who achieve a MRD level of less than 1 in 10,000 versus those who fail to achieve a MRD level less than 1 in 10,000. The Kaplan-Meier method will be used to calculate estimates of DFS while methods that account for competing events will be used to estimate cumulative incidence of relapse. Comparisons of outcomes will be accomplished using the log-rank test, Grey's test, and multivariate Cox regressions that adjust for treatment arm, and clinical, demographic, and laboratory features, such as diagnostic WBC, cytogenetic class, and race. Assuming MRD results are available for 200 patients (100 ADE and 100 ADE + Bortezomib) and 40% of patients have MRD, then there will be 80% power to detect a 20% difference in DFS plateaus (46% vs 66%, 1.9 hazard ratio) between those with and without MRD collapsed across treatment arms. Analysis for a particular treatment arm will be 80% power to detect a 28% difference in DFS plateaus (41% vs 69%, 2.4 hazard ratio) between those with and without MRD.

9.2.3.15 Power Calculations Aim 1.2.13: determine the prevalence and prognostic significance of molecular abnormalities of *WT1*, *RUNX1*, *MLL-PTD*, *TET2*, *c-CBL*, *KIT*, and other novel AML associated genes in pediatric AML

The proportions of patients with *WT1*, *RUNX1*, *MLL-PTD*, *TET2*, *c-CBL*, *KIT*, and other abnormalities will be estimated along with corresponding confidence intervals. Assuming that 900 patients will have molecular results available, this study will provide a worst case precision of ±3.3% at a prevalence of 50%. This precision will improve as the prevalence moves away from 50%, with a ± 2.0% precision at a prevalence of 10%. The Kaplan-Meier method will be used to calculate estimates of OS, EFS, and DFS. Comparisons of outcomes will be accomplished using the log-rank test and multivariate Cox regressions that adjust for treatment arm, and clinical, demographic, and laboratory features, such as diagnostic WBC, cytogenetic class, and race. The table below provides the difference in EFS plateaus and hazard ratios (HR) this study will have 80% power to detect between those with and without the mutation if the specified prevalence of mutation is observed.

Prevalence of Mutation	Difference in EFS plateaus (HR)
5%	20% (1.74)
10%	15.5% (1.51)
15%	13% (1.45)
20%	11.25% (1.38)

9.2.3.16 Power Calculations Aim 1.2.14: Correlate the expression of CD74 antigen as well as PSMB5 gene expression and mutation with response to Bortezomib

Descriptive statistics will be used to summarize expression of CD74 antigen as well as PSMB5 gene expression and mutation. The Kaplan-Meier method will be used to calculate estimates of OS and DFS. Outcomes will be compared for subjects with expression levels above and below the median expression levels using, for example, the log-rank test and multivariate Cox regressions that adjust for treatment arm, and clinical, demographic, and laboratory features, such as diagnostic WBC, cytogenetic class, and race.

9.2.3.17 Power Calculations Aim 1.2.15: Evaluation of Protein Expression and Unfolded Protein Response in Patients with AML

Bulk leukemia cells were collected and analyzed from 35 of the initial 80 patients enrolled on study (43%). Based on this collection rate, with an enrollment of 1150 evaluable patients, we estimate that we will evaluate samples from 624 patients (312/treatment group). Leukemia initiation cells (LIC) isolation (CD34+CD38+) was successfully completed for 18 of the 35 evaluable samples. Based on this data, we conservatively estimate that we will successfully enrich for LIC in 25% of eligible patients (n=156, 78/treatment group).

9.2.3.17.1 Protein Expression: The objective of this biology aim is to provide a more global assessment of protein cell stress pathways and AML cell signaling following ADE +/- bortezomib treatments. Data in this analysis arise from a clinical trial with multiple arms, 3 sampling time points and 2 cellular subsets of individual samples (bulk tumor and LIC). Therefore, many different comparisons may be of interest.

The statistical analysis will be performed in a stepwise manner:

- 1) We will first look at dynamic changes in protein activations following chemotherapy in myeloblasts and AML-LIC cells. 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.
- 2) Once clinical outcomes become available, we will analyze the difference in protein profiles between ADE and ADEB treatment groups as they relate to CR. This part will include different unsupervised methods and group comparisons.
- 3) Finally, we will create a classifier(s) to predict clinical outcome. We will use outcome variables CR, MRD, relapse risk, and 2-year EFS.

As with other high-dimensional data, RPPA data analysis will include low-level and high-level analysis:

- 1) In the low-level analysis, relative protein levels will be determined by interpolation of dilution curves from the global "standard curve" using the R package "SuperCurve".⁵² (<http://bioinformatics.mdanderson.org/Software/OOMPA/Current/SuperCurve-manual.pdf>). After normalization for protein loading and appropriate transformation, assessments of quality will be performed.
- 2) 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.⁵³ Cluster stability will be assessed using reproducibility measures, including GAP,⁵⁴ as well as robustness and discrepancy indices.⁵⁵ 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 Benjamini-Hockberg correction to account for multiple comparisons.⁵⁶ 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,⁵⁷ Random Forest,⁵⁸ 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 methods⁵⁹ to combine different models for the purpose of producing more accurate classification and regression models.

Comparison of protein expression profiles between responders and non-responders (e.g., CR after Induction I and EFS at 2 years) will also be assessed using an unpaired t-test in each group. We would expect to be able to detect fold changes as small as 1.54 (tumor cell group) and 1.86 (LIC group) between responders and non-responders at an alpha level of 0.05 and power of 80%.

9.2.3.17.2 Unfolded protein response: We will assess UPR activity at baseline, before and after treatment, and across AML molecular subtypes. Differences in transcript expression will be compared using 2-sample t-tests, paired t-tests for pre-/post- treatment expression, or mixed-effects modeling when multiple post-treatment measures are obtained. Baseline UPR activation, and UPR induction following treatment, will be compared between cytogenetic and molecular subtypes by analysis of variance. Fold-differences in Grp78 and CHOP median expression, and between spliced and non-spliced sXBP1, will be reported for each common cytogenetic phenotype. Samples were collected and analyzed from 35 of the initial 80 patients enrolled (43%). Based on this collection rate, with an enrollment of 1150 evaluable patients, we estimate that we will collect samples from 624 patients (312/treatment group). Because of the varying numbers of patients that are expected to be seen with each molecular abnormality, the power to detect differences will be dependent on the number of patients within each category. Chemotherapy responders and non-responders will be compared within each treatment group using t-tests or a nonparametric analogue to compare baseline UPR activity or induction after treatment. While the UPR transcript expressions will be separately analyzed, we will look for any significant correlations between the transcripts. Given the projected sample numbers and using variability data from preliminary analyses, fold-changes as small as 1.23 in the tumor cell group and 1.35 in the LIC group would be detectable using a conservative standard deviation of 1.0 (log-2 scale) at an alpha level of 0.05 and 80% power.

9.2.3.17.3 Correlation of protein data with clinical variables: We expect to analyze 624 pediatric 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. 1) *Determine if there is a protein classifier prognostic of clinical outcome*: After assignment of each patient to a training set or test set (n=312 each), supervised clustering analysis will be performed based on each clinical outcome variable, including CR, MRD, relapse risk and 2 year EFS. Multivariate analysis will be performed to determine if classifiers are independent predictors of response (or outcome) when controlling for known prognostic variables. 2) *Determine if any of the protein classifiers are independent of known outcome predictors*. Supervised clustering will be repeated for each prognostic variable in pediatric AML, including age ($\leq 1y$ vs. $> 1y$), cytogenetics (low risk vs. high risk), WBC count ($< 150,000$ vs. $\geq 150,000$ cells/ μ L) and MRD status at the end of induction ($< 0.1\%$ = MRD negative, $\geq 0.1\%$ = MRD positive). This will determine if putative RPPA protein classifiers provide an independent assessment of either ADE response or relapse risk.

9.2.3.18 Power Calculations Aim 1.2.16: Expression levels of wild type FLT3, and correlate with outcome and *in vitro* sensitivity to FLT3 inhibition

Descriptive statistics will be used to summarize FLT3 expression in those without FLT3/ITD+. The Kaplan-Meier method will be used to calculate estimates of OS, EFS, and DFS. Outcomes will be compared for subjects with expression levels above and below the median expression levels using, for example, the log-rank test and multivariate Cox regressions that adjust for treatment arm, and clinical, demographic, and laboratory features, such as diagnostic WBC, cytogenetic class, and race. We will identify the 30-50 subjects with highest wild type (WT) FLT3 expression and 30-50 subjects with lowest WT FLT3 expression. In these patients we will investigate the ability of sorafenib to induce cell apoptosis and cell

death. The IC₅₀ of sorafenib will be calculated for each case, and descriptive statistics will be used to characterize the IC₅₀ for the high and low expression groups. Appropriate statistics will be used to compare these parameters between the two groups, and to test the hypothesis that cases with high FLT3 expression will be more sensitive (i.e., will have a lower IC₅₀) than cases with low FLT3 expression. For example, with 30 cases in each group, assuming a intragroup standard deviation of 10 nM, we will be able to detect an IC₅₀ difference of 7.4 nM between groups (alpha of 0.05, power of 80%).

9.2.3.19 Power Calculations Aim 1.2.18: Development of aGVHD Prediction Algorithm

Simulation studies performed on the pilot data set indicate that a sample size of 250 patients will have appropriate statistical power to define the sensitivity and specificity of the prediction algorithm. Specifically, the mean and covariance matrix for the four (log-transformed) biomarker levels was estimated. Assuming a multivariate normal distribution for the measured biomarkers, a dataset of (log-transformed) biomarker levels for *N* hypothetical patients was created. Two-thirds of patients were randomly selected as a training set and the remaining one-third of patients as a validation set. A logistic regression model for the probability of developing aGVHD by Day 56 using the log-transformed biomarker levels was fit. Thresholds for the resulting fitted probabilities such that 50% of aGVHD- patients in the training set had a fitted probability no larger than that threshold, i.e. a specificity of 0.50, were set. The fitted logistic regression model and threshold was applied to the validation set to determine the specificity and sensitivity of in the validation set. This simulation was repeated 1,000 times to assess inherent variability. The following table displays the means sensitivity and specificity values with 95% confidence intervals across a range of sample sizes. Evaluating these test characteristics, a sample size of 250 was selected.

<i>N</i>	Training Set		Validation Set	
	Mean Specificity (95% CI)	Mean Sensitivity (95% CI)	Mean Specificity (95% CI)	Mean Sensitivity (95% CI)
200	0.50 n/a	0.69 (0.52, 0.84)	0.47 (0.31, 0.64)	0.65 (0.42, 0.86)
250	0.50 n/a	0.68 (0.52, 0.82)	0.48 (0.32, 0.64)	0.65 (0.43, 0.84)
300	0.50 n/a	0.68 (0.53, 0.80)	0.48 (0.32, 0.63)	0.66 (0.44, 0.82)

9.3 Interim Monitoring

9.3.1 Bortezomib Toxic Mortality and Adult Respiratory Distress Syndrome Monitoring

Continuous monitoring of toxicity will be performed in the first 100 non- HR FLT3/ITD patients randomized to receive Bortezomib. It is expected that a small percentage of patients will experience toxic mortality (TM). Interim monitoring will be performed by determining the probability of observing the observed number of treatment related deaths out of the number of patients that have completed that phase of therapy, if the expected TM rate is the true rate. If this probability is smaller than 0.01 at an interim analysis, then treatment modification or study termination will be considered. The tables below provide the type I error rates and power of rejecting the null (expected) TM rate when the true incidence is that given in the Table below and a boundary of 0.06 is used in the final analysis that includes all 100 patients.

Course	Expected TM rate	Type I error	Excess TM rate	Power
Induction I	2%	0.063	8%	91.9%
Induction II	1%	0.027	7%	91.3%
Intensification I	1%	0.059	7%	92.7%
Intensification II	2%	0.032	8%	69.1%

It is expected that a small percentage of patients will experience Grade 4 or higher ARDS. Continuous monitoring will be performed by determining the probability of observing the observed number of Grade 4 or higher ARDS out of the number of patients that have completed that phase of therapy, if the expected ARDS rate is the true rate. If this probability is smaller than 0.01 at an interim analysis, then treatment modification or study termination will be considered. The tables below provide the type I error rates and power of rejecting the null (expected) ARDS rate when the true incidence is that given in the Table below and a boundary of 0.06 is used in the final analysis that includes all 100 patients.

Course	Expected ARDS rate	Type I error	Excess ARDS rate	Power
Induction I	3%	0.050	10%	89.7%
Induction II	1%	0.027	7%	91.3%
Intensification I	1%	0.059	7%	92.7%
Intensification II	3%	0.043	10%	83.6%

Update as of Amendment #3A: Interim monitoring after the 100 non-HR FLT3/ITD patients was completed and stopping rules for adult respiratory distress syndrome and treatment related mortality were not triggered.

9.3.2 Interim Monitoring for Bortezomib Efficacy

Monitoring for efficacy with respect to EFS from study entry will utilize monitoring based on the Lan-DeMets criterion with α -spending function α^2 (truncated at 3 standard deviations) and 2.5% type I error. The study will also be monitored for futility using lower boundaries based on testing the alternative hypothesis at the 0.005 level. Formal monitoring analyses will be performed every 12 months with the first analysis performed after 25% of the expected information has been observed.

9.3.3 Monitoring of Sorafenib Arm C

9.3.3.1 Toxic Mortality for Sorafenib Patients during Chemotherapy Courses

Sorafenib dose modification or termination will be considered if 3 or more of the first 10 patients enrolled on Arm C cohort 3 experience a toxic mortality (TM) during any of the four courses of chemotherapy. Sorafenib dose modification or termination will be considered with probability 0.10 if the true TM rate is 10% and with probability 0.83 if the true TM rate is 40%.

9.3.3.2 Toxic Mortality for Sorafenib Patients during Maintenance

Sorafenib dose modification or termination during maintenance will be considered if 3 or more of the first 10 patients enrolled on Arm C cohort 3 experience a toxic mortality (TM) during Sorafenib Maintenance therapy. Sorafenib dose modification or termination will be considered with probability 0.10 if the true TM rate is 10% and with probability 0.83 if the true TM rate is 40%.

9.3.3.3 Sorafenib Discontinuation for Cardiac Toxicity

Sorafenib dose modification or termination will be considered if 3 or more of the subsequent 10 patients enrolled on Arm C cohort 3 have permanent sorafenib discontinuation for cardiac toxicity during any phase of treatment. Sorafenib dose modification or termination will be considered with probability 0.07 if the true permanent discontinuation rate is 10% and with probability 0.83 if the true permanent discontinuation rate is 40%.

9.4 Gender and Minority Accrual Estimates

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	174	161	335
Not Hispanic or Latino	706	709	1415
Ethnic Category: Total of all subjects	880	870	1,750
Racial Category			
American Indian or Alaskan Native	3	3	6
Asian	52	41	93
Black or African American	125	99	224
Native Hawaiian or other Pacific Islander	0	3	3
White	700	724	1424
Racial Category: Total of all subjects	880	870	1750

This distribution was derived from AAML0531.

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize the 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 NCI website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. 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).

All suspected or confirmed dose limiting toxicities should be reported to the Study Chair within 24 hours of toxicity ascertainment. All suspected or dose limiting toxicities should also be submitted through the CTEP-AERS mechanism. Additionally, all reportable toxicities are to be submitted to eRDES on the appropriate case report forms. See [Section 11](#).

10.2 Response Criteria for Patients with Acute Myeloid Leukemia

The following definitions for response will be used:

10.2.1 Complete Remission (CR)

The bone marrow is regenerating normal hematopoietic cells and contains < 5% leukemic blasts by morphology and no evidence of extramedullary disease (EMD). This designation occurs at the end of Induction II.

10.2.2 Refractory Disease/Induction Failure (RD)

Two consecutive cellular bone marrow evaluations (separated by at least 2 weeks) that contain $\geq 5\%$ leukemic blasts by morphology **or** evidence of EMD at the end of Induction II. Patients with CNS EMD at end of Induction I may stay on protocol therapy if CSF cytology is negative.

Presence of CNS leukemia as determined by CSF examination after 6 intrathecal cytarabine treatments in either Induction I or Induction II.

Presence of CNS leukemia as determined by CSF examination at the start of Induction II after treatment for CNS leukemia at start of Induction I.

10.2.3 Relapse

Morphologic relapse after CR is defined as a reappearance of leukemic blasts in the peripheral blood or $\geq 5\%$ blasts in the bone marrow not attributable to any other cause (e.g., bone marrow regeneration) after documented CR at end of Induction II. In the setting of recent treatment, if there are no circulating blasts and the bone marrow contains 5% to 20% blasts, **a repeat bone marrow performed at least a week later is necessary** to distinguish relapse from bone marrow regeneration. Should local flow cytometric analyses suggest relapse (by the reappearance of a similar immunophenotype to the original leukemia) in the presence of $< 5\%$ blasts, or $\geq 5\%$ blasts in a regenerating marrow, **a repeat bone marrow(s) performed at least a week later is necessary** to confirm relapse by morphologic methods. In such instances the date of recurrence is defined as the first date that more than 5% leukemic blasts were observed in the marrow.

Molecular and/or genetic relapse is characterized by reappearance of a cytogenetics or molecular abnormality after documented CR at end of Induction II. A bone marrow examination performed at least a week later is necessary to confirm cytogenetic or molecular relapse.

Extramedullary disease relapse is defined as appearance of cytologically proven extramedullary disease after documented CR in Induction II.

Recurrence of disease prior to the end of Induction II and still present at the end of Induction II will be considered refractory disease in accordance with [Section 10.2.2](#). Recurrence of disease prior to the end of Induction II but absent at the end of Induction II would be classified as a complete remission in accordance with [Section 10.2.1](#).

10.2.4 Unevaluable

Aplastic or severely hypocellular marrow with any blast percentage. In this instance, marrow evaluation should be repeated every 7 to 21 days until response determination can be made.

10.3 **Graft Failure following Allogeneic SCT**

10.3.1 Primary Graft Failure:

Failure to achieve an ANC $> 500/\mu\text{L}$ by Day 28 post-HSCT (Day 42 for cord blood or haploidentical donors) **and** $< 20\%$ donor cells as assessed by bone marrow chimerism studies **and** absence of leukemia relapse (which would be considered a relapse event).

10.3.2 Secondary Graft Failure:

Hematopoietic recovery followed by severe neutropenia (ANC $\leq 500/\mu\text{L}$) for 7 consecutive measurements on different days that is not caused by recurrent leukemia, active viral infection, or drugs. Improvement is defined as an ANC $> 500/\mu\text{L}$ for 7 days.

Marrow or peripheral blood stem cell reinfusion carried out any time after Day 0 because of inadequate hematopoietic function will be considered graft failure regardless of ANC values and marrow cellularity.

Acute and Chronic GVHD:

The staging of acute GVHD will follow National Marrow Donor Program (NMDP) guidelines ([Section 20.5](#)) but will include weekly capture of symptoms for the first 100 days post transplant. Details regarding the definition and diagnosis of chronic GVHD are listed in [Section 20.5](#).

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 that 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 Expedited 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

- o Death attributable to a CTCAE term.
- o Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- o 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.
- o Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with grade 5.
- o Death due to progressive disease should be reported as ***Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"*** under the system organ class (SOC) of the same name. 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, Fetal Death, 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) 230-0159. 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.

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 **Fetal Death**

Fetal death, defined in CTCAE as “*A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation*”, needs to be reported expeditiously, as **Grade 4 “Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)”**. Do NOT report a fetal death 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 “*A disorder characterized by cessation of life occurring during the first 28 days of life*” needs to be reported expeditiously, as **Grade 4 “General disorders and administration - Other (neonatal loss)”** 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 (eg, 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 NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting and are located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTCAE.

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 (eg, 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) 230-0159 (back-up: (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.				
Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	7 Calendar Days			24-Hour Notification 5 Calendar Days
Not resulting in				

Hospitalization ≥ 24 hrs	Not Required	7 Calendar Days	
<p>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.</p>			
<p>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.</p>			
<p>¹SAEs 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:</p> <ul style="list-style-type: none"> • All Grade 4, and Grade 5 AEs <p>Expedited 7 calendar day reports for:</p> <ul style="list-style-type: none"> • Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization • Grade 3 adverse events 			

11.10 Protocol Specific Additional Instructions and Reporting Exceptions

The following toxicities **DO NOT** require expedited reporting on this trial:

- Grades 1-4 hemoglobin, leukocytes, lymphocytes, neutrophils/granulocytes (ANC/AGC) or platelets, +/- hospitalization
- Grade 3 nausea, vomiting, diarrhea, anorexia, or fatigue
- Grade 3 alanine aminotransferase increased, aspartate aminotransferase increased that returns to Grade ≤ 1 or baseline within 35 days of the start of each course
- Grade 3 hyperglycemia, hypokalemia, hypophosphatemia, hypomagnesemia
- Grade 3 mucositis/stomatitis
- Grade 1-4 fever, fever and neutropenia, or infection, +/- hospitalization, with the exception of zoster (varicella) or herpetic infections.

The following toxicities **DO** require expedited reporting via the CTEP-AERS abbreviated pathway:

- All suspected or dose limiting toxicities (as defined in [Sections 4.7](#) and [Section 5](#) [*toxicities related to bortezomib and sorafenib*]) should be submitted through the CTEP-AERS mechanism.

11.11 Reporting of Adverse Events for commercial agents – CTEP-AERS abbreviated pathway

The following are expedited reporting requirements for adverse events experienced by patients on study who have not 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.

Table B

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 Days¹

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 <u>not</u> 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.

Patients enrolled on Arm D (note enrolling on Arm C) do NOT require routine reporting of study specific adverse events.

Patients enrolled on Arm C require routine reporting of study specific adverse events as outlined below.

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,
- All Grade 3 and higher non hematological Adverse Events,
- All grades of the following cardiac events: left ventricular systolic dysfunction, heart failure, electrocardiogram QT corrected interval prolonged,
- Dose limiting toxicities as defined in [Section 5](#) [*toxicities related to bortezomib and sorafenib*].
- All grades of peripheral neuropathy and gastrointestinal ileus.

11.13 Definition of Onset and Resolution of Adverse Events

Note: These guidelines below are for reporting adverse events through eRDE and do not alter the guidelines for CTEP-AERS reporting.

11.13.1 If an adverse event occurs more than once in a course (cycle) of therapy only the most severe grade of the event should be reported.

11.13.2 If an adverse event progresses through several grades during one course of therapy, only the most severe grade should be reported.

11.13.3 The duration of the AE is defined as the duration of the highest (most severe) grade of the Adverse Effects.

11.13.4 The resolution date of the AE is defined as the date at which the AE returns to baseline or less than Grade 1, whichever level is **higher** (note that the resolution date may therefore be different from the date

at which the grade of the AE decreased from its highest grade). If the AE does not return to baseline the resolution date should be recorded as "ongoing."

11.13.5 An adverse event that persists from one course to another should only be reported once unless the grade becomes more severe in a subsequent course. An adverse event which resolves and then recurs during a different course, must be reported each course it recurs.

12.0 RECORDS AND REPORTING

The Case Report Forms and the submission schedule are posted on the COG web site with each protocol under "*Data Collection/Specimens*".

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 Data and Safety Monitoring Committee

To protect the interests of patients and the scientific integrity for all clinical trial research by the Children's Oncology Group, the COG Data and Safety Monitoring Committee (DSMC) reviews reports of interim analyses of study toxicity and outcomes prepared by the study statistician, in conjunction with the study chair's report. The DSMC may recommend the study be modified or terminated based on these analyses.

Toxicity monitoring is also the responsibility of the study committee and any unexpected frequency of serious events on the trial are to be brought to the attention of the DSMC. The study statistician is responsible for the monitoring of the interim results and is expected to request DSMC review of any protocol issues s/he feels require special review. Any COG member may bring specific study concerns to the attention of the DSMC.

The DSMC approves major study modifications proposed by the study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results or other trials reported, increasing target sample size, etc.). The DSMC determines whether and to whom outcome results may be released prior to the release of study results at the time specified in the protocol document.

12.3 CRADA/CTA

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 AND SPECIMEN REQUIREMENTS

13.1 Immunophenotyping Studies

Immunophenotyping studies may be done at the local institution as part of the initial diagnostic work-up of acute leukemia. Although these immunophenotyping studies will be performed at multiple institutions and the nature of the technique and interpretation of results preclude the central review process, these data will be captured in this study as they are contributory to the diagnosis of AML. This information should be included in the bone marrow pathology report (to include the institutional morphologic assessment, immunophenotyping and cytochemistry). Flow cytometry immunophenotyping may be reported separately, if so, the complete flow cytometry report should be submitted. Immunophenotyping studies are essential in distinguishing AML from ALL. The 2008 world health organization (WHO) classification requires assessment of a certain set of cytoplasmic and surface antigens, with or without use of cytochemistry to define myeloid, lymphoid or mixed/ambiguous lineage (see Table 13.1.1). We strongly encourage the use of these markers:

- 1) MPO (by cytochemistry, flow cytometry or immunohistochemistry)
- 2) Cytoplasmic CD3 by flow cytometry
- 3) CD19 (by flow cytometry), and at least 2 additional B-cell markers such as CD10, CD79a or cytoplasmic CD22.
- 4) At least 3 markers of monocytic lineage: CD11c, CD14, CD64, lysozyme (by flow or immunohistochemistry) or NSE cytochemistry.

Although no single myeloid-lineage-associated antigen is expressed by the leukemic blast cells in every patient with AML, the CD33 antigen is the most frequent, detectable in approximately 70%-80% of cases.¹²⁹⁻¹³¹ Many patients with AML have blast cells that express other antigens associated with myeloid lineage differentiation (including CD11b, CD13, CD14, CD15, CD36, and glycoprotein Ib). However, a significant portion of patients with AML also express antigens associated with lymphoid-lineage differentiation.¹³⁰⁻¹³³ Use of the above recommended markers will aid in identifying acute leukemias with ambiguous or mixed myeloid/lymphoid lineages according to the WHO guidelines. Expression of certain antigens correlate with the morphologic subtype, such as CD11c, CD14, CD64, CD4, lysozyme in FAB M4 and M5 acute leukemias with monocytic differentiation, glycoprotein IIb/IIIa, glycoprotein Ib and Factor VIII antigen by megakaryoblastic leukemias (FAB M7)^{134,135} and glycoprotein expression by erythroblastic leukemias (FAB M6).¹³⁶

In this study, AML blasts will be classified based upon the 2008 WHO morphologic, immunophenotypic, and genetic criteria.¹¹⁷

Table 13.1.1

ASSIGNMENT OF LINEAGE IN MIXED PHENOTYPE ACUTE LEUKEMIA	
Lineage	Criteria
Myeloid lineage	<ul style="list-style-type: none"> Myeloperoxidase (by flow cytometry, immunohistochemistry or cytochemistry) <i>OR</i> <ul style="list-style-type: none"> Monocytic differentiation (express at least 2 of the following: CD11c, CD14, CD64, lysozyme or NSE)
T lymphoblastic	<ul style="list-style-type: none"> Cytoplasmic CD3 by flow cytometry <i>OR</i> <ul style="list-style-type: none"> Surface CD3 (rare)
B lymphoblastic	<ul style="list-style-type: none"> Strong CD19 and strong expression of at least one of the following: CD79a, cytoplasmic CD22 or CD10 <i>OR</i> <ul style="list-style-type: none"> Weak CD19 and strong expression of at least 2 of the following: CD79a, cytoplasmic CD22, CD10

For Patients with Myeloid Sarcoma (Chloroma):

If touch preps are able to be prepared from the tumor, the institutional pathologist should distribute/triage order(s) for FISH to be performed accordingly (e.g., RUNX1T1-RUNX1, CBFβ).

AML Reference Laboratory Immunophenotyping

Research monoclonal antibody studies will be performed at the AML Reference Laboratory at the Fred Hutchinson Cancer Research Center in Seattle. The Reference Laboratory will help local institutions with immunophenotyping studies in difficult cases, but the Reference Laboratory does not have as its charge the routine immunophenotyping of all AML samples.

13.2 Cytomorphologic Studies

13.2.1 Central Pathology Review

The purpose of central review is to establish concordance between the institution and the designated reviewer, as well as possible evaluation of clinically and biologically important subsets that may be appropriate for subset specific therapy. For this study, acute myeloid leukemia will be classified according to the WHO criteria

13.2.2 Instructions for Labeling Slides

Bone Marrow Slides (aspirate smears, core biopsy, and clot section) - Please label bone marrow slides with the patient's COG registration number and the surgical pathology identification (SPID) number (e.g. specific specimen number) from the matching bone marrow report.

Peripheral Blood Slides (Smears) - If there is a procedure or report number on the institutional laboratory report, please label the peripheral blood slides with the patient's COG Patient ID Number and the report (or procedure) number from the institutional report. If there is not a number on the report then please label the slide with the date the blood was drawn, patient initials and the COG Patient ID Number.

13.2.3 Required Materials for Pathology Review

Submit slides, all pertinent institutional reports and the AAML1031 pathology transmittal form to the Biopathology Center as described below. These samples should be submitted within 7 days of the procedure.

All Patients at the time of Disease Diagnosis Evaluation and Diagnosis of Refractory Disease or Relapse.

The following materials are required for patients with bone marrow involvement*:

- 1) A completed pathology report, bone marrow aspirate/biopsy report, and flow cytometry report.
- 2) One (1) peripheral blood smear (Wright-Giemsa stained).
- 3) A concurrent complete blood count including white blood cell differential (manual or automated)
- 4) One (1) representative bone marrow aspirate smear (Wright-Giemsa stained).
- 5) One (1) H&E stained slide from bone marrow clot section and/or one (1) stained slide from core biopsy.
- 6) Four (4) unstained slides from aspirate smears.
- 7) Four (4) unstained slides from clot section and/or core biopsy (preferably on charged slides).
- 8) Cytogenetic report, and molecular testing for chromosomal translocations (if performed).

RECOMMENDED ADDITIONAL MATERIALS:

- 1) Additional aspirate smears (stained and/or unstained)
- 2) Cytochemical stains (myeloperoxidase, non-specific esterase, PAS)
- 3) Immunohistochemical stains (ex. CD34, CD74, CD117, myeloperoxidase)
- 4) Tissue blocks

For patients with myeloid sarcoma (chloroma) submit the following materials for central pathology review*:

- 1) One (1) H&E stained slide and six (6) unstained recuts from the biopsy material of the myeloid sarcoma. Slides must be sent from a representative block with remaining diagnostic material.
- 2) Immunohistochemistry stained slides that were used to establish the diagnosis (this is strongly recommended, but not required; these special stained slides will be sent back at the conclusion of review, if requested by contributor).
- 3) A copy of pathology report (including results of special stains, flow cytometry and cytogenetics, if performed).

* Patients can be included without these specimens on approval of the Chair or Vice-chair of the study. This approval will be contingent on the adequacy of alternate materials (e.g., peripheral blood) for performance of all tissue based techniques for the study.

CENTRAL PATHOLOGY REVIEW MATERIALS WILL BE RETAINED BY COG AND WILL NOT BE RETURNED UNLESS OTHERWISE INDICATED ABOVE.

Ship materials by regular mail or using your institution's courier account to the Biopathology Center:

COG Biopathology Center – AAML1031
Nationwide Children's Hospital
700 Children's Drive, WA1340**
Columbus, OH 43205
Phone: 614-722-2894
Fax: 614-722-2897

** Be sure to include the room number. Packages received without the room number may be returned to the sender.

14.0 FLT3 MOLECULAR GENETIC ANALYSIS GUIDELINES AND REQUIREMENTS

Specimens for mutation analysis must be obtained prior to therapy initiation. The result of the mutation analysis will be used for risk assignment. NPM and CEBP α testing will no longer be done as part of the protocol as per Amendment #7A but remain advised as part of good clinical practice. All patients that do not have adequate specimens submitted will remain in their strata based upon other criteria.

For patients enrolled after Amendment #7A, adequate specimen must be submitted in order to determine ITD allelic ratio. Per Amendment #7A, patients with unknown FLT3/ITD status at End of Induction I on Arm D will be removed from study with no further data submission.

Results of mutation analysis are not required to be completed prior to therapy initiation, but samples must be collected prior to therapy initiation.

14.1 NIH-Funded Laboratory for Mutation Analysis at Diagnosis

In order to provide uniform mutation analysis that is used for risk-based therapy, all FLT3 mutation analysis will be performed as part of the protocol therapy at a single site through NIH funding by the Molecular Oncology Laboratory at Seattle Cancer Care Alliance (SCCA) **free of charge to the patient.**

14.2 FLT3 Mutation Analysis

As previously described, diagnostic DNA samples from each patient will be used to amplify appropriate region of the gene of interest gene using specifically designed fluorescent labeled primers.¹³⁷ **PCR products** will be resolved by microcapillary electrophoresis and the wild type and ITD products quantitated by GenescanTM or Genemapper software.¹³⁸ The ITD allelic ratio is determined for each patient by dividing the ITD product peak by the wild type product peak. High Risk status is defined as an allelic ratio > 0.4.

14.3 Specimen Requirements

Diagnostic bone marrow is required for FLT3 testing. Peripheral blood is only an acceptable alternative when bone marrow is unavailable. CEBP α and NPM1 testing remain advised but will need to be sent from the treating institution as a clinical test.

Bone marrow aspirate: collect a minimum of 1 - 3 mL of bone marrow in an EDTA vacutainer (purple/lavender top). Clearly label tube as bone marrow aspirate (BMA) along with draw date and draw time, and the patient name and DOB.

Peripheral blood (*only* if marrow specimen is not available): collect a minimum of 5 mL of whole blood in an EDTA vacutainer (purple/lavender top). Clearly label tube as blood (PB) along with draw date and draw time, and the patient name and DOB. ***Please state percent peripheral blast if a diagnostic specimen.***

Note: For patients with myeloid sarcoma, submit biopsy material (slides, paraffin block, etc), if available, in addition to the requirements described above.

Follow shipping instructions on the SCCA Molecular Oncology Requisition form.

14.4 Shipping

Samples should be shipped to SCCA at the address listed below. All specimens must be accompanied by a Molecular Oncology Requisition Form (see Specimen Shipping Forms on the protocol website). Diagnostic marrow/peripheral blood specimens are to be shipped priority overnight to the following address:

Molecular Oncology
Seattle Cancer Care Alliance
1100 Fairview Avenue North, Rm. D2-281
Seattle, WA 98109
Phone: (206) 667-2592
Fax: (206) 667-6092

See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information. The Lab hours are Monday - Friday 8:00 am – 4:30 pm, closed on weekends and holidays. For Saturday deliveries, laboratory needs be informed of the shipment. Please see the Molecular Oncology Requisition Form for details regarding sample collection, handling and shipping, and include this form with every request for specimen testing.

Please allow 5 - 7 business days for samples to be processed. Results will be communicated by fax.

Storage: If sample cannot be shipped immediately (i.e., sample is drawn on a Saturday, Sunday or holiday), store sample at room temperature and ship on the next business day.

14.5 Specimens Shipping Options

Diagnostic FLT3 mutational analysis specimens and optional biology research specimens (as described in [Appendix VII](#)) may be combined into one FedEx shipment at baseline. However, each specimen **must** be placed into **separate** biohazard bags (with the appropriate specimen transmittal form). Clearly label each bag with “Mutation analysis” or “AML Reference Lab” and send to SCCA lab address in [Section 14.4](#).

15.0 MRD ANALYSIS GUIDELINES AND REQUIREMENTS

Specimen submission for MRD analysis are required, must be obtained prior to therapy initiation as well as end of Induction I, and must be sent directly to Hematologies Inc. The result of the MRD analysis will be used for risk assignment. All patients that do not have adequate specimens submitted will remain in their strata based upon other criteria.

Results of diagnostic MRD evaluations are not required to be completed prior to therapy initiation, but samples must be collected prior to therapy initiation.

Submission of an additional sample at the end of Induction II, Intensification I, Intensification II and when a patient is determined to have relapsed/refractory disease is strongly encouraged. The results of these optional MRD analyses will not be returned.

15.1 NIH-Funded Laboratory for MRD Testing at Diagnosis

In order to provide uniform MRD analysis that is used for risk-based therapy, MRD testing will be performed as part of the protocol therapy at a single site through NIH funding. MRD analysis will be performed by Hematologies Inc. **free of charge to the patient.**

International sites should submit to Hematologies Inc. for MRD analysis. All samples will be processed free of charge, regardless of the country of origin.

15.2 MRD Analysis

All diagnostic and post remission specimens will undergo immunophenotypic characterization with

4-color multidimensional flow cytometry (MDF). Presence of post remission residual disease will be determined by identification of population of cells with aberrant cell surface markers and quantified as percent of total nucleated cells present in the sample. This data will be used to stratify those with and without minimal residual disease (MRD) after induction therapy.

15.3 Specimen Requirements

Bone marrow specimen is required for MRD testing. Peripheral blood is only an acceptable alternative when bone marrow is unobtainable.

Bone marrow aspirate: collect a minimum of 2 - 4 mL of bone marrow in a preservative-free sodium heparin vacutainer (green top). Clearly label tube as bone marrow aspirate (BMA) along with draw date and draw time, and the patient name and DOB. In cases where specimen is expected to take more than 24 hours to arrive, please add equal volume of RPMI 1640 medium to the marrow specimen.

Peripheral blood (*only* if marrow specimen is not obtainable): collect a minimum of 5 mL of whole blood in a preservative-free sodium heparin vacutainer (green top). Clearly label tube as blood (PB) along with draw date and draw time, and the patient name and DOB. ***Please state percent peripheral blast if a diagnostic specimen.***

Note: For patients with myeloid sarcoma, submit biopsy material (slides, paraffin block, etc) at diagnosis, if available, in addition to the requirements described above. For the end of Induction I time point, submit re-biopsy if available. Otherwise only submit samples as described above.

Optional timepoints: Specimens submitted for the optional timepoints should be labelled with the COG # and date/time of specimen collection only.

15.4 Shipping

Samples should be shipped to Hematologics Inc. Seattle, WA at the address listed below. All specimens sent to Hematologics Inc. must be accompanied by an MRD Requisition Form (see Specimen Shipping Forms on the protocol website). All specimens must be shipped priority overnight to the following address:

Hematologics, Inc.
3161 Elliot Ave. Suite 200
Seattle, WA 98121
Phone: (800) 860-0934 or (206) 223-2700
Fax: (206) 223-5550
Weekends and After Hours: (206) 264-4459

See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information. **Weekend Specimens:** The lab is staffed 6 days a week. For Saturday delivery, please use a *Saturday delivery* sticker and check the *Saturday delivery* box on the address label. Both sticker and checked box are necessary to insure proper handling.

Please allow 2 - 4 business days for samples to be processed. Results will be communicated by fax and entered into eRDE.

Storage: If sample cannot be shipped immediately (i.e., sample is drawn on a Saturday, Sunday or holiday), store sample with equal volume of RPMI medium at room temperature and ship on the next business day.

16.0 CYTOGENETIC ANALYSIS GUIDELINES AND REQUIREMENTS

16.1 Cytogenetic Analysis Overview

It is strongly recommended that all patients enrolled on AAML1031 have a cytogenetics study performed by a COG-approved laboratory at the time of initial diagnosis, relapse, and when refractory status is confirmed (for patients with normal diagnostic cytogenetic results: see [Section 16.1.1](#)). If the diagnostic cytogenetics/FISH findings are unknown/undetermined, the patient will be treated the same as patients with low risk cytogenetics.

The institutional CRA must inform the cytogenetics laboratory that the patient has been enrolled in a COG Myeloid study and that the cytogenetics/FISH data must be submitted within 2 weeks after registration on the AAML1031 protocol. See [Appendix V](#) for cytogenetics procedures and for the Sample Authorization Form for reflexive FISH testing.

Central cytogenetics review will only be performed for patients enrolled on Arm C after Amendment #7A.

16.1.1 Cytogenetic Analysis for Refractory Patients

Perform cytogenetic studies when patient is deemed refractory to protocol therapy *only if* diagnostic cytogenetics studies were normal. Note: Repeat the conventional G-banding cytogenetics study but no FISH unless indicated by new abnormality on G-banding. If there was FISH but no G-banding at diagnosis, then repeat G-banding, but not FISH unless FISH was abnormal. If no G-banding or FISH at diagnosis, both tests should be repeated.

DIAGNOSIS: KARYOTYPE/FISH	REFRACTORY DISEASE: KARYOTYPE/FISH
ABNORMAL RESULT	NOT NECESSARY TO REDO CYTOGENETICS
NORMAL G-BANDING, NORMAL FISH	YES G-BANDING, NO FISH
NORMAL G-BANDING, ABNORMAL FISH	YES G-BANDING, YES FISH
NO G-BANDING, NORMAL FISH	YES G-BANDING, NO FISH
NO G-BANDING, NO FISH	YES G-BANDING, YES FISH

16.2 Specimen Collection and Submission

16.2.1

It is strongly recommended that specimens for cytogenetic analysis be sent to a COG-approved institutional cytogenetics laboratory. If a COG institution does not have a COG-approved laboratory, the institution may send the samples for karyotyping/FISH studies to any COG-approved cytogenetics laboratory on a fee-for-service basis. Prior arrangements for performing cytogenetics and/or FISH studies should be made with the laboratory. An authorization form for the reflexive FISH testing signed and dated (by the physician or designees just as with any order) sent to the cytogenetics laboratory with the sample will simplify and enhance the ability to obtain FISH testing and results.

16.2.2

A minimum of 2 mL (optimal volume, 3 mL) of fresh whole bone marrow aspirated through a needle into a syringe containing sodium heparin (preservative-free is preferable) is recommended in all cases. A first or second draw, or a draw from a repositioned needle, is best to ensure a sufficient number of leukemic cells in the aspirate. The specimens should be kept at **room temperature** and transported to the institutional cytogenetics laboratory as quickly as possible (**always within 24 hours of collection**). If shipping is done

by overnight courier to an approved laboratory, that laboratory should be contacted to obtain instructions on transport. Some laboratories request specimens to be transferred in a 15 mL conical tube filled with RPMI-1640 and 15% heat-inactivated fetal calf serum. Specimens should be kept at ambient temperature.

16.2.3

If bone marrow cannot be aspirated, a bone marrow core biopsy should be submitted.

16.2.4

Peripheral blood (3 - 5 mL) collected in sodium heparin should be submitted as a back-up to the bone marrow when the marrow sample is suboptimal or unobtainable, if more than 20% circulating blasts are identified, or when a constitutional abnormality (e.g., trisomy 21) is a possibility. For documenting a constitutional abnormality, a PHA stimulated blood study should be performed.

For patients with myeloid sarcoma:

In addition to the requirements described above, if touch preps are able to be prepared from the tumor, the institutional pathologist should distribute/triage order(s) for FISH to be performed accordingly (e.g., MLL, RUNX1T1-RUNX1, CBF3).

16.2.5

Results of these studies should be submitted electronically to the corresponding coordinator of the Myeloid COG cytogenetics committee for central review (see below).

16.2.6

Requirements for Data Submission

The following are required for each case: a completed COG Cytogenetics/FISH Reporting Form (found on the eRDE) and 2 original karyotypes (different cells), with corresponding full-size metaphase spreads of each abnormal clone or 2 karyotypes of normal cells with corresponding full-size metaphase spreads in the case of normal cytogenetic analysis. This information must be sent to the appropriate coordinating cytogeneticist **within 2 weeks after registration on the AAML1031 protocol** (see [Appendix V](#)) and within 1 month of disease relapse, or confirmed refractory status (for patients with normal diagnostic cytogenetics results; see [Section 16.1.1](#)). Reports must be sent electronically (preferred as PowerPoint presentation). If the laboratory is unable to send an electronic file with the documentation, please contact the COG cytogenetics coordinator for your area for advice. Reporting forms must be filled out for all cases, whether or not the cytogenetic analyses were successful. A separate form is required for each specimen (i.e., bone marrow and blood) analyzed in each case. Published data on FISH screening report an overall incidence of occult aberrations in AML with normal karyotypes between 3% to 10%.¹³⁹⁻¹⁴¹ Thus, for all cases with only normal karyotypes FISH must be performed to evaluate for inv(16)/t(16;16), t(8;21), t(15;17) and 11q23. However, if the sample did not yield sufficient quantity of metaphases for analysis, -7 and -5/5q- should also be performed in addition to the inv(16)/t(16;16), t(8;21), t(15;17) and 11q23. Published data indicate that 10%-25% of patients who have one of these recurring translocations may have a concomitant deletion encompassing a region adjacent to the breakpoint on one of the involved derivative chromosomes. Such deletions may portend a less favorable prognosis.¹⁴²⁻¹⁴⁴ Thus, for all cases in which a recurring chromosomal abnormality [t(8;21), inv(16)/t(16;16), 11q23] is identified, it is recommended that FISH be performed with probes specific to that rearrangement, to evaluate any variant pattern that might include a deletion associated with a gene fusion. FISH forms should also be completed and sent to the coordinating cytogeneticist with images documenting the abnormal or normal FISH patterns. If the laboratory is not able to perform FISH, contact the COG cytogenetic coordinators for advice. Any discrepancies in interpretation between cytogenetics/FISH results from the laboratory and the coordinator will be discussed between the coordinators, with consult of additional cytogenetics committee members as necessary. The COG cytogenetics committee decision will prevail.

Cytogenetic Coordinators

Please send above materials by e-mail (preferably as a PowerPoint file) to the following COG Cytogenetics Laboratories.

WEST OF MISSISSIPPI RIVER

(INCLUDE MINNESOTA AND WISCONSIN),
AUSTRALIA, NEW ZEALAND, WESTERN CANADA
SEND TO:

Betsy Hirsch, Ph.D.

Telephone: 612-273-4952/3171

E-mail: hirsc003@umn.edu

EAST OF MISSISSIPPI RIVER

(EXCLUDE MINNESOTA AND WISCONSIN),
EUROPE, EAST CANADA
SEND TO:

Susana C. Raimondi, Ph.D.

Telephone: 901-595-3537/3536

E-mail: susana.raimondi@stjude.org

Recommendations for Case Analysis by Institutional, COG-Approved Cytogenetics Laboratories

See [Appendix V](#).

17.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

A Sample Submission Schedule is posted on the AAML1031 protocol website: <https://members.childrensoncologygroup.org/prot/AAML1031/AAML1031SampleSubmissionSchedule.pdf> This document provides an overview of the AAML1031 sample submission requirements and shipping information.

17.1 Pharmacokinetic and Pharmacodynamic Studies

17.1.1 **Biology Aim 1.2.6:** Pharmacokinetics of Bortezomib

Blood draws for measurement of plasma bortezomib concentrations were performed on Day 8 of Induction II of therapy. The bortezomib PK study was open to patients 2 - 16 years of age, randomized to Arm B. This study was closed to new patient participation on June 25, 2013, as accrual goals were met.

17.1.2 **Biology Aim 1.2.7:** Pharmacokinetics and Pharmacokinetic-Pharmacodynamics of Sorafenib

17.1.2.1 Pharmacokinetics of Sorafenib and N-Oxide metabolite

Steady state pharmacokinetics of sorafenib and N-oxide metabolite will be performed during Induction I, Induction II, Intensification I and Intensification II for all subjects receiving sorafenib who provide consent and assent. Additional samples will be collected during the maintenance sorafenib phase. The purpose of the pharmacokinetic study is to characterize the concentration x time profile and trough concentrations at steady state for sorafenib and the N-oxide metabolite in children and adolescents with AML receiving multiagent chemotherapy. The pharmacokinetic samples are separate from PK-PD study using the PIA assessment of FLT3. For adequate PK profiling at steady state, it is important to obtain one plasma sample prior to the administration of any sorafenib (during Induction I, prior to Day 10), and 3 additional samples during each Induction and Intensification course in subjects receiving sorafenib. All samples will be analyzed by HPLC MS/MS to quantify the sorafenib and N-oxide metabolite concentrations. This study is for research, no adjustment in individual patient dosing will be made based on the plasma concentrations. See [Appendix X](#) for collection, labelling and shipment instructions.

17.1.2.2 PK-PD of Sorafenib by PIA Assay

Plasma will be separated by centrifugation from blood samples collected during sorafenib administration and cryopreserved. Aliquots of 4×10^6 TF1/ITD cells (human AML cell line transfected with FLT3/ITD construct) will then be incubated for 1 hour with 0.5 mL of plasma from each time point. The cells will then be washed, lysed and analyzed for FLT3 phosphorylation by FLT3 immunoprecipitation and sequential immunoblotting with 4G10 anti-phosphotyrosine antibody and anti-FLT3 antibody. Densitometric analysis will be used to calculate the PIA (the percent inhibition of FLT3 phosphorylation of each time point relative

to the pre-treatment sample). These results will be used to determine whether a given dose of sorafenib is biologically active. Another 0.5 mL of plasma from the same time points will be collected for PK measurement to facilitate PK-PIA correlation. See [Appendix XI](#) for collection, labelling and shipment instructions.

17.1.3 Pharmacokinetics for Busulfan Dose Adjustments

17.1.3.1 1st Dose Pharmacokinetics

Please note: Pharmacokinetic studies must be performed in a CLIA, NATA, IANZ-certified laboratory or equivalent authorized accrediting body for non-US sites.

Target first dose pharmacokinetics should be performed in all patients regardless of busulfan dosing schedule.

Daily dosing should target an area under the curve (AUC) of 3,600 to 6,000 (micromole/liter)*minute per dose.

Every six hour dosing should target an AUC of 900 to 1,500 (micromole/liter)*minute.

Instructions for Q24 hr dosing: Busulfex concentrations will be determined in plasma by collecting blood into a green top sodium heparin tube. Samples should be collected at the end of the first 3 hour infusion, 195 minutes after start of infusion (or end of infusion plus 15 minutes), and 4, 5, 6, and 8 hours after the start of the first infusion. Samples should not be drawn from the lumen used to infuse busulfan. See table in [Section 7.3.1](#).

Institutional pharmacokinetic studies are acceptable. If pharmacokinetics cannot be performed at a local laboratory, samples can be shipped to the Seattle Cancer Care Alliance (as **fee for service**) or other regional laboratories experienced in this assay. Results are usually available in time for adjusting doses 3 and 4.

Instructions for Q6hr dosing: Busulfex concentrations will be determined in plasma by collecting blood into a green top sodium heparin tube. Samples should be collected at the end of the first 2 hour infusion, 135 minutes after start of infusion, 150 minutes after start of infusion, and 3, 4, 5, and 6 hours after the start of the first infusion. Samples should not be drawn from the lumen used to infuse busulfan. See table in [Section 7.3.1](#).

For Q6 hr infusion: In case infusion runs more or less than 2 hours, draw 1 sample immediately when infusion ends. Then, draw the next 2 samples 15 minutes apart and continue to draw 3, 4, 5, and 6 hours samples by counting from beginning of the infusion.

Institutional pharmacokinetic studies are acceptable. If pharmacokinetics cannot be performed at a local laboratory, samples may be shipped to the Seattle Cancer Care Alliance (as **fee for service**) or other regional laboratories experienced in this assay. Results are usually available in time for adjusting doses 7 through 16. Brief instructions are as follows:

Collect 1 - 3 mL of blood into sodium heparin tubes (green top) according to the schedule above. Place labeled samples immediately on wet ice and refrigerate. Centrifuge samples as soon as possible at 4°C. Separate plasma from RBCs. Store plasma at -20°C. Plasma tubes must be labeled with the patient's name, medical records number, and the date and actual clock time that the sample was drawn.

If using the SCCA Pharmacokinetics Laboratory, sample requisition form, collection schedule, and shipping instructions can also be found at: <http://www.seattlecca.org/busulfan-lab-samples.cfm>. Sites will be required to set up an institutional account with the SCCA PK laboratory for billing.

17.1.3.3 Guidelines for Adjusting Busulfan Dosing Based on Results of First Dose Pharmacokinetic Results

Once the results of the area under the curve (AUC) analysis of the first busulfan dose are available, subsequent doses will be adjusted to achieve an overall exposure target AUC of 900 - 1,500 (micromole/liter)*minute. The amount by which the dose is increased or decreased should be decided by the patient's attending physician in conjunction with the institutional toxicology laboratory director, or the Seattle Cancer Care Alliance Pharmacokinetics Laboratory (if using the SCCA laboratory).

In cases where the pharmacokinetics are performed locally, a second AUC analysis should be performed after administration of the first modified dose, and further dose adjustments made accordingly, whenever feasible.

17.2 Leukemia Biology Studies

17.2.1 **Biology Aim 1.2.10:**

Evaluate the prognostic significance of molecular MRD and its contribution to risk identification with MDF based MRD in patients with translocations amenable to quantitative RT-PCR (e.g., t(8;21), inv(16), t(9;11), WT1 expression).

As described in [Section 2.7.2](#) transcripts for Inv(16), t(8;21), t(9;11) and WT1 gene will be quantified at the end of each course and correlated with MRD by MDF and clinical outcome. See [Appendix VII](#) for further details.

17.2.2 **Biology Aim 1.2.11:**

To determine the leukemic involvement of the hematopoietic early progenitor cell and its role in defining response to therapy. In order to assess the early progenitor involvement of the leukemia, diagnostic specimens from patients that have known molecular abnormalities (FLT3, KIT, RAS, etc) will be sorted for the CD34+/CD33- early progenitor population. DNA will be extracted from the flow sorted cells and subjected to mutation analysis. Based on this method we will define the early progenitor involvement of the specific mutations. Presence or absence of early progenitor involvement will be correlated with disease response. See [Appendix VII](#) for further details.

17.2.3 **Biology Aim 1.2.12:**

To define the leukemic stem cell population in patients with AML.

In order to assess the ability of bortezomib to deplete the Leukemia Stem Cell (LSC) population in AML, diagnostic specimens will be sorted by 10-color flow to characterize an individualized leukemia initiating cell (LIC) population. The LIC population will be quantified at diagnosis and after the first course of treatment with either ADE or ADE + bortezomib. In patients with a sortable LIC population, RNA will be extracted from the flow sorted cells and subjected to low density TILDA mutation analysis. Based on this method we will determine the ability of bortezomib to deplete LSC in AML. If the patient is MRD+ after Induction I, a bone marrow sample should also be sent following Induction II. See [Appendix VIII](#) for further details.

17.2.4 **Biology Aim 1.2.13:**

To determine the prevalence and prognostic significance of molecular abnormalities of *WT1*, *RUNX1*, *MLL-PTD*, *TET2*, *c-CBL*, *KIT*, and other novel AML associated genes in pediatric AML.

Functional regions of the abovementioned genes will be amplified and genomic alterations will be evaluated by direct sequencing or by capillary electrophoresis. Presence of mutations will be correlated with disease characteristics and outcome. See [Appendix VII](#) for further details.

17.2.5 **Biology Aim 1.2.14:**

Correlate the expression of CD74 antigen as well as PSMB5 gene expression and mutation with response to bortezomib.

CD74 expression determination: Diagnostic marrow specimens from all patients enrolled on the study will be evaluated for CD74 expression by MDF. Expression level of CD74 will be subsequently correlated with CR and relapse rates in patients with and without exposure to bortezomib to determine whether CD74 exposure correlates with response to bortezomib.

PSMB5 mutations and expression: Diagnostic DNA from all consented patients will be subjected to amplification of individual exons for this gene and directly sequenced. All mutations will be confirmed and submitted to COG statistical office for future clinical correlation. RNA extracted from diagnostic specimens will be converted to cDNA and expression of *PSMB5* gene will be assessed by TaqMan using commercially available primers and probes. All expression data will be normalized to that in normal bone marrow as a point of reference and presented as a ratio of expression in normal marrow. All *PSMB5* expression data will be forwarded to COG statistical office for correlation with response to bortezomib. The aim is to evaluate sequence variations in *PSMB5* gene in patients treated in AAML1031 and correlate the mutations with response to Bortezomib. See [Appendix VII](#) for further details.

17.2.6 **Biology Aim 1.2.15:**

To evaluate the changes in protein expression and unfolded protein response (UPR) in patients with HR AML. Consenting patients treated with ADE or ADE with bortezomib and with a peripheral blast count > 1,000 cells/ μ L will have peripheral blood samples drawn prior to the start of therapy (Day 1, hour 0) and at 2 time points after the initiation of therapy (10 hours and 24 hours) to examine for changes in protein expression patterns using reverse phase protein lysate array (RPPA). Analysis will also assess cell stress pathways leading to myeloblast cell death, including analysis of the UPR proteins by RT-PCR, and analysis of autophagy by MDC flow cytometry. The goal is to determine if there are pre-treatment proteins predictive of either clinical response or proteins predictive of bortezomib drug resistance. See [Appendix IX](#) for collection, labelling and shipment instructions.

17.2.7 **Biology Aim 1.2.16:**

Determine the expression level of wild type FLT3, and correlate with outcome and *in vitro* sensitivity to FLT3 inhibition.

The quantitative expression level of surface wild type FLT3 will be measured prospectively on the baseline MRD samples by including the CD135 antibody in the diagnostic panel (see [Section 15.0](#)). The quantitative expression level of FLT3 mRNA will be measured prospectively by qRT-PCR using RNA isolated from diagnostic marrow blasts in consenting patients. The *in vitro* cytotoxic response of high vs. low expressing blasts will be performed retrospectively on viably cryopreserved diagnostic marrow blasts in consenting patients. High vs. low expressors will be selected from the leukemia bank based on CD135 and/or qPCR expression. Vials of cryopreserved cells will be thawed, exposed to a dose range of sorafenib and assayed using WST-1 and annexin V binding assays. See [Appendix VII](#) for further details.

17.2.8 **Biology Aim 1.2.17:**

To collect biology specimens at diagnosis, treatment time points, and relapse for future biology studies.

17.2.9 **Biology Aim 1.2.18**

To create a pediatric-specific algorithm to predict the occurrence of Grade 2 - 4 acute graft-versus-host disease prior to its clinical manifestations.

A combination of pre-transplant clinical variables and serum GVHD biomarker concentrations in the first weeks after SCT will be used to create an algorithm for the prediction of GVHD. Consenting patients treated with stem cell transplant will have peripheral blood drawn post-SCT on Days +7, +14 and +28 and serum concentrations of GVHD biomarkers (e.g. IL-2Ra, TNFR1, REG3a, and Elafin) will be measured by ELISA. See [Appendix XIII](#) for collection, labelling and shipment instructions.

18.0 QUALITY OF LIFE AND PARENTAL STRESS ASSESSMENTS

Closed to new patient accrual as of 05-15-2015. The COG memo posted on the study page 05-08-2015.

Participation in the Health Related Quality of Life and parental stress assessments is optional but strongly encouraged for patients between the ages of 2 years old and 18 years old (≥ 2 years and ≤ 18 years) with a parent/guardian who is able to speak and read English. Only the parent/guardian must be able to speak and read English to participate. That is, if the child does not speak or read English, the patient is still eligible for the HRQOL study and parents should complete the Parent-Proxy forms and the Pediatric Inventory for Parents (PIP). If both the parent/guardian and child speak and read English, both should complete their forms if the child agrees to self-report. Those under the age of 2 years and those 19 years old and over will not be eligible. Assessments will be administered at multiple time-points in order to assess short and long-term HRQOL and parental stress.

Measures of HRQOL will be the Pediatric Quality of Life (PedsQL) 4.0 Generic Core Scale,^{51,52} the PedsQL 3.0 Acute Cancer Module,⁵² and the PedsQL Multidimensional Fatigue Scale.^{52,53} These have been found to be reliable and valid in this population.⁵² For HRQOL assessments, parents or guardians will provide proxy assessments for all patients while for patient's ≥ 5 years of age who can understand English, self-report also will be completed if the child is able and agrees.

Parental stress will be assessed using the Pediatric Inventory for Parents (PIP) scale.^{54,55} The PIP was developed to measure parenting stress related to caring for a child with an illness. PIP has been found to be a reliable and valid tool assessing parental stress in the pediatric oncology population.⁵⁵ This instrument will only be completed by parents or guardians who are able to speak and read English.

See [Section 18.1.1](#) for details of questionnaires used.

18.1 Time points for Assessment

The HRQOL and parental stress assessments will be administered at multiple time-points in order to assess short and long-term HRQOL and parental stress. The first assessment will serve as a baseline and will be given within the first 2 weeks of treatment initiation. Although it would be optimal to obtain the baseline assessment prior to treatment initiation in Induction I, it is not feasible to reliably obtain such an assessment because the child may be ill at presentation and because treatment initiation often is a priority. The second and third assessments will be given on or after Day 21 of Induction II and Intensification I as one is recovering, to assess HRQOL prior to SCT and assess changes over time. To describe changes following consolidative therapy, assessments will be made 1 and 4 months following the start of Intensification II (chemotherapy only arm) or start of the preparative regimen for those who undergo SCT.

At 12 months from diagnosis, assessments will be made and then yearly until 3 years from diagnosis. These time-points should give an overall assessment of HRQOL and parental stress following completion of therapy. We will also see how HRQOL and parental stress changes with continued follow-up and possible development of late-effects of therapy.

Note: If a patient is removed from protocol therapy **prior** to treatment completion, no additional assessments are required. Patients that have completed planned therapy, continue to submit assessments until 3 years from diagnosis according to the schedule below.

The assessment time points are:

Time point	Timing
1	Within 14 days of Induction initiation (will serve as baseline)
2	On or after Day 21 of Induction II but prior to the start of Intensification I.
3	On or after Day 21 of Intensification I but prior to the start of Intensification II
4	1 month (\pm 7 days) from start of Intensification II for chemotherapy arms or preparative regimen for SCT
5	4 months (\pm 1 month) from start of Intensification II for chemotherapy arms or preparative regimen for SCT
6	12 months (\pm 1 month) from date of diagnosis
7	24 months (\pm 3 months) from date of diagnosis
8	36 months (\pm 3 months) from date of diagnosis

Age (Years)	Assessments			
	PedsQL - Generic	PedsQL - Cancer Module	PedsQL - Fatigue	PIP
2 - 4	X	X	X	X
5 - 7	X	X	X	X
8 - 12	X	X	X	X
13 - 18	X	X	X	X
Time to complete	68 questions: 15 - 20 minutes			42 questions: 8 - 12 minutes

18.1.1 Assessment

For all consenting participants, the appropriate questionnaires based on age will be determined and downloaded from the COG protocol web site by the institutional CRA.

The assessments will be performed by the CRA and family at the appropriate time-points described above. Completed forms will be reviewed by the CRA for completeness. Responses will be entered through the RDE system and the original forms retained by the institution. Questionnaires will be logged. Families that do not submit a questionnaire (parental or child form) at a particular time point for any reason will still continue to participate in the remaining questionnaire time points.

18.2 **Health Related Quality Of Life (HRQOL) and Parental Stress Questionnaires**

18.2.1 Pediatric Quality of Life Inventory (PedsQL)

Quality of life (QOL) will be measured by the PedsQL™ Scales. This instrument is a modular instrument for measuring HRQOL in children and adolescents between the ages of 2 and 18 years of age. The PedsQL™ 4.0 Generic Core Scales are multidimensional child self-report and parent proxy-report scales developed as a generic core measure of QOL. The Generic Core Scales measure physical functioning, emotional functioning, social functioning, and school functioning. In addition, there is a cancer disease-specific module that measures pain and hurt, nausea, procedural anxiety, treatment anxiety, worry, cognitive problems, perceived physical appearance, and communication. Validity of the PedsQL™ 4.0

Generic Core Scales and the Acute Cancer Module were established by known group comparisons and correlations with other measures of disease burden. Reliability was established by internal consistency reliability. Alpha coefficients for both self-report and proxy-report were greater than 0.90. Item response distributions were across the full-scale range, with no floor effects and minimal ceiling effects.¹⁴⁵

The PedsQL™ Multidimensional Fatigue Scale was designed to measure fatigue with the following scales: summary score, general fatigue, sleep/rest fatigue and cognitive fatigue. It has previously demonstrated good to excellent self-report measurement properties in pediatric patients ages 2 – 18 years with cancer (e.g., leukemia and brain tumor), as well as healthy children and adolescents. Across the ages, the PedsQL multidimensional fatigue scale total score for both child self-report and parent proxy-report approached or exceeded an alpha of 0.90. It has been recommended for individual patient analysis, making the PedsQL multidimensional fatigue scale total score a suitable outcome measure. It has recently been studied in the young adult population as well.⁵¹⁻⁵³

18.2.2 Pediatric Inventory for Parents (PIP)

The Pediatric Inventory for Parents (PIP) is a 42 item parent self-report rating of stress associated with caring for a child with a medical illness. It was initially developed for the oncology population but its use has extended to other medical illnesses. The PIP has measures in 4 domains: communication, medical care, role functioning, and emotional functioning. Each item is rated on two 5 point Likert scales assessing the frequency of the stressor and the difficulty of the issue/stressor for the parent. It has been found to have adequate internal consistency (alpha = .80 – .96) and construct validity.^{54,55,146}

19.0 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 (see Administrative Policy 3.9).

General Guidelines

The radiation therapy (RT) guidelines for this study were developed specifically for patients with AML who develop chloroma. RT is not required for any patient enrolled on this protocol; however, when RT may be indicated, care should be taken to use advanced treatment methods to ensure that the targeted volume receives the protocol specified dose and that normal tissue irradiation is limited and well documented. To achieve these goals, at a minimum, CT-based treatment planning is required. Patients who relapse after treatment on this protocol may eventually undergo total body irradiation. Knowledge of the dose administered to normal tissues will be essential to provide optimal care.

Required Benchmark and Questionnaires

RT using photons (either 3-D conformal [3-D CRT] or intensity modulated [IMRT]) and electrons will be allowed in this study. Protons are not allowed. Centers participating in this protocol using 3-D CRT are required to complete the 3-D benchmark; those using IMRT must complete the IMRT questionnaire and benchmark or phantom (QARC or RPC). Benchmark materials and questionnaires may be obtained from the Quality Assurance Review Center (www.qarc.org) and must be submitted before patients on this

protocol can be evaluated. For information regarding the IMRT phantoms, please contact the RPC (<http://rpc.mdanderson.org/rpc>).

Guidelines and Requirements for the Use of IMRT

Investigators using IMRT will be required to comply with the guidelines developed for the use of IMRT in National Cancer Institute sponsored cooperative group trials. These guidelines are available through www.qarc.org. These guidelines require that the protocol explicitly state their requirements and methods for localization and immobilization; the use of volumetric imaging; target and organ motion management; nomenclature, definitions and rationale for targets and organs at risk; target volume coverage and normal tissue dose constraints; effects of heterogeneity in tissues; and quality assurance.

19.1 Indications for Radiation Therapy

- No patient is required to receive radiation therapy on this protocol.
- Radiation therapy may be considered for patients who present with a chloroma that may result in irreversible loss of function.

19.2 Timing

- If the patient presents with chloroma that is producing or threatens to produce an irreversible neurologic deficit (e.g., visual impairment or myelopathy), radiation therapy may be given at the beginning of induction therapy.

19.3 Emergency Radiation Therapy

Radiation therapy may be delivered on an emergent basis to patients with spinal cord compression, loss of vision or other function-threatening conditions. The decision to irradiate emergently should be made by the treating physicians. Although it is recommended to complete a continuous course of treatment once initiated, it will be the decision of the treatment team to deliver the entire course of the emergency radiotherapy or stop early if symptoms quickly resolve.

19.4 Equipment and Methods of Delivery and Verification

Equipment	Photons (any energy)	Electrons (any energy)	IMRT (4-10MV)
Linear Accelerator*	X	X	X

* For tumors adjacent or included in lung tissue, photon beam energy should be ≤ 10 MV.

19.4.1 Treatment planning

CT-treatment planning is required for all patients irradiated on this protocol. Slices no more than 0.5 cm thick (0.2 - 0.3 cm is recommended) shall be taken through out the extent of the irradiated volume. CT (volumetric) based planning is required to optimize dose to the PTV while protecting normal tissues. Organs within the irradiated volume should be contoured including those required by treatment site ([Section 19.9](#)). A DVH is necessary to determine target coverage and evaluate dose to normal tissues. In the event that a patient must start emergently with a non-volumetric treatment plan, a volumetric plan will be accomplished as soon as is reasonably possible and the previously utilized beams should be incorporated into the composite plan.

19.4.2 In-room verification of spatial positioning

2D or volumetric imaging may be used to verify correct patient positioning. 2D portal imaging using an electronic portal imaging device (MV or kV) is the most common method, particularly when the target volume possesses a fixed spatial relationship with visualized bony anatomy. Film is discouraged but is acceptable. For IMRT and 3-D CRT treatments, a pair of images (usually orthogonal AP and lateral) is

required to verify that the isocenter is in correct alignment relative to the treatment plan; these may be MV or kV images. Volumetric imaging for position verification may be in-room kV or MV cone beam or conventional CT imaging.

19.4.3 Imaging submission requirements

For 2D imaging, the portal and isocenter setup verification images should be submitted along with the digitally reconstructed radiographs from the treatment plan. Images should be sent in the form of screen captures or in hardcopy format. For volumetric imaging, submit representative axial slices showing the treatment scan registered to the planning scan and indicating correct positioning either by isocenter location or overlay of the isodoses.

19.5 Target Volumes

19.5.1 Standard tumor and target volume definitions

International Commission on Radiation Units and Measurements (ICRU) Reports 50 and 62 (www.icru.org) define prescription methods and nomenclature that will be utilized for this study. Treatment planning will be based on the following definitions and applies to the treatment site:

Photons

- *Gross tumor volume (GTV)* is the volume at the first sign of visible or palpable disease.
- *Clinical target volume (CTV)* includes the GTV and sites with potential occult tumor involvement including lymph nodes adjacent to the GTV that may be clinically involved. The CTV margin for this protocol will be 1cm. The CTV may be anatomically confined at barriers to tumor infiltration.
- *Planning target volume (PTV)* is the CTV surrounded by a geometric margin to account for variability in set-up, breathing or motion during treatment. The PTV margin for cranial including head and neck sites will be 0.5cm. The PTV margin for extracranial sites should be 0.5 - 0.7 cm

19.6 Target Dose

19.6.1 Dose Definition

Photon dose is to be specified in centigray (cGy)-to-muscle.

19.6.2 Prescribed dose and fractionation

The protocol-specified dose per fraction is 150 or 200 cGy. The treatment should be limited to one fraction per day. Five fractions should be given per week. There may be an exception if a department is closed on a major holiday but an effort should be made to minimize days missed. The dose per fractionation may be reduced from 200 cGy to 150 cGy when large volumes are treated (*i.e.*, whole abdomen and pelvis) or when tolerance is poor (*i.e.*, mucositis or diarrhea). Changes to the fractionation regimen should be noted in the treatment record and submitted information.

Table 19.6.2 Protocol-specified dose for chloroma

Fractionation	Total Dose (cGy)
Gross disease (fractionation 200 cGy/day)	2,000
Gross disease (fractionation 150 cGy/day)	2,050

19.6.3 Dose uniformity

At least 95% of the protocol-specified dose should encompass 100% of the PTV and no more than 10% of the PTV should receive greater than 110% of the protocol dose as evaluated by DVH. The 100% isodose should be equal to the protocol specified dose. Wedges, compensators and other methods of generating more uniform dose distributions are encouraged.

19.6.4 Tissue heterogeneity

Calculations must take into account tissue heterogeneity and should be performed with CT-based treatment planning to generate dose distributions and treatment calculations from CT densities. When IMRT is used in lung, the heterogeneity correction algorithm must be approved by QARC. For questions about heterogeneity corrections or approved algorithms, please contact QARC (www.QARC.org).

19.6.5 Environment of care - Interruptions, delays and dose modifications

There will be no planned rests or breaks from treatment, and once radiation therapy has been initiated, treatment will not be interrupted except for any life-threatening infection or severe hematologic toxicity defined as ANC < 300/ μ L or platelets < 40 000/ μ L during the course of treatment. Under these circumstances, radiation therapy shall be delayed until the counts have recovered. Blood product support should be instituted according to institutional/protocol guidelines. The reason for any interruptions greater than 3 treatment days should be recorded in the patient's treatment chart and submitted with the QA documentation. There should be no modifications in dose fractionation due to age or field size.

19.7 **Treatment Technique**

19.7.1 Beam Configuration

Every attempt should be made to minimize dose to organs at risk without compromising coverage of the target volume. Three-dimensional conformal therapy (coplanar or non-coplanar) or IMRT are required to minimize dose to normal tissues.

19.7.2 Field Shaping

Field shaping will be done with either customized cerrobend blocking or multileaf collimation.

19.7.3 Simulation including patient positioning and immobilization

19.7.3.1 Patient positioning

Reproducible setups are critical and the use of immobilization devices is strongly encouraged. The patient may be treated in any appropriate, stable position. Consideration should be given to implications for inter- and intra-fraction motion when using non-standard positioning.

19.7.3.2 Immobilization devices

Standard immobilization devices for the torso, extremities or head and neck are to be used. For IMRT delivery approaches, the methods used for localization and immobilization of both patient and tumor are critical. The imaging studies should provide a clear assessment of the target volume with the patient in the treatment position.

19.7.4 Special considerations

Anesthesia or sedation may be required in certain patients, such as very young patients, to prevent movement during simulation and daily treatments.

19.7.5 Motion Management and Margins to Account for Target Volume and Organ Motion

Considering motion of normal tissues and target volumes is important. The internal target volume (ITV) is defined as the CTV surrounded by the internal motion (IM) component of the PTV and is meant to account

for potential motion of the CTV. The planning organ at risk volume (PRV) includes the organs at risk (OAR) surrounded by a margin to compensate for physiologic change in the target volume. If adequate clinical data do not exist to define the IM component of the PTV or the PRV margin, the following suggestions are provided:

- A margin of at least 0.5 cm should be added to any OAR to form the PRV.
- For a CTV susceptible to physiologic motion, a margin of at least 0.5 cm should be added to the CTV prior to PTV margin expansion or a PTV margin of 1.0 cm should be chosen.
- For tumors of the thorax or abdomen, an assessment should be made to determine the extent of motion present. PTV margins should include this motion as a component.
- IMRT may be used for tumors of the thorax only if the degree of tumor motion is assessed and can be limited to 0.5 cm in any direction. If required to achieve this goal, techniques for managing or suppressing tumor motion shall be applied.
- A description of the method used and evidence (i.e., observed motion during fluoroscopy, motion of surrogate markers using camera systems, or analysis of 4-D CT) of the remaining tumor motion should be submitted on the Motion Management Reporting Form with the Quality Assurance Documentation materials as noted in [Section 19.10](#).

19.8 Organs at Risk

The organs at risk guidelines in this section are recommendations. If the recommended doses to the organs at risk are exceeded because of target volume coverage requirements or other conditions, an explanation should be included in the quality assurance documentation. In some cases, photon IMRT may be the preferred treatment method to meet these recommendations and the required target volume coverage guidelines.

Table 19.8: Organs at risk dose recommendations

Organ	Volume (%)	Dose (cGy)
Single organs		
Lens	100%	600
Paired organs		
Kidney (bilateral)	50%	2400
Kidney (bilateral)	100%	1440
Lung (bilateral)	20%	2000
Lung (bilateral)	100%	1500

Paired organs - % refers to one of the paired organs unless specified as bilateral (kidney, lung) in which both of the paired organs are included in the %.

19.9 Dose Calculations and Reporting

19.9.1 Prescribed Dose

The prescribed dose for each target volume and/or phase of treatment shall be calculated and submitted on the RT-1/IMRT Dosimetry Summary Form. If IMRT is used, the monitor units generated by the IMRT planning system must be independently checked prior to the patient's first treatment. Measurements in a QA phantom can suffice for a check as long as the patient's plan can be directly applied to a phantom geometry. The total prescribed dose shall be calculated and reported on the RT-2 Radiotherapy Total Dose Record.

19.9.2 Normal Tissue Dosimetry

The total dose to the critical organs indicated should be calculated whenever they are directly included in a radiation field. The total dose shall be calculated and reported on the RT-2 Radiotherapy Total Dose Record form and the appropriate dose-volume histograms should be submitted. If IMRT is used, a DVH must be submitted for a category of tissue called “unspecified tissue,” which is defined as tissue contained within the skin, but which is not otherwise identified by containment within any other structure.

Table 19.9.2: Required normal tissue DVH data according to primary treatment site(s)

Treatment Area	Required DVH
Head	Optic Nerve
	Optic Chiasm
	Pituitary
	Right and Left Cochlea
Neck	Thyroid
Chest	Lung
	Heart
Abdomen	Liver
	Right and Left Kidney
Pelvis	Bladder
	Rectum
Spinal Cord	Spinal Cord

19.10 Quality Assurance Documentation

Key Points

- No on-treatment review will be required for this study.
- Non-IMRT case: within 1 week of the completion of radiotherapy submit the forms indicated below and include information for each treated site(s).
- IMRT case: within 1 week of the completion of radiotherapy submit forms indicated below and treatment data in digital format.

IMRT ONLY

Institutions are required to submit the treatment plan in digital format. An institution’s treatment planning system must have the capability of exporting data in 1 of 2 formats:

- RTOG Data Exchange Format, Version 3.20 or later (specifications at http://itc.wustl.edu/exchange_files/tapeexch400.htm); or
- DICOM 3.0 in compliance with the Advanced Technology Consortium's (ATC) DICOM 3.0 Conformance Statement. A list of commercial systems that are known to have this capability are listed on the ATC Website (http://atc.wustl.edu/credentialing/atc_compliant_tps.html).
- The data may be submitted on a CD or sent electronically via ftp to QARC. Instructions for digital submissions may be found on the QARC Website - www.qarc.org, under Digital Data, RT Treatment Planning.

Please submit the following for the Treatment Volume:

External Beam Treatment Planning System

- Digitally reconstructed radiographs (DRR) or simulator films for each treatment field and orthogonal (anterior/posterior and lateral) images for isocenter localization for each group of concurrently treated beams. When using IMRT, orthogonal isocenter images are sufficient.

- Isodose distributions for the composite treatment plan in the axial, sagittal and coronal planes at the center of the treatment or planning target volume. The planning target volume, isocenter and the normalization method must be clearly indicated.
- Dose volume histograms (DVH) for the composite treatment plan for all target volumes and required organs at risk. A DVH shall be submitted for the organs at risk specified in [Section 19.8](#). When using IMRT, a DVH shall be submitted for a category of tissue called “unspecified tissue”. This is defined as tissue contained within the skin, but which is not otherwise identified by containment within any other structure.
- Treatment planning system summary report that includes the monitor unit calculations, beam parameters, calculation algorithm, and volume of interest dose statistics.
- Beams-eye-view (BEV) of portals showing collimator, beam aperture, target volume and critical structures are required when not using IMRT.

Digital Data

- Submission of the treatment plan in digital format is required. Please refer to www.QARC.org and click on "Digital Data" for guidelines regarding digital submission. All submissions, including those that are digital, require hard copy submission of the other items included in this list. If there are any problems with digital data submission, please contact QARC.

Supportive Data

- All diagnostic imaging used to plan the target volume. This includes CT or MRI PRIOR to attempted surgical resection of the primary tumor. Digital format is preferred.
- Radiotherapy record (treatment chart) including prescription and daily and cumulative doses to all required areas and organs at risk.
- Documentation of an independent check of the calculated dose when IMRT is used.
- If the recommended doses to the organs at risk are exceeded, an explanation should be included for review by the QARC and the radiation oncology reviewers.
- If emergency RT is administered, documentation should be provided in the form of the RT-2 Total Dose Record Form and the radiotherapy record (treatment chart).

Forms

- RT1/IMRT Dosimetry Summary Form.
- Motion Management Reporting Form (if applicable, see [Section 19.7](#)).
- RT-2 Radiotherapy Total Dose Record Form.

NON-IMRT

Forms

- RT-2 Radiotherapy Total Dose Record Form.
- Radiotherapy record (treatment chart) including prescription and daily and cumulative doses to all required areas and organs at risk.

These data should be forwarded to:

Quality Assurance Review Center
Building A, Suite 201
640 George Washington Highway
Lincoln, RI 02865-4207
Phone: (401) 753-7600
Fax: (401) 753 7601

Questions regarding the dose calculations or documentation should be directed to:

COG Protocol Dosimetrist
Building A, Suite 201
Quality Assurance Review Center
640 George Washington Highway
Lincoln, RI 02865-4207

20.0 HEMATOPOIETIC TRANSPLANT 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).

All transplants performed on COG trials must occur at FACT-accredited SCT programs (see administrative policy 3.3).

20.1 Pre-SCT Conditioning

Refer to [Sections 4.24 - 4.25](#).

20.2 Admission Criteria (see [Section 7.3](#))

Documented remission after first intensification (BMA/Biopsy)
7/8 or 8/8 allele HLA-match related donor
Cardiac (Echo/ECG): Shortening fraction by echocardiogram $\geq 28\%$ or ejection fraction $\geq 55\%$
Electrolytes: Within normal institutional limits
Pulmonary Function Tests if age allows: DLCO $> 50\%$
Renal: Creatinine Clearance / GFR ≥ 60 mL/minute/1.73m²
Lumbar Puncture: No leukemic infiltrate
Documented HSV and CMV titers
Hepatitis B Surface Antigen Serology, and Hepatitis C Serology
HIV by serology: Negative
Hepatic: Transaminases < 2.5 x normal; Total Bilirubin < 2 mg/dL

20.2.1 HLA Typing

At the time of diagnosis, high resolution HLA typing at HLA-A, B, C and DRB1 should be performed on all patients, their biological parents, and is recommended for any full siblings. For full siblings in whom neither parent, recipient or donor are homozygous at one of the 4 loci, high resolution typing in that sibling may be deferred if not routinely done at an institution. High resolution typing is recommended at diagnosis for all children, even those with no siblings, so that it is available for rapid alternative donor search in case of induction failure, persistent disease after Induction I, high-risk cytogenetics, or relapse.

20.3 Bone Marrow Harvest, Processing, Dose and Infusion

Bone marrow will be harvested and infused on Day 0. Peripheral blood stem cells are not permitted to be used with MFD SCT on this study. A minimum of 2×10^8 nucleated bone marrow cells/kg of the recipients' ideal weight must be collected, but 4×10^8 nucleated bone marrow cells/kg is desirable. There will be no manipulation of the bone marrow to deplete T cells. Bone marrow should be processed to deplete red blood cells if there is a major ABO mismatch (e.g., O type in recipient and A type in donor) or plasma depleted

in the case of a minor ABO incompatibility (e.g., O type in donor and A type in patient) to avoid an acute hemolytic transfusion reaction. Bone marrow will be infused according to standard operating procedure.

Donor bone marrow grafts should be assessed for quantity of total nucleated cells and reported.

20.4 Supportive Care Guidelines

(Also see [Appendix I](#))

20.4.1 Blood Product Support

Irradiated and leukodepleted packed red blood cell and platelet transfusions will be given as indicated clinically at the discretion of the managing attending physician.

20.4.2 Infectious Prophylaxis

Trimethoprim-sulfamethoxazole (TMP-SMX): 2-3 mg/kg/dose PO or IV BID to begin on day of admission through Day -2 daily, then twice weekly on consecutive days starting on Day 21 and continuing for 1 year. Dapsone or pentamidine may be used in patients who are allergic to TMP-SMX (Bactrim) or in patients with myelosuppression attributed to TMP-SMX (Bactrim).

Fluconazole: 5 mg/kg dose, maximum of 400 mg/dose, daily QD IV or PO for the first 28 days.

Acyclovir: Standard doses per institutional guidelines for 1 year after SCT. Acyclovir will be used in patients who have been exposed to HSV, VZV or CMV or whose donors have been infected by CMV.

Weekly CMV screening from Day +14 through Day +100 and pre-emptive treatment with ganciclovir.

20.5 COG Stem Cell Committee Consensus Guidelines for Establishing Organ Stage and Overall Grade of Acute Graft versus Host Disease (GVHD)

20.5.1 Reporting Requirements for Acute GVHD in COG Studies

In an attempt to standardize reporting of acute GVHD, the COG Stem Cell Transplantation Committee has adopted a modification of guidelines that were originally developed at the University of Michigan.

Table 1 outlines standard criteria for GVHD organ staging. However, confounding clinical syndromes (such as non-GVHD causes of hyperbilirubinemia) may make staging GVHD in a given organ difficult. In addition, timing of organ specific symptoms affects whether that symptom is more or less likely to be true GVHD. Please refer to **Tables 2 and 3** to assist you in deciding whether to attribute these clinical findings to GVHD, especially in situations where a biopsy is not possible. For additional help, please see the text which follows the tables.

Table 4 reviews the approach to assessing GVHD as acute, chronic, or the overlap between the two.

Finally, *engraftment syndrome* will be reported separately from the GVHD scoring presented below.

20.5.2 Engraftment Syndrome

A clinical syndrome of fever, rash, respiratory distress, and diarrhea has been described just prior to engraftment in patients undergoing unrelated cord blood and mismatched transplantation. If in the judgment of the local investigator a patient experiences this syndrome, details of the event should be reported when requested in the study CRFs.

Modified Glucksberg Staging Criteria for Acute Graft versus Host Disease

Table 1. Organ Staging (See tables and text below for details)

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dL	Adult: < 500 mL/day Child: < 10 mL/kg/day
1	Maculopapular rash < 25% BSA	2 - 3 mg/dL	Adult: 500 - 999 mL/day Child: 10 - 19.9 mL/kg/day <i>Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.</i>
2	Maculopapular rash 25% – 50% BSA	3.1 - 6 mg/dL	Adult: 1,000 - 1,500 mL/day Child: 20 - 30 mL/kg/day
3	Maculopapular rash > 50% BSA	6.1 - 15 mg/dL	Adult: > 1,500 mL/day Child: > 30 mL/kg/day
4	Generalized erythroderma plus bullous formation and desquamation > 5% BSA	> 15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

For GI staging: The “adult” stool output values should be used for patients > 50 kg in weight. Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is presumed to be 50% of total stool/urine mix.

For Stage 4 GI: the term “severe abdominal pain” will be defined as:
 (a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
 (b) Pain that significantly impacts performance status, as determined by the treating MD.

If colon or rectal biopsy is +, but stool output is < 500 mL/day (< 10 mL/kg/day), then consider as GI Stage 0.

There is no modification of liver staging for other causes of hyperbilirubinemia

Overall Clinical Grade (based on the highest stage obtained):

Grade 0: No Stage 1 - 4 of any organ

Grade I: Stage 1 - 2 skin and no liver or gut involvement

Grade II: Stage 3 skin, or Stage 1 liver involvement, or Stage 1 GI

Grade III: Stage 0 - 3 skin, with Stage 2 - 3 liver, or Stage 2 - 3 GI

Grade IV: Stage 4 skin, liver or GI involvement

Table 2. Evaluating Liver GVHD in the Absence of Biopsy Confirmation

Establishing liver GVHD with no skin or GI GVHD		
No Skin/GI GVHD Day 0 - 35	Assume no liver GVHD unless proven by biopsy	
No Skin/GI GVHD Day 36 - 100	If NO other etiology identified, NO improvement with stopping hepatotoxic medications/TPN: Stage as liver GVHD	If other etiology identified or improves with stopping hepatotoxic drugs/TPN: Do not stage as liver GVHD
Establishing liver GVHD with skin or GI GVHD and other cause of hyperbilirubinemia		
Skin and/or GI GVHD present	Worsening bilirubin level (includes worsening just prior to onset of skin or GI tract GVHD) OR stable elevated bilirubin despite resolution of non-GVHD cause of increased bilirubin: Stage as liver GVHD	Stable or improving bilirubin after diagnosis of skin or GI GVHD, irrespective of treatment: Do not stage as liver GVHD
Changing liver GVHD stage with other cause of hyperbilirubinemia		
Skin and GI GVHD stable, improving, or absent	Liver GVHD staging is carried forward without increase in stage until other disease process resolves (e.g., if TTP is diagnosed in the presence of Stage 2 liver GVHD, the liver GVHD Stage 2 is carried forward despite rising bilirubin level until TTP is resolved. If there is no liver GVHD – Stage 0 – and new onset TTP, the Stage 0 is carried forward until TTP is resolved).	
Skin and/or GI GVHD worsening	<p>Liver GVHD is staged according to the Glucksberg criteria. The elevated bili is attributed to GVHD alone.</p> <p>Thus, when skin or GI GVHD is worsening, there is no downgrading of liver GVHD staging for other causes of hyperbilirubinemia. (e.g., if TTP is diagnosed in the presence of Stage 2 liver GVHD and worsening skin or GI GVHD, the liver is staged according to the actual bilirubin level even if some of the rise in bilirubin is attributed to TTP).</p> <p>Similarly, even if there is no liver GVHD at onset of a new process, (such as TPN cholestasis), but skin or GI GVHD worsen during that process, then liver GVHD is diagnosed and staged according to the height of the bilirubin.</p> <p>There is one exception to this: the diagnosis of TTP, with high LDH and unconjugated bilirubin precludes the diagnosis and staging of new liver GVHD in the absence of a confirmatory liver biopsy.</p>	

Table 3. Evaluating GI GVHD in the Absence of Biopsy Confirmation

Establishing GI GVHD with new onset diarrhea and no skin or liver GVHD		
No Skin/liver GVHD Day 0 through engraftment	Assume no GI GVHD unless proven by biopsy	
No Skin/liver GVHD Engraftment through Day 100	NO other etiology of diarrhea identified: Stage as GI GVHD	Any other etiology of diarrhea identified: Do not stage as GI GVHD

Establishing GI GVHD with pre-existing diarrhea and skin or liver GVHD		
Skin and/or liver GVHD present	Worsening diarrhea (includes worsening just prior to onset of skin or liver GVHD) OR persistent diarrhea despite resolution of non-GVHD cause: Stage as GI GVHD	Improving diarrhea after the diagnosis of skin or liver GVHD (irrespective of treatment) OR persistent diarrhea without resolution of underlying non-GVHD cause: Do not stage as GI GVHD

20.5.3 Differentiating Acute GVHD, Chronic GVHD, and Overlap Syndrome

There is often confusion differentiating acute from chronic GVHD, especially in the setting of reduced intensity transplants, DLI and new prophylactic treatments. The NIH Working Group recently published new classifications for GVHD:

Table 4. Acute GVHD, Chronic GVHD, and Overlap Syndrome

Category	Time of Symptoms after HCT or DLI	Presence of Acute GVHD features	Presence of Chronic GVHD features
Acute GVHD			
Classic acute GVHD	≤ 100 d	Yes	No
Persistent, recurrent, or late-onset acute GVHD	➤ 100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

- Scoring of acute GVHD may need to occur past Day 100. In particular, patients should continue to be scored for acute GVHD when classic acute GVHD symptoms (maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea - particularly if bloody and ileus) persist past Day 100 or if identical symptoms previously scored as acute GVHD resolve and then recur within 30 days during immunosuppression taper but past Day 100.
- Those patients being scored as having acute GVHD should NOT have diagnostic or distinctive signs of chronic GVHD.
- Patients with both acute and chronic symptoms should be diagnosed as having Overlap Syndrome and scored according to their chronic GVHD score.

20.5.4 Further Explanation of Criteria presented in Tables 2 and 3

20.5.4.1 Assessment of Skin GVHD

Presence or Absence of Skin GVHD: Skin GVHD will be considered present if a rash characteristic of acute GVHD develops after allogeneic marrow transplantation involving more than 25% of the body surface not clearly attributable to causes such as drug administration or infection. The extent of the body surface area involved can be estimated by the “Rule of Nines”. In estimating the extent of skin GVHD, the area involved is calculated for individual anatomic areas, such as the arm or leg, and then the total is derived from a simple summation. Areas that are non-blanching should not be considered involved regardless of the overlying color of the rash (red, brown, etc). Limited distribution erythema (with the exception of palms and soles) in the absence of associated rash elsewhere on the body will not be considered GVHD.

20.5.4.2 Assessment of Liver GVHD

Assessing for the Presence or Absence of Liver GVHD

- A. Hyperbilirubinemia (total bilirubin \geq 2.0 mg/dL) in the **absence** of other signs of acute GVHD in the skin or GI tract:
- i) Day 0 - 35: If hyperbilirubinemia alone is present with no other signs of acute GVHD in other organ systems, acute GVHD will not be diagnosed based solely on laboratory abnormalities. Acute GVHD will be diagnosed if findings on histopathology studies of liver from a biopsy or autopsy are confirmatory.
 - ii) Day 35 - 100: If hyperbilirubinemia (must be conjugated bilirubin) is not improving or is exacerbated (especially if serum alkaline phosphatase is increased), in the absence of acute GVHD in other organ systems, no other etiologies are identified, and does not improve with discontinuation of hepatotoxic drugs, acute GVHD will be diagnosed. However, it is distinctly unusual to develop ascites or a coagulopathy in the early stages of acute GVHD of the liver alone. In the absence of histopathology studies of liver from a biopsy or autopsy specimen, ascites or a coagulopathy secondary to liver dysfunction will be considered to indicate the presence of another disease process (e.g., veno-occlusive disease). Recommended non-invasive studies to define an etiology for hyperbilirubinemia are:
 - a. Imaging of liver (CT or ultrasound)
 - b. Hepatitis screen (only if ALT is elevated)
 - c. PT
 - d. Blood cultures
 - e. Review of medication list for potentially hepatotoxic drugs
 - f. Review of risk factors for viral liver infection (HSV, CMV, VZV, adenovirus, EBV, HBV, HCV)
 - g. Hemolysis screen
- B. Pre-existing hyperbilirubinemia clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems.
- i) If pre-existing non-GVHD liver disease (documented clinically, by lab assessment, or by imaging studies) is stable or improving at the onset of signs of acute GVHD in other organs, then acute GVHD of the liver will not be considered to be present unless proven by liver

- biopsy or autopsy.
- ii) If hyperbilirubinemia worsens several days before or at the time of onset of signs of acute GVHD in other organ systems, GVHD will be considered to be present unless histopathology studies of liver are available and negative on a biopsy during that time interval or autopsy results exclude GVHD.
 - iii) If hyperbilirubinemia persists and is not improving after resolution of a pre-existing non-GVHD liver disease process (e.g. localized infection of liver, systemic sepsis, biliary tract obstruction) when signs of acute GVHD are present in other organ systems or no other intervening cause has been diagnosed, then acute GVHD will be considered to be present in the absence of a new, clearly identifiable cause of non-GVHD liver disease or unless a liver biopsy or autopsy specimen is negative.
- C. Prior acute GVHD in liver with new onset of a disease process that exacerbates pre-existing or recently resolved hyperbilirubinemia:
- i) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia and acute liver GVHD has been diagnosed and has been stable, improving, or resolved, then the liver will not be restaged for acute GVHD until the resolution or stabilizing of the concurrent disease process (i.e., the liver stage prior to the onset of the new disease process will be carried forward until the new disease process resolves). Example: Acute GVHD of the liver and gut is diagnosed on Day 20. Treatment of acute GVHD results in falling bilirubin levels to liver Stage 1. Sepsis or TTP develops with transient worsening of the hyperbilirubinemia. The liver stage is not increased, despite a higher bilirubin level, because the cause of worsening hyperbilirubinemia is attributed to sepsis or TTP.
 - ii) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia in the presence of already worsening acute liver GVHD or GVHD of the skin or GI tract is simultaneously worsening, then the liver GVHD will be staged according to the actual bilirubin level, even though another cause of hyperbilirubinemia is present.

20.5.4.3 Assessment of GVHD of the Gastrointestinal Tract

Assessing for the Presence or Absence of GVHD of the Gastrointestinal Tract

- A. Diarrhea (≥ 500 mL/day in adults or > 10 mL/kg in pediatric patients) in the absence of other signs of acute GVHD in other organ systems
- i) Day 0 - engraftment: If diarrhea alone is present without other signs of acute GVHD in other organ systems, acute GVHD will not be considered present. Diarrhea will be attributed to acute GVHD if histopathology studies of gastrointestinal tract from a biopsy or autopsy are diagnostic.
 - ii) Engraftment - Day 100: If diarrhea persists and is not improving, is exacerbated, or develops *de novo* in the absence of acute GVHD in other organ systems, histopathology studies of gut biopsies or from autopsy specimens are not available, and no other etiologies are clearly identified, acute GVHD will be considered to be the cause. A stool specimen should be examined to rule out infectious causes (e.g., rotavirus, adenovirus, and *C. difficile* toxin). It is recommended, if at all possible, that biopsies be obtained for diagnostic purposes.

- B. Pre-existing diarrhea clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems:
- i) If pre-existing diarrhea caused by a process other than GVHD has been documented clinically or by lab assessment and is stable or improving at the onset of signs of acute GVHD in the skin or liver, then acute GVHD of the intestine will not be considered to be present in the absence of biopsy confirmation or autopsy report.
 - ii) If diarrhea or gastrointestinal symptoms are already present, but worsen significantly at the time of onset of signs of acute GVHD in the skin or liver, GVHD will be considered present, unless biopsy or autopsy are negative.
 - iii) If diarrhea persists after resolution of a pre-existing disease process with signs of acute GVHD present in other organ systems, GVHD will be considered present, unless biopsy or autopsy are negative.
- C. Prior or present acute GVHD in other organ systems with new onset of diarrhea:

If diarrhea is **clearly** attributable to an etiology other than acute GVHD (e.g., infection) and a history of acute GVHD exists or acute GVHD is present in other organ systems and is stable, then the gastrointestinal tract will not be evaluable for acute GVHD until the resolution or stabilizing of the other disease process (e.g., infection) in the absence of biopsy or autopsy confirmation.

- D. Persistent anorexia, nausea or vomiting in the absence of signs of acute GVHD in other organ systems:

Persistent anorexia, nausea or vomiting in the absence of other known causes of these symptoms will be considered Stage 1 acute GVHD if confirmed by endoscopic biopsy.

If a biopsy is not possible (e.g., secondary to thrombocytopenia) but the clinical findings are compatible with acute GVHD, then the patient will be treated and recorded as having acute GVHD.

Staging of the Gastrointestinal Tract for the Severity of Acute GVHD

The severity of gastrointestinal tract GVHD will be staged according to modified Glucksberg criteria. To minimize errors caused by large day-to-day variation, diarrhea volume is measured as an average over 3 days and reported as the volume in ml per day. When urinary mixing is noted the stool volume will be considered half of the total volume unless nursing staff is able to give a better estimate from direct observation. Abdominal cramps are considered significant for staging if the severity results in a clinical intervention (e.g., analgesia, fasting, etc.). Blood in the stools is considered significant if the blood is visible or hematochezia/melena is present and not clearly attributed to a cause other than GVHD (e.g. epistaxis/hemorrhoids).

APPENDIX I: SUPPORTIVE CARE GUIDELINES

General Guidelines

The following guidelines are intended to give general direction for optimal patient care and to encourage uniformity in the treatment of this study population. They are provided for institutional consideration. Notify Study Chair of any unexpected or unusually severe complications. Please also see the COG Supportive Care Guidelines at: https://members.childroncologygroup.org/prot/reference_materials.asp

Hospitalization/Hospital Environment

Hospitalization following each course of chemotherapy is strongly recommended until the absolute phagocyte count (sum of the neutrophils, bands and monocytes) is rising for 2 successive days, and the patient is afebrile and clinically stable. An additional discharge criterion of an absolute neutrophil count (ANC) of at least 200/ μ L is also suggested.

It is recommended that patients should be assigned to rooms with special air filtration systems such as high efficiency particulate air filters (HEPA) or clean-air rooms with constant positive pressure airflow if at all possible.

Central Venous Access

It is recommended that all patients have a double lumen central venous access line placed prior to the beginning of therapy.

Hyperleukocytosis and Metabolic Derangement

Patients with high peripheral blast counts ($> 100,000/\mu$ L) may have increased problems related to metabolic abnormalities, bleeding, and hyperviscosity. Transfusion with packed red blood cells should be given very cautiously, since it may increase viscosity and increase the risk of leukostasis. In these patients, the platelet count should be maintained at least greater than 20,000/ μ L.¹⁴⁷

Continuation of AML Therapy

AML therapy can proceed when there is documented evidence of response to antimicrobial therapy for infections complications, absence of fever or other signs or symptoms of infection, and other starting criteria have been met.

Antiemetics

Corticosteroids should not be used as antiemetics or to prevent infusion toxicity of Amphotericin B.

Nutrition

Active measures should be used to prevent weight loss of greater than 10% of pre-illness body weight. If possible, enteral feedings are preferred to parenteral.

Chemotherapy dosing for obese patients should be based on actual body weight rather than ideal body weight or an adjusted body weight **except in [Sections 4.24.3](#) SCT conditioning.**¹⁴⁸

Mucosal Evaluation and Care

Mucositis is expected and may be severe; liberal use of pain medications for this condition is encouraged. For patients with poor oral hygiene, consultation by dentistry is recommended prior to initiating therapy.

Suppression of Menstruation

Menstruating females may receive depo-provera or another suppressant during the entire course of this protocol. Suppression of menses should be continued until the platelet count is 50 000/ μ L without transfusion support.

Blood Product Support

Irradiation

Blood products should be irradiated following the current FDA guidelines found at: <http://www.fda.gov/cber/gdlns/gamma.htm>

Investigators in Canadian institutions need to follow the CSA standards for Blood and Blood Components CAN/CSA-Z902-04 issued in March 2004 and available at <http://www.shopcsa.ca>.

Infection Prophylaxis

Management of Fever and Neutropenia

Patients with an ANC < 500/ μ L (or < 1,000 / μ L and falling) and an oral temperature > 38°C twice in 12 hours or \geq 38.3°C once, should have empiric systemic antibiotics initiated immediately. The specific choice of empiric antibiotics should be guided by the resistance patterns seen at the individual institution. However, the antibiotic regimen should contain activity against Gram negative organisms and *Pseudomonas aeruginosa* in particular.¹⁴⁹ Examples of acceptable monotherapy directed at Gram negative organisms include ceftazidime and carbapenems. Acceptable combinations directed against Gram negative organisms include an anti-pseudomonal extended spectrum penicillin plus an aminoglycoside. In addition, the initial regimen should include agents with activity against viridans group *Streptococcus*, as these organisms are a common cause of fulminant sepsis in children with AML.^{150,151} Examples of such antibiotics with anti-streptococcal coverage include beta lactams, first generation cephalosporins, clindamycin and vancomycin.

Broad-spectrum antibacterial antibiotics, once started, should be continued until there is evidence of ANC recovery, or for a minimum of 14 days if there is no evidence of ANC recovery.^{149,152} Additional therapy will be dictated by the patient's clinical status.

Management of Possible or Documented Fungal Infection

Patients with AML are at particularly high risk of invasive fungal infection and these organisms are a major cause of infection-related mortality.¹⁵⁰ Antifungal prophylaxis can reduce morbidity and fungal infection-related mortality in severely neutropenic chemotherapy recipients. Evidence for benefit is strongest for those with > 15% rate of systemic fungal infection, prolonged neutropenia and SCT recipients.^{153,154}

The persistence of fever for 5 days despite broad spectrum antibiotic therapy or the emergence of a new fever in neutropenic patients warrants the investigation for invasive fungal infection and initiation of empiric antifungal therapy.¹⁴⁹

Investigation of invasive fungal infection should include computerized tomography (CT) of sinuses and chest and imaging (CT or ultrasound) of the abdomen along with blood and urine cultures. Radiologic investigations in those with invasive fungal infections may be negative in the setting of profound neutropenia and, therefore, imaging at recovery of neutrophils also may be considered.

In the event of suspected fungal lesions, biopsy whenever possible is strongly recommended in order to determine the causative organism. Antifungal options include Amphotericin B (convention or lipid products), caspofungin, voriconazole, and micafungin as well as combination therapy. The choice of

specific agent(s) and length of therapy will be dictated by the suspected or confirmed fungal species, site of infection clinical status and concomitant drug restrictions (see [Section 4.2](#)). Consultation with Infectious Disease is recommended.

Pneumocystis Prophylaxis

The administration of trimethoprim-sulfamethoxazole 150 mg/m²/day trimethoprim or 5 mg/kg/day trimethoprim (maximum 320 mg/day trimethoprim) orally divided twice daily for 2 or 3 consecutive days per week is recommended to prevent infection with *Pneumocystis jiroveci*.¹⁵⁵

For children who are intolerant of trimethoprim-sulfamethoxazole, acceptable alternatives are dapsone (2 mg/kg/day, maximum 100 mg/day) orally once daily and aerosolized pentamidine for children old enough to cooperate with administration (≥ 5 years) (300 mg inhaled monthly). For children in whom trimethoprim-sulfamethoxazole, dapsone and inhaled pentamidine cannot be administered, intravenous pentamidine (4 mg/kg/dose intravenous every 2 to 4 weeks) should be given.¹⁵⁵

Prophylaxis against PCP should begin as soon as possible after the initiation of chemotherapy and continue for at least 3 months following discontinuation of chemotherapy. See the COG Supportive Care Guidelines for additional drugs.

Prevention of Viral Infections

It is suggested that physicians follow their institutional guidelines regarding the prevention and treatment of viral infections such as cytomegalovirus and herpes simplex virus in those undergoing initial chemotherapy and following SCT.

Use of leuko-reduced and/or cytomegalovirus negative blood products for cytomegalovirus negative recipients are strongly encouraged.

Prophylactic acyclovir can reduce the recurrence of mucocutaneous HSV infection in both immunocompetent and immunocompromised hosts. The efficacy of prophylactic acyclovir has primarily been restricted to reduction of recurrent mucocutaneous HSV infection and not with other clinical endpoints such as duration of fever, use of antibiotics and mortality.¹⁵⁶

Pulmonary Toxicity

Cytarabine may result in pulmonary toxicity characterized by pulmonary infiltrates and shortness of breath similar to acute-respiratory distress syndrome (ARDS). Bortezomib has also been associated with pulmonary toxicity that resembles ARDS. Therefore, surveillance for pulmonary toxicity is extremely important, and aggressive intervention for pulmonary symptoms is indicated.

If a patient should develop respiratory distress and/or oxygen saturation of $< 92\%$ on room air:

- Infection, congestive heart failure, and other potential causes for respiratory distress should be evaluated. Consultation with a pulmonologist and bronchoscopy are encouraged if clinically indicated.
- Repeat imaging using a PA/LAT CXR and a CT of the chest (with contrast if appropriate) is encouraged.
- See [Section 5.7](#) for dose modifications if pulmonary toxicity is considered drug induced.

Treatment of sorafenib related Rash/Hand-foot syndrome

Patients who develop hand-foot syndrome may receive topical emollients (such as Aquaphor) as well as topical or oral steroids or antihistamines if appropriate. Hand foot syndrome may include skin pain without rash that impacts activities of daily living.

Oral administration of vitamin B 6 (pyridoxine) can also be used for these patients - BSA < 0.5m²: 50 mg per day; BSA 0.5 - 1.0 m²: 100 mg per day; BSA 1.1 - 1.5 m²: 200 mg per day, and BSA > 1.5m²: 300 mg per day.

Treatment of sorafenib related hypertension.

The algorithm used in [Section 5.2.2](#) will be used to grade and manage sorafenib related hypertension for patients less than 18 years. For patients 18 years and older, refer to CTCAE v4 guidelines for grading of hypertension and utilize institutional standards to define BP normalization following medical management. Should initiation of antihypertensive therapy be required, single agent therapy (consider a calcium channel blocker such as amlodipine or nifedipine) should be started and the blood pressure should be monitored at least twice weekly until BP is within the 95th percentile for age, height and gender per [Appendix VI](#).

APPENDIX II: SORAFENIB DOSING NOMOGRAM

AAML1031 Sorafenib Dosing Nomogram			
BSA Range (m²)	Dose Level 0 100 mg/m ² /day once daily (DOSE/WEEK)	Dose Level 1 200 mg/m ² /day once daily (DOSE/WEEK)	Dose Level 2 300 mg/m ² /day divided BID (DOSE/WEEK)
≤ 0.35	50 mg x 4 days/week, 0 x 3 day/week (200 mg)	50 mg x 5 days/week, 100 mg x 2 days/week (450 mg)	50 mg twice daily x 7 days/week (700 mg)
0.36 - 0.4	50 mg x 5 days/week, 0 x 2 day/week (250 mg)	50 mg x 4 days/week, 100 mg x 3 days/week (500 mg)	50 mg twice daily x 6 days/week + 100 mg twice daily x 1 day/week (800 mg)
0.41 - 0.45	50 mg x 6 days/week, 0 x 1 day/week (300 mg)	50 mg x 2 days/week, 100 mg x 5 days/week (600 mg)	50 mg twice daily x 5 days/week + 100 mg twice daily x 2 days/week (900 mg)
0.46 - 0.55	50 mg x 7 days/week (350 mg)	100 mg x 7 days/week (700 mg)	50 mg q AM +100 mg q PM x 7 days/week (1050 mg)
0.56 - 0.6	50 mg x 6 days/week, 100 mg x 1 day/week (400 mg)	100 mg x 6 days/week, 200 mg x 1 day/week (800 mg)	50 mg twice daily x 2 days/week + 100 mg twice daily x 5 days/week (1200 mg)
0.61 - 0.7	50 mg x 5 days/week, 100 mg x 2 days/week (450 mg)	100 mg x 5 days/week, 200 x 2 days/week (900 mg)	100 mg twice daily x 7 days/week (1400 mg)
0.71 - 0.8	50 mg x4 days/week, 100 mg x3 days/week (500 mg)	150 mg x 7 days/week (1050mg)	100 mg twice daily x 5 days/week + 150 mg twice daily x 2 days/week (1600 mg)
0.81 - 0.9	50 mg x 2 days/week, 100 mg x 5 days/week (600 mg)	150 mg x 4 days/week, 200 mg x 3 days/week (1200 mg)	100 mg q AM + 150 mg q PM x 7 days/week (1750 mg)
0.91 - 1.05	100 mg x 7 days/week (700 mg)	200 mg x 7 days/week (1400 mg)	150 mg twice daily x 7 days/week (2100 mg)
1.06 - 1.2	100 mg x 6 days/week, 200 mg x1 day/week (800 mg)	200 mg x 3 days/week, 250 mg x 4 days/week (1600 mg)	150 mg q AM + 200 mg q PM x 7 days/week (2450 mg)
1.21 - 1.35	100 mg x 5 days/week, 200 mg x 2 days/week (900 mg)	250 mg x 7 days/week (1750 mg)	200 mg twice daily x 7 days/week (2800 mg)
1.36 - 1.6	100 mg x 4 days/week, 200 mg x 3 days/week (1000 mg)	300 mg x 7 days/week (2100 mg)	200 mg q AM + 250 mg q PM x 7 days/week (3150 mg)
1.61 - 1.7	100 mg x 2 days/week, 200 mg x 5 days/week (1200 mg)	350 mg x 7 days/week (2450 mg)	250 mg twice daily x 7 days/week (3500 mg)
1.71 - 1.85	100 mg x 1 day/week, 200 mg x 6 days/week (1300 mg)	350 mg x 3 days/week 400 mg x 4 days/week (2650 mg)	250 mg q AM + 300 mg q PM x 7 days/week (3850 mg)
≥ 1.86	200 mg x 7 days/week (1400 mg)	400 mg x 7 days/week (2800 mg)	300 mg twice daily x 7 days /week (4200 mg)

APPENDIX III: INSTRUCTIONS FOR CHEMOTHERAPY DRUG PREPARATION, ADMINISTRATION AND SAFE HANDLING

Patient Name: _____ Cycle#/Course#: _____ Date Range: _____

Sorafenib

Sorafenib is a chemotherapeutic drug that requires safe handling. This information sheet will help you to safely prepare, administer, store, and dispose the drug. Please read the information before preparing and taking your drug. If you have any questions, please contact:

WHAT DO I NEED?

Your dose is:

- Sorafenib _____ mg by mouth
_____ time(s) per day
on the following days: _____

You should use the following combination of tablets for each dose:

_____ of the 50 mg tablets
_____ of the 200 mg tablets

AND:

- Sorafenib _____ mg by mouth
_____ **time(s) per day**
on the following days: _____

You should use the following combination of tablets for each dose:

_____ of the 50 mg tablets
_____ of the 200 mg tablets

Supplies:

- Disposable pad or paper towels to cover the work area
- Disposable gloves and mask
- Disposable cup for mixing drug with water
- Disposable spoon
- A container to collect waste (zip top plastic bag or medical waste bag or container)
- Tap or bottled water (drinking water)
_____ mL of water for mixing
_____ mL of water for rinsing
(based on dose in table to the right)

HOW DO I PREPARE THE DRUG?

[**Caution:** If you are pregnant, could become pregnant, or are breast-feeding, we suggest that you DO NOT prepare or administer this drug without FIRST checking with your health care provider.]

1. Choose a quiet working space away from food, windows, fans or heat ducts.
2. Clean the working space with damp paper towels.
3. Place a disposable pad or paper towel on the clean working space and place all needed items and drug on the pad or paper towel.
4. Wash your hands with soap and water and dry thoroughly.
5. It is suggested that you put on disposable gloves.

Drug preparation instructions:

(Refer to the section on “How do I Take/Give the Drug?” on the next page for specific instructions)

1. Note: The suspension must be administered within one hour after preparation.
2. Fill the disposable cup with the volume of water appropriate for the dose as indicated in the table below:

Sorafenib dose	Amount of mixing water needed
50 mg to 199 mg	30 mL (1 oz)
200 mg to 400 mg	60 mL (2 oz)

3. Place the number of tablets required for the dose into the water. Record the time.
4. Begin stirring with the plastic spoon after approximately 5 minutes. The tablets will begin to form a suspension.
5. Continue stirring until the tablets are completely disintegrated (dissolved). The tablet coating may initially form a thin film; however, this has no impact on dosing accuracy.
6. The suspension is ready for administration after about 10 minutes.

HOW DO I TAKE/GIVE THE DRUG?

- Take/Give sorafenib at approximately the same time each day.
- Take/Give sorafenib on an empty stomach, at least one hour before or two hours after food.
- Do not consume grapefruit juice during this study as it interacts with sorafenib.
- Just prior to administration, stir the suspension thoroughly and administer the dose to the patient.
- After drinking the suspension, rinse the cup several times with the total volume of water indicated in the table below to capture all of the remaining drug particles, and administer to subject.

Sorafenib dose	Amount of rinsing water needed
50 mg to 199 mg	90 mL (3 oz)
200 mg to 400 mg	180 mL (6 oz)

- When you are finished, place the dirty gloves, spoon, cup, and other tools used to mix the drug in a plastic zip top bag or the waste container provided by your nurse or pharmacist.
- If a dose is skipped, DO NOT replace the skipped dose. Wait for the next dose to take the drug. Do not double the dose to catch up.
- If the dose is vomited, do not take another dose.

HOW DO I STORE THE DRUG BOTTLE?

Store the medication in the bottle at room temperature away from food and out of the reach of children or pets.

WHAT SHOULD I DO WITH LEFT-OVER DRUG AND USED SUPPLIES?

If the patient could not take the dose or part of the dose, DO NOT keep or store the left-over portion. Place the remaining drug from this dose in the waste container and seal. Store the waste container out of the reach of children or pets. Return the waste container to the clinic at the next visit. Notify your study team at the next opportunity.

WHAT DO I DO WITH THE EXPIRED OR NOT USED TABLETS?

Return the unused drug to your study team at the clinic or hospital at the next visit.

WHAT PRECAUTION/SAFETY MEASURES DO I NEED TO TAKE?

If drug gets into eyes, hold eyelids open while flushing with water for at least 15 minutes. Call your doctor or nurse immediately at

and/or contact the Poison Control Center at

If you spilled the drug on your skin, remove contaminated clothing. Wash area with soap and large amount of water. Seek medical attention (see contact information above) if the skin becomes red or irritated or if you are concerned.

APPENDIX IV: LIST 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 frequently updated medical references.

CYP3A4 substrates	Strong Inhibitors¹	Moderate Inhibitors	Weak Inhibitors	Inducers
alfentanil ^{4,5}	atazanavir	aprepitant	alprazolam	armodafinil
amiodarone ⁴	boceprevir	conivaptan	amiodarone	barbiturates
aprepitant ⁵	clarithromycin	crizotinib	amlodipine	bosentan
atorvastatin ⁵	cobicistat	diltiazem	atorvastatin	carbamazepine
benzodiazepines	darunavir	dronedarone	bicalutamide	deferasirox
bortezomib	delavirdine	erythromycin	cilostazol	echinacea
brentuximab	grapefruit ³	fluconazole	cimetidine	efavirenz
budesonide ⁵	grapefruit juice ³	fosamprenavir	ciprofloxacin	etravirine
bupirone ⁵	indinavir	grapefruit ³	cyclosporine	fosphenytoin
calcium channel blockers	itraconazole	grapefruit juice ³	fosaprepitant	glucocorticoids ²
cisapride	ketoconazole	imatinib	fluvoxamine	modafinil
citalopram/escitalopram	lopinavir/ritonavir	mifepristone	isoniazid	nafcillin
conivaptan ⁵	nefazodone	nilotinib	nicardipine	nevirapine
glucocorticoids ²	nelfinavir	verapamil	propofol	oxcarbazepine
crizotinib	posaconazole		quinidine	phenobarbital
cyclosporine ⁴	ritonavir		ranolazine	phenytoin
cyclophosphamide	saquinavir			pioglitazone
dapsone	telaprevir			primidone
darifenacin ⁵	telithromycin			rifabutin
darunavir ⁵	voriconazole			rifampin
dasatinib ⁵				rifapentin
dihydroergotamine				ritonavir
docetaxel				St. John's wort
doxorubicin				topiramate
dronedarone ⁵				
eletriptan ⁵				
ergotamine ⁴				
eplerenone ⁵				
erlotinib				
esomeprazole				
estrogens				
etoposide				
everolimus ⁵				
felodipine ⁵				
fentanyl ⁴				
fosaprepitant ⁵				
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 ⁵ modafinil montelukast nefazodone nilotinib nisoldipine ⁵ omeprazole ondansetron paclitaxel pazopanib quetiapine ⁵ quinidine ⁴ saquinavir ⁵ sildenafil ⁵ 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, fruit juices and herbal supplements (star fruit, Seville oranges, pomegranate, ginkgo, goldenseal) may inhibit CYP3A4 isozyme, however, the degree of that inhibition is unknown.

²Dexamethasone is considered a weak CYP3A4 inducer.

³The effect of grapefruit juice (strong vs moderate CYP3A4 inhibition) varies widely among brands and is concentration-, dose-, and preparation-dependent.

⁴Narrow therapeutic range substrates

⁵Sensitive substrates

**APPENDIX V: SAMPLE SUBMISSION FOR CYTOGENETIC/FISH STUDIES
(A COPY OF THIS SECTION MUST BE SENT TO THE RECOMMENDED INSTITUTIONAL,
COG-APPROVED CYTOGENETICS LABORATORY)**

CHROMOSOME ANALYSIS

- Bone marrow should be studied by cytogenetics methods in all cases of acute myeloid leukemia.
- A back-up blood specimen should be studied when the bone marrow aspirate or bone marrow core biopsy is inadequate or unobtainable (in which case short-term unstimulated cultures are established to examine spontaneously dividing [presumably leukemic] cells) or when a constitutional chromosomal abnormality is a possibility (phytohemagglutinin-stimulated cultures should be established to examine presumably nonleukemic [constitutional] lymphocytes).
- Short-term (15 - 48 hours) unstimulated cultures are recommended for each bone marrow sample. Analysis of direct preparations is successful in some laboratories. Mitogen-stimulated cultures of bone marrow samples should not be initiated.
- All preparations must be G-banded. (Q- or R-banding will not be accepted as a stand-alone banding method.)
- Complete analysis of 20 G-banded metaphases is required for each case, except as noted below. Complete analysis is defined as follows: the chromosomes in each metaphase cell have been counted; each chromosome has been examined to determine whether the banding pattern is normal, and all abnormalities present in the cell have been defined. Analysis may be accomplished by examining metaphase spreads under the microscope or by imaging. Sometimes analysis of 20 metaphases is not possible because of poor in vitro growth or a very limited quantity of specimen. However, limited characterization of the abnormal clone can still be informative. Such informative cases will be considered acceptable. A minimum analysis of 20 metaphases is required for a normal case.
- Identification of clones will follow the criteria of the Second International Workshop on Chromosomes in Leukemia, as stated in the: General Report (Cancer Genet. Cytogenet 2:93-96, 1980): at least 2 metaphases with identical structural abnormalities or extra chromosomes, or at least three metaphases with identical missing chromosomes will constitute a clone. Nonclonal abnormalities (excluding random loss) should also be recorded.
- Karyotypes are to be designated according to the guidelines described in ISCN 2009, An International System for Human Cytogenetic Nomenclature (2009), LG Shaffer, ML Slovak and LJ Campbell (eds); S. Karger, Basel, 2009.

FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

- FISH using commercially available probes will be required for the following cases A.) Normal B) Insufficient Quantity. C) Abnormal case with an inv(16)/t(16;16), t(8;21) or 11q23 (see below). The laboratory is expected to follow the standards and guidelines for FISH put forth by the American College of Medical Genetics. If the laboratory is unable to perform FISH tests, contact cytogenetics coordinators for advice.
- A) For all cases for which G-banding reveals a normal karyotype, FISH with *RUNX1T1/RUNX1*, *CBFB*, or *CBFB-MYH11*, *PML-RARA* and *MLL* (probes must be performed to rule out a cryptic

t(8;21), inv(16) or t(16;16), t(15;17) and 11q23 abnormality. FISH testing should be performed on 1 day cultures (unstimulated sample).

- B) For all cases with failed cytogenetics, FISH with *RUNX1T1/RUNX1*, *CBFB* or *CBFB-MYH11*, *PML-RARA*, *MLL*, 7 (LSI, D7Z1/D7S486), and 5 (LSI, *EGR1/D5S23*, D5S721). The threshold for a positive FISH finding for -7, -5/5q- will be >30%. If possible, morphologic and immunophenotypic data should be obtained as such data can be suggestive of a particular abnormality and thereby help the laboratory director to prioritize these studies.
- C) When G-banding reveals a known recurring chromosomal rearrangement, specifically one involving an t(8;21), inv(16)/t(16;16), or abnormal 11q23, FISH is recommended with a probe set targeting the involved loci (i.e., *RUNX1T1/RUNX1*, *CBFB* or *CBFB-MYH11*, *MLL*) should be performed on metaphase or interphase cells to determine whether there is an associated deletion involving the regions 3' or 5' of the participating genes, and to establish the pattern that can be used to monitor this patient's disease. When G-banding reveals an abnormality of 12p, FISH with a probe that can assess *ETV6* status (*ETV6* breakapart or *ETV6-RUNX1*) in order to detect *ETV6* rearrangement or deletion.

CYTOGENETIC STUDY/FISH SUBMISSION

Steps to obtain the FORMS by the institution's CRA

1. www.childrensoncologygroup.org
2. COG members
3. Protocols ► Protocol Reference Materials ► Generic Forms
4. Under COG ► Cytogenetics Reporting/FISH Forms

The COG Forms should be completed by the designated individual in the Cytogenetics Laboratory and signed by the Cytogenetics Director. It is highly recommended to scan the Forms and e-mail with the appropriate documentation (PowerPoint presentation preferred) to COG reviewers for the Myeloid Committee. If the laboratory is unable to send an electronic file with the documentation, please contact the COG cytogenetics coordinator for your area for advice

Because the chromosome analysis results should be finalized and will be used for risk stratification and to guide therapy by Day 28 of a patient's enrollment, **the case should be sent to the appropriate reviewer by Day 14 but only for those patients identified as having HR FLT3/ITD (AR >0.4) who were consented to Arm C. As of Amendment #7A, cytogenetic review will not be conducted on those patients removed from protocol therapy after they were determined to be ineligible for Arm C.**

Cytogenetic Coordinators

Please send above materials by e-mail (preferably as PowerPoint file) to the following COG Cytogenetics Laboratories:

WEST OF MISSISSIPPI RIVER
(INCLUDE MINNESOTA AND WISCONSIN),
AUSTRALIA, NEW ZEALAND, WESTERN
CANADA
SEND TO:
Betsy Hirsch, Ph.D.
Telephone: 612-273-4952/3171
E-mail: hircs003@umn.edu

EAST OF MISSISSIPPI RIVER
(EXCLUDE MINNESOTA AND
WISCONSIN), EUROPE, EAST CANADA
SEND TO:
Susana C. Raimondi, Ph.D.
Telephone: 901-595-3537/3536
E-mail: susana.raimondi@stjude.org

CYTOGENETICS REVIEW

The region's cytogenetics coordinator will review each case when it is submitted. She/he will determine whether each case is adequate in terms of the numbers of metaphase cells analyzed, quality of banding, and interpretation of the karyotypes. If the coordinator agrees with the submitting laboratory, the results of the study will be entered into the appropriate eRDE. If the coordinator does not agree and the results are significant for patient stratification, she/he will send the case to another member of the COG Cytogenetics Review Committee for rapid review. If the coordinator does not agree with the submitting laboratory but the results are not significant for patient stratification, the case will be taken to the next central review session to be reviewed there. If the case is determined as not adequate it will be registered as unknown cytogenetics.

(A SIGNED AND DATED COPY OF THIS AUTHORIZATION FORM FOR REFLEXIVE FISH TESTING MUST BE SENT TO THE CYTOGENETICS LABORATORY, TOGETHER WITH THE BONE MARROW SAMPLE)

AUTHORIZATION FORM FOR REFLEXIVE FISH TESTS

Patients are stratified for AAML1031 treatment by cytogenetic groups. To achieve the most accurate diagnosis/subgrouping.

A). REQUIRED FISH if a case is cytogenetically normal or inadequate

t(8;21)	[<i>RUNX1T1/RUNX1</i>]
inv(16)/t(16;16)	[<i>CBFB</i> or <i>CBFB-MYH11</i>]
t(15;17)	[<i>PML-RARA</i>]
11q23	[<i>MLL</i>]

B). REQUIRED FISH if a case is cytogenetically inadequate

-7:	[<i>LSI, D7Z1/D7S486</i>]
-5/5q-:	[<i>LSI, EGR1/D5S23, D5S721</i>]
t(8;21)	[<i>RUNX1T1/RUNX1</i>]
inv(16)/t(16;16)	[<i>CBFB</i> or <i>CBFB-MYH11</i>]
t(15;17)	[<i>PML-RARA</i>]
11q23	[<i>MLL</i>]

C). RECOMMENDED FISH if a case has a cytogenetically detectable

t(8;21)	[<i>RUNX1T1/RUNX1</i>]
inv(16)/t(16;16)	[<i>CBFB</i> or <i>CBFB-MYH11</i>]
11q23	[<i>MLL</i>]
Abnormality of 12p	[<i>ETV6</i> breakapart or <i>ETV6-RUNX1</i>]

It is authorized to perform reflexive FISH testing to rule-out cryptic aberrations and/or to evaluate molecular deletions.

Patient Registration # _____

Print Name of Attending Physician

Print Name of Institution and City, State

Signature of attending physician or designee: _____

Date: _____

APPENDIX VI: BLOOD PRESSURE LEVELS FOR CHILDREN BY AGE AND HEIGHT PERCENTILE

BLOOD PRESSURE (BP) LEVELS FOR BOYS AGED 1-17 YEARS

Age (years)	BP Percentile	Systolic Blood Pressure, mm Hg							Diastolic Blood Pressure, mm Hg						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
2	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
3	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
4	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
5	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
6	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
7	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
8	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
9	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
10	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
11	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
12	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
13	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
14	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
15	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
16	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
17	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89

Instructions for using this BP Chart:

1. Measure the patient’s blood pressure using an appropriate size cuff.
2. Select appropriate chart for a female or male patient.
3. Using the “age” row and “height” column determine if the BP is within the ULN.
4. See [Section 5.2.2](#) for definition of dose-limiting hypertension, management and grading of hypertension, and [Appendix I](#) for medical treatment of sorafenib related hypertension.

This table was taken from “The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents”.¹⁵⁷

BLOOD PRESSURE (BP) LEVELS FOR GIRLS AGED 1-17 YEARS

Age (years)	BP Percentile	Systolic Blood Pressure, mm Hg							Diastolic Blood Pressure, mm Hg						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
2	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
3	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
4	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
5	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
6	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
7	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
8	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
9	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
10	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
11	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
12	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
13	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
14	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
15	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
16	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
17	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86

Instructions for using this BP Chart:

1. Measure the patient’s blood pressure using an appropriate size cuff.
2. Select appropriate chart for a female or male patient.
3. Using the “age” row and “height” column determine if the BP is within the ULN.
4. See [Section 5.2.2](#) for definition of dose-limiting hypertension, management and grading of hypertension, and [Appendix I](#) for medical treatment of sorafenib related hypertension.

This table was taken from “The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents”.¹⁵⁷

APPENDIX VII: LEUKEMIA BIOLOGY & GENE POLYMORPHISM STUDIES

Leukemia Biology Studies and Specimen Banking:

Submit 3 - 10 mL of bone marrow in EDTA containing tubes (purple/lavender top tubes) at the times specified in [Section 7.4](#). Peripheral blood in the same amount (i.e., 3–10 mL) may be substituted if bone marrow is unobtainable. When marrow is unattainable for mutation analysis, peripheral blood should be obtained in preservative-free heparin tubes (green top tubes).

Gene Polymorphism Study:

Submit 5 mL of peripheral blood in purple/lavender top tubes at sent at the time of diagnosis and at the time of the bone marrow exam at the end of Induction I.

Shipping: Samples should be sent to:

Fred Hutchinson Cancer Research Center
1100 Fairview Ave. N., D2-367
Seattle, WA 98109
(206) 667-2286
Contact person: Sommer Castro
Email: scastro@fhcrc.org
Director: Irwin Bernstein, M.D.
Email: ibernste@fhcrc.org

See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information. Saturday deliveries are permissible; Sunday deliveries are not permissible. **Note:** If a Saturday delivery is planned, please notify Sommer Castro at the number listed above and clearly mark the package “For Saturday Delivery.”

Ship each serial specimen individually by overnight air freight on the day of its harvest. Maximum time from sample collection to shipment should be no greater than 24 hours.

Exceptions:

- 1) Samples collected on weekends or holidays should be shipped the first working day following collection.
- 2) Although diagnostic marrow samples should be sent in as soon as possible, diagnostic samples will be accepted if they are later than 24 hours from sample collection to shipment (only post-Induction samples require the 24-hour time limit).

Store and ship samples at room temperature. Send fresh. If delay in shipment is not avoidable, please add equal volume of RPMI medium (1 mL RPMI for 1 mL of specimen).

Labelling: Label the tubes with the patient’s COG ID number, protocol ID number, BPC #, date and time of collection, phase of therapy, and source of material (i.e., bone marrow or blood). No refrigeration is necessary, with the exception of the Day 1 samples collected for protein expression array. **Utilize AML Specimen Transmittal forms specific for AAML1031.** PACK TUBES CAREFULLY! Wrap in an absorbable material, place in an airtight container (i.e., ziplock bag), and place entire specimen into an appropriate shipping container.

Peripheral Blood/Genotyping studies: These samples will be stored for future use if the patient/family consents to the use of lab samples for future studies on cancer or unrelated diseases.

Banking Specimens: If the patient consents, any specimens left over on this study after required tests are performed will be banked for future research studies.

APPENDIX VIII: LEUKEMIA INITIATING CELL (LIC) STUDY

Sample collection time point: End Induction I

NOTE: If a patient is MRD+ at the end of Induction I, a second 5 cc sample should be sent to the University of Rochester for LIC assessment after Induction II.

Specimen Requirements:

- Collect 5 cc BM into a syringe and transfer the specimen immediately into a 10 mL green heparin tube.
- Mix well by inverting several times immediately after injecting into tube. Up to 5 mL of BM can be placed in one 10 mL green top tube.
- Use multiple syringes and tubes as necessary. Reposition the bone marrow aspirate needle at least once during the diagnostic procedure to ensure the maximum quality of bone marrow.
- Label each tube with Patient ID, Patient's initials, Protocol number (AAML1031), date of sample collection, institution and type of specimen (bone marrow).
- Each sample should be clearly labeled to include the 6 digit COG number as well as the 4 digit treatment accession number; the study number (AAML1031), date and time sample was drawn.
- **Store in refrigerator until shipment.**

Shipping Requirements:

- Prior to sample collection, please contact Dr. Michael Becker at (585) 273-3083 or a Becker Lab representative at (585) 273-5548 to notify them of pending collection. If time is available the Becker lab will send a shipping container prior to collection of the end of Induction I bone marrow.
- In the absence of a shipping box from the Becker lab, place collection tubes in a container and place the container in a Styrofoam box.
 - The sample must be placed in a leak proof **primary receptacle** (e.g.: vacutainer).
 - Multiple fragile primary receptacles must be individually wrapped or separated to prevent contact.
 - The primary receptacle must be placed into a leak proof **secondary container** (e.g.: ziplock bag) in such a way that under normal conditions of transport, they cannot break or leak. *Please place each sample in a separate secondary container to prevent cross-contamination.*
 - **Absorbent material**, such as paper towels or absorbent pads or pillows, must be placed in the secondary container with sufficient capacity to absorb the entire contents of the primary receptacle(s).
 - The secondary containers must be placed into an **outer package** with suitable cushioning material and the total amount of diagnostic specimen must not exceed 4 L (liquid) or 4 kg (solid) per package.
 - The secondary packaging must be labeled with the universal biohazard symbol.
 - The outer packaging must be clearly and durably marked with the words "**Diagnostic Specimen**".
 - The outer packaging must be marked with the name, address, and phone number of both the sender and recipient.
 - In addition to the above list of requirements for ground transportation, the following requirements apply when shipping via FedEx or other air courier:

- a. All packages shipped via aircraft must display a 2 inch diamond with "UN3373" inside of the diamond.
 - b. Outer packages must be rigid (*bags and envelopes are not allowed*).
 - c. The outer packaging must be at least 4 inches in the smallest overall external dimension.
- **Please ship the sample with an ice pack placed in either the shipping container provided by the Becker lab or the Styrofoam box. During the non-winter months (April - October) add an additional ice pack to assure the samples stays cold during shipment.**
 - Package sample as appropriate for biologic material.
 - For all samples:
 - a. Include a copy of the AAML1031 LIC Specimen Transmittal Form (posted on the protocol website) with each shipment.
 - b. **DocuSAM a recent bone marrow immunophenotype report with the first peripheral blood sample.** If this is not possible, please send the report that day via fax (585) 276-2596 or email to Dr. Becker at Michael_becker@urmc.rochester.edu. (Please strip unnecessary identifiers)
 - **Ship the sample on the same day it was obtained by Federal Express Priority Overnight delivery to:**

Dr. Michael W. Becker c/o Susan Murray
J.P. Wilmot Cancer Center, Room 30852
575 Elmwood Avenue
Rochester, NY, 14620
 - See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information.
 - Notify Dr. Michael Becker at (585) 273-3083 or a Becker Lab representative at (585) 273-5548 prior to sample shipment
 - **Do not ship samples for delivery on a weekend or holiday.** If samples are collected on a Friday, refrigerate over the weekend and ship on Monday.
 - **Samples must be shipped within 24 hours** (except for samples collected on Friday, see above).

APPENDIX IX: PROTEIN EXPRESSION (RPPA) AND UNFOLDED PROTEIN RESPONSE (UPR) STUDY

As of 2/17/2017, the number of required specimens for analysis of RPPA and Unfolded Protein Response (UPR) has been met. No samples should be submitted. (See memo from 02/17/2017

<https://cogmembers.org/site/News/newsitem.aspx?lid=18690>)

Eligible samples:

Sample 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 1,000 myeloblasts/μL**. To calculate the absolute blast percentage, multiply the total WBC by the % peripheral blasts:

$$(WBC)(\% \text{ blast})(1,000) = \text{absolute blast count}/\mu\text{L}$$

As an example, if the patient has a WBC of 10 and 50% blasts, the absolute blast count is:

$$(10)(.5)(1,000) = 5,000/\mu\text{L}$$

If the initial % blasts is unknown, send samples only if the total WBC is more than 10,000 and notify the Horton lab of the % blast as soon as available (contact info provided below).

The Horton lab can accept Saturday shipments if we are contacted ahead of time. Please contact Gaye Jenkins or Dr Horton (832-824-4676 or 832-824-4269) for alternative address and shipping information for Saturday delivery.

Sample collection time points:

	Day 1, Hour 0	Day 1, Hour 10	Day 1, Hour 24
Peripheral Blood (Induction 1 only)	5 mL* (before start of systemic chemotherapy)	5 mL*	5 mL* (collected 24 hours after the start of the chemotherapy infusion)

* **Sample Collection:** Divide 5 mL sample into the CellSave tubes (3 mL) and heparin tubes (2 mL). Either lithium heparin or sodium heparin is acceptable (however please do **not** use heparin tubes with plasma separator gel, i.e., plasma separator tubes or PST tubes). Hour 10 samples should be collected 10 hours after the start of systemic chemotherapy.

Shipping Note: Although not preferred, 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 upon IRB approval.
 - 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 (AAML1031), date and time sample was drawn.
- On the AAML1031 Specimen Transmittal Form in the RDE 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.

- The institutional immunophenotype report should be submitted via the eRDEs Document Imaging System (DocuSAM).

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 small ice pack in the ThermoSafe container in the space provided.**
- Include a copy of the AAML1031 Specimen Transmittal Form (as a CRF in RDE) with each shipment.
- **If possible, please DocuSAM 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

- See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information.
- Notify Gaye Jenkins or Horton lab representative **prior** to shipment of the sample. Phone: (832) 824-4676 or (832) 824-1236. 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 X: SORAFENIB PHARMACOKINETIC (PK) STUDY

Sample collection time points:

Baseline (Any Day 1 - 10)	3 mL prior to any sorafenib therapy		
Induction I (Day 21 + 3)	3 mL pre-sorafenib dose	3 mL 3-4 hours post sorafenib dose	3 mL 6-8 hours post sorafenib dose
Induction II (Day 21 + 3)	3 mL pre-sorafenib dose	3 mL 3-4 hours post sorafenib dose	3 mL 6-8 hours post sorafenib dose
Intensification I (Day 21 + 3)	3 mL pre-sorafenib dose	3 mL 3-4 hours post sorafenib dose	3 mL 6-8 hours post sorafenib dose
Intensification II (Day 21 + 3)	3 mL pre-sorafenib dose	3 mL 3-4 hours post sorafenib dose	3 mL 6-8 hours post sorafenib dose
Maintenance:	Baseline (pre-sorafenib), Month 2*, 4*, 6*, 8*, 12* Also obtain specimen within 2 weeks (±5 days) of dose adjustment		

* ± 2 weeks

Note: Days are counted from the start of each course of therapy and correspond with TDM day; steady state PK samples should be obtained as outlined in [Appendix X](#), at approximately Day 21 (+3 days) of the indicated treatment cycles.. PK should only be collected on days that sorafenib is administered.

Specimen Requirements:

- **Peripheral blood samples:** 3 mL of whole blood will be collected in a heparinized tube (Becton Dickinson 6334 Green Top). If blood samples are taken via an indwelling central venous cannula, an appropriate amount of fluid should be removed from the cannula to clear the line before each blood sample is taken.
- **Plasma:** should be separated by centrifugation (10 minutes at 1,000 g or 2,500 rpm) within 6 hours after collection. Transfer separated plasma to a standard freezer tube/cryovial and label tube with the registration number, subject COG ID number, date and time of sampling, and sample number. Plasma samples should be frozen and stored in the dark at -70°C.

Shipping Requirements:

Frozen plasma samples may be batched for shipment. Frozen samples must be packed in dry ice sufficient to last at least 3 days, and shipped via FedEx or other express courier from **Monday through Thursday** along with the [pharmacokinetic worksheet](#) (below) to:

Ganesh Moorthy, PhD
Division of Clinical Pharmacology & Therapeutics
The Children’s Hospital of Philadelphia
CTRB-4200
3501 Civic Center Blvd
Philadelphia, PA 19104
Email: moorthyg@email.chop.edu
Phone: 215-590-0142

Alternate contact:
Elizabeth Fox, MD
Email: foxe@email.chop.edu
Phone: 267-425-3010
Fax: 267-425-0113
Page: 877-314-9558
Cell: 267-648-1274

See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information. Dr. Moorthy or Fox should be notified by phone or e-mail prior to the day of the shipment to facilitate sample tracking and receipt.

PK SPECIMEN COLLECTION WORKSHEET:

COG Registration #: _____ Study ID: **AAML1031**

BASELINE PK sample in ALL PATIENTS who consent to PK (Blank PK sample)				
Sample #	Prior to Start of Any Sorafenib	Hour	Target Date/Time	Date/Time Obtained
IND I-1	Induction I	Pre-sorafenib		

Induction I (Obtain samples on one day between day 21-24) Sorafenib Dose (mg/m²): _____ BSA (m²): _____

Sample #	Day	Hour	Target Date/Time	Date/Time Obtained
	Day Prior to PK: Sorafenib Dose Administered (mg) :			Date Time
IND1-2	Day 21 (+3)	Pre-sorafenib dose		
	Day of PK: Sorafenib Dose Administered (mg) :			Date Time
IND1-3	Day 21 (+3)	3-4 hr post dose		
IND1-4	Day 21 (+3)	6-8 hr post dose		

Induction II (Obtain samples on one day between day 21-24) Sorafenib Dose (mg/m²): _____ BSA (m²): _____

Sample #	Day	Hour	Target Date/Time	Date/Time Obtained
	Day Prior to PK: Sorafenib Dose Administered (mg):			Date Time
IND2-1	Day 21 (+3)	Pre-sorafenib dose		
	Day of PK: Sorafenib Dose Administered (mg):			Date Time
IND2-2	Day 21 (+3)	3-4 hr post dose		
IND2-3	Day 21 (+3)	6-8 hr post dose		

Intensification I (Obtain samples on one day between day 21-24) Sorafenib Dose (mg/m²): _____ BSA (m²): _____

Sample #	Day	Hour	Target Date/Time	Date/Time Obtained
	Day Prior to PK: Sorafenib Dose Administered (mg)			Date Time
INT1-1	Day 21 (+3)	Pre-sorafenib dose		
	Day of PK: Sorafenib Dose Administered (mg)			Date Time
INT1-2	Day 21 (+3)	3-4 hr post dose		
INT1-3	Day 21 (+3)	6-8 hr post dose		

IntensificationII (Obtain samples on one day between day 21-24) Sorafenib Dose (mg/m²): _____ BSA(m²): _____

Sample #	Day	Hour	Target Date/Time	Date/Time Obtained
	Day Prior to PK: Sorafenib Dose Administered (mg) :			Date Time
INT2-1	Day 21 (+3)	Pre-sorafenib dose		
	Day of PK: Sorafenib Dose Administered (mg) :			Date Time
INT2-2	Day 21 (+3)	3-4 hr post dose		
INT2-3	Day 21 (+3)	6-8 hr post dose		

PK SPECIMEN COLLECTION WORKSHEET (con't):

COG Registration #: _____

Study ID: AAML1031

Maintenance (All samples are obtained as troughs, prior to dose of sorafenib)

Sample #	Month		Sorafenib dose PRIOR to PK sample				Sample Obtained
			Dose Level (mg/m ²)	Actual Dose (mg)	BSA (m ²)	Date & time prior dose	Date/Time
MNT M1	Month 1	Pre-sorafenib dose					
MNT M2	Month 2 ± 2 weeks	Pre-sorafenib dose					
MNT M4	Month 4 ± 2 weeks	Pre-sorafenib dose					
MNT M6	Month 6 ± 2 weeks	Pre-sorafenib dose					
MNT M8	Month 8 ± 2 weeks	Pre-sorafenib dose					
MNT M12	Month 12 ± 2 weeks	Pre-sorafenib dose					
DM	Date of Dose Modification: _____ _ New Dose Level (mg/m ² /day): _____ Dose (mg) admin. prior to sample: _____						

APPENDIX XI: SORAFENIB PIA STUDY

NOTE: ALL “POST-COURSE” SAMPLES SHOULD BE DRAWN JUST BEFORE SCHEDULED DOSES OF SORAFENIB (I.E., AT TROUGH TIME POINTS)

Sample Collection Time Points for Sorafenib + Chemotherapy Courses:

Course	Days					
	Pre-Course	During chemotherapy	Post-Chemotherapy			
Induction I	1	Deleted per Amendment #6A	Deleted per Amendment #6A	14 ± 1	21±3	28 ± 1
Induction II	1			14 ± 1	21±3	28 ± 1
Intensification I	1			14 ± 1	21±3	28 ± 1
Intensification II	1			14 ± 1	21±3	28 ± 1
Note: Numbers above refer to treatment day according to treatment delivery maps (TDMs)						
	Pre-Course	Months				
Maintenance	1	2, 4*, 6*, 8*, 12* Also obtain specimen within 2 weeks of dose adjustment				

* ± 2 weeks.

Specimen Requirements:

- Peripheral Blood: At each time point, 3 mL of blood must be drawn into green-top (sodium heparin) vacutainer(s).
- Each blood collection tube must be labeled as “Course ___ (IND1/IND2/INT1/INT2), Day: ___ PK/PIA sample” with patient initials, COG ID number, sample date and collection time recorded to the nearest minute using a 24-hour clock. A completed Specimen Transmittal Form must accompany the sample(s) when shipped.
- Any missing samples, and samples not drawn, must be noted on the Specimen Transmittal Form.

Shipping Requirements:

Samples should be maintained at room temperature (no refrigeration or icing of samples is necessary) and shipped via overnight courier service to:

Dr. Patrick Brown
 Johns Hopkins Oncology
 Cancer Research Building I, Room 262
 1650 Orleans Street
 Baltimore, MD 21231
 Laboratory phone: (410) 955-8688
 Pager no: (410) 434-0732
 E-mail: pbrown2@jhmi.edu

See the AAML1031 Sample Submission Schedule posted on the protocol webpage for courier information. Samples can be shipped 7 days a week. Saturday deliveries are permissible. If a Saturday delivery is required, please notify Dr. Brown of the planned shipment via email or pager **prior** to the time of shipment and clearly mark the package “For Saturday Delivery”.

APPENDIX XII: YOUTH INFORMATION SHEETS**INFORMATION SHEET REGARDING RESEARCH STUDY AAML1031
(for children from 7 - 12 years of age)**

*A Study of Adding Sorafenib to the Usual Treatment in Children
with AML (Acute Myeloid Leukemia)*

1. We have been talking with you about your illness, acute myeloid leukemia (AML). AML is a type of cancer that grows in the center of your bones. The center of your bones is called bone marrow. It is where your body makes blood. 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 AML. 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 AML.
3. All children who are part of this study will get the usual treatment for AML. The normal treatment is cancer-fighting medicines. Sometimes a stem cell transplant is also done. A stem cell transplant is done to replace blood cells that are killed by cancer treatment. If a test finds you have a lot of a certain change in a gene in your cancer cells you may switch to getting a different medicine, called sorafenib in addition to the usual treatment for AML. A gene is one of the building blocks of DNA. Your doctor will tell you which group you are in. We do not know if treatment with the extra medicine will be better than the usual treatment. That is why we are doing this study.
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 a better chance of getting rid of the leukemia for as long as possible. But, we do not 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”. There is a risk that you will have more bad effects from the medicines if you are treated with sorafenib along with the usual medicines. If this happens, you may need treatment for the bad effects. Other things may happen to you that we do not yet know about.
6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study any time. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions 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 react to treatment. Almost all of these samples will be taken when other standard blood tests and bone marrow tests are done. You would need some extra blood tests done on the days you take the experimental medicine, as well as for some of the special biology tests, so there would only be a few extra needle sticks. We would also like to save any leftover samples 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 AAML1031
(for teens from 13 - 17 years of age)**

A Study of Adding Sorafenib to Standard Treatment in Children and Teens with AML (Acute Myeloid Leukemia)

1. We have been talking with you about your illness, acute myeloid leukemia (AML). AML is a type of cancer that grows in the center of your bones. The center of your bones is called bone marrow. It is where your body makes blood. After doing tests, we have found that you have this type of cancer.
2. Now we want to ask you to take part in a research study because you have just been diagnosed with AML. 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 find better ways to treat children and teens with AML.
3. All children and teens that are part of this study will be treated with the usual treatment for AML. The usual treatment includes chemotherapy (anti-cancer medicine). Sometimes it also includes a stem cell transplant. A stem cell transplant is done to replace blood cells that are killed by cancer treatment. If you are found to have a high amount of a certain change in one of the genes in your cancer cells, you may switch to getting a different medicine, called sorafenib in addition to the usual chemotherapy for AML. Study doctors want to find out if adding sorafenib to the usual chemotherapy will be better for treating leukemia. That is why we are doing this study.
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 a better chance at getting rid of the leukemia for as long as possible. However, we do not 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”. There is a risk of more side effects from the chemotherapy if you are treated with sorafenib in addition to the usual chemotherapy. If this happens, you may need treatment for the side effects. Some risks of taking sorafenib are tiredness, diarrhea and painful hands or feet. Study doctors will check you closely to see if any side effects are happening. Other things may happen to you that we do not yet know about.
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 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. You would need some extra blood tests done on the days you take the experimental medicine sorafenib, as well as for some of the special biology tests, so there would only be a few extra procedures. 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 XIII: BIOMARKERS FOR THE PREDICTION OF GVHD STUDY

As of 2-24-2017, the funding to collect and analyze the optional samples for Biomarkers for the Prediction of GVHD study (Appendix XIII) has been depleted. Samples for this optional study should no longer be submitted. Please see memo dated 2/24/2017 (https://members.childrensoncology_group.org/prot/AAML1031/AAML1031Mem2_24_17.pdf).

Participation in the GVHD prediction study is limited to patients receiving stem cell transplant on AAML1031. This study is optional and patient consent is required.

Sample Collection Schedule: Day +7, Day +14, and Day +28 following SCT infusion (Day 0).

Sample Collection Instructions:

- Collect 3 - 6 mL of peripheral blood in a red top tube (no reagent).
- Place sample upright in a rack for 30 minutes at room temperature.
- Label sample with the COG ID, study ID (AAML1031), date of collection, Day post SCT (i.e., Day +7, Day +14, and Day +28).
- Sample should be collected Monday through Thursday. Avoid collecting samples on Fridays, weekends or holidays. It is permissible to collect sample \pm 2 days from the target day in order to accommodate the Monday through Thursday schedule and the restriction of weekend deliveries.

Shipping Instructions:

- **Ship each sample on the day of collection.**
- Complete aGVHD Biomarker Specimen Transmittal Form (see Specimen Shipping Forms on the AAML1031 protocol website) and include a copy of the transmittal form with each shipment.
- Ship sample at room temperature via overnight delivery only on Monday through Thursday, excluding holidays.
- Ship specimen using the courier account provided in the sample submission schedule on the AAML1031 protocol website.
- Please ship the sample at room temperature in the Styrofoam box.
- Package sample as appropriate for biologic material.
- The sample must be placed in a leak proof **primary receptacle** (e.g.: red top tube).
- If multiple primary receptacles are to be shipped in the same package, please wrap each receptacle separately.
- The primary receptacle must be placed into a leak proof **secondary container** (e.g.: zip-lock bag) in such a way that under normal conditions of transport, they cannot break or leak. *Please place each sample in a separate secondary container to prevent cross contamination.*
- **Absorbent material**, such as paper towels or absorbent pads or pillows, must be placed in the secondary container with sufficient capacity to absorb the entire contents of the primary receptacle(s).
- The secondary containers must be placed into an **outer package** with suitable cushioning material and the total amount of diagnostic specimen must not exceed 4 L (liquid) or 4 kg (solid) per package.
- The secondary packaging must be labeled with the universal biohazard symbol.
- The outer packaging must be marked with the name, address, and phone number of both the sender and recipient.

- In addition to the above list of requirements for ground transportation, the following requirements apply when shipping via FedEx or other air courier:
 - a. All packages shipped via aircraft must display a 2 inch diamond with "UN3373" inside of the diamond.
 - b. Outer packages must be rigid (*bags and envelopes are not allowed*).
 - c. The outer packaging must be at least 4 inches in the smallest overall external dimension.

Shipping Address:

Dr. Andrew Harris c/o Joel Whitfield
4725 Med Sci 2
1150 West Medical Center Drive
Ann Arbor, MI 48109
Tel: (734) 647-9524
(no fax; please scan and email documents if necessary)

Laboratory Contact:

Joel Whitfield
Email: jrwhitf@umich.edu
Tel: (734) 647-9524
(no fax; please scan and email documents if necessary)

Questions regarding the GVHD Prediction Study may be directed to:

Email: andrew.harris@hsc.utah.edu
Phone: (801) 662-4700
Pager: (801) 914-6184
Fax: (801) 662-4707

APPENDIX XIV: CTEP AND CTSU 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).

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.

Submitting Regulatory Documents:

ONLINE: www.ctsu.org (members' section) → Regulatory Submission Portal

EMAIL: CTSUSubregulatory@ctsu.coccg.org (for regulatory document submission only)

FAX: 215-569-0206

MAIL: CTSU Regulatory Office

1818 Market Street, Suite 1100

Philadelphia, PA 19103

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 XV: 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.

Bortezomib

Drugs that may interact with bortezomib
<ul style="list-style-type: none"> • Antibiotics <ul style="list-style-type: none"> ○ Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin, tetracycline • Antidepressants and antipsychotics <ul style="list-style-type: none"> ○ Aripiprazole, citalopram, clozapine, escitalopram, fluoxetine, nefazodone, sertraline • Antifungals <ul style="list-style-type: none"> ○ Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole • Antiretrovirals and antivirals <ul style="list-style-type: none"> ○ Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, telaprevir • Anti-seizure medications <ul style="list-style-type: none"> ○ Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone • Heart medications <ul style="list-style-type: none"> ○ Amiodarone, clopidogrel, diltiazem, nicardipine, verapamil • Some chemotherapy (be sure to talk to your doctor about this) • Many other drugs, including the following: <ul style="list-style-type: none"> ○ Aprepitant, bosentan, cimetidine, cyclosporine, deferasirox, esomeprazole, haloperidol, ivacaftor, lomitapide, mifepristone, omeprazole, pimozide

Food and supplements that may interact with bortezomib**
<ul style="list-style-type: none"> • 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"

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Sorafenib

Drugs that may interact with sorafenib
<ul style="list-style-type: none"> • Antibiotics <ul style="list-style-type: none"> ○ Ciprofloxacin, levofloxacin, moxifloxacin, clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin • Antidepressants and antipsychotics

<ul style="list-style-type: none"> ○ Aripiprazole, citalopram, clozapine, escitalopram, nefazodone, paliperidone, paroxetine, quetiapine, risperidone, sertraline, thioradizine, trazodone, ziprasidone ● Antifungals <ul style="list-style-type: none"> ○ Itraconazole, ketoconazole, posaconazole, voriconazole, terfenadine ● Arthritis medications <ul style="list-style-type: none"> ○ Leflunomide, tofacitinib ● Antiretrovirals and antivirals <ul style="list-style-type: none"> ○ Atazanavir, boceprevir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, rilpivirine, ritonavir, saquinavir, Stribild, telaprevir, tipranavir ● Anti-seizure medications <ul style="list-style-type: none"> ○ Carbamazepine, oxcarbazepine, phenobarbital, phenytoin, primidone ● Heart and cholesterol medications <ul style="list-style-type: none"> ○ Amiodarone, clopidogrel, digoxin, dronedenarone, fenofibric acid, fluvastatin, gemfibrozil, irbesartan, losartan, procainamide, sotalol ● Some chemotherapy (be sure to talk to your doctor about this) ● Many other drugs, including the following: <ul style="list-style-type: none"> ○ Acetaminophen, bosentan, chlorpromazine, granisetron, haloperidol, methadone, mifepristone, natalizumab, omeprazole, pentamidine, pimozide, pioglitazone, tacrolimus, rosiglitazone, treprostinil, warfarin
--

Food and supplements that may interact with sorafenib**
<ul style="list-style-type: none"> ● Echinacea ● Grapefruit, grapefruit juice, Seville oranges, star fruit ● Milk thistle ● St. John's Wort ● Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Busulfan

Drugs that may interact with busulfan
<ul style="list-style-type: none"> ● Acetaminophen ● Itraconazole ● Metronidazole ● Thioguanine ● Fosphenytoin or phenytoin (in some cases these drugs are used with busulfan on purpose)

Food and supplements that may interact with busulfan**
<ul style="list-style-type: none"> ● Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Cytarabine IV

Drugs that may interact with cytarabine
<ul style="list-style-type: none"> • Clozapine • Digoxin • Flucytosine • Leflunomide

Food and supplements that may interact with cytarabine**
<ul style="list-style-type: none"> • Echinacea

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Daunorubicin

Drugs that may interact with daunorubicin
<ul style="list-style-type: none"> • 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**
<ul style="list-style-type: none"> • Echinacea • Grapefruit, grapefruit juice, Seville oranges, star fruit • St. John's Wort • Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Etoposide

Drugs that may interact with etoposide
<ul style="list-style-type: none"> • Antibiotics <ul style="list-style-type: none"> ○ Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin • Antidepressants and antipsychotics <ul style="list-style-type: none"> ○ Aripiprazole, clozapine, nefazodone • Antifungals <ul style="list-style-type: none"> ○ Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole • Arthritis medications <ul style="list-style-type: none"> ○ 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:**
 - Atovaquone, bosentan, sitaxentan, aprepitant, dexamethasone, mifepristone, natalizumab, pimoziide, ivacaftor, deferasirox, lomitapide

- | Food and supplements that may interact with etoposide** |
|--|
| <ul style="list-style-type: none"> ● Echinacea ● Glucosamine ● St. John's Wort ● Grapefruit, grapefruit juice, Seville oranges, star fruit |

***Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Fludarabine

- | Drugs that may interact with fludarabine |
|---|
| <ul style="list-style-type: none"> ● Clozapine ● Leflunomide ● Natalizumab ● Pentostatin ● Tofacitinib |

- | Food and supplements that may interact with fludarabine** |
|---|
| <ul style="list-style-type: none"> ● Echinacea |

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided. Talk to your doctor before starting any new medications or herbal supplements and before making a significant change in your diet.*

Methotrexate

- | Drugs that may interact with methotrexate |
|---|
| <ul style="list-style-type: none"> ● 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, warfarin

Food and supplements that may interact with methotrexate**

- Alcohol
- Echinacea
- Some vitamins, including those that contain folic acid or high doses of vitamin C

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Methylprednisolone

Drugs that may interact with methylprednisolone

- Antibiotics
 - Azithromycin, clarithromycin, erythromycin, telithromycin, rifampin
- Antidepressants and antipsychotics
 - Aripiprazole, nefazodone
- Antifungals
 - Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole
- Arthritis medications
 - Leflunomide, tofacitinib
- Antiretrovirals and antivirals
 - Atazanavir, boceprevir, darunavir, delaviridine, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, telaprevir
- Anti-seizure medications
 - Carbamazepine, phenobarbital, phenytoin, primidone
- Some chemotherapy (be sure to talk to your doctor about this)
- Many other drugs, including the following:
 - Aprepitant, cyclosporine, deferasirox, lomitapide, mifepristone, natalizumab, warfarin

Food and supplements that may interact with methylprednisolone**

- Echinacea

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

Mitoxantrone

Drugs that may interact with mitoxantrone

- Aripiprazole
- Clozapine
- Cyclosporine
- Dofetilide
- Leflunomide
- Natalizumab

- Pimozide
- Tofacitinib

Food and supplements that may interact with mitoxantrone**

- Echinacea

**** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.**

Tacrolimus

Drugs that may interact with tacrolimus

- **Antibiotics**
 - Clarithromycin, erythromycin, nafcillin, rifabutin, rifampin, telithromycin
- **Antidepressants and antipsychotics**
 - Citalopram, clozapine, escitalopram, fluvoxamine, lurasidone, nefazodone, paliperidone, quetiapine, thioridazine, ziprasidone
- **Antifungals**
 - Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole
- **Anti-inflammatory, arthritis, or pain medications**
 - Leflunomide, tofacitinib
- **Anti-rejection medications**
 - Cyclosporine, sirolimus
- **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, disopyramide, procainamide, sotalol, verapamil
- **Kidney medications**
 - Amiloride, spironolactone, triamterene
- **Stomach and reflux medications**
 - Dexlansoprazole, esomeprazole, lansoprazole, omeprazole
- **Some chemotherapy (be sure to talk to your doctor about this)**
- **Many other drugs, including the following:**
 - Ambrisentan, bosentan, sitaxentan, aprepitant, colchicine, dexamethasone, mifepristone, natalizumab, pimozide

Food and supplements that may interact with tacrolimus**

- Echinacea
- St. John's Wort
- Grapefruit, grapefruit juice, Seville oranges, star fruit

**** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.**

REFERENCES

1. Yamaguchi O, Watanabe T, Nishida K, et al: Cardiac-specific disruption of the c-raf-1 gene induces cardiac dysfunction and apoptosis. *J Clin Invest* 114:937-43, 2004
2. Levis M, Pham R, Smith BD, et al: In vitro studies of a FLT3 inhibitor combined with chemotherapy: sequence of administration is important to achieve synergistic cytotoxic effects. *Blood* 104:1145-50, 2004
3. Chintalgattu V, Ai D, Langley RR, et al: Cardiomyocyte PDGFR-beta signaling is an essential component of the mouse cardiac response to load-induced stress. *J Clin Invest* 120:472-84, 2010
4. Duran JM, Makarewich CA, Trapanese D, et al: Sorafenib cardiotoxicity increases mortality after myocardial infarction. *Circ Res* 114:1700-12, 2014
5. Serve H, Krug U, Wagner R, et al: Sorafenib in combination with intensive chemotherapy in elderly patients with acute myeloid leukemia: results from a randomized, placebo-controlled trial. *J Clin Oncol* 31:3110-8, 2013
6. Ravandi F, Arana Yi C, Cortes JE, et al: Final report of phase II study of sorafenib, cytarabine and idarubicin for initial therapy in younger patients with acute myeloid leukemia. *Leukemia* 28:1543-5, 2014
7. Ravandi F, Cortes JE, Jones D, et al: Phase I/II Study of Combination Therapy With Sorafenib, Idarubicin, and Cytarabine in Younger Patients With Acute Myeloid Leukemia. *J Clin Oncol*, 2010
8. Yee KW, Schittenhelm M, O'Farrell AM, et al: Synergistic effect of SU11248 with cytarabine or daunorubicin on FLT3 ITD-positive leukemic cells. *Blood* 104:4202-9, 2004
9. Schittenhelm MM, Kampa KM, Yee KW, et al: The FLT3 inhibitor tandutinib (formerly MLN518) has sequence-independent synergistic effects with cytarabine and daunorubicin. *Cell Cycle* 8:2621-30, 2009
10. Woods WG: Curing childhood acute myeloid leukemia (AML) at the half-way point: Promises to keep and miles to go before we sleep. *Pediatr Blood Cancer* 46:565-9, 2006
11. Gibson BE, Wheatley K, Hann IM, et al: Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. *Leukemia* 19:2130-8, 2005
12. Alonzo T HP, Gerbing R, Gamis A, Raimondi S, Hirsch B, Cooper T, Franklin J, Pardo L, Loken M, Meshinchi S: Combination of conventional cytogenetics, molecular profiling and flow cytometric response data allows creation of a two-tier risk group system for risk-based therapy allocation in childhood AML – a report from Children's Oncology Group. [Abstract]. *Blood* 16(21):761, 2010
13. Richardson PG, Mitsiades C, Schlossman R, et al: Bortezomib in the front-line treatment of multiple myeloma. *Expert Rev Anticancer Ther* 8:1053-72, 2008
14. Guzman ML, Swiderski CF, Howard DS, et al: Preferential induction of apoptosis for primary human leukemic stem cells. *Proc Natl Acad Sci U S A* 99:16220-5, 2002
15. 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. *Clin Cancer Res* 14:1446-54, 2008
16. Horton TM, Gannavarapu A, Blaney SM, et al: Bortezomib interactions with chemotherapy agents in acute leukemia in vitro. *Cancer Chemother Pharmacol* 58:13-23, 2006
17. 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 Chemother Pharmacol* 60:245-55, 2007
18. Pigneux A, Mahon FX, Moreau-Gaudry F, et al: Proteasome inhibition specifically sensitizes leukemic cells to anthracyclin-induced apoptosis through the accumulation of Bim and Bax pro-apoptotic proteins. *Cancer Biol Ther* 6:603-11, 2007

19. 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). *J Clin Oncol* 22:4804-9, 2004
20. 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* 13:1516-22, 2007
21. 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. *Pediatr Blood Cancer* 55:254-9, 2010
22. Wilhelm S, Carter C, Lynch M, et al: Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 5:835-44, 2006
23. Lee SH, Paietta E, Racevskis J, et al: Complete resolution of leukemia cutis with sorafenib in an acute myeloid leukemia patient with FLT3-ITD mutation. *Am J Hematol* 84:701-2, 2009
24. Metzelder S, Wang Y, Wollmer E, et al: Compassionate use of sorafenib in FLT3-ITD-positive acute myeloid leukemia: sustained regression before and after allogeneic stem cell transplantation. *Blood* 113:6567-71, 2009
25. Safaian NN, Czibere A, Bruns I, et al: Sorafenib (Nexavar) induces molecular remission and regression of extramedullary disease in a patient with FLT3-ITD+ acute myeloid leukemia. *Leuk Res* 33:348-50, 2009
26. Ravandi F, Cortes JE, D J, et al: Phase I/II Study of Combination Therapy With Sorafenib, Idarubicin, and Cytarabine in Younger Patients With Acute Myeloid Leukemia. *J Clin Oncol*, 2010
27. Inaba H, Rubnitz JE, Coustan-Smith E, et al: Phase I pharmacokinetic and pharmacodynamic study of the multikinase inhibitor sorafenib in combination with clofarabine and cytarabine in pediatric relapsed/refractory leukemia. *J Clin Oncol* 29:3293-300, 2011
28. Watt TC, Cooper T: Sorafenib as treatment for relapsed or refractory pediatric acute myelogenous leukemia. *Pediatr Blood Cancer* 59:756-7, 2012
29. Chen MH, Kerkela R, Force T: Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics. *Circulation* 118:84-95, 2008
30. Force T, Krause DS, Van Etten RA: Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nat Rev Cancer* 7:332-44, 2007
31. Izumiya Y, Shiojima I, Sato K, et al: Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload. *Hypertension* 47:887-93, 2006
32. Shiojima I, Sato K, Izumiya Y, et al: Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J Clin Invest* 115:2108-18, 2005
33. Rollig C, Muller-Tidow C, Huttmann A, et al: Sorafenib versus placebo in addition to standard therapy in younger patients with newly diagnosed acute myeloid leukemia: results from 267 patients treated in the randomized placebo-controlled SAL-Sorami Trial. *Blood* 124 (21), 2014
34. Todd Michael Cooper, Jemily Malvar, Jeannette Cassar, et al: A Phase I Study Of AC220 (Quizartinib) In Combination With Cytarabine and Etoposide In Relapsed/Refractory Childhood ALL and AML: A Therapeutic Advances In Childhood Leukemia & Lymphoma (TACL) Study. *Blood* 122 (21), 2013
35. Metzelder S, Wang Y, Wollmer E, et al: Compassionate use of sorafenib in FLT3-ITD-positive acute myeloid leukemia: sustained regression before and after allogeneic stem cell transplantation. *Blood* 113:6567-71, 2009
36. Winkler J, Rech D, Kallert S, et al: Sorafenib induces sustained molecular remission in FLT3-ITD positive AML with relapse after second allogeneic stem cell transplantation without exacerbation of acute GVHD: A case report. *Leukemia Research* 34:e270-e272, 2010

37. Metzelder S, Finck A, Fey M, et al: Sorafenib Monotherapy Is Effective In Relapsed and Refractory FLT3-ITD Positive Acute Myeloid Leukemia, Particularly After Allogeneic Stem Cell Transplantation. *ASH Annual Meeting Abstracts* 116:3314-, 2010
38. Sharma M, Ravandi F, Bayraktar UD, et al: Treatment of FLT3-ITD-positive acute myeloid leukemia relapsing after allogeneic stem cell transplantation with sorafenib. *Biol Blood Marrow Transplant* 17:1874-7, 2011
39. Metzelder SK, Schroeder T, Finck A, et al: High activity of sorafenib in FLT3-ITD-positive acute myeloid leukemia synergizes with allo-immune effects to induce sustained responses. *Leukemia*, 2012
40. Widemann BC, Kim A, Fox E, et al: A phase I trial and pharmacokinetic study of sorafenib in children with refractory solid tumors or leukemias: a Children's Oncology Group Phase I Consortium report. *Clin Cancer Res* 18:6011-22, 2012
41. Leung W, Hudson MM, Strickland DK, et al: Late effects of treatment in survivors of childhood acute myeloid leukemia. *J Clin Oncol* 18:3273-9, 2000
42. Mulrooney DA, Dover DC, Li S, et al: Twenty years of follow-up among survivors of childhood and young adult acute myeloid leukemia: a report from the Childhood Cancer Survivor Study. *Cancer* 112:2071-9, 2008
43. Nicholson HS, Zhou T, Chen Z, et al: Quality of Life in Survivors of Childhood and Adolescent Acute Myelogenous Leukemia (AML) Does Not Differ by Treatment (Bone Marrow Transplant (BMT) vs. Chemotherapy). A Report from the Children's Oncology Group (COG). *ASH Annual Meeting Abstracts* 106:701, 2005
44. Redaelli A, Stephens JM, Brandt S, et al: Short- and long-term effects of acute myeloid leukemia on patient health-related quality of life. *Cancer Treat Rev* 30:103-17, 2004
45. Watson M, Buck G, Wheatley K, et al: Adverse impact of bone marrow transplantation on quality of life in acute myeloid leukaemia patients; analysis of the UK Medical Research Council AML 10 Trial. *Eur J Cancer* 40:971-8, 2004
46. Clarke SA, Eiser C, Skinner R: Health-related quality of life in survivors of BMT for paediatric malignancy: a systematic review of the literature. *Bone Marrow Transplant* 42:73-82, 2008
47. Kazak AE, Barakat LP: Brief report: parenting stress and quality of life during treatment for childhood leukemia predicts child and parent adjustment after treatment ends. *J Pediatr Psychol* 22:749-58, 1997
48. Vannatta K, Zeller M, Noll RB, et al: Social functioning of children surviving bone marrow transplantation. *J Pediatr Psychol* 23:169-78, 1998
49. Barrera M, Boyd-Pringle LA, Sumbler K, et al: Quality of life and behavioral adjustment after pediatric bone marrow transplantation. *Bone Marrow Transplant* 26:427-35, 2000
50. Varni JW, Seid M, Knight TS, et al: The PedsQL 4.0 Generic Core Scales: sensitivity, responsiveness, and impact on clinical decision-making. *J Behav Med* 25:175-93, 2002
51. Varni JW, Seid M, Kurtin PS: PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. *Med Care* 39:800-12, 2001
52. Varni JW, Burwinkle TM, Katz ER, et al: The PedsQL in pediatric cancer: reliability and validity of the Pediatric Quality of Life Inventory Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer* 94:2090-106, 2002
53. Varni JW, Burwinkle TM, Szer IS: The PedsQL Multidimensional Fatigue Scale in pediatric rheumatology: reliability and validity. *J Rheumatol* 31:2494-500, 2004
54. Streisand R, Kazak AE, Tercyak KP: Pediatric-Specific Parenting Stress and Family Functioning in Parents of Children Treated for Cancer. *Children's Health Care* 32:245 - 256, 2003
55. Streisand R, Braniecki S, Tercyak KP, et al: Childhood illness-related parenting stress: the pediatric inventory for parents. *J Pediatr Psychol* 26:155-62, 2001

56. van der Pal HJ, van Dalen EC, Hauptmann M, et al: Cardiac function in 5-year survivors of childhood cancer: a long-term follow-up study. *Arch Intern Med* 170:1247-55, 2010
57. Tukenova M, Guibout C, Oberlin O, et al: Role of cancer treatment in long-term overall and cardiovascular mortality after childhood cancer. *J Clin Oncol* 28:1308-15, 2010
58. Reulen RC, Winter DL, Frobisher C, et al: Long-term cause-specific mortality among survivors of childhood cancer. *JAMA* 304:172-9, 2010
59. Sanchez-Medina J, Gonzalez-Ramella O, Gallegos-Castorena S: The effect of dexrazoxane for clinical and subclinical cardiotoxicity in children with acute myeloid leukemia. *J Pediatr Hematol Oncol* 32:294-7, 2010
60. Woods WG, Neudorf S, Gold S, et al: A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission. *Blood* 97:56-62, 2001
61. Lange BJ, Smith FO, Feusner J, et al: Outcomes in CCG-2961, a children's oncology group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children's oncology group. *Blood* 111:1044-53, 2008
62. Horan JT, Alonzo TA, Lyman GH, et al: Impact of disease risk on efficacy of matched related bone marrow transplantation for pediatric acute myeloid leukemia: the Children's Oncology Group. *J Clin Oncol* 26:5797-801, 2008
63. McDonald GB: Hepatobiliary complications of hematopoietic cell transplantation, 40 years on. *Hepatology* 51:1450-60, 2010
64. Ozkaynak MF, Weinberg K, Kohn D, et al: Hepatic veno-occlusive disease post-bone marrow transplantation in children conditioned with busulfan and cyclophosphamide: incidence, risk factors, and clinical outcome. *Bone Marrow Transplant* 7:467-74, 1991
65. Sencer SF, Haake RJ, Weisdorf DJ: Hemorrhagic cystitis after bone marrow transplantation. Risk factors and complications. *Transplantation* 56:875-9, 1993
66. McDonald GB, Slattery JT, Bouvier ME, et al: Cyclophosphamide metabolism, liver toxicity, and mortality following hematopoietic stem cell transplantation. *Blood* 101:2043-8, 2003
67. Gandhi V, Plunkett W: Cellular and clinical pharmacology of fludarabine. *Clin Pharmacokinet* 41:93-103, 2002
68. Bornhauser M, Thiede C, Platzbecker U, et al: Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res* 7:2254-62, 2001
69. de Lima M, Couriel D, Thall PF, et al: Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood* 104:857-64, 2004
70. Andersson BS, de Lima M, Thall PF, et al: Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. *Biol Blood Marrow Transplant* 14:672-84, 2008
71. Pulsipher MA, Boucher KM, Wall D, et al: Reduced-intensity allogeneic transplantation in pediatric patients ineligible for myeloablative therapy: results of the Pediatric Blood and Marrow Transplant Consortium Study ONC0313. *Blood* 114:1429-36, 2009
72. Resnick IB, Aker M, Tsirigotis P, et al: Allogeneic stem cell transplantation from matched related and unrelated donors in thalassemia major patients using a reduced toxicity fludarabine-based regimen. *Bone Marrow Transplant* 40:957-64, 2007
73. Bradley MB, Satwani P, Baldinger L, et al: Reduced intensity allogeneic umbilical cord blood transplantation in children and adolescent recipients with malignant and non-malignant diseases. *Bone Marrow Transplant* 40:621-31, 2007
74. Irvani M, Evazi MR, Mousavi SA, et al: Fludarabine and busulfan as a myeloablative conditioning regimen for allogeneic stem cell transplantation in high- and standard-risk leukemic patients. *Bone Marrow Transplant* 40:105-10, 2007

75. Horn B, Baxter-Lowe LA, Englert L, et al: Reduced intensity conditioning using intravenous busulfan, fludarabine and rabbit ATG for children with nonmalignant disorders and CML. *Bone Marrow Transplant* 37:263-9, 2006
76. Sievers EL, et al: Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 patients with acute myeloid leukemia. *Blood* 101(9):3398-406, 2003
77. Meshinchi S, et al: Minimal Residual Disease Detection by Four-Color Multidimensional Flow Cytometry Identifies Pediatric AML Patients at High Risk of Relapse. *ASH Annual Meeting Abstracts* 110(11):1429, 2007
78. Rosenberg AR, et al: Sub-Morphologic Evidence of Disease Prior to Stem Cell Transplantation Correlates with Inferior Post Transplant Outcome in Childhood Acute Myeloid Leukemia. *ASH Annual Meeting Abstracts* 114(22):328, 2009
79. Pollard JA, Alonzo TA, Gerbing RB, et al: FLT3 internal tandem duplication in CD34+/CD33-precursors predicts poor outcome in acute myeloid leukemia. *Blood* 108:2764-9, 2006
80. Bonnet D, Dick JE: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3:730-7, 1997
81. Jordan CT, Upchurch D, Szilvassy SJ, et al: The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. *Leukemia* 14:1777-84, 2000
82. Majeti R, Chao MP, Alizadeh AA, et al: CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* 138:286-99, 2009
83. Frankel A, Liu JS, Rizzieri D, et al: Phase I clinical study of diphtheria toxin-interleukin 3 fusion protein in patients with acute myeloid leukemia and myelodysplasia. *Leuk Lymphoma* 49:543-53, 2008
84. Yalcintepe L, Frankel AE, Hogge DE: Expression of interleukin-3 receptor subunits on defined subpopulations of acute myeloid leukemia blasts predicts the cytotoxicity of diphtheria toxin interleukin-3 fusion protein against malignant progenitors that engraft in immunodeficient mice. *Blood* 108:3530-7, 2006
85. 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
86. Guzman ML, Upchurch D, Grimes B, et al: Expression of tumor-suppressor genes interferon regulatory factor 1 and death-associated protein kinase in primitive acute myelogenous leukemia cells. *Blood* 97:2177-9, 2001
87. Majeti R, Becker MW, Tian Q, et al: Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. *Proc Natl Acad Sci U S A* 106:3396-401, 2009
88. Guzman ML, Swiderski CF, Howard DS, et al: Preferential induction of apoptosis for primary human leukemic stem cells. *Proc Natl Acad Sci U S A* 99:16220-5, 2002
89. van Rhenen A, Moshaver B, Kelder A, et al: Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission. *Leukemia* 21:1700-7, 2007
90. Bakker AB, van den Oudenrijn S, Bakker AQ, et al: C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia. *Cancer Res* 64:8443-50, 2004
91. Pollard JA, et al: Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood* 115(12):2372-9, 2010
92. Pollard JA, et al: Prevalence and Prognostic Implications of WT1 Mutations in Pediatric AML A: Report from Children's Oncology Group. *ASH Annual Meeting Abstracts* 112(11):143, 2008
93. Lapillonne H, et al: High WT1 Expression After Induction Therapy Predicts High Risk of Relapse and Death in Pediatric Acute Myeloid Leukemia. *J Clin Oncol* 24(10):1507-1515, 2006

94. Noronha SA, et al: WT1 expression at diagnosis does not predict survival in pediatric AML: a report from the Children's Oncology Group. *Pediatr Blood Cancer* 53(6):1136-9, 2009
95. Ho PA, et al: Molecular alterations of the IDH1 gene in AML: a Children's Oncology Group and Southwest Oncology Group study. *Leukemia*, 2010
96. Wagner K, et al: Impact of IDH1 R132 Mutations and an IDH1 Single Nucleotide Polymorphism in Cytogenetically Normal Acute Myeloid Leukemia: SNP rs11554137 Is an Adverse Prognostic Factor. *J Clin Oncol*, 2010
97. Abdel-Wahab O, Mullally A, Hedvat C, et al: Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. *Blood* 114:144-147, 2009
98. Loh ML, Sakai DS, Flotho C, et al: Mutations in CBL occur frequently in juvenile myelogenous leukemia. *Blood* 114:1859-1863, 2009
99. Lu S, et al: Overexpression of the PSMB5 gene contributes to bortezomib resistance in T-lymphoblastic lymphoma/leukemia cells derived from Jurkat line. *Exp Hematol* 36(10):1278-84, 2008
100. Lu S, et al: Different mutants of PSMB5 confer varying bortezomib resistance in T lymphoblastic lymphoma/leukemia cells derived from the Jurkat cell line. *Exp Hematol* 37(7):831-7, 2009
101. Mori S, Cortes J, Kantarjian H, et al: Potential role of sorafenib in the treatment of acute myeloid leukemia. *Leuk.Lymphoma* 49:2246-2255, 2008
102. 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
103. Guzman ML, Jordan CT: Considerations for targeting malignant stem cells in leukemia. *Cancer Control* 11:97-104, 2004
104. Richardson PG, Hideshima T, Mitsiades C, et al: Proteasome inhibition in hematologic malignancies. *Ann.Med* 36:304-314, 2004
105. Obeng EA, Carlson LM, Gutman DM, et al: Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. *Blood* 107:4907-4916, 2006
106. 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
107. Fels DR, Ye J, Segan AT, et al: Preferential cytotoxicity of bortezomib toward hypoxic tumor cells via overactivation of endoplasmic reticulum stress pathways. *Cancer Res* 68:9323-9330, 2008
108. Davies SM, Wang D, Wang T, et al: Recent decrease in acute graft-versus-host disease in children with leukemia receiving unrelated donor bone marrow transplants. *Biol Blood Marrow Transplant* 15:360-6, 2009
109. Jagasia M, Arora M, Flowers ME, et al: Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood* 119:296-307, 2012
110. Ferrara JL, Harris AC, Greenson JK, et al: Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. *Blood* 118:6702-8, 2011
111. Paczesny S, Braun TM, Levine JE, et al: Elafin is a biomarker of graft-versus-host disease of the skin. *Sci Transl Med* 2:13ra2, 2010
112. Paczesny S, Krijanovski OI, Braun TM, et al: A biomarker panel for acute graft-versus-host disease. *Blood* 113:273-8, 2009
113. Choi SW, Kitko CL, Braun T, et al: Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival. *Blood* 112:1539-42, 2008
114. Kitko CL, Mineishi S, Braun T, et al: Day 7 TNFR1 levels following reduced intensity allogeneic hematopoietic stem cell transplant (HCT) predict for acute GVHD (abstract). *Biol Blood Marrow Transpl.* In press, 2009
115. Paczesny S, Braun T, Lugt MV, et al: Three Biomarker Panel at Day 7 and 14 Can Predict Development of Grade II IV Acute Graft Versus Host Disease. *Blood* 116(21):296-7, 2010

116. Levine JE, Paczesny S, Sarantopoulos S: Clinical applications for biomarkers of acute and chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 18:S116-24, 2012
117. Vardiman JW, Thiele J, Arber DA, et al: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 114:937-95, 2009
118. 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
119. Perrone G, Hideshima T, Ikeda H, et al: Ascorbic acid inhibits antitumor activity of bortezomib in vivo. *Leukemia* 23:1679-86, 2009
120. 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
121. Smith GA, Damon LE, Rugo HS, et al: High-dose cytarabine dose modification reduces the incidence of neurotoxicity in patients with renal insufficiency. *J Clin Oncol* 15(2):833-839, 1997
122. 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.,
123. Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.,
124. Gastrointestinal hemorrhage may include 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.,
125. Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.,
126. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 53:457-481, 1958
127. Burnham KP, Anderson DR: Multimodel inference - understanding AIC and BIC in model selection. *Sociol Method Res*. 33(2):261-304, 2004
128. Sposto R, Sather HN: Determining the duration of comparative clinical trials while allowing for cure. *J Chron Dis* 38:683-690, 1985
129. Kuerbitz SJ, Civin CI, Krischer JP, et al: Expression of myeloid-associated and lymphoid-associated cell-surface antigens in acute myeloid leukemia of childhood: a Pediatric Oncology Group study. *J Clin Oncol* 10:1419-29, 1992
130. Smith FO, Lampkin BC, Versteeg C, et al: Expression of lymphoid-associated cell surface antigens by childhood acute myeloid leukemia cells lacks prognostic significance. *Blood* 79:2415-22, 1992
131. Creutzig U, Harbott J, Sperling C, et al: Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87. *Blood* 86:3097-108, 1995
132. Pui CH, Dahl GV, Melvin S, et al: Acute leukaemia with mixed lymphoid and myeloid phenotype. *Br J Haematol* 56:121-30, 1984
133. Cross AH, Goorha RM, Nuss R, et al: Acute myeloid leukemia with T-lymphoid features: a distinct biologic and clinical entity. *Blood* 72:579-87, 1988
134. Ruiz-Arguelles GJ, Marin-Lopez A, Lobato-Mendizabal E, et al: Acute megakaryoblastic leukaemia: a prospective study of its identification and treatment. *Br J Haematol* 62:55-63, 1986
135. van der Reijden HJ, van Rhenen DJ, Lansdorp PM, et al: A comparison of surface marker analysis and FAB classification in acute myeloid leukemia. *Blood* 61:443-8, 1983

136. Villevall JL, Cramer P, Lemoine F, et al: Phenotype of early erythroblastic leukemias. *Blood* 68:1167-74, 1986
137. Meshinchi S, Woods WG, Stirewalt DL, et al: Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. *Blood* 97:89-94, 2001
138. Thiede C, Steudel C, Mohr B, et al: Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* 99:4326-4335, 2002
139. Stark B, Jeison M, Gabay LG, et al: Classical and molecular cytogenetic abnormalities and outcome of childhood acute myeloid leukaemia: report from a referral centre in Israel. *Br.J.Haematol* 126:320-337, 2004
140. Cuneo A, Bigoni R, Cavazzini F, et al: Incidence of cryptic chromosome aberrations detected by fluorescent in situ hybridization in acute myeloid leukemia with normal karyotype. *Leukemia* 16:745-751, 2002
141. Frohling S, Skelin S, Liebisch C, et al: Comparison of cytogenetic and molecular cytogenetic detection of chromosome abnormalities in 240 consecutive adult patients with acute myeloid leukemia. *J Clin Oncol* 20:2480-2485, 2002
142. Huntly BJ, Bench AJ, Delabesse E, et al: Derivative chromosome 9 deletions in chronic myeloid leukemia: poor prognosis is not associated with loss of ABL-BCR expression, elevated BCR-ABL levels, or karyotypic instability. *Blood* 99:4547-4553, 2002
143. Huntly BJ, Guilhot F, Reid AG, et al: Imatinib improves but may not fully reverse the poor prognosis of patients with CML with derivative chromosome 9 deletions. *Blood* 102:2205-2212, 2003
144. Morel F, Ka C, Le Bris MJ, et al: Deletion of the 5'ABL region in Philadelphia chromosome positive chronic myeloid leukemia: frequency, origin and prognosis. *Leuk Lymphoma* 44:1333-1338, 2003
145. Varni JW, Seid M, Kurtin PS: Measurement model for the Pediatric Quality of Life Inventory Version 4.0. Mapi Research Institute, 1998
146. Logan DE, Radcliffe J, Smith-Whitley K: Parent factors and adolescent sickle cell disease: associations with patterns of health service use. *J Pediatr Psychol* 27(5):475-84, 2002
147. Avvisati G, Tirindelli MC, Annibaldi O: Thrombocytopenia and hemorrhagic risk in cancer patients. *Crit Rev Oncol Hematol* 48:S13-6, 2003
148. Rogers PC, Meacham LR, Oeffinger KC, et al: Obesity in pediatric oncology. *Pediatr Blood Cancer*, 2005
149. Hughes WT, Armstrong D, Bodey GP, et al: guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 34:730-51, 2002
150. Feusner JH, Alonzo TA, Dinndorf P, et al: Infectious Complications of Intensive Chemotherapy on Children's Cancer Group Protocol 2961 for Pediatric Acute Myeloid Leukemia, American Society of Clinical Oncology. Chicago. . 2003
151. Gamis AS, Howells WB, DeSwarte-Wallace J, et al: Alpha hemolytic streptococcal infection during intensive treatment for acute myeloid leukemia: a report from the Children's cancer group study CCG-2891. *J Clin Oncol* 18:1845-55, 2000
152. Rubin M, Hathorn JW, Pizzo PA: Controversies in the management of febrile neutropenic cancer patients. *Cancer Invest* 6:167-84, 1988
153. Bow EJ, Laverdiere M, Lussier N, et al: Antifungal prophylaxis for severely neutropenic chemotherapy recipients: a meta analysis of randomized-controlled clinical trials. *Cancer* 94:3230-46, 2002
154. Kanda Y, Yamamoto R, Chizuka A, et al: Prophylactic action of oral fluconazole against fungal infection in neutropenic patients. A meta-analysis of 16 randomized, controlled trials. *Cancer* 89:1611-25, 2000

155. American Academy of Pediatrics Red book: Report of the committee on infectious diseases (ed 26th). Elk Grove Village, IL, American Academy of Pediatrics. . 2003
156. Bergmann OJ, Mogensen SC, Ellermann-Eriksen S, et al: Acyclovir prophylaxis and fever during remission-induction therapy of patients with acute myeloid leukemia: a randomized, double-blind, placebo-controlled trial. *J Clin Oncol* 15:2269-74, 1997
157. Adolescents NHBPEPWGoHBPiCa: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. *Pediatrics* 114:555-576, 2004