CHILDREN’S ONCOLOGY GROUP

AAML0431

The Treatment of Down Syndrome Children with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS) Under the Age of 4 Years

A Groupwide Phase 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

Jeffrey W. Taub, M.D.
Division of Hematology/Oncology
Children’s Hospital of Michigan
3901 Beaubien Blvd.
Detroit, Michigan  48201
Phone:  (313) 745-5515
Fax:  (313) 745-5237
Email:  jtaub@med.wayne.edu

For Statistics and Data Center Contact Person see:  http://members.childrensoncologygroup.org
<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>STUDY COMMITTEE</td>
<td>4</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>6</td>
</tr>
<tr>
<td>EXPERIMENTAL DESIGN SCHEMA</td>
<td>7</td>
</tr>
<tr>
<td>1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)</td>
<td></td>
</tr>
<tr>
<td>1.1 Primary Aims</td>
<td>8</td>
</tr>
<tr>
<td>1.2 Secondary Aims</td>
<td>8</td>
</tr>
<tr>
<td>2.0 BACKGROUND</td>
<td></td>
</tr>
<tr>
<td>2.1 Event Free Survival and Relapse Rates in Down Syndrome Children</td>
<td>9</td>
</tr>
<tr>
<td>2.2 Age as Prognostic Indicator for Down Syndrome AML Patients</td>
<td>9</td>
</tr>
<tr>
<td>2.3 Toxicities in Down Syndrome AML Patients</td>
<td>10</td>
</tr>
<tr>
<td>2.4 Total Cumulative Drug Doses of Prior Down Syndrome AML Protocols</td>
<td>10</td>
</tr>
<tr>
<td>2.5 Rationale for Trial Design</td>
<td>11</td>
</tr>
<tr>
<td>2.6 Down Syndrome Leukemia Phenotype and GATA1 Mutations</td>
<td>12</td>
</tr>
<tr>
<td>2.7 AML and Minimal Residual Disease Detection</td>
<td>13</td>
</tr>
<tr>
<td>2.8 Down Syndrome AML Cells and Drug Sensitivity</td>
<td>13</td>
</tr>
<tr>
<td>2.9 Gene Expression and Down Syndrome AML</td>
<td>13</td>
</tr>
<tr>
<td>2.10 Polymorphisms in Phase I and Phase II Detoxification Genes and DNA Repair Pathways and Susceptibility to Leukemia and Outcome of Therapy</td>
<td>14</td>
</tr>
<tr>
<td>3.0 STUDY ENROLLMENT AND PATIENT ELIGIBILITY</td>
<td></td>
</tr>
<tr>
<td>3.1 Study Enrollment</td>
<td>15</td>
</tr>
<tr>
<td>3.2 Patient Criteria</td>
<td>15</td>
</tr>
<tr>
<td>4.0 TREATMENT PLAN</td>
<td></td>
</tr>
<tr>
<td>4.1 Induction I</td>
<td>18</td>
</tr>
<tr>
<td>4.2 Induction II</td>
<td>19</td>
</tr>
<tr>
<td>4.3 Induction III</td>
<td>22</td>
</tr>
<tr>
<td>4.4 Induction IV</td>
<td>24</td>
</tr>
<tr>
<td>4.5 Intensification I</td>
<td>26</td>
</tr>
<tr>
<td>4.6 Intensification II</td>
<td>28</td>
</tr>
<tr>
<td>5.0 DOSE MODIFICATIONS FOR TOXICITIES</td>
<td></td>
</tr>
<tr>
<td>5.1 CNS toxicity</td>
<td>32</td>
</tr>
<tr>
<td>5.2 Cardiac Toxicity</td>
<td>32</td>
</tr>
<tr>
<td>5.3 Hepatic Toxicity</td>
<td>32</td>
</tr>
<tr>
<td>5.4 Renal Toxicity</td>
<td>32</td>
</tr>
<tr>
<td>5.5 Allergy to Etoposide</td>
<td>33</td>
</tr>
<tr>
<td>6.0 DRUG INFORMATION</td>
<td></td>
</tr>
<tr>
<td>7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED</td>
<td></td>
</tr>
<tr>
<td>7.1 Required, Recommended and Optional Clinical, Laboratory and Disease Evaluations</td>
<td>33</td>
</tr>
<tr>
<td>7.2 Follow-up Studies</td>
<td>34</td>
</tr>
<tr>
<td>8.0 SUPPORTIVE CARE GUIDELINES</td>
<td></td>
</tr>
<tr>
<td>8.1 Tumor Lysis Syndrome</td>
<td>35</td>
</tr>
<tr>
<td>8.2 Hyperleukocytosis</td>
<td>35</td>
</tr>
<tr>
<td>8.3 Venous Access Lines</td>
<td>35</td>
</tr>
<tr>
<td>8.4 Prophylaxis for Patients with Congenital Heart Defects</td>
<td>35</td>
</tr>
</tbody>
</table>
9.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA
9.1 Criteria for Removal from Protocol Therapy
9.2 Off Study Criteria

10.0 STATISTICAL CONSIDERATIONS
10.1 Statistical Design
10.2 Patient Accrual and Expected Duration of Trial
10.3 Statistical Analysis Methods
10.4 Gender and Minority Accrual Estimates

11.0 EVALUATION CRITERIA
11.1 Common Terminology Criteria for Adverse Events (CTCAE)
11.2 Response Criteria for Patients with Acute Myeloid Leukemia

12.0 ADVERSE EVENT REPORTING REQUIREMENTS
12.1 Purpose
12.2 Determination of Reporting Requirements
12.3 Reporting of Adverse Events for Commercial Agents - AdEERS abbreviated pathway
12.4 Routine Adverse Event Reporting
12.5 Reporting Secondary AML/MDS

13.0 RECORDS AND REPORTING
13.1 CDUS

14.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS
14.1 Central Review of Diagnosis
14.2 Local Cytogenetic Analysis and Data Submission to Central Laboratory

15.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS
15.1 DNA/RNA Extraction and Drug Sensitivity Assays for Consenting Patients
15.2 Minimal Residual Disease Detection for Consenting Patients
15.3 Pharmacokinetic Studies during High-dose Ara-C (Induction II) for Consenting Patients
15.4 Banking Specimens

APPENDIX I: PERFORMANCE STATUS SCALES/SCORES

APPENDIX II: LIST OF ANTICONVULSANTS BASED ON CYP3A4/ENZYME INDUCTION

REFERENCES

SAMPLE INFORMED CONSENT/PARENTAL PERMISSION FOR PARTICIPATION IN RESEARCH
STUDY COMMITTEE

STUDY CHAIR
Jeffrey W. Taub, M.D.
Pediatric Oncology
Children’s Hospital of Michigan
3901 Beaubien Blvd.
Detroit, MI 48201
Phone: (313) 745-5515
Fax: (313) 745-5237
E-mail: jtaub@med.wayne.edu

STUDY CHAIR
Alan Gamis, M.D.
Hematology/Oncology
The Children’s Mercy Hospital
Department of Hematology/Oncology
2401 Gillham Rd
Kansas City, MO 64108
Phone: (816) 234-3265
Fax: (816) 855-1700
E-mail: agamis@cmh.edu

STUDY VICE CHAIR
Prasad Mathew, M.D.
Pediatric Oncology
University of New Mexico
1 University of New Mexico School of medicine
MSC 10 5590 ACC 3rd fl.
Albuquerque, NM 87131
Phone: (505) 272-4461
Fax: (505) 272-8699
E-mail: pmathew@salud.unm.edu

STUDY VICE CHAIR
David Richmond Head, M.D.
Pathology
Vanderbilt Children's Hospital
Vanderbilt Clinical Labs
1161 21st. Avenue S., 4605 TBC
Nashville, TN 37232
Phone: (615) 322-0126
Fax: (615) 343-8420
E-mail: david.head@vanderbilt.edu

STUDY STATISTICIAN
Todd Alonzo, Ph.D.
Biostatistics
Childrens Oncology Group
440 Huntington Drive; 4th Floor
Arcadia CA 91006
Phone: (626) 241-1522
Fax: (626) 445-4334
E-mail: talonzo@childrensoncologygroup.org

Betsy Hirsch, Ph.D.
Cytogenetics
University of Minnesota Cancer Center
Division of Laboratory Medicine
420 Delaware St., SE Box 609
Minneapolis, MN 55455
Phone: (612) 273-4952
Fax: (612) 273-4689
E-mail: hirscc003@umn.edu

Robert Gerbing, M.S.
Statistics
Children's Oncology Group - Operations Center
440 Huntington Drive; 4th Floor
Arcadia CA 91006
Phone: (626) 241-1526
Fax: (626) 445-4334
E-mail: rgerbing@childrensoncologygroup.org

Johann Hitzler, M.D., F.R.C.P.C.
Hematology/Oncology
Hospital for Sick Children
555 University Avenue
Toronto ON M5G1X8
Canada
Phone: (416) 813-8887
Fax: (416) 813-5327
E-mail: johann.hitzler@sickkids.ca

STUDY COMMITTEE MEMBERS
Gita Vasers Massey, M.D.
Hematology/Oncology
Virginia Commonwealth Univ Health System-MCV
MCV Station
P.O. Box 980121
Richmond, VA 23298-0121
Phone: (804) 828-9605
Fax: (804) 828-6455
E-mail: GMassey2@mcvh-vcu.edu

Deborah Lynn Robinson, B.S.N., M.S.N.R.
Nursing
Washington University Medical Center
1 Children's Place
Pediatrics
2426 Christopher Winds Lane
St Louis, MO 63129
Phone: (314) 454-6045
Fax: (314) 454-2780
E-mail: dlr3005@bjc.org
April D. Sorrell, M.D.
Hematology/Oncology
City of Hope National Medical Center
Peds Hematology & Stem Cell Transplantation
1500 E. Duarte Rd
Duarte, CA 91010-3000
Phone: (626) 301-8442 x64331
Fax: (626) 256-8723
E-mail: asorrell@coh.org

AGENT NSC#
Cytarabine #063878
Daunorubicin #82151
Etoposide #141540
L-asparaginase #109229
Thioguanine #000752

SEE SECTIONS 14 AND 15 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 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**

Down Syndrome (DS) children with Acute Myeloid Leukemia (AML) have extremely high event-free survival (EFS) rates and lower relapse rates compared to non-DS children with AML based on past Pediatric Oncology Group (POG) and Children’s Cancer Group (CCG) studies. In the prior CCG-2891 study, age was reported to be a prognostic factor for DS AML patients. DS children between the ages of 0-2 years had significantly higher EFS rates compared to children between the ages of 2-4 years, while DS children older than 4 years had the worse outcome. However, in the recently closed COG-A2971 study, no significant difference was observed for the age groups (< 2 years and ≥ 2 to < 4 years), with the overall survival (OS) for the 0-4 year old group was 88.1% and the EFS rate was 77.9%. The current study is designed to improve the EFS rates of DS AML patients < 4 years of age at diagnosis compared to COG-A2971 and attempt to decrease potential treatment-related toxicity. The remission induction backbone of the COG-A2971 study using continuous infusion cytarabine (Ara-C)/daunorubicin and oral 6-thioguanine will be used for the first, third and fourth courses of induction therapy. Cytarabine (Ara-C) therapy will be intensified during induction therapy by administering the Capizzi II arm (high-dose Ara-C/L-asparaginase) as the second course of therapy, which was previously administered as the intensification therapy (overall fifth course of therapy) on CCG-2891 and COG-A2971. Two courses of intensification therapy will consist of etoposide and Ara-C. The number of intrathecal chemotherapy treatments will be reduced from seven to two and the cumulative anthracycline dose reduced by 25%, based on the high incidence of congenital cardiac defects in DS children and potential risks for developing cardiomyopathies. Biological studies will include: i) analysis of the frequency of GATA1 mutations, their relationship to the megakaryocytic phenotype, and treatment outcome; ii) minimal residual disease monitoring by flow cytometry and its relationship to remission status and outcome; iii) in vitro pharmacology and in vivo pharmacokinetic studies and their relationship to age, GATA1 gene status and outcome; iv) gene expression profiles analyzed by microarrays and the relationship to leukemia phenotype and outcome.
EXPERIMENTAL DESIGN SCHEMA

Study Entry
< 4 years of age at diagnosis

Induction I  CI-TAD + IT AraC

Induction II  Capizzi II Course

Induction III  CI-TAD + IT AraC

Induction IV  CI-TAD

Intensification I  VP/Ara-C

Intensification II  VP/Ara-C

Follow-Up

BMA/MRD At Diagnosis

BMA/MRD^1 Day 14

BMA/MRD Day 28^2

If cellular, moderately cellular, ≥ 20% blasts

If cellular, moderately cellular, ≥ 20% blasts

BMA/MRD^1 Day 14 and 28 if PR or RD after Induction I Day 28 marrow

If RD

PR, RD or Relapse

Off Protocol Therapy

BMA = Bone Marrow Aspirate.
MRD = Minimal Residual Disease (if consent is obtained).
CI-TAD: Continuous Infusion Cytarabine (Ara-C)/Daunorubicin + 6-Thioguanine
CR = Complete Response (See Section 11.2)
PR = Partial Response (See Section 11.2)
RD = Refractory Disease (See Section 11.2)
Capizzi II: Cytarabine (Ara-C)/L-asparaginase
VP/Ara-C: Etoposide/Cytarabine (Ara-C)
IT AraC: Intrathecal Cytarabine

1) See Section 4.0 for BMA Evaluations.
2) If Day 28 marrow is aplastic/severely hypocellular, repeat bone marrow one week later (see Section 4.0)
1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To determine the event-free survival (EFS) and overall survival (OS) rates of Down syndrome (DS) children with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS) < 4 years of age at diagnosis.

1.1.2 To determine if the EFS rate of DS AML patients aged < 4 years at diagnosis can be increased compared to the EFS rate on COG A2971 with an intensification of cytarabine (Ara-C) therapy during induction therapy.

1.1.3 To determine if the number of intrathecal chemotherapy treatments can be reduced in the treatment of DS AML patients < 4 years of age at diagnosis.

1.1.4 To determine if the total cumulative anthracycline can be reduced in the treatment of DS AML patients < 4 years of age at diagnosis.

1.2 Secondary Aims

1.2.1 To determine the types and degrees of treatment-related toxicity of DS AML patients.

1.2.2 To determine the prevalence of leukemia phenotype and GATA1 mutations of DS patients < 4 years of age at diagnosis.

1.2.3 To determine the relationship of GATA1 mutations with leukemia phenotype and EFS rates of DS patients < 4 years of age at diagnosis.

1.2.4 To determine the relationship of Minimal Residual Disease (MRD) monitored by flow cytometry and remission status during and after completion of therapy based on bone marrow morphology.

1.2.5 To examine parameters of in vitro drug sensitivity and in vivo Ara-C pharmacokinetics.

1.2.6 To examine gene expression profiles by microarrays and the relationship to leukemia phenotype and outcome.

1.2.7 To examine the relationship of functional polymorphisms in Phase I and Phase II detoxification genes and DNA repair pathways that may modify susceptibility to leukemia and outcome of therapy in DS children.

1.2.8 To assess the effect of karyotypic abnormalities on survival.

1.2.9 To establish a DS leukemia cell bank for future biological studies.
2.0 BACKGROUND

2.1 Event Free Survival and Relapse Rates in Down Syndrome Children

Since the early 1990’s, it has been recognized that Down syndrome (DS) children with Acute Myeloid Leukemia (AML) have extremely high event-free survival (EFS) rates and lower relapse rates compared to non-DS children with AML. Studies from both the Pediatric Oncology Group (POG) and Children’s Cancer Group (CCG) have highlighted this unique clinical observation which appeared to coincide with the utilization of high-dose cytarabine (Ara-C) therapy (e.g. 3 grams/m²/dose) for DS AML patients.1-7 Common clinical features of the DS AML patients was the predominance of the megakaryocytic leukemia (AMKL) phenotype. The high EFS rate of DS patients is partly on account of increased sensitivity to Ara-C and daunorubicin.8,9 Lower-dose Ara-C regimens have also been used successfully for the treatment of DS AML/Myelodysplastic Syndrome (MDS) patients.10-12

A summary of the outcome of DS AML patients treated on recent clinical trials is summarized in Table 1. These results demonstrate the high EFS rates and low relapse rates of DS AML patients in the recent treatment era.

Table 1 Treatment Outcome of Down Syndrome Children with AML/MDS

<table>
<thead>
<tr>
<th>Protocol</th>
<th>N=</th>
<th>EFS</th>
<th>Relapse</th>
<th>Rx deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>POG 8498</td>
<td>12</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>POG 8821</td>
<td>34</td>
<td>68%</td>
<td>15%</td>
<td>18%</td>
</tr>
<tr>
<td>(Age &lt;2 years)</td>
<td></td>
<td>79%</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>CCG-2861/2891</td>
<td>109</td>
<td>69% (88%*)</td>
<td>9%</td>
<td>14%</td>
</tr>
<tr>
<td>NOPHO4</td>
<td>38</td>
<td>83%</td>
<td>11%</td>
<td>0%</td>
</tr>
<tr>
<td>AML-BFM98</td>
<td>58</td>
<td>89%</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>Japan11</td>
<td>33</td>
<td>80%</td>
<td>9%</td>
<td>9%</td>
</tr>
</tbody>
</table>

CCG-2861 included DS AML patients treated with intensively timed induction; *Disease free survival with conventional induction therapy; NR: not reported

2.2 Age as Prognostic Indicator for Down Syndrome AML Patients

Recently age has been reported to be a prognostic factor for DS AML patients. The 161 DS AML/MDS patients treated on the CCG-2891 study with standard timed induction had significantly better 8-year EFS (77±7% v 21±4% standard and 40±4% intensive induction; \( P < 0.0001 \)) compared to non-DS patients.13 Remission induction consisted of 4 cycles of continuous infusion Ara-C/daunorubicin/etoposide and oral dexamethasone/6-thioguanine [each cycle for 4 days] followed by one course of the Capizzi II regimen with high-dose Ara-C/L-asparaginase followed by low-dose maintenance therapy for 3 months. Multivariate analysis in DS children revealed that only age at diagnosis of 2 years or older was a risk factor for greater relapse risk (odds ratio, 4.9; \( P = 0.006 \)) and worse survival. Children between ages 0 to 2 years (n = 94) had a 6-year EFS of 86±7%; those from 2 to 4 years (n = 58), 68±12%; and those older than 4 years (n = 9), 33±31%. Remission failures were the primary reason for worse 6-year EFSs (1% in those 0 to 2 years v 14% if > 2 years; \( P = 0.002 \)).

In the recent COG-A2971 study, the induction therapy for Arm B patients with AML utilized the identical dosing of Ara-C/daunorubicin/6-thioguanine for the 4 cycles of induction therapy with the elimination of etoposide and dexamethasone, followed by one course of the Capizzi II regimen and two additional intrathecal treatments at the end of the protocol without the use of low-dose maintenance therapy. This protocol was recently closed (10/1/04) to further enrollment of newly diagnosed AML patients, though patients with the transient myeloproliferative disorder previously enrolled on Arm A of the study could crossover to receive therapy on Arm B if they developed either AML or myelodysplastic syndrome. Preliminary results of the A2971 study have not demonstrated a difference in overall (OS) survival and
EFS rates between the 0-2 and 2-4 year old groups; the 2.5 year estimated OS and EFS rates for the different age categories are: i) < 2 years (89±7% and 83±8%); ii) 2-4 years (82±12% and 79±13%) and iii) > 4 years (33±38% and 33±38%). For the entire 0 to < 4 year old DS group, the OS was 86.5% and the EFS rate was 81.7%.

### 2.3 Toxicities in Down Syndrome AML Patients

Increased toxicity including treatment-related deaths, particularly infection-related, of DS AML patients have been reported in several studies including the POG 8821 study.\(^3,14,15\) In view of the high incidence of congenital cardiac defects in DS children, potential concerns exist particularly with the use of anthracyclines. No cardiac toxicity was reported in the POG 8498 (Phase II) study with a cumulative daunorubicin dose of 135 mg/m\(^2\), while 5 of 34 patients (4 patients < 2 years of age) treated on POG 8821 (cumulative daunorubicin dose of 350 mg/m\(^2\)), developed either cardiomyopathies or reduced cardiac function. Of these 5 patients, one had an underlying congenital heart defect (repaired A-V canal), while an additional 9 patients with underlying congenital heart defects, were not reported to have developed cardiac toxicity. Unpublished data from the POG 9421 AML study also suggested an increased frequency of cardiac-related late effects which used a total cumulative anthracycline dose of ~375 mg/m\(^2\) [daunorubicin: 135 mg/m\(^2\); mitoxantrone: 80 mg/m\(^2\) with an approximate conversion factor of 3:1], with 15/62 (24%) of DS patients developing cardiomyopathies [Gita Massey-personal communication]. Based on a review of 6,493 children treated on POG protocols from 1974-1990 with anthracycline chemotherapy, multivariate analysis identified that DS children had a relative risk of 3.4 to develop cardiac toxicity.\(^16\) This suggests that DS children have an increased risk of anthracycline-induced cardiac toxicity, potentially due to the localization of the superoxide dismutase and carbonyl reductase genes on chromosome 21, which are involved in oxygen radical and anthracycline metabolism, respectively.

For the CCG-2891 study, there were no significant differences in Grade III/IV toxicity at any site between DS and non-DS patients treated with the high-dose Ara-C (Capizzi II cycle).\(^13\) Non-DS patients experienced significantly greater hepatic toxicity [7.4% v 2%; \(P = 0.036\)], while DS patients experienced greater mucositis [9.6% v 3.7%; \(P = 0.023\)] and skin toxicity [3.7% v 0.4%; 0.024] compared to the non-DS patients.

### 2.4 Total Cumulative Drug Doses of Prior Down Syndrome AML Protocols

Table 2 summarizes the total cumulative drug doses of prior DS AML protocols from POG, CCG, BFM and Japanese groups. The cumulative drug doses for the AAML0431 study are included in the table.

<table>
<thead>
<tr>
<th></th>
<th>AAML0431 ( &lt; 4 years)</th>
<th>POG 8498 Phase II</th>
<th>POG 8821</th>
<th>POG 9421</th>
<th>AML-BFM98</th>
<th>CCG 2891</th>
<th>CCG/COG A2971</th>
<th>Japanese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>27.8 g/m(^2)</td>
<td>40.7 g/m(^2)</td>
<td>48.1 g/m(^2)</td>
<td>20.7 g/m(^2)</td>
<td>23-29 g/m(^2)</td>
<td>27.2 g/m(^2)</td>
<td>27.2 g/m(^2)</td>
<td>1.4-5.6 g/m(^2)</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>240 mg/m(^2)</td>
<td>135 mg/m(^2)</td>
<td>350 mg/m(^2)</td>
<td>135 mg/m(^2)</td>
<td>220 mg/m(^2)*</td>
<td>350 mg/m(^2)</td>
<td>350 mg/m(^2)</td>
<td>100-400 mg/m(^2)</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td></td>
<td></td>
<td>80 mg/m(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>750 mg/m(^2)</td>
<td>3000 mg/m(^2)</td>
<td>3000 mg/m(^2)</td>
<td>1000 mg/m(^2)</td>
<td>950 mg/m(^2) *</td>
<td>1600 mg/m(^2)</td>
<td></td>
<td>900-3600 mg/m(^2)</td>
</tr>
<tr>
<td>Total Cycles</td>
<td>6</td>
<td>7+POMP x 4; Cl Ara-C x 4</td>
<td>9</td>
<td>5</td>
<td>5 + maintenance</td>
<td>5 + maintenance</td>
<td>5 + intrathecal</td>
<td>2-8</td>
</tr>
</tbody>
</table>

CI: continuous infusion; *cumulative anthracycline dose of idarubicin and mitoxantrone
2.5 **Rationale for Trial Design**

This study is designed to optimize the treatment of DS AML patients and improve the EFS rates compared to historical controls. The treatment arm for patients ages < 4 years will intensify Ara-C therapy during induction therapy to try and improve EFS rates compared to the COG A2971 protocol.

2.5.1 **Specific hypotheses**

The specific hypotheses for this study are as follows:

A) The use of high dose Ara-C therapy with L-asparaginase (Capizzi II regimen) administered as the second course of induction therapy and the addition of a second course of Intensification therapy can improve the EFS rate compared to COG A2971 for DS AML patients < 4 years of age at diagnosis.

B) The number of intrathecal chemotherapy treatments can be reduced from 7 to 2 based on the extremely low incidence of CNS involvement of DS patients.

C) The total cumulative anthracycline dose can be reduced by 25% in view of potential risks for the development of cardiomyopathies.

The following is the percentage of DS children with AML who fit in the age strata based on the COG A2971 study: Age < 2 years: 64%; Age ≥ 2 to < 4 years: 31%; Age ≥ 4 years: 5%.

2.5.2 **Total Cumulative drug doses for first two Induction courses from prior protocols**

Table 3 summarizes the total cumulative drug doses of the first two courses of induction therapy from prior POG and CCG protocols, as well as the cumulative drug doses for the AAML0431 study.

**Table 3: Summary of the Total Cumulative Drug Doses for the First Two Cycles of Induction Therapy of POG, CCG, and COG AML Protocols**

<table>
<thead>
<tr>
<th></th>
<th>AAML0431</th>
<th>POG 8498 Phase II</th>
<th>POG 8821</th>
<th>POG 9421</th>
<th>CCG 2891</th>
<th>CCG/COG A2971</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>24.8 g/m²</td>
<td>18.7 g/m²</td>
<td>18.7 g/m²</td>
<td>10.7 g/m²</td>
<td>1.6 g/m²</td>
<td>1.6 g/m²</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>80 mg/m²</td>
<td>135 mg/m²</td>
<td>135 mg/m²</td>
<td>135 mg/m²</td>
<td>160 mg/m²</td>
<td>160 mg/m²</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>800 mg/m²</td>
<td></td>
</tr>
</tbody>
</table>

For the POG studies, the first two cycles of therapy were defined as induction therapy while the first four cycles of therapy for the CCG studies were defined as induction therapy.

2.5.3 **Analysis of prior DS AML studies**

An analysis of the prior POG and CCG DS AML studies raise several issues in the design of a new therapeutic protocol:

2.5.3.1 Patients Age < 4 Years

In order to improve the EFS rate of DS patients aged < 4 years of age at diagnosis compared to historical controls, increasing the total number of cycles of therapy and/or increasing the dose intensity can be attempted if this would likely not result in a significant increase in morbidity and/or mortality. For the CCG 2891 and COG A2971 studies, the first two cycles (out of a total of 4 identical cycles of induction therapy), the total cumulative Ara-C dose was 1.6 g/m².

Based on the unique pattern of increased drug sensitivity of DS AML cells, particularly to Ara-C and
daunorubicin, it is conceivable that earlier exposure to high-dose Ara-C (3 g/m²), based cycles as utilized during the second induction cycle for the POG 8498, 8821 and 9421 studies or the Capizzi II regimen used in CCG-2891/2971 protocols (for the overall fifth cycle of therapy) may improve the induction rate for this age group. Of the 29 evaluable DS patients between the ages of 2-4 years treated on the POG 8498, 8821 and 9421 studies, 27 (93%) achieved a CR after two cycles of induction therapy. In this age group, there was one induction failure and one partial response (M2 bone marrow). DS patients treated on the CCG-2891 study had comparable degrees of toxicity compared to non-DS patients during the intensification phase with the Capizzi II regimen, except for significantly higher rates of mucositis and skin toxicity.

For this protocol, CI-TAD will be administered for Cycles 1, 3 and 4 of induction therapy while the Capizzi II regimen will be administered as the second cycle of induction therapy.

Patients will be treated with a total of 6 cycles of therapy (Induction x 4; Intensification x 2). The two cycles of intensification therapy will be administered based on a modification of the Japanese regimen using etoposide and continuous infusion Ara-C. The Japanese protocol used repetitive courses of Ara-C (continuous infusion x 7 days), etoposide x 3 days and daunorubicin x 2 days for a total of 2-8 courses. The overall EFS rate of the 33 MDS/AML patients (all < 4 years of age) was 80% ± 7% at 8 years. There were 3 relapses on this protocol and 3 deaths from cardiac toxicity (n = 2) and septic shock (n = 1). The daunorubicin dose for these two cycles [total cumulative dose: 100 mg/m²] is being eliminated to minimize potential cardiac toxicity.

2.5.3.2 All Patients < 4 Years
For the AAML0431 study, the overall total cumulative daunorubicin dose will be 240 mg/m², which is comparable to the cumulative anthracycline dose of the AML-BFM 98 protocol [220-240 mg/m²], which reported an EFS rate of 89% in 66 patients.

Based on the extremely low rate of CNS involvement in DS AML cases, a total of two intrathecal chemotherapy treatments will be administered for the study (Induction I and Induction III), instead of the seven treatments administered on COG A297 (Induction: 4; Intensification: 3).

2.5.3.3 Patients Age ≥ 4 Years
The outcome of DS patients ≥ 4 years of age has been demonstrated in POG, CCG, NOPHO and BFM protocols to have a significantly inferior outcome. As this age group represents a small proportion of the total DS AML population (5% of DS patients on COG A2971), a separate protocol will not be designed for this group, though it will be recommended that they be enrolled on the current frontline COG AML study.

2.6 Down Syndrome Leukemia Phenotype and GATA1 Mutations
AMkL is the most common FAB subtype of DS AML/MDS cases as reported in the POG 8498 (58%), POG 8821 (47%), POG 9421 (91%), CCG-2891 (70%), AML-BFM98 (97%), Toronto (100%) and Japan (100%) studies. The discrepancy in frequency of AMkL cases is likely due to variations by individual institutions or by laboratories in the identification of megakaryoblasts by either morphology or the identification of surface expression of platelet associated membrane antigens (glycoprotein IIb/IIIa) using CD41/61 monoclonal antibodies.

Recently, somatic mutations in exon 2 of the GATA1 gene (which encodes a zinc-finger transcription factor that is essential for normal erythroid and megakaryocytic differentiation) have been detected exclusively in almost all DS AMkL and transient myeloproliferative disorder (TMD) cases and not in any non-DS AML or non-AMkL DS leukemia case analyzed. This is the most specific abnormality other than trisomy 21, linked exclusively to DS AMkL. It is conceivable that the presence of a GATA1 mutation is synonymous with the AMkL phenotype in DS children.
2.7 AML and Minimal Residual Disease Detection

Prospective monitoring of minimal residual disease (MRD) detection is gaining widespread use in clinical AML protocols including the St. Jude AML 2002 protocol in which therapy is based on a more sensitive measure of bone marrow remission status compared to bone marrow morphology. The most commonly utilized MRD detection method in AML utilizes flow cytometry, while the use of PCR–based assays to detect leukemia-associated AML translocations is only applicable in approximately 30% of AML cases (and fewer DS AML cases which typically lack translocations). It is unlikely that GATA1 mutations can be widely used as a MRD marker in DS AMkL cases. MRD testing in DS AML cases may provide important information to better identify why DS AML patients have extremely high EFS rates and also identify the small proportion of patients who do not have a favorable treatment response (e.g. remission induction failure) which could necessitate an intensification of therapy. Currently, the relationship of MRD in DS is unknown. In a recent St. Jude study, a DS AML patient who remained in clinical remission off therapy, had persistent MRD positivity, indicating the significance of MRD in DS needs to be tested in a larger population.

2.8 Down Syndrome AML Cells and Drug Sensitivity

Studies from Children’s Hospital of Michigan and POG have identified factors which contribute to the significantly higher event-free survival (EFS) rates of DS AML patients. (i) DS blast cells generated significantly higher (median 5.2-fold) levels of the active intracellular Ara-C metabolite, Ara-CTP following in vitro incubations with ³H-Ara-C and were 4.5-fold more sensitive to Ara-C [median IC₅₀: 77.5 nm] compared to a large sample (n = 362) of non-DS leukemia cells [median IC₅₀: 350.9 nm]. The genes encoding the Ara-C metabolizing enzymes cytidine deaminase (CDA; catalyzes the deamination of Ara-C to the inactive metabolite Ara-U) and deoxycytidine kinase (dCK; rate limiting enzymes which phosphorylates Ara-C to the active metabolite Ara-CTP) were expressed at 2.7-fold lower and 2.6-fold higher levels, respectively, in DS compared to non-DS AML cells. In these studies, all of the DS AML samples were determined to be the AMkL phenotype in a central POG reference laboratory, suggesting that the increased sensitivity of DS AML cases is related to the M7 phenotype. The relationship of in vitro drug sensitivity and age of DS AML patients has not been determined to date.

The uniform detection of GATA1 mutations in DS AMkL/TMD cases raises an intriguing possibility that GATA1 mutations in DS TMD and AMkL blasts results in differential regulation of target genes (e.g. CDA which deaminates Ara-C to the inactive metabolite Ara-U) that contribute to the extremely high EFS rates when utilizing Ara-C based chemotherapy. Stable transfection of the wild-type GATA1 coding cDNA into the DS AMkL cell line, CMK (which contains a mutated GATA1 gene), resulted in a 3-fold decreased generation of Ara-CTP and decreased Ara-C sensitivity following incubation with ³H-Ara-C accompanied by increased CDA expression, compared to wild-type and mock-transfected CMK cells, confirming a relationship between GATA1 mutations and Ara-C sensitivity.

DS leukemia cells were also 12-fold more sensitive to daunorubicin [median IC₅₀: 5.8 nm] compared to a large sample of non-DS leukemia cells [median IC₅₀: 71.2 nm]. Studies from groups in the Netherlands and Finland have also reported the increased in vitro sensitivity of DS leukemia cells to Ara-C and daunorubicin as well as to 6-thioguanine and etoposide. Overexpression of chromosome 21-localized genes, including cystathionine-β-synthase (CBS) have also been linked to the increased Ara-C sensitivity of DS leukemia cells and potential overexpression of superoxide dismutase linked to increased daunorubicin sensitivity.

2.9 Gene Expression and Down Syndrome AML

Several recent studies have used microarray analysis to characterize gene expression profiles of pediatric AML patients linked to FAB phenotypes, leukemia-associated translocations and treatment outcome. In a study from St. Jude Children’s Research Hospital, AMkL cases displayed specific gene clustering profiles which included two DS AMkL samples, though based on sample size, no DS vs. non-DS AMkL
comparison was performed.\textsuperscript{27}

One study has compared the gene expression profiles of DS TMD ($n = 9$) and DS AMkL ($n = 7$) patients utilizing the Affymetrix HG U133A microarray.\textsuperscript{28} Supervised clustering identified a number of differentially expressed genes which were confirmed by real-time PCR. The genes included: i) CDKN2C which is a mediator of GATA1-regulated cell cycle arrest and was overexpressed in AMkL compared to TMD cases; ii) the neuroblastoma-associated oncogene, MYCN, which was expressed at significantly higher levels in TMD cases compared to AMkL; and iii) PRAME (preferentially expressed antigen in melanoma) which was identified as a specific marker for AMkL and was not expressed in TMD cases. This study, however, failed to link these specific genes with any of the unique features of DS TMD/AMkL cases including the spontaneous regression of TMD, the progression of 20-30\% of TMD cases to AMkL and the high EFS rates of DS AMkL patients.

An additional study from The Hospital for Sick Children compared DS TMD and DS AMkL patients using an institution designed microarray and identified a different set of genes compared to the former study.\textsuperscript{29}

\section*{2.10 Polymorphisms in Phase I and Phase II Detoxification Genes and DNA Repair Pathways and Susceptibility to Leukemia and Outcome of Therapy}

Common functional genetic polymorphisms in drug-metabolizing enzymes may result in impaired detoxification of carcinogens and/or chemotherapeutic agents. A variety of enzymatic mechanisms are involved in the metabolism of genotoxic agents, including oxidative activation by Phase I enzymes and detoxification through conjugation by Phase II enzymes. In addition, other polymorphic enzyme systems including those that metabolize endogenous oxidants and repair DNA are also potentially involved in host response to mutagenic stress. A growing number of studies have identified a potential role for polymorphisms in the genes encoding the glutathione-S-transferases (GSTs), NAD(P)H:quinone oxidoreductase, myeloperoxidase, N-acetyltransferase (NATs), cytochrome P450 (CYP) 1A1 and 3A4, methylenetetrahydrofolate reductase, cystathionine-beta-synthase (CBS), Glutathione peroxidase 1 (GPX1), O6-alkylguanine alkyltransferase (AGT), X-Ray Cross Complementing (XRCC1 and XRCC3), Excision Repair Cross-Complementing 1 (ERCC1), Fanconi Anemia A (FAA), and others in the etiology of acute leukemias and therapy-related complications.\textsuperscript{30} A previous Children’s Cancer Group (CCG) study, examined the frequency of the polymorphic genes GSTM1 and GSTT1 in 232 white (non-Hispanic) children with AML.\textsuperscript{31} Absence of GSTM1 or GSTT1 enzyme activity is due to homozygous inherited deletion of the gene, reducing detoxification of carcinogens such as epoxides and alkylating agents and potentially increasing cancer risk. In this study the frequency of GSTM1 null genotype was significantly increased in AML/MDS cases compared to controls (64\% vs 47\%; OR 2.0, (95\% CI 1.3-3.1); $P = 0.001$), while the frequency of GSTT1 null genotype in AML/MDS cases was not statistically different from controls. A test for homogeneity across FAB subtypes revealed a statistically significant difference among subtypes ($P = 0.04$, 8df) for GSTM1 only. In particular, there was an increased frequency of GSTM1 null genotypes in FAB groups M3 (82\%, $n = 22$, OR 5.1 (95\% CI 1.6-21.3)) and M4 (72\%, $n = 53$, OR 2.9, (95\% CI 1.4-6.0)).
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 eRDE 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).*

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.ctsu.org). Any other regulatory documents needed for access to the study enrollment screens will be listed for the study on the CTSU Member’s Website under the RSS Tab.

IRB/REB approval documents may be faxed (1-215-569-0206), Emailed (CTSURegulatory@ctsu.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-CTSU. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

3.1.3 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 eRDE system. If you have problems with enrollment, refer to online help in the Applications area of the COG website.

3.1.4 Timing
Study enrollment must take place within five (5) calendar days of beginning protocol therapy. If enrollment takes place before starting therapy, the date protocol therapy is projected to start must be no later than five (5) calendar days after enrollment.

3.1.5 Bilingual Services
To allow non-English speaking patients to participate in the study, bilingual health care services will be provided in the appropriate language.

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

3.2.1 Age
Patients < 4 years of age at diagnosis are eligible for this study. AML patients with DS ≥ 4 years of age will not be eligible for enrollment on this study and should be encouraged to be enrolled on the front-line COG AML study.

3.2.2 Diagnosis (Newly diagnosed)
- Patients must have a confirmed diagnosis of DS or DS mosaicism confirmed by karyotype or chromosomal analysis performed locally.
- Children with newly diagnosed AML based on FAB criteria. Patients with promyelocytic leukemia are excluded.
- Patients with a diagnosis of MDS with < 30% blasts will be eligible.
- Institutional immunophenotype is required for study entry. Immunophenotyping including CD41 or CD61, CD33, CD34, CD14, CD7 and Gly-A is strongly recommended.

3.2.3 Patients with a History of Transient Myeloproliferative Disorder
Patients > 90 days old at diagnosis of AML/MDS with a history of TMD (which may or may not have required chemotherapy intervention) are eligible if they:
  i) have ≥ 30% blasts in the bone marrow, regardless of the time since resolution, or
  ii) are > 8 weeks since TMD resolution with ≥ 5% blasts in the bone marrow.

3.2.4 Prior Therapy
Children who have previously received chemotherapy or radiation therapy or any antileukemic therapy are not eligible for this protocol. Exceptions include IT cytarabine given at diagnosis, and prior therapy for TMD.

3.2.5 Concomitant Medications Restrictions
There are no concomitant medications restrictions on this protocol.

3.2.6 Organ Function Requirements:

3.2.6.1 Adequate renal function defined as:
  - Creatinine clearance or radioisotope GFR ≥ 70mL/min/1.73m² or
  - A serum creatinine based on age/gender as follows:

<table>
<thead>
<tr>
<th>Age</th>
<th>Maximum Serum Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>1 month to &lt; 6 months</td>
<td>0.4</td>
</tr>
<tr>
<td>6 months to &lt; 1 year</td>
<td>0.5</td>
</tr>
<tr>
<td>1 to &lt; 2 years</td>
<td>0.6</td>
</tr>
<tr>
<td>2 to &lt; 6 years</td>
<td>0.8</td>
</tr>
<tr>
<td>6 to &lt; 10 years</td>
<td>1</td>
</tr>
<tr>
<td>10 to &lt; 13 years</td>
<td>1.2</td>
</tr>
<tr>
<td>13 to &lt; 16 years</td>
<td>1.5</td>
</tr>
<tr>
<td>≥ 16 years</td>
<td>1.7</td>
</tr>
</tbody>
</table>

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.
3.2.6.2 Adequate liver function defined as:
- Total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN) for age, and
- SGOT (AST) or SGPT (ALT) $< 2.5 \times$ upper limit of normal (ULN) for age.

3.2.6.3 Adequate cardiac function defined as:
- Shortening fraction of $\geq 27\%$ by echocardiogram, or
- Ejection fraction of $\geq 50\%$ by radionuclide angiogram.

3.2.6.4 Adequate pulmonary function defined as:
- No evidence of dyspnea at rest,
- No exercise intolerance, and
- A pulse oximetry $> 94\%$.

3.2.7 Regulatory

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

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

The following instructions pertain to bone marrow evaluations and peripheral blood count recovery.

I Bone Marrow Evaluations

The evaluations below will be made following the specified bone marrow aspirations. Bone marrow for MRD evaluation will be taken in addition to the required marrow at these time points only if patient consent is obtained. Refer to Section 7.1 and BMA/MRD as indicated on the Experimental Design Schema.

A) Induction I Day 14 BMA:

All patients will have a bone marrow aspirate (BMA) performed on Day 14 of Induction I to determine the extent of residual leukemia.

1) Patients will proceed to Induction II therapy regardless of peripheral blood count recovery if Day 14 marrow:
   a) is cellular or moderately cellular, and
   b) continues to show evidence of residual leukemia (≥ 20% blasts).

   These patients will not require a Day 28 BMA Evaluation.

2) All other patients will have a BMA performed on Day 28 in order to determine remission status. Induction II chemotherapy will not be given until the Day 28 bone marrow is performed.

   If the Day 14 marrow is aplastic/severely hypocellular and blasts are present, the percentage of blasts will be recorded.

B) Induction I Day 28 BMA:

1) If BMA is cellular or moderately cellular with a Complete Response (CR), Induction II will be administered when ANC > 1000/µL and platelets > 100,000/µL (CBC is to be repeated every 4 days until counts are adequate).

   If a patient has a CR [see Section 11.2.1] after Day 28 marrow, the BMA will only be repeated at the end of induction therapy (after Induction IV) and at the End of Therapy (after Intensification II).

2) If the Day 28 bone marrow is aplastic/severely hypocellular and the patient shows Partial Response (PR) or Refractory Disease (RD) [see Section 11.2.2 and 11.2.3], the bone marrow is to be repeated one week later and, if necessary, weekly thereafter. If CR is confirmed at the time of repeat BMA, the patient will proceed to Induction II chemotherapy when the marrow is cellular or moderately cellular with ANC > 1000/µL and platelets > 100,000/µL.
3) Patients with PR or RD (regardless of cellularity) after Day 28 BMA will proceed to up to two successive BMA evaluations. These patients will proceed to Induction II if marrow is cellular or moderately cellular and in CR with ANC > 1000/µL and platelets > 100,000/µL. If after two successive Day 28 marrows patient has ≥ 5% blasts, proceed to Induction II regardless of count recovery. These patients must have repeat marrows performed on Days 14 and 28 following Induction II therapy.

C) Induction IV BMA

Patients with a PR or RD after Induction IV will be taken Off Protocol Therapy.

II Peripheral Blood Count Recovery

A) All patients in remission (< 5% blasts) and with an ANC > 1000/µL and platelets > 100,000/µL are eligible to begin the next course of therapy except when noted in item #C below. Repeat blood counts every 4 days until counts are adequate. For all courses, if the counts have not recovered by Day 49, repeat the marrow studies. Biopsies are necessary if the aspirate is inadequate.

B) If the platelet count is rising progressively to ≥ 75,000/µL but has not reached 100,000/µL by three weeks after the ANC has reached 1,000/µL, patients may proceed to the next course of therapy.

C) Note: Patients with a Partial Response (PR) or Refractory Disease (RD) will not require normal blood counts before starting the Capizzi II Course of Induction II chemotherapy. Adequate counts are required before receiving Intensification as outlined in items #A and #B above.

4.1 Induction I

Criteria to start Induction I are the patient eligibility criteria described in Section 3.2. Induction I is a minimum of 28 days (unless Day 14 BMA indicates that the patient should proceed directly to Induction II, see Section 4.0).

Intrathecal Cytarabine (IT ARAC): IT

The first dose of intrathecal (IT) Ara-C may be given at the time of the diagnostic marrow and lumbar puncture if there is no uncertainty about the diagnosis. If IT Ara-C is not given at time of diagnostic LP, then administer on Day 1 of Induction I. If IT cytarabine is given prior to diagnosis, a separate institutional consent must be obtained.

Age-based dosing:

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to &lt; 13 months</td>
<td>20 mg</td>
</tr>
<tr>
<td>13 to &lt; 25 months</td>
<td>30 mg</td>
</tr>
<tr>
<td>25 to &lt; 36 months</td>
<td>50 mg</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>70 mg</td>
</tr>
</tbody>
</table>

For CNS positive patients: If CNS disease is present [defined as any number of blasts on a cyto spin prep in an atraumatic (< 100 RBCs) lumbar puncture], IT Ara-C will be given twice weekly until the CSF is clear plus two additional intrathecal treatments. Patients with intradural CNS chloromas will be considered as having CNS disease. Patients with CNS leukemia after 6 doses of IT Ara-C will be taken Off Protocol Therapy.
Cytarabine (ARAC IV): Continuous IV infusion for 96 hours

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>6.67 mg/kg/24 hrs</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>200 mg/m²/24 hrs</td>
</tr>
</tbody>
</table>

Days: 1, 2, 3, 4

Note compatibility information: cytarabine and daunorubicin are compatible when mixed in the same bag and in Dextrose 5% sodium chloride 0.45% (D₅₀.⁴₅NS) or Dextrose 5% in Water (D₅W). Concentrations and conditions are described in Trissle’s Handbook on Injectable Drugs.³²

DAUNOrubicin (DAUN): Continuous IV infusion for 96 hours

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>0.67 mg/kg/24 hrs</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>20 mg/m²/24 hrs</td>
</tr>
</tbody>
</table>

Days: 1, 2, 3, 4

Note: See compatibility information above.

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.

Thioguanine (TG): PO BID

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>1.65 mg/kg/dose twice daily</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>50 mg/m²/dose twice daily</td>
</tr>
</tbody>
</table>

Days: 1, 2, 3, 4

Administer dose in AM and at bedtime on an empty stomach (at least 1 hour before or 2 hours after food or drink except water). Round thioguanine doses to nearest 20 mg.

The therapy delivery map for Induction I is on the next page. See Section 4.2 for criteria to begin Induction II.
4.1.1 **AAML0431: Induction I**

Induction I is a minimum of 28 days (unless patient meets exception described in Section 4.0 & 4.2 at Day 14 BMA).

Criteria to start protocol therapy are described in Section 3.2. Extensive treatment details are in Section 4.1. Induction I is 28 days long (except for patients with cellular or moderately cellular marrow at Day 14 BMA and ≥ 20% blasts who will begin Induction II after Day 14 BMA results). This TDM is on 1 page.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ROUTE</th>
<th>DOSAGE</th>
<th>DAYS</th>
<th>IMPORTANT NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathecal Cytarabine</td>
<td>IT</td>
<td>Age (months)</td>
<td>Dose</td>
<td>If given at diagnostic LP, separate institutional consent should be signed. For CNS+ patients: see * below &amp; Section 4.1 for add’l IT treatments.</td>
</tr>
<tr>
<td>(IT ARAC)</td>
<td></td>
<td>0 to &lt; 13 mo.</td>
<td>20 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 to &lt; 25 mo.</td>
<td>30 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 to &lt; 36 mo.</td>
<td>50 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 36 mo.</td>
<td>70 mg</td>
<td></td>
</tr>
<tr>
<td>Cytarabine (ARAC IV)</td>
<td>Continuous IV infusion for 96 hours</td>
<td>Age (months)</td>
<td>Dose</td>
<td>Daunorubicin and Ara-C may be administered together in compatible IV fluids. See Section 4.1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 36 mo.</td>
<td>6.67 mg/kg/24 hrs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 36 mo.</td>
<td>200 mg/m²/24 hrs</td>
<td></td>
</tr>
<tr>
<td>DAUNorubicin (DAUN)</td>
<td>Continuous IV infusion for 96 hours</td>
<td>Age (months)</td>
<td>Dose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 36 mo.</td>
<td>0.67 mg/kg/24 hrs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 36 mo.</td>
<td>20 mg/m²/24 hrs</td>
<td></td>
</tr>
<tr>
<td>Thioguanine (TG)</td>
<td>PO</td>
<td>Age (months)</td>
<td>Dose</td>
<td>Administer dose in AM &amp; at bedtime on an empty stomach. Round TG doses to nearest 20 mg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 36 mo.</td>
<td>1.65 mg/kg/dose, BID</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 36 mo.</td>
<td>50 mg/m²/dose, BID</td>
<td></td>
</tr>
</tbody>
</table>

**IMPORTANT NOTES**

- **OBSERVATIONS**
  - a. History, PE, PS, CBC with differential & platelets, electrolytes (Ca++, Mg++, PO₄), Bun, Cr, SGPT, SGOT; bili (total & direct)
  - b. BMA (biopsy is req. if aspirate cannot be obtained)
  - c. Optional: MRD (submit sample for each BMA, see Section 15.2)

**OTBAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**

<table>
<thead>
<tr>
<th>Date Due</th>
<th>Date Given</th>
<th>Day</th>
<th>IT ARAC mg</th>
<th>ARAC IV mg</th>
<th>DAUN mg</th>
<th>TG mg</th>
<th>Studies</th>
<th>Comments (Include any held doses, or dose modifications)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b*, c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Directions for starting next course are described in Section 4.2.

*If not given at time of diagnostic LP. If given at time of diagnostic LP, record date:

**CNS + Pts:** Record dates of add’l IT Ara-C:

Obtain studies prior to start of therapy.

*See Sect. 4.0 for evaluation of BMAs & add’l BMAs req. if CR is not achieved by Day 28.

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 8 FOR SUPPORTIVE CARE.
4.2 **Induction II**

See Section 4.0 and below for criteria to begin Induction II. Induction II is a minimum of 28 days.

**When to Proceed to Induction II:**
Induction II therapy will be given regardless of peripheral blood count recovery if the Induction I Day 14 marrow is cellular or moderately cellular, and continues to show evidence of residual leukemia (≥ 20% blasts).

All other patients will proceed to Induction I Day 28 BMA evaluation:
- If BMA is cellular or moderately cellular and in CR, Induction II will be administered when ANC > 1000/µL and platelets > 100,000/µL (CBC is to be repeated every 4 days until counts are adequate). See Section 4.0, II.
- If the marrow is aplastic/severely hypocellular, the patient will proceed with up to two successive BMA evaluations. If CR is confirmed at the time of either of the repeat BMA evaluations, the patient will proceed to Induction II when the marrow is cellular or moderately cellular with ANC > 1000/µL and platelets > 100,000/µL.
- Patients with PR or RD (regardless of cellularity) after Day 28 BMA will proceed to up to two successive BMA evaluations. These patients will proceed to Induction II if marrow is cellular or moderately cellular and in CR with ANC > 1000/µL and platelets > 100,000/µL. If after two successive Day 28 marrows patient has ≥ 5% blasts, proceed to Induction II without achieving ANC > 1000/µL and platelets > 100,000/µL. These patients must have repeat marrows performed on Days 14 and 28 following Induction II therapy.

**High Dose Cytarabine (HD ARAC):** IV infusion over 3 hours, twice a day

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>100 mg/kg/dose, BID</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>3000 mg/m²/dose, BID</td>
</tr>
</tbody>
</table>

**Days:** 1, 2, 8, 9
Administer the diluted solution at Hours 0-3 and Hours 12-15.
Administer steroid eye drops such as dexamethasone, 2 drops in both eyes every 6 hours beginning immediately before the first dose of cytarabine and continuing until 24 hours after the last dose. If the patient does not tolerate steroid eye drops, administer artificial tears on an every 2-4 hour schedule.

**L-Asparaginase *E.Coli* (LASP):** IM

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>200 units/kg/dose</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>6000 units/m²/dose</td>
</tr>
</tbody>
</table>

**Days:** 2 & 9
Administer at Hour 18 on Days 2 and 9 after the 4th and 8th dose of Ara-C.

**Special precautions:**
1. Asparaginase is contraindicated with a history of severe pancreatitis with prior asparaginase therapy, serious thrombosis with prior asparaginase therapy, or serious hemorrhagic events with prior asparaginase therapy.
2. Asparaginase may affect coagulation factors and predispose to bleeding and/or thrombosis. Caution should be used when administering any concurrent anticoagulant therapy.

The therapy delivery map for Induction II is on the next page. See Section 4.3 for criteria to begin Induction III.
### 4.2.1 AAML0431: Induction II

Induction II is a minimum of 28 days.

Criteria to start Induction II and extensive treatment details are described in Section 4.2. This TDM is on 1 page.

#### DRUG

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ROUTE</th>
<th>DOSAGE</th>
<th>DAYS</th>
<th>IMPORTANT NOTES</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
</table>
| High Dose       | IV infusion over 3 hours (2 doses per day) | Age (months) Dose | 1, 2, 8, 9 | Administer at Hours 0-3 and Hours 12-15. Use eye drops as described in Section 4.2. | a. History, PE, PS, CBC with differential & platelets, electrolytes (Ca++, Mg++, PO4), Bun, Cr, SGPT, SGOT, bili (total & direct)  
| Cytarabine (HD ARAC) | | < 36 mo. 100 mg/kg/dose, BID | | | b. Amylase, lipase, fibrinogen  
| | | ≥ 36 mo. 3000 mg/m²/dose, BID | | | c. BMA (biopsy is req. if aspirate cannot be obtained)  
| | | | | | d. Optional: MRD (submit sample for each BMA, see Section 15.2)  
| | | | | | e. Optional: Ara-C PK (see Section 15.3)  
| | | | | | OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE |
| L-Asparaginase E. coli (LASP) | IM | Age (months) Dose | 2, 9 | Administer at Hour 18 on Days 2 and 9 after the 4th and 8th dose of Ara-C." | a. History, PE, PS, CBC with differential & platelets, electrolytes (Ca++, Mg++, PO4), Bun, Cr, SGPT, SGOT, bili (total & direct)  
| | | < 36 mo. 200 units/kg/dose | | | b. Amylase, lipase, fibrinogen  
| | | ≥ 36 mo. 6000 units/m²/dose | | | c. BMA (biopsy is req. if aspirate cannot be obtained)  
| | | | | | d. Optional: MRD (submit sample for each BMA, see Section 15.2)  
| | | | | | e. Optional: Ara-C PK (see Section 15.3)  
| | | | | | OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE |

#### Studies

- **Enter calculated dose above and actual dose administered below**

<table>
<thead>
<tr>
<th>Date Due</th>
<th>Date Given</th>
<th>Day</th>
<th>HD ARAC mg</th>
<th>LASP units</th>
<th>Studies</th>
<th>Comments (Include any held doses, or dose modifications)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>mg</td>
<td>mg</td>
<td>-</td>
<td>a*, b*, e^</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>mg</td>
<td>mg</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>mg</td>
<td>mg</td>
<td>-</td>
<td>b^</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>mg</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>mg</td>
<td>mg</td>
<td>-</td>
<td>c^, d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>mg</td>
<td>mg</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* Obtain studies prior to start of therapy.  
^ Perform on Day 14 & Day 28 only if previous marrow indicated a PR or RD. Patients with RD at Day 28 will be taken Off Protocol Therapy.  
^ Performed with the first dose of Ara-C. See Section 15.3 for collection schedule.  
^ Perform on Day 1 then as clinically indicated. Fibrinogen on Days 1 & 8.

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 8 FOR SUPPORTIVE CARE.
4.3 **Induction III**

Induction III will be administered when ANC > 1000/µL and platelets > 100,000/µL. Also see Section 4.0, II. Patients with refractory disease at the time of Induction II therapy Day 28 BMA will be taken Off Protocol Therapy. Induction III is a minimum of 28 days.

**Intrathecal Cytarabine (IT ARAC): IT**

Age-based dosing:

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to &lt; 13 months</td>
<td>20 mg</td>
</tr>
<tr>
<td>13 to &lt; 25 months</td>
<td>30 mg</td>
</tr>
<tr>
<td>25 to &lt; 36 months</td>
<td>50 mg</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>70 mg</td>
</tr>
</tbody>
</table>

IT Ara-C is administered on Day 1 of Induction III (or with Induction II Day 28 BMA, if applicable).

**Cytarabine (ARAC IV):** Continuous IV infusion for 96 hours

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>6.67 mg/kg/24 hrs</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>200 mg/m²/24 hrs</td>
</tr>
</tbody>
</table>

**DAUNOrubicin (DAUN):** Continuous IV infusion for 96 hours

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>0.67 mg/kg/24 hours</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>20 mg/m²/24 hours</td>
</tr>
</tbody>
</table>

**Note compatibility information:** cytarabine and daunorubicin are compatible when mixed in the same bag and in Dextrose 5% sodium chloride 0.45% (D₅0.45NS) or Dextrose 5% in Water (D₅W). Concentrations and conditions are described in Trissle’s Handbook on Injectable Drugs.³²

**Thioguanine (TG): PO BID**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>1.65 mg/kg/dose, twice daily</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>50 mg/m²/dose, twice daily</td>
</tr>
</tbody>
</table>

**Days:** 1, 2, 3, 4

**Note:** See compatibility information above.

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

The therapy delivery map for Induction III is on the next page. See Section 4.4 for criteria to begin Induction IV.
### 4.3.1 AAML0431: Induction III

Induction III is a minimum of 28 days.

Criteria to start Induction III and extensive treatment details are described in Section 4.3. This TDM is on 1 page.

#### DRUG

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ROUTE</th>
<th>DOSAGE</th>
<th>DAYS</th>
<th>IMPORTANT NOTES</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathecal Cytarabine (IT ARAC)</td>
<td>IT</td>
<td>Age (months)</td>
<td>1</td>
<td>May be administered with end of Induction II BMA, if applicable.</td>
<td>a. History, PE, PS, CBC with differential &amp; platelets, electrolytes (Ca++, Mg++, PO4), Bun, Cr, SGPT, SGOT, bili (total &amp; direct)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dose</td>
<td></td>
<td></td>
<td>b. CSF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 to &lt; 13 mo.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 to &lt; 25 mo.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 to &lt; 36 mo.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>70 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 36 mo.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytarabine (ARAC IV)</td>
<td>Continuous IV infusion for 96 hours</td>
<td>Age (months)</td>
<td>1, 2, 3, 4</td>
<td>Daunorubicin and Ara-C may be administered together in compatible IV fluids. See Section 4.3.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.67 mg/kg/24 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 mg/m²/24 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAUNorubicin (DAUN)</td>
<td>Continuous IV infusion for 96 hours</td>
<td>Age (months)</td>
<td>1, 2, 3, 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.67 mg/kg/24 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 mg/m²/24 hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thioguanine (TG)</td>
<td>PO</td>
<td>Age (months)</td>
<td>1, 2, 3, 4</td>
<td>Administer dose in AM &amp; at bedtime on an empty stomach. Round TG doses to nearest 20 mg.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.65 mg/kg/dose, BID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg/m²/dose, BID</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Important Notes

- * May be administered with end of Induction II BMA, if applicable.
- ** Obtain studies prior to start of therapy.
- @ Collect when IT AraC is administered.

** Enter calculated dose above and actual dose administered below

<table>
<thead>
<tr>
<th>Date Due</th>
<th>Day</th>
<th>IT ARAC mg</th>
<th>ARAC IV mg</th>
<th>DAUN mg</th>
<th>TG mg</th>
<th>Studies</th>
<th>Comments (Include any held doses, or dose modifications)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>mg*</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>a*, b**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Directions on starting next course are described in Section 4.4.

* May be administered with end of Induction II BMA, if applicable. ** Obtain studies prior to start of therapy. @ Collect when IT AraC is administered.

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 8 FOR SUPPORTIVE CARE.
4.4  Induction IV

Induction IV will be administered when ANC > 1000/µL and platelets > 100,000/µL. Also see Section 4.0, II. Induction IV is a minimum of 28 days.

**Cytarabine (ARAC IV):** Continuous IV infusion for 96 hours

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>6.67 mg/kg/24 hrs</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>200 mg/m²/24 hrs</td>
</tr>
</tbody>
</table>

Days: 1, 2, 3, 4

**Note compatibility information:** cytarabine and daunorubicin are compatible when mixed in the same bag and in Dextrose 5% sodium chloride 0.45% (D₅0.45NS) or Dextrose 5% in Water (D₅W). Concentrations and conditions are described in Trissle’s Handbook on Injectable Drugs.³²

**DAUNOrubicin (DAUN):** Continuous IV infusion for 96 hours

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>0.67 mg/kg/24 hours</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>20 mg/m²/24 hours</td>
</tr>
</tbody>
</table>

Days: 1, 2, 3, 4

**Note:** See compatibility information above.

**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.

**Thioguanine (TG):** PO BID

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose:</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>1.65 mg/kg/dose twice daily</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>50 mg/m²/dose twice daily</td>
</tr>
</tbody>
</table>

Days: 1, 2, 3, 4

Administer dose in AM and at bedtime on an empty stomach (at least 1 hour before or 2 hours after food or drink except water). Round thioguanine doses to nearest 20 mg.

The therapy delivery map for Induction IV is on the next page. See Section 4.5 for criteria to begin Intensification I. Patients with a PR or RD following the BMA after Induction IV will be Off Protocol Therapy.
4.4.1 **AAML0431: Induction IV**

Induction IV is a minimum of 28 days.

Criteria to start Induction IV are described in Section 4.4. Extensive treatment details are in Section 4.4. This TDM is on 1 page.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ROUTE</th>
<th>DOSAGE</th>
<th>DAYS</th>
<th>IMPORTANT NOTES</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine (ARAC IV)</td>
<td>Continuous IV infusion for 96 hours</td>
<td>Age (months) Dose 6.67 mg/kg/24 hrs 200 mg/m²/24 hrs</td>
<td>1, 2, 3, 4</td>
<td>Daunorubicin and AraC may be administered together in compatible IV fluids. See Section 4.4</td>
<td>a. History, PE, PS, CBC with differential and platelets, electrolytes (Ca⁺⁺, Mg⁺⁺, PO₄), Bun, Cr, SGPT, SGOT, bili (total &amp; direct)</td>
</tr>
<tr>
<td>DAUNorubicin (DAUN)</td>
<td>Continuous IV infusion for 96 hours</td>
<td>Age (months) Dose 0.67 mg/kg/24 hrs 20 mg/m²/24 hrs</td>
<td>1, 2, 3, 4</td>
<td></td>
<td>b. BMA (biopsy is req. if aspirate cannot be obtained)</td>
</tr>
<tr>
<td>Thioguanine (TG)</td>
<td>PO</td>
<td>Age (months) Dose 1.65 mg/kg/dose, BID 50 mg/m²/dose, BID</td>
<td>1, 2, 3, 4</td>
<td>Administer dose in AM &amp; at bedtime on an empty stomach. Round TG doses to nearest 20 mg.</td>
<td>c. Optional: MRD (submit sample for each BMA, see Section 15.2)</td>
</tr>
</tbody>
</table>

**OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**

<table>
<thead>
<tr>
<th>Ht cm</th>
<th>Wt kg</th>
<th>BSA m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enter calculated dose above and actual dose administered below</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Directions on starting next course are described in Section 4.5. b, c

* Obtain studies prior to start of therapy.  "Patients with a PR or RD after Induction IV will be Off Protocol Therapy.

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 8 FOR SUPPORTIVE CARE.
4.5 **Intensification I**

Intensification I will be administered when ANC > 1000/µL and platelets > 100,000/µL. Also see Section 4.0. II. Intensification I is a minimum of 28 days.

**Cytarabine (ARAC IV):** Infuse the diluted solution by continuous IV infusion X 7 days (168 hours)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>3.3 mg/kg/24 hrs</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>100 mg/m²/24 hrs</td>
</tr>
</tbody>
</table>

**Days:** 1-7 continuously

**Etoposide (ETOP):** IV infusion over 1 hour. Slow rate of administration if hypotension occurs.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>4.2 mg/kg/dose</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>125 mg/m²/dose</td>
</tr>
</tbody>
</table>

**Days:** 1, 2, 3

Infuse diluted solution (concentration ≤ 0.4 mg/mL). The use of an in-line filter during the infusion is suggested.

The therapy delivery map for Intensification I is on the next page. See Section 4.6 for criteria to begin Intensification II.
4.5.1 **AAML0431: Intensification I**

Intensification I is a minimum of 28 days.

Criteria to start Intensification I and extensive treatment details are described in Section 4.5. This TDM is on 1 page.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ROUTE</th>
<th>DOSAGE</th>
<th>DAYS</th>
<th>IMPORTANT NOTES</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine (ARAC IV)</td>
<td>Continuous IV infusion X 7 days (168 hours)</td>
<td>Age (months) Dose</td>
<td>1 through 7 continuously</td>
<td></td>
<td>a. History, PE, PS, CBC with differential and platelets, electrolytes (Ca++, Mg++, PO₄), Bun, Cr, SGPT, SGOT, bili (total &amp; direct) Obtain other studies as required for good patient care</td>
</tr>
<tr>
<td>Etoposide (ETOP)</td>
<td>IV infusion over 1 hour</td>
<td>Age (months) Dose</td>
<td>1, 2, 3</td>
<td>Slow rate of infusion if hypotension occurs.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ht cm</th>
<th>Wt kg</th>
<th>BSA m²</th>
<th>Date Due</th>
<th>Date Given</th>
<th>Day</th>
<th>ARAC IV mg</th>
<th>ETOP mg</th>
<th>Studies</th>
<th>Comments (Include any held doses, or dose modifications)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enter calculated dose above and actual dose administered below

1. mg
2. mg
3. mg
4. mg
5. mg
6. mg
7. mg

Directions on starting next course are described in Section 4.6.

* Obtain studies prior to start of therapy

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 8 FOR SUPPORTIVE CARE.
4.6 **Intensification II**

Intensification II will be administered when ANC > 1000/µL and platelets > 100,000/µL. Also see Section 4.0, II. Intensification II is a minimum of 28 days.

**Cytarabine (ARAC IV):** Infuse the diluted solution by continuous IV infusion X 7 days (168 hours)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>3.3 mg/kg/24 hrs</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>100 mg/m²/24 hrs</td>
</tr>
</tbody>
</table>

**Days:** 1-7 continuously

**Etoposide (ETOP):** IV infusion over 1 hour. Slow rate of administration if hypotension occurs.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 36 months</td>
<td>4.2 mg/kg/dose</td>
</tr>
<tr>
<td>≥ 36 months</td>
<td>125 mg/m²/dose</td>
</tr>
</tbody>
</table>

**Days:** 1, 2, 3

Infuse diluted solution (concentration ≤ 0.4 mg/mL). The use of an in-line filter during the infusion is suggested.

Intensification II ends on Day 28. Protocol therapy is completed after blood count recovery (ANC > 1000/µL and platelets > 100,000/µL and ≥ 7 days from the last platelet transfusion) post Intensification II.
### 4.6.1 AAML0431: Intensification II

Intensification II is a minimum of 28 days.

Criteria to start Intensification II and extensive treatment details are described in Section 4.6. This TDM is on 1 page.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ROUTE</th>
<th>DOSAGE</th>
<th>DAYS</th>
<th>IMPORTANT NOTES</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>Continuous IV</td>
<td>Age (months)</td>
<td>1 through 7 continuously</td>
<td>a. History, PE, PS, CBC with differential &amp; platelets, electrolytes (Ca$$, Mg$$, PO4), Bun, Cr, SGPT, SGOT, bili (total&amp; direct)</td>
<td>b. BMA (biopsy is req. if aspirate cannot be obtained)</td>
</tr>
<tr>
<td>(ARAC IV)</td>
<td>infusion X 7 days</td>
<td>&lt; 36 mo.</td>
<td>3.3 mg/kg/24 hours</td>
<td>c. Optional: MRD (submit sample for each BMA, see Section 15.2)</td>
<td>d. Optional: BM or blood for correlative biology studies (see Section 15.1)</td>
</tr>
<tr>
<td></td>
<td>(168 hours)</td>
<td>≥ 36 mo.</td>
<td>100 mg/m$$^2$/24 hours</td>
<td>** Obtain other studies as required for good patient care</td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>IV infusion over</td>
<td>Age (months)</td>
<td>1, 2, 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ETOP)</td>
<td>1 hour</td>
<td>&lt; 36 mo.</td>
<td>4.2 mg/kg/dose</td>
<td>Slow rate of infusion if hypotension occurs.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 36 mo.</td>
<td>125 mg/m$$^2$/dose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Observations

- **a.** History, PE, PS, CBC with differential & platelets, electrolytes (Ca$$, Mg$$, PO4), Bun, Cr, SGPT, SGOT, bili (total& direct)
- **b.** BMA (biopsy is req. if aspirate cannot be obtained)
- **c.** Optional: MRD (submit sample for each BMA, see Section 15.2)
- **d.** Optional: BM or blood for correlative biology studies (see Section 15.1)

**OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE**

<table>
<thead>
<tr>
<th>Date Due</th>
<th>Date Given</th>
<th>Day</th>
<th>ARAC IV mg</th>
<th>ETOP mg</th>
<th>Studies</th>
<th>Comments (Include any held doses, or dose modifications)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td>a**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>3.3 mg</td>
<td></td>
<td></td>
<td>Protocol therapy is completed after blood count recovery.** b, c, d</td>
</tr>
</tbody>
</table>

* Obtain studies prior to start of therapy ** See Section 7.1 for End of Therapy Evaluations.

SEE PROTOCOL SECTION 5 FOR DOSE MODIFICATIONS. SEE SECTION 8 FOR SUPPORTIVE CARE.
5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 CNS toxicity
Patients who experience ≥ Grade 3 CNS toxicity from high-dose cytarabine (3000 mg/m²/dose) should not receive further high-dose cytarabine therapy. The most common neurotoxicity is an acute cerebellar syndrome that may manifest itself as ataxia, nystagmus, or dysarthria. However, seizures and encephalopathy have also occurred following therapy with high dose cytarabine.

5.2 Cardiac Toxicity
Patients with clinical evidence of congestive heart failure should receive no additional daunorubicin. In the event of a fractional shortening of < 27%, consideration should be given to discontinuing daunorubicin therapy. Cardiac toxicities described in Section 12.4 are to be reported.

5.3 Hepatic Toxicity

5.3.1 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, and is between 2 and 3 mg/dL, give 50% of the calculated dose of daunorubicin and etoposide. If the direct bilirubin is between 3 and 5 mg/dL give 25% of the daunorubicin and etoposide doses, and notify the Study Chair. If the direct bilirubin is > 5 mg/dL hold the daunorubicin and etoposide and notify the Study Chair. Full dose of these agents may resume when the direct bilirubin has fallen to < 1.2 mg/dL.

5.4 Renal Toxicity
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, timed urine collection for creatinine clearance, or calculated by the Schwartz formula before proceeding with HD cytarabine therapy (doses of 1 g/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. In patients with impaired renal function, the following initial dose modification of etoposide should be considered based on measured or calculated 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 CL Cr, 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.

Estimated Creatinine Clearance (in mL/min/1.73 m²)** = \( (k)(L)/Pcr \)

Where
- \( L \) = child’s length in cm
- \( Pcr \) = plasma (or serum) creatinine (in mg/dL)

**Values**

<table>
<thead>
<tr>
<th>( k ) Values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>low birth weight infant</td>
</tr>
<tr>
<td>0.45</td>
<td>term infant</td>
</tr>
<tr>
<td>0.55</td>
<td>child</td>
</tr>
<tr>
<td>0.55</td>
<td>adolescent female</td>
</tr>
<tr>
<td>0.70</td>
<td>adolescent male</td>
</tr>
</tbody>
</table>

**The conversion formula for serum/plasma creatinine when reported in \( \mu \text{Mol/L} \) units:**

\( (k \times \text{ht})/(sCr \text{ in } \mu \text{Mol/L} / 88.4) \)

5.5 **Allergy to Etoposide**

Etoposide allergic reactions may be managed with pre-medications such as diphenhydramine 1 mg/kg (maximum dose 50 mg) IV, ranitidine 1 mg/kg IV, hydrocortisone 1-4 mg/kg IV, and by slowing the rate of the infusion. For those reactions which are unable to be controlled with pre-medication and the slowing of the rate of etoposide infusion, etoposide phosphate may be substituted in the same molar equivalent dose and at the same or a slower rate. Etoposide phosphate is a prodrug of etoposide. Each 100 mg of etoposide is equivalent to 113.5 mg of etoposide phosphate. Pre-medication for etoposide phosphate is recommended.

6.0 **DRUG INFORMATION**

See the consent document for toxicities. All other information is available on the COG website in the manual titled “Drug Information for Commercial Agents used by the Children’s Oncology Group” at: https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

7.0 **EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED**

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).
## 7.1 Required, Recommended and Optional Clinical, Laboratory and Disease Evaluations

All baseline studies must be performed prior to starting protocol therapy unless otherwise noted below. Obtain other evaluations prior to start of phase unless otherwise indicated.

<table>
<thead>
<tr>
<th>STUDIES TO BE OBTAINED</th>
<th>Study Entry</th>
<th>Induction I</th>
<th>Induction II, III, IV</th>
<th>Intensification I, II</th>
<th>End Of Therapy</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>History/Physical Exam/Performance Scale</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chest X-ray (CXR)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echo/EKG or MUGA scan</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC with Differential &amp; Platelets</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrolytes (Ca++, Mg++, PO₄)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN, Creatinine (Cr)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>AST (SGOT)/ALT (SGPT), Bilirubin (Total &amp; Direct)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein, Albumin, LDH, Uric Acid</td>
<td>Xₕ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase, Lipase, Fibrinogen</td>
<td>X</td>
<td></td>
<td>X₅</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIC&lt;sup&gt;C&lt;/sup&gt;: PT/PTT, D-dimer</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicella serology</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hep B by surface antigen/</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hep C by serology</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Oxygen Saturation</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Marrow Slides for Central Review&lt;sup&gt;D&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X²</td>
<td></td>
</tr>
<tr>
<td>Cytogenetics&lt;sup&gt;D&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X²</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Institutional Immunophenotyping&lt;sup&gt;H&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histocytochemistry (MPO, ANB, ANA)</td>
<td>X₅</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
<td>X</td>
<td>X&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X&lt;sup&gt;k&lt;/sup&gt;</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MRD&lt;sup&gt;M&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X&lt;sup&gt;k&lt;/sup&gt;</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Pharmacokinetics (PK)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Biology Studies&lt;sup&gt;1&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A: Repeat uric acid as clinically indicated.
B: Amylase and Lipase on Day 1 of Induction II and then as clinically indicated. Fibrinogen on Days 1 and 8 of Induction II.
C: Disseminated intravascular coagulation.
D: Per the instructions in Section 14.1.
E: **Recommended but not required.**
F: Submit 5 mL of bone marrow for cytogenetics per the instructions in Section 14.2. Peripheral blood should be submitted as a back-up to the bone marrow when the marrow sample is suboptimal or unobtainable.
G: Perform at time of IT chemo administered with Induction III therapy.
H: Institutional immunophenotype (flow cytometry analysis) is required at study entry. CD41 or CD61, CD33, CD34, CD14, CD7 and Gly-A are strongly recommended. CD14 should be combined with either CD41 or CD61 to rule out non-specific platelet adherence to monocytic cells. Additional recommended antigen markers include: CD36, CD10, CD19, CD13, CD15, CD11b, and CD56. Submit report via the COG Document Imaging System.
I: Bone marrow biopsy is required if bone marrow aspiration cannot be obtained.
J: Perform on Day 14 and Day 28 of Induction I (see Section 4.0 for instructions and successive BMA evaluations).
K: Perform on Day 14 and Day 28 of Induction II if previous marrow was PR or RD. Perform after Induction IV for all patients. Patients with refractory disease at the time of Induction II Day 28 BMA will be taken Off Protocol Therapy. Patients with a PR or RD after Induction IV will be taken Off Protocol Therapy.
L: Perform on Day 28 of Intensification II.
M: Collect 2-3 mL of anticoagulated bone marrow and 5 mL of blood (in preservative free heparin/green top tube). Only submit bone marrow for Study Entry sample. See Section 15.2 for specimen collection and shipping instructions.
N: Performed with the first dose of cytarabine in Induction II. See Section 15.3 for specimen collection and shipping instructions.
O: Bone marrow or peripheral blood sample sent for GATA1 analysis, gene expression and in vitro pharmacology studies for consenting patients. See instructions in Section 15.1.

* **Optional biology study: requires patient/family consent.**
7.2 Follow-up Studies

<table>
<thead>
<tr>
<th>STUDIES TO BE OBTAINED*</th>
<th>Monthly for first 12 months from completion of therapy</th>
<th>Every 3 months for the next 12 months (2 years from completion of therapy)</th>
<th>Every 6 months until five years following completion of therapy</th>
<th>Yearly</th>
</tr>
</thead>
<tbody>
<tr>
<td>History/Physical Exam/Performance Scale</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Echo/EKG or MUGA scan</td>
<td></td>
<td>X**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC with Differential &amp; Platelets</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* For patients who complete chemotherapy
** Perform yearly for the first five years, then per institutional criteria.

8.0 SUPPORTIVE CARE GUIDELINES

These are provided for institutional consideration. Investigator discretion should be used, and individual considerations made for specific patient situations and institutional practices. Care providers are referred to Supportive Care of Children with Cancer, 3rd ed., Arnold J. Altman, MD.34

Patients enrolled on this COG protocol may be eligible for enrollment on COG supportive care protocols.

8.1 Tumor Lysis Syndrome
In patients with large tumor burdens monitor serum electrolytes carefully to prevent hyperuricemia and hyperphosphatemia. Patients should receive IV hydration at 3000 mL/m²/day before the initiation of therapy. Recombinant urate oxidase if available or allopurinol should be administered.

8.2 Hyperleukocytosis
In patients with WBC > 100 x 10⁹/µL or symptoms of hyperviscosity, leukopheresis or exchange transfusion may be necessary.

8.3 Venous Access Lines
Patients should have indwelling double lumen catheters inserted prior to the initiation of therapy. Totally implanted Portacaths are not recommended based on the need for multiple venous accesses during therapy.

8.4 Prophylaxis for Patients with Congenital Heart Defects
Patients should receive bacterial subacute endocarditis prophylaxis prior to procedures according to the American Heart Association guidelines.

8.5 Nutrition
Aggressive measures, including enteral and parental feedings, should be instituted to prevent a weight loss of > 10% of body weight.

8.6 Pneumocystis Prophylaxis
The administration of trimethoprim-sulfamethoxazole (TMP/SMX) to prevent Pneumocystis carinii pneumonia (PCP) (also called Pneumocystis jiroveci pneumonia) should begin on Day 28 of therapy and be administered by mouth on 3 consecutive days/week during Induction and Intensification at the dosing schedule of trimethoprim 2.5 mg/kg/dose (75mg/m²/dose) twice daily, maximum 160 mg/dose. For patients who cannot tolerate TMP/SMZ, daily oral atovaquone or dapsone, or monthly aerosolized or intravenous
pentamidine may be substituted.

8.7 **Mucosal Care**
Mucositis is expected to be severe; liberal use of pain medications for this condition is encouraged. In patients with poor hygiene, consultation by Oral Surgery is recommended prior to the initiation of therapy since multiple dental extractions may be necessary. Stomatitis and esophagitis due to Herpes virus may be confused with drug–induced mucositis and viral cultures should be obtained frequently. Anaerobic coverage should be included in the antibiotic regimen.

8.8 **Conjunctivitis Prophylaxis**
Dexamethasone ophthalmic solution (0.1%) or prednisolone (1%) eye drops, 2 drops to both eyes every six hours, should be used during high-dose Ara-C administration (3000 mg/m²/dose) and for 24 hours after completion to prevent conjunctival irritation. If the patient does not tolerate steroid eye drops, the physician may administer artificial tears on a q 2 to 4 hour schedule to prevent conjunctival and corneal pain.

8.9 **Fever and Management of Neutropenia and Fever**
In patients with ANC of ≤ 500/µL (or < 1000 and falling) and oral temperature ≥ 38°C (100.4°F) twice in a 12 hour period or ≥ 38.3°C (101°F) once, empiric systemic antibiotics should be started immediately. Broad spectrum monotherapy with cefepime or duotherapy with an aminoglycoside and an extended spectrum penicillin to cover major gram negative pathogens is recommended. Antibiotic coverage with vancomycin for suspected cases of alpha hemolytic streptococcus (particularly after high-dose Ara-C therapy) and staphylococci is strongly recommended. In patients with viridans streptococcal sepsis, the following antibiotics are recommended: Cefepime 1500 mg/m²/dose or 50 mg/kg/dose (maximum dose of 2000 mg) IV every 8 hours; Vancomycin 400 mg/m²/dose IV or 10-15 mg/kg/dose IV every 6 or 8 hours.

Patients who have suspected catheter-related infection, have evidence of sepsis (including shock, hypotension, rigors, septic emboli, unexplained respiratory distress or hypoxemia, or poor peripheral perfusion), are known to be colonized by Pseudomonas aeruginosa, or have received a parentally administered cephalosporin within the previous 7 days should also receive the following: Tobramycin 2.5 mg/kg/dose every 8 hours.

The persistence of fever after 7 days of broad-spectrum antibiotic coverage, or the emergence of a new fever in neutropenic patients with negative blood cultures, warrants the initiation of antifungal therapy.

8.10 **Growth Factors**
Prophylactic use of hematopoietic growth factors is not recommended. However, filgrastim (G-CSF; 5-10 µg/kg/day) is recommended for patients who have documented or suspected fungal infections or bacterial sepsis and should be continued until the ANC > 500/µL for 2 consecutive days.

8.11 **Drug Interactions**
Etoposide is a major substrate of cytochrome P450 3A4 (CYP3A4) and a minor substrate of CYP1A2 CYP2E1. It is a weak inhibitor of CYP2C9 and CYP3A4. The clinical outcome and significance of CYP450 interactions with etoposide is not certain. Strong CYP3A4 inhibitors (such as fluconazole, voriconazole, itraconazole, and ketoconazole) or inducers (such as rifampin and St. John’s wort) should be avoided or used with close monitoring for toxicity. Strong CYP3A4 inducing anticonvulsant drug (such as phenytoin, phenobarbital and carbamazepine) should be avoided. A list of weak or non-inducing anticonvulsants is available in Appendix II. Aprepitant also interacts with CYP3A4 and should be used with caution. Additional inducers or inhibitors of CYP450 isoenzymes can be found at [http://medicine.iupui.edu/clinpharm/ddis/](http://medicine.iupui.edu/clinpharm/ddis/).
8.12 Blood Product Support

8.12.1 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

All blood products should be irradiated, leukocyte-depleted and CMV-negative if possible. Platelets should
be given to keep the platelet count > 10,000/µL and packed red blood cells should be given to prevent
symptomatic anemia.

9.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY
CRITERIA

9.1 Criteria for Removal from Protocol Therapy

a) Patients with persistent CNS leukemia after 6 doses of intrathecal Ara-C during Induction I.
b) Patients with a Partial Response (PR), Refractory Disease (RD), or Relapse after Induction IV.
c) Patients with refractory disease following Induction II Day 28 BMA.
d) Refusal of further protocol therapy by patient/parent/guardian.
e) Completion of planned therapy.
f) Physician determines it is in the patient’s best interest.
g) Development of a second malignant neoplasm.
h) Adverse Events requiring removal from protocol 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.

9.2 Off Study Criteria

a) Death.
b) Lost to follow-up.
c) Enrollment onto another COG study with tumor therapeutic intent (e.g., at recurrence).
d) Withdrawal of consent for any further data submission.
e) The tenth (10th) anniversary of study entry.

10.0 STATISTICAL CONSIDERATIONS

10.1 Statistical Design
This study is a non-randomized study of children less than 4 years of age with Down syndrome and AML.

10.2 Patient Accrual and Expected Duration of Trial
Based on accrual rates for DS AML patients on previous CCG and POG studies, accrual is expected to be
about 57 patients per year. Thus, a total of 205 patients are expected to be accrued in 3.6 years. Analysis
will be performed after an additional year of follow-up.

10.3 Statistical Analysis Methods
The primary endpoint of interest will be the event-free survival (EFS), the time from on study to
Induction failure, relapse, or death. Other outcome measures will include the induction remission rate,
overall survival from on study, and percentage of patients experiencing grade 3 or 4 toxicity, time to
count recovery, number and type of infections, length of hospitalization, and number of days in the ICU.

10.3.1 Sample size requirements
We wish to be reasonably confident of being able to detect a decrease of 35% in the risk of EFS events in patients on this study as a result of an intensification of cytarabine (Ara-C) therapy during induction therapy. We will assume the null hypothesis that the A2971 EFS experience for children less than 4 years of age with Down syndrome and AML follows an exponential mixture cure model. Since A2971 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. EFS for patients 0-4 years old on A2971 is estimated to be about 78%. We will assume the null hypothesis that the EFS experience on this study follows the exponential cure model. Power of 80% is obtained to detect a relative risk of failure of 0.65 (1-sided testing at the 5% level of statistical significance) if 205 eligible patients are entered. This accrual is expected to be completed in approximately 3.6 years.

10.3.2 Group sequential monitoring
Let \( K \) be the number of failures observed in the available follow-up and \( R \) be the sum of the null cumulative hazard to time \( t_i \), where \( t_i \) is the follow-up for patient \( i \). Then \( T = K - R \) is approximately normally distributed with independent increments and may be used for interim monitoring using standard group sequential boundaries.

Monitoring for efficacy will utilize monitoring based on the Lan-DeMets criterion with an \( \alpha \)-spending function \( \alpha t^2 \) (truncated at 3 standard deviations) and 5% type I error. Formal monitoring analyses of differences in the number of events observed in the available follow-up and the expected number of events under the null hypothesis for the available follow-up will be performed once a year after at least 25% of the expected information has been observed.

A futility analysis will be performed by testing the hypothesis that the relative risk of failure on this study is 0.65 compared to the A2971 experience, with consideration of suspension of accrual should this hypothesis be rejected at any of the interim looks at a significance level of 0.005.

10.3.3 Statistical Considerations for Secondary Objectives

10.3.3.1 Leukemia phenotype and GATA1 mutation analysis
The prevalence of AMkL phenotype will be estimated as the proportion of patients with phenotype data available determined to have the AMkL phenotype. The half-width of corresponding 95% confidence intervals (CI) for patients are provided in Table 4 for various observed prevalences assuming all patients and only 80% of patients have phenotype data available, respectively. EFS will be estimated for those with and without AMkL using the approach of Kaplan and Meier. Differences in these estimates will be tested for significance using the log-rank statistic test. Table 4 summarizes the differences in EFS plateaus between patients with and without AMkL phenotype that this study will have 80% power to detect assuming all patients and only 80% of patients have phenotype data available, respectively.

The prevalence of GATA1 mutations will be estimated as the proportion of patients with phenotype data available determined to have the GATA1 mutations. The half-width of corresponding 95% confidence intervals (CI) for patients are provided in Table 4 for various observed prevalences assuming all patients and only 80% of patients have mutation data available, respectively. Assuming all patients have mutation data available, Table 4 summarizes the differences in EFS plateaus between patients with and without GATA1 mutations that this study will have 80% power to detect assuming all patients and only 80% of patients have mutation data available, respectively.

Table 4. Half-width of 95% confidence intervals (CI) and differences in EFS plateaus for which the study will have 80% power to detect for various prevalences of AMkL phenotype, GATA1 mutations, and genotypes if all patients have phenotype and mutation data available.
Corresponding estimates are provided in parentheses assuming only 80% of patients have phenotype and mutation data available.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>CI half width</th>
<th>Difference in EFS plateaus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>6.7% (7.5%)</td>
<td>18% (21%)</td>
</tr>
<tr>
<td>0.5</td>
<td>6.8% (7.7%)</td>
<td>18% (21%)</td>
</tr>
<tr>
<td>0.6</td>
<td>6.7% (7.5%)</td>
<td>18% (21%)</td>
</tr>
<tr>
<td>0.7</td>
<td>6.3% (7.0%)</td>
<td>18% (21%)</td>
</tr>
<tr>
<td>0.8</td>
<td>5.5% (6.1%)</td>
<td>21% (23%)</td>
</tr>
<tr>
<td>0.9</td>
<td>4.1% (4.6%)</td>
<td>27% (30%)</td>
</tr>
<tr>
<td>0.95</td>
<td>3.0% (3.3%)</td>
<td>35% (39%)</td>
</tr>
</tbody>
</table>

10.3.3.2 Minimal Residual Disease Analysis
The proportions of patients in morphologic remission with positive MRD after Induction I, Induction IV, and End of Therapy will be estimated. Assuming that 90% of the patients will be in morphologic remission after Induction I, the maximum half-width of the corresponding 95% confidence intervals will be 7.2%. There will be better precision if the observed proportions with positive MRD are different from 50%. For the AML patients achieving morphologic remission after Induction I, the cumulative incidence of relapse (relapse or death due to progressive disease) will be estimated for those with and without MRD. Deaths from non-progressive disease will be considered competing events. Differences in the cumulative incidence of relapse for those with and without MRD will be tested using Gray’s test.

10.3.3.3 Drug Sensitivity
Ara-C pharmacokinetic studies will be performed when high dose Ara-C is administered in the Capizzi II chemotherapy course during Induction II. Pharmacokinetic data will be determined by the R-Strip (MicroMath) curve fitting program. Reiterative curvilinear regression analysis will be performed by the SPSS statistical package. Descriptive statistics will be used to summarize pharmacokinetic parameters, such as peak plasma concentration, area under the concentration time curve, and half-life of elimination.

10.3.3.4 Gene Expression Profiles
Gene expression profile analysis using diagnostic blast samples (and relapsed blast samples if available) will be performed as previously described. These analyses will attempt to identify differentially expressed genes which may be associated with clinical and biological subgroups of DS AML patients based on: i) Leukemia phenotype [e.g. AMkL compared to non-AMkL cases]; ii) Blast samples with GATA1 mutations compared to wild–type GATA1, and iii) Treatment response comparing patients who remain in first CR to patients who relapse or are Induction failures.

10.3.3.4.1 Estimated Number of Samples required for analysis
Determination of the necessary number of samples depends both on the magnitude and variability in the gene expression differences. The profiles of clinical specimens are clearly complex, but the number of samples needed for this study will be determined by the availability of samples from enrolled patients.

10.3.3.4.2 Biostatistical Approaches
The scope of the biostatistical challenge posed by this methodology is enormous. The basic informatics approach that has been devised uses a system of cluster analysis to arrange genes according to similarities in patterns of expression. Software written by Michael Eisen and colleagues will be used for the initial analysis of the data (GenePix, Cluster, and Tree View). This software is available at the following web site: http://www.microarrays.org and http://www.axon.com/GN_GenePixSoftware.html.

A hierarchical clustering algorithm is used to assemble the genes into a dendrogram or tree structure with branches containing genes with similar patterns of expression. This ordered representation can be graphically displayed with colors that reflect the qualitative and quantitative relationships of the
expressed genes. In the current version, a log-ratio of 1.0 indicating no change in gene expression is designated by a black color, increased expression is in red, and decreased expression is in increasing intensities of green.

In order to eliminate or filter as much “noise” as possible from the data sets, it is adjusted to remove array batch, date of amplification, and date of hybridization bias by three-way ANOVA with a simple additive model,

$$Y_{ijkl} = \mu + B_i + H_j + A_k + \epsilon_{ijkl},$$

where $Y_{ijkl}$ is the $l$th observation of the $i$th batch, the $j$th date of hybridization and the $k$th date of amplification. The errors were assumed to be independent and normally distributed with mean zero and variance $\sigma^2$. The residuals were taken as the true signals after accounting for biases.\(^{40}\)

Two types of supervised analysis will be used to study these samples. One is significance analysis of microarrays (SAM), a statistical method used in microarray analysis that calculates a score for each gene and thus identifies genes with a statistically significant association with an outcome variable such as survival.\(^{41}\) Our second type of supervised analysis is prediction analysis of microarrays (PAM), a statistical technique that identifies a subgroup of genes that best characterized a predefined class and uses this gene set to predict the class of new samples.\(^{42}\) We will use these two methods of analysis to find a cluster of genes related to clinical outcome.

10.3.3.5 Analyses of drug-metabolizing enzyme polymorphisms

As a secondary objective, we will measure functional polymorphisms in Phase I and Phase II detoxification genes and DNA repair pathways that may modify susceptibility to leukemia and outcome of therapy in children with Down syndrome. These investigations are funded by NCI R01-CA111778 (PI: Perentesis) and include control comparison groups of 270 patients with Down syndrome without leukemia from the Cincinnati Children’s Hospital Thomas Center for Down Syndrome Services, and 492 patients with leukemia but without Down syndrome enrolled on recent COG leukemia clinical trials.

The prevalence of polymorphisms in the children with Down syndrome and AML will be estimated as binomial proportions and corresponding confidence intervals will be calculated. The half-width of corresponding 95% confidence intervals are provided in Table 4 for various observed prevalences assuming all patients and only 80% of patients have genotype data available, respectively. On-study characteristics of patients with different genotypes will be compared. The significance of observed differences in proportions will be tested using the Chi-squared test and Fisher’s exact test when data are sparse. For continuous data, the Mann-Whitney test will be used to compare the medians of distributions.\(^{43}\)

For the analyses of genotypes and outcome of therapy, differences in induction remission rates, induction failure rates, and induction death rates will be assessed using the Chi-squared test and Fisher’s exact test when data are sparse. Other outcome measures will include the EFS, OS, relapse free survival (RFS), and toxic related mortality (TRM). The Kaplan-Meier method will be used to calculate estimates of OS, EFS, RFS, and TRM.\(^{36}\) Corresponding confidence intervals will be calculated using Greenwood’s formula.\(^{44}\) The log-rank statistic will be used to test for differences in OS, EFS, RFS, and TRM.\(^{42}\) In addition, trend tests will be performed to compare outcomes for the genotypes.

Table 4 summarizes the difference in EFS plateaus between patients with and without the genotype that this study will have 80% power to detect assuming all patients and only 80% of patients have genotype data available, respectively. For example, assuming that 80% of the patients have genotype data available and approximately half have at least one low activity MTHFR allele, this study will have 80% power to detect 21% difference in EFS plateaus between children with and without at least one low activity MTHFR allele. Preliminary analyses suggest that it is plausible that such a difference in plateaus could be observed. Power for other polymorphisms will be a function of the observed percentage of children with
each genotype and the outcomes for each genotype.

10.3.3.6 Karyotypic abnormalities
To assess the effect of karyotypic abnormalities on survival. The Kaplan-Meier method will be used to calculate estimates of OS and EFS for specific karyotypic abnormalities. The log-rank test will be used to test for differences in OS and EFS between karyotypic abnormalities.

10.4 Gender and Minority Accrual Estimates

The gender and minority distribution of the study population is expected to be:

<table>
<thead>
<tr>
<th>Ethnic Category: Total of all subjects</th>
<th>Sex/Gender</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>92</td>
<td>82</td>
</tr>
<tr>
<td>Racial Category: Total of all subjects</td>
<td>106</td>
<td>99</td>
</tr>
</tbody>
</table>

This distribution was derived from COG A2971.

11.0 EVALUATION CRITERIA

11.1 Common Terminology Criteria for Adverse Events (CTCAE)
This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. The descriptions and grading scales found in the revised CTCAE version 4.0 will be utilized for reporting beginning October 1\(^{st}\), 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0, which can be downloaded from the CTEP web site ([http://ctep.cancer.gov](http://ctep.cancer.gov)). Additionally, toxicities are to be reported on the appropriate data collection forms.

11.2 Response Criteria for Patients with Acute Myeloid Leukemia
The following definitions for response will be used:

11.2.1 Complete Response (CR)
The bone marrow is regenerating normal hematopoietic cells. The bone marrow is found to have < 5% blast cells by morphology and no evidence of extramedullary disease (EMD). In addition, there is no evidence of Auer rods, leukemia-specific karyotype, or circulating blasts.

11.2.2 Partial Response (PR)
This classification is used when patients fail to qualify for the Complete Response (CR) or Refractory Disease (RD) categories.
11.2.3 Refractory Disease (RD)
Presence of $\geq 20\%$ blasts by morphologic evaluation by bone marrow aspirate with at least moderate cellularity. If the marrow is aplastic/severely hypocellular and peripheral blasts have cleared, a marrow examination should be repeated in one week to document refractory disease.

11.2.4 Relapse
During or following therapy, the bone marrow is found to have $\geq 20\%$ blast cells with or without EMD, after a CR marrow has been documented, or evidence of EMD following a complete remission. Also included are patients with $< 20\%$ blasts with a confirmed recurrence of a karyotypic abnormality characteristic of AML (t(8;21), inv16, t(16;16) or the unequivocal presence of megakaryoblasts.

11.2.5 Unevaluable
Aplastic or severely hypocellular marrow with any blast percentage. In this instance, marrow evaluation should be repeated weekly until response determination can be made.

12.0 ADVERSE EVENT REPORTING REQUIREMENTS

12.1 Purpose
Adverse event 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.

12.2 Determination of Reporting Requirements
Reporting requirements may include the following considerations: 1) the characteristics of the adverse event including the *grade* (severity); 2) the *relationship to the study therapy* (attribution); and 3) the *prior experience* (expectedness) of the adverse event.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases an agent obtained commercially may be used for indications not included in the package label. In addition, NCI may on some occasions distribute commercial supplies for a trial. Even in these cases, the agent is still considered to be a commercial agent and the procedures described below should be followed.

Determine the prior experience Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered *unexpected*, for reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known toxicities for each commercial agent as provided in the Drug Information for Commercial Agents Used by the Children’s Oncology Group posted on the COG website; or
- the drug package insert.

12.3 Reporting of Adverse Events for Commercial Agents - AdEERS abbreviated pathway
Commercial reporting requirements are provided in Table B. The commercial agent(s) used in this study are listed in the front of this protocol immediately following the Study Committee roster.

- COG requires the AdEERS report to be submitted within 5 calendar days of learning of the event.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.
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.

AdEERS Reporting Requirements for Adverse Events That Occur During Therapy With a Commercial Agent or Within 30 Days¹

<table>
<thead>
<tr>
<th>Attribution</th>
<th>Grade 4</th>
<th>Grade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexpected</td>
<td>Expected</td>
</tr>
<tr>
<td>Unrelated or Unlikely</td>
<td></td>
<td>AdEERS</td>
</tr>
<tr>
<td>Possible, Probable, Definite</td>
<td>AdEERS</td>
<td>AdEERS</td>
</tr>
</tbody>
</table>

¹This 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 which can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via AdEERS.

12.4 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 AdEERS reporting.

The NCI defines both routine and expedited AE reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all Grade 3 or higher non-hematological and all grades of the following cardiac Adverse Events: prolonged QTc interval and left ventricular systolic dysfunction.

Note: The following AE term has been updated in CTCAE version 4. Beginning 10/01/10 use the following updated term:

- **Prolonged QTc interval** is replaced with **electrocardiogram QT corrected interval prolonged** (see CTCAE v.4 category “Investigations”).

12.5 Reporting Secondary AML/MDS

All cases of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) that occur in patients on NCI-sponsored trials following their chemotherapy for cancer must be reported to the Investigational Drug Branch (IDB) of the NCI Cancer Therapy Evaluation Program (CTEP) and included as part of the second malignant neoplasm reporting requirements for this protocol. Submit the following information within two weeks of an AML/MDS diagnosis occurring after treatment for cancer on NCI-sponsored trials:

PLEASE NOTE: As of 10/1/2010 Reporting Secondary AML/MDS will be via AdEERS using CTCAE v 4, replacing the directions below.

- a copy of the pathology report confirming the AML/MDS; and
- a copy of the cytogenetics report (if available).

Submit the information via fax to: NCI (fax # 301-230-0159)
Note: If a patient has been enrolled in more than one NCI-sponsored study, the NCI/CTEP Secondary AML/MDS Report Form must be submitted for the most recent trial.

13.0 RECORDS AND REPORTING
See the Case Report Forms posted on the COG web site with each protocol under “Data Collection/Specimens”. A submission schedule is included.

13.1 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.

14.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS

14.1 Central Review of Diagnosis
Specimens will be submitted for Central Review upon study entry and are recommended to be submitted at the time of relapse, if applicable.

14.1.1 Specimen requirements for Central Review are as follows:

1. One Wright & Giemsa stained and 6 unstained bone marrow aspirate smears.
2. Flow cytometry report.
3. Pathology report from the referring institution.
4. One H&E stained and 6 unstained sections of bone marrow core biopsy (if obtained).
5. One H&E stained and 6 unstained sections of bone marrow clot section (if available).
6. One Wright & Giemsa stained and 2 unstained peripheral blood smears (if available).
7. Specimen Transmittal Form.

Please label all materials for Central Review with the patient’s COG patient identification number and the surgical pathology identification number (SPID) found on the corresponding report.

14.1.2 Please send all the above mentioned materials to:

Biopathology Center – AAML0431
Nationwide Children’s Hospital
700 Children’s Drive, WA1340
Columbus, OH 43205
Phone: (614) 722-2894
Fax: 614-722-2897

The Biopathology Center will forward the materials to Dr. David Richmond Head, E-mail: david.head@vanderbilt.edu

14.2 Local Cytogenetic Analysis and Data Submission to Central Laboratory

14.2.1 Specimen Collection for Local Cytogenetics Analysis
Collect approximately 5 mL of bone marrow for cytogenetics in a sodium heparin tube (green top vacutainer) or utilize transport media provided by your cytogenetics laboratory. It is best to use the specimen from the first or second draw for cytogenetics analysis in order to capture the dividing abnormal cells. It is recommended that cytogenetics laboratories keep leftover cytogenetic pellets in order to
evaluate equivocal results.

**Please note:** 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.

It is required that a specimen be sent to the cytogenetics laboratory for your institution at study entry. Submission of an additional specimen is recommended at relapse, but is not required. A case will be considered normal when a +21, mosaic +21 or Robertsonian translocation is present. A case will be considered abnormal when an acquired clonal chromosomal abnormality is present, in addition to the constitutional +21, mosaic +21, or Robertsonian translocation.

### 14.2.2 Data Submission to Central Laboratory Following Local Cytogenetics Analysis
Submit the following to one of the central COG Cytogenetics Laboratories listed below after completion of local cytogenetic studies:

1. COG Cytogenetics Reporting Form (CYTOGFRM FISHFRM.pdf available from Generic Forms on COG Web site).
2. If abnormal, two different abnormal karyotypes from each cell line.
3. If normal, two normal karyotypes.
4. If FISH was performed, please send the following:
   a) images to document the findings, and a
   b) COG FISH Reporting Form (CYTOGFRM FISHFRM.pdf available from Generic Forms on COG Web site).

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

### 15.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

#### 15.1 DNA/RNA Extraction and Drug Sensitivity Assays for Consenting Patients
Diagnostic, end of therapy (Following Intensification II), and relapse (if applicable) bone marrow and/or peripheral blood samples from consenting patients will have genomic DNA and total RNA extracted for the following biology studies:

- a) Analysis of GATA1 mutations (Dr. Hans Hitzler, Hospital for Sick Children).
- b) Analysis of gene expression profiles by microarray (Dr. N. Lacayo, Stanford University).
- c) Validation of selected genes (identified by microarray) by real-time PCR and expression of genes encoding Ara-C metabolizing enzymes.
- d) Analysis of polymorphisms in Phase I and Phase II detoxification genes and DNA repair pathway genes (Dr. J. Perentesis, Cincinnati Children’s Hospital Medical Center).

Diagnostic bone marrow and/or peripheral blood samples will also be analyzed for *in vitro* drug sensitivity patterns to Ara-C and daunorubicin.

#### 15.1.1 Specimen Collection
A total of 3-5 mL of bone marrow should be acquired for these studies. If the peripheral blast count is ≥ 50%,
3-5 mL of peripheral blood can be sent for the studies; however, bone marrow is preferable to peripheral blood. Samples should be collected in preservative free heparin and shipped at ambient temperature to the laboratory (please, NO Saturday deliveries). Samples should be labeled with the COG number, BPC number, collection date, and specimen type.

15.1.2 Ship bone marrow and/or peripheral blood samples by Federal Express Priority Overnight using Account Number 2504-6481-9. Include a COG specimen transmittal form.

Send to: Mr. Steven Buck or Dr. Mark L. Stout
Children’s Hospital of Michigan
Division of Hematology/Oncology
Room 3X60
3901 Beaubien Blvd.
Detroit, Michigan 48201
313-745-5603 or 745-5554
sbuck@med.wayne.edu  mstou@med.wayne.edu

15.2 Minimal Residual Disease Detection for Consenting Patients
At the designated time points indicated below, collect 2-3 mL of anticoagulated bone marrow (preservative-free heparin) from consenting patients. Place in a 15 mL sterile conical centrifuge tube and add 5 mL of sterile RPMI-1640 with 20% fetal calf serum. Samples should be labeled with the COG number, BPC number, collection date, and specimen type. Seal centrifuge tube with paraffin or equivalent.

If bone marrow is unattainable at diagnosis (dry tap), peripheral blood containing leukemic blasts should be submitted along with any bone marrow obtained. 5 mL of peripheral blood collected in preservative-free heparin should be added to RPMI 1640 with 20% FCS, processed and packaged for shipment as described for bone marrow specimens. Do not freeze MRD samples.

NOTE: At diagnosis, only bone marrow is required to be sent for MRD analysis, while paired bone marrow and peripheral blood samples are required for all subsequent MRD samples.

15.2.2 Schedule of MRD Sample Collection:
If a patient achieves a CR after Induction I, collect MRD samples according to the following schedule:

<table>
<thead>
<tr>
<th>Study Entry</th>
<th>Induction I</th>
<th>Induction IV</th>
<th>Intensification II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 28*</td>
<td>Day 28</td>
</tr>
</tbody>
</table>

* This sample is not required if Day 14 (Induction I) bone marrow is cellular with ≥ 20% blasts.

If a patient does not achieve a CR after Induction I (begins Induction II with PR or RD), additional bone marrow evaluations will be performed on Day 14 and 28 of Induction II. For these patients, MRD samples will be collected according to the following schedule:

<table>
<thead>
<tr>
<th>Study Entry</th>
<th>Induction I</th>
<th>Induction II</th>
<th>Induction IV</th>
<th>Intensification II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 14</td>
<td>Day 28</td>
<td>Day 28</td>
</tr>
</tbody>
</table>

It is expected that patients will have a minimum of 5 MRD samples collected (7 samples if the Day 28 Induction I bone marrow is not CR).

15.2.3 At relapse (if applicable), paired bone marrow and peripheral blood samples should be submitted as described above.
15.2.4 Send MRD samples by Federal Express Priority Overnight using Account Number 2504-6481-9. Include a COG Specimen Transmittal form and ship at room temperature to:

Dario Campana, M.D., Ph.D.
Department of Oncology
St. Jude Children’s Research Hospital
Thomas Tower, Room D4046, 262 Danny Thomas Place
Memphis, TN 38105
Phone (901) 595-2528 or (901) 595-2527

15.3 Pharmacokinetic Studies during High-dose Ara-C (Induction II) for Consenting Patients

15.3.1 Protocol for Collection of Plasma for Ara-C Pharmacokinetics in Down Syndrome Patients for Consenting Patients:

1. When a patient is scheduled for treatment with high dose Ara-C (Capizzi II regimen) for Induction II please call Dr. Stout (313-745-5603) at least four days prior to treatment in order that heparinized vacucontainers containing tetrahydrouridine (inhibits cytidine deaminase) can be sent for the pharmacokinetic studies, providing patient consent has been obtained for these studies.

2. A separate venous sampling site (preferably on the opposite side of the infusion site) should be established before infusion starts and kept patent with heparinized IV fluid.

Note: A separate line should be established only if feasible for the patient and with consent. If a separate IV collection site cannot be established, samples are drawn from a central port.

3. Before Ara-C infusion starts, draw 3 mL of blood and collect into the labeled provided heparinized tube (“Enzyme Analysis”), mix well, centrifuge, collect plasma, and freeze as cold as possible until shipment with other samples.

4. Thirty minutes prior to the end of the three-hour Ara-C infusion, again take 1 mL of blood from the sampling catheter and place into the provided THU heparinized labeled tube and place on ice. Have this cold centrifuged, collect the plasma, label this tube and freeze sample (–20°C). Label this tube as steady state (ss). The steady state sample is the most critical time point.

Note: For patients that do not have a separate line, the sample may be collected between 30-5 minutes before the end of infusion. The infusion must be stopped, the port cleared with a heparinized saline wash, 1-2 mL of blood removed and discarded, and then 1-1.5 mL of blood taken for the sample. **Speed is of the essence because the initial distribution half life is only 7-9 minutes.** Washing and clearing the port is also essential; if this is not done, sampling would be of the infusion bag and not the central venous site. After this is accomplished the remaining collection samples can be taken easily from the port site.

5. Collect the remaining timed samples taking 1 mL of blood into the appropriately labeled THU heparinized tube. Have these samples cold centrifuged, remove the plasma, label the time on this along with patients initials and store frozen as outlined in #4 above. Collect blood at 30 minutes prior to stopping drug, 5, 10 and 30 minutes post infusion and at 1, 4, and 8 hours post infusion. The 5 and 10-minute samples may be batched centrifuged while the 30-minute and hourly samples should be individually processed.

6. If there is no lab facility available, the samples will be well mixed and shipped on wet-ice or equivalent for next day delivery.

**NOTE:** These tubes will be Vacutainer-type but will have the vacuum broken to add the THU inhibitor under sterile conditions. Blood samples, therefore, must be drawn into sterile syringes
and the sample transferred to the prepared tubes. All sample tubes should be labeled with the time after end of infusion, COG number, BPC number, collection date, and specimen type. All samples after blood draw are always kept on ice until processed and frozen. The five and ten-minute samples can be centrifuged together.

If times cannot be followed, be sure to record accurately the post-infusion time the sample was drawn.

15.3.2 After collection, send samples on dry ice by Federal Express Priority Overnight using Account Number 2504-6481-9. Include a COG Specimen Transmittal Form and send to:

Dr. Mark L. Stout
Children’s Hospital of Michigan
Division of Hematology/Oncology
Room 3X60
3901 Beaubien Blvd.
Detroit, Michigan 48201
313-745-5603
mstou@med.wayne.edu

15.3.3 Flow Sheet

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TIME</th>
<th>TUBE TYPE &amp; LABEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>Before Ara-C Infusion</td>
<td>No THU – Green Top 3 mL (Enzyme Analysis)</td>
</tr>
<tr>
<td>Plasma</td>
<td>30 mins prior to end of infusion</td>
<td>THU – Green Top (SS)</td>
</tr>
<tr>
<td>Plasma</td>
<td>5 mins post-infusion</td>
<td>THU – Green Top (5-PI)</td>
</tr>
<tr>
<td>Plasma</td>
<td>10 mins post-infusion</td>
<td>THU – Green Top (10-PI)</td>
</tr>
<tr>
<td>Plasma</td>
<td>30 mins post-infusion</td>
<td>THU – Green Top (30-PI)</td>
</tr>
<tr>
<td>Plasma</td>
<td>1 Hr post-infusion</td>
<td>THU – Green Top (1 Hr-PI)</td>
</tr>
<tr>
<td>Plasma</td>
<td>4 Hr post-infusion</td>
<td>THU – Green Top (4 Hr-PI)</td>
</tr>
<tr>
<td>Plasma</td>
<td>8 Hr post-infusion</td>
<td>THU – Green Top (8 Hr-PI)</td>
</tr>
</tbody>
</table>

The remaining samples require 1mL of peripheral blood for each time point.

15.4 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 I: PERFORMANCE STATUS SCALES/SCORES

**Performance Status Criteria**
Karnofsky and Lansky performance scores are intended to be multiples of 10

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

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.*
APPENDIX II: LIST OF ANTICONVULSANTS BASED ON CYP3A4/ENZYME INDUCTION

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Trade Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabapentin</td>
<td>Neurontin</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Lamictal</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>Keppra</td>
</tr>
<tr>
<td>Tiagabine</td>
<td>Gabitril</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Topamax</td>
</tr>
<tr>
<td>Valproic Acid</td>
<td>Depakote, Depakene</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>Zonegran</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Trade Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Tegretol</td>
</tr>
<tr>
<td>Felbamate</td>
<td>Felbatol</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Dilantin</td>
</tr>
<tr>
<td>Primidone</td>
<td>Mysoline</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>Trileptal</td>
</tr>
</tbody>
</table>
REFERENCES

34 Altman AJ: Supportive Care of Children with Cancer: Current Therapy & Guidelines from the Children's Oncology Group. 3rd edition. The Johns Hopkins University Press, Baltimore, MD, 2004
35 Woolson RF: Rank tests and a one-sample log rank test for comparing observed survival data to a standard population. Biometrics 37:687–696, 1981
41 Tusher VG, Tibshirani R, Chu G: Significance analysis of microarrays applied to the ionizing
43 Mann HB, Whitney DR: On a test of whether one of two random variables is stochastically larger than the other. Ann Math Statistics 18:50-60, 1947
This model informed consent form has been reviewed by the DCT/NCI and is the official consent document for this study. Institutions should use the sections of this document which are in bold type in their entirety. Editorial changes to these sections may be made as long as they do not change information or intent. If the institutional IRB insists on making deletions or more substantive modifications to any of the sections in bold type, they must be justified in writing by the investigator at the time of the institutional audit.

SAMPLE INFORMED CONSENT/PARENTAL PERMISSION FOR PARTICIPATION IN RESEARCH

AAML0431, The Treatment of Down Syndrome Children with Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS) Under the Age of 4 Years

If you are a parent or legal guardian of a child who may take part in this study, permission from you is required. The assent (agreement) of your child may also be required. When we say “you” in this consent form, we mean you or your child; “we” means the doctors and other staff.

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS STUDY?

This study is called a clinical trial. A clinical trial is a research study involving treatment of a disease in human patients. This study is organized by Children’s Oncology Group (COG). COG is an international research group that conducts clinical trials for children with cancer. More than 200 hospitals in North America, Australia, New Zealand, and Europe are members of COG.

You are being asked to allow your child to take part in this study because your child has Down syndrome (DS) and has recently been diagnosed with Acute Myeloid Leukemia (AML) or Myelodysplastic Syndrome (MDS).

AML is a cancer of the bone marrow, the spongy tissue inside large bones where blood cells are made. In AML, the bone marrow makes large numbers of immature white blood cells called blasts. These blast cells crowd out the normal cells of the bone marrow. They may flood the bloodstream and invade vital organs such as the brain, testes, ovaries, or skin. These cancerous AML cells can sometimes form a solid tumor called a chloroma.

Many patients have MDS before they get AML. MDS is a disease in which the body makes fewer blood cells than usual. Bone marrow in MDS patients does not produce enough healthy blood cells. MDS can develop into leukemia.

It is common to enroll children and adolescents with cancer in a clinical trial that seeks to improve cancer treatment over time. Clinical trials include only people who choose to take part. You have a choice between a standard treatment for AML or MDS and this clinical trial.

Please take your time to make your decision. Discuss it with your friends and family. We encourage you to include your child in the discussion and decision to the extent that she or he is able to understand and take part.

WHAT IS THE CURRENT STANDARD OF TREATMENT FOR THIS DISEASE?

The standard treatment for AML and MDS is to use a combination of cancer-fighting drugs.
called chemotherapy. Chemotherapy destroys the leukemia cells in the blood and bone marrow. The standard treatment regimen consists of two phases of therapy, called Induction and Intensification. In the Induction phase we try to remove all visible signs of leukemia and allow normal blood cells to be restored. This is called remission. Induction treatment is usually repeated for 3 courses of therapy (each course is 28 days). The next phase of treatment is called Intensification. Intensification chemotherapy is used to kill the few remaining leukemia cells that may have survived Induction. Intensification is usually 3 courses of therapy, and includes high dose cytarabine (one of the chemotherapy drugs) in the last course. Patients with AML or MDS may also be treated with up to 7 doses of cytarabine that is injected into the spinal fluid. More information about standard chemotherapy can be found in the COG Family Handbook for Children with Cancer.

WHY IS THIS STUDY BEING DONE?

Research has shown that children with DS are more likely to develop leukemia than children who do not have DS. However, they are also known to respond better to chemotherapy than children with AML who do not have DS.

The overall goal of this study is to see if we can increase the cure rate and decrease the side effects of therapy. Side effects are unintended and unwanted results of treatment.

In this study, we will test the effects good and/or bad of changing the order of one of the chemotherapy treatments, high dose cytarabine (Ara-C). Subjects in this study will receive high dose cytarabine earlier in the treatment schedule than in past studies.

Since DS patients do well on chemotherapy, study doctors want to see if it is possible to lower the side effects of chemotherapy without lowering the effectiveness of the treatment. Study doctors would like to know the effects good and/or bad of reducing the following chemotherapy treatments:
1) the number of treatments given in the spinal fluid (called “intrathecal”) and
2) the number of doses of daunorubicin, one of the chemotherapy drugs.

A secondary goal of the study is to learn more about the biology of AML and MDS in DS patients. These tests are optional and will be done only if you agree. The optional biology tests are described in detail at the end of the consent and you will be asked if you want your child to participate. Briefly, the biology studies will:
- test for genetic changes in the leukemia cells, and genetic factors which might affect a subject’s likelihood of getting leukemia and outcome with treatment.
- look for very small amounts of cancer cells in the blood and bone marrow, called Minimal Residual Disease (MRD). Researchers want to find out if measuring MRD can be used in the future to decide how great the risk of the cancer coming back is for a person and predict how a subject will do with treatment.
- see what happens to high dose cytarabine (one of the chemotherapy drugs) in the body and how much of the drug remains active over an 8-hour period. These are called pharmacokinetic (PK) tests.
- collect blood and bone marrow specimens and store them in a cell bank for future research into Down syndrome.

In summary, the goals of this study are:
1. To see if changing the order of high dose cytarabine in the treatment plan has an affect on the cure rate for DS patients.
2. To see if lowering the number of treatments into the spinal fluid and the number of doses of daunorubicin will be as effective as standard treatment with fewer side effects.

3. To understand the biology of AML and MDS better with the optional biology tests.

HOW MANY CHILDREN WILL TAKE PART IN THE STUDY?

About 205 children are expected to take part in this study.

WHAT WILL HAPPEN TO MY CHILD IN THIS STUDY THAT IS RESEARCH?

Treatment Plan

All of the chemotherapy drugs given in this study have been used before to treat children with AML and MDS. The drugs being used are not new but the way they are being given on this study is new. All subjects participating in this study will get the new treatment. The treatment on this clinical trial takes about 6 months.

Chemotherapy will be given in the following stages:

- Induction 1: chemotherapy given for 4 days followed by about 3 weeks of rest.
- Induction 2: chemotherapy is given for 9 days followed by about 3 weeks of rest.
- Induction 3: chemotherapy is given for 4 days followed by about 3 weeks of rest.
- Induction 4: chemotherapy is given for 4 days followed by about 3 weeks of rest.
- Intensification 1: chemotherapy is given over 7 days followed by 3 weeks of rest.
- Intensification 2: chemotherapy is given over 7 days followed by 3 weeks of rest.

The treatment given in Induction 2 is usually given as the fifth treatment, but in this study it is the second treatment.

Daunorubicin, one of the standard chemotherapy drugs, is being omitted from Intensification 1 and 2 in this study.

The 7 standard therapy intrathecal (or IT) cytarabine injections will be omitted from Induction 2 and 4 and Intensification 1 and 2. Subjects will instead only be given IT cytarabine injections as part of Induction 1 and 3 for a total of 2 injections.

You will receive no payment or money for taking part in this study. If you agree to participate in the optional biology studies described later in this form, there are no plans for you to profit from any new products developed from research done on your specimens.
Diagram of treatment

Study Entry
Less than 4 years old at diagnosis.

Bone Marrow Test

Induction 1

Bone Marrow Test
Day 14

Bone Marrow Test
Day 28

Induction 2

Bone Marrow Test
Day 14 & Day 28
(Only if leukemia cells present after Induction 1)

If there is no response to therapy (leukemia cells are not being destroyed)

Induction 3

Bone Marrow Test

Induction 4

Bone Marrow Test
Leukemia cells present?

Intensification 1

Off therapy*

Intensification 2

Bone Marrow Test

Follow-Up Tests

* Your child's doctor will talk to you about other treatment.
**Drug Therapy: Before your child begins the study**
Before entering this study, your child had his or her bone marrow and spinal fluid tested for cancer cells. At the time that the spinal fluid was tested, your child may have received a drug called cytarabine inserted with a needle directly into the spinal fluid (intrathecal or IT). The spinal fluid is taken out and this drug is put in often during the same procedure. If your child had this done, and if there are no further signs of cancer cells in the spinal fluid, then your child will not need IT cytarabine during Induction I of this study. If this drug was not given at the time the spinal fluid was taken out, then your child will receive IT cytarabine on this study during the first day of treatment.

**Drug Therapy: During the study**
If cancer cells are found in the spinal fluid after IT cytarabine is given, the subject will be considered as having Central Nervous System (CNS) disease. A patient will also be considered as having CNS disease if they have a chloroma, or solid tumor sometimes found in patients that have AML. CNS disease is very rarely seen in Down syndrome patients, and it is treated by giving additional IT cytarabine treatment. See Attachment #1 for more information on CNS disease treatment.

Subjects will receive six courses of therapy: Induction 1, Induction 2, Induction 3, Induction 4, Intensification 1, and Intensification 2. The purpose of Induction therapy is to destroy as many cancer cells as possible in the blood and bone marrow. The purpose of Intensification is to kill any remaining cancer cells that may not be active but could begin to re-grow and cause the cancer to return (called relapse).

All courses of therapy last for 28 days (4 weeks). Your child will be hospitalized for all courses of therapy.

**Methods for Giving Drugs**
Various methods will be used to give drugs to subjects.
- **PO** - Drug is given by tablet or liquid swallowed through the mouth
- **IV** - Drug is given using a needle inserted into a vein. It can be given by IV push over several minutes or by IV infusion over minutes or hours.
- **IM** - Drug is given by inserting a needle injected into the muscle (IM shot).
- **IT** - Drug used to treat the brain and spinal cord is given using a needle inserted into the spinal fluid (intrathecally, IT).

### Induction 1 (28 Days)

<table>
<thead>
<tr>
<th>Drug</th>
<th>How the drug will be given</th>
<th>Day(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>IT</td>
<td>Day 1 (if not given at diagnosis)</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>IV over 96 hours</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>IV over 96 hours</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>PO</td>
<td>1, 2, 3, 4</td>
</tr>
</tbody>
</table>
### Induction 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>How the drug will be given</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>IV over 3 hours (2 doses per day)</td>
<td>1, 2, 8, 9</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>IM</td>
<td>2, 9</td>
</tr>
</tbody>
</table>

### Induction 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>How the drug will be given</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>IV over 96 hours</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>IV over 96 hours</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>PO</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Intrathecal Cytarabine</td>
<td>IT</td>
<td>1</td>
</tr>
</tbody>
</table>

### Induction 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>How the drug will be given</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>IV over 96 hours</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>IV over 96 hours</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>PO</td>
<td>1, 2, 3, 4</td>
</tr>
</tbody>
</table>

### Intensification 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>How the drug will be given</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>IV infusion X 7 days (168 hours)</td>
<td>1 through 7 continuously</td>
</tr>
<tr>
<td>Etoposide</td>
<td>IV over 1 hour</td>
<td>1, 2, 3</td>
</tr>
</tbody>
</table>

### Intensification 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>How the drug will be given</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>IV infusion X 7 days (168 hours)</td>
<td>1 through 7 continuously</td>
</tr>
<tr>
<td>Etoposide</td>
<td>IV over 1 hour</td>
<td>1, 2, 3</td>
</tr>
</tbody>
</table>

Slides of bone marrow and blood will be taken at study entry and sent to central review centers with reports to confirm the diagnosis. This is part of the Children’s Oncology Group quality control.
**Bone Marrow Tests**

Bone marrow tests will be done according to the following timetable:

<table>
<thead>
<tr>
<th>Study Vector</th>
<th>Induction I*</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 28</td>
<td>Induction I</td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>Induction II**</td>
<td>Day 28</td>
</tr>
<tr>
<td>Day 28</td>
<td>Induction IV</td>
<td></td>
</tr>
</tbody>
</table>

* If the Day 14 bone marrow test shows that you still have a high number of blasts present, and the non-blast cells in your bone marrow appear normal, you will not receive a Day 28 bone marrow test.

** If the bone marrow looks free of leukemic cells after Induction I, no further marrow will be collected during Induction II. If leukemic cells are present in the marrow after Induction I, then patients will have bone marrow aspirations at these times.

*** End of therapy is after Intensification

For more information on bone marrow evaluations see Attachment #1 and the *COG Family Handbook for Children with Cancer.*

**HOW LONG IS THE STUDY?**

Your child will be asked to take study drugs for approximately six months if enrolled on this study. After your child is finished taking study drugs, the study doctor will ask you to visit the office for follow-up exams every month for the first 12 months, every 3 months for the next 12 months, every 6 months until five years following completion of therapy, and then once a year after that. Echocardiograms will be given once a year for the first five years. After five years, they will be given according to your doctor’s orders. Echocardiograms use sound waves to take pictures of the heart. These tests would most likely be given even if your child was not on this study. See the *COG Family Handbook for Children with Cancer* for more information on echocardiograms and follow up tests for children that have received cancer treatment.

We will continue to collect some medical information about how your child is doing for 10 years.

Your doctor or the study doctor may decide to take someone off this study under the following circumstances:

- if he/she believes that it is in the person’s best interest
- if the subject’s disease comes back during treatment
- if the subject experiences side effects from the treatment that are considered too severe
- if new information becomes available that shows that another treatment would be better for the person

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study doctor and your regular doctor first. Your study doctor or regular doctor will be able to help your child stop treatment in the safest way for your child.

**WHAT ARE THE RISKS OF THE STUDY AND HOW ARE THE RISKS DIFFERENT FROM TREATMENT?**

**Treatment Risks**

All people who receive cancer treatment are at risks of having side effects. In addition to killing tumor cells, cancer chemotherapy can damage normal tissue and produce side effects. Side effects are usually reversible when the medication is stopped but occasionally persist and cause serious complications. A person can die from these side effects and other complications.

Common side effects include nausea, vomiting, hair loss, and fatigue. Drugs may be
given to prevent or decrease nausea and vomiting. Hair loss is usually temporary but on very rare occasions it may be permanent. Some chemotherapy may lead to sterility. Sterility is the inability to have children. There is also the possibility that a second cancer may develop years later as a result of the chemotherapy. The risks of the individual drugs given as standard treatment are listed on the tables in Attachment #2. Side effects can be increased when chemotherapy drugs are combined.

The most common serious side effect from cancer treatment is lowering of the number of blood cells resulting in anemia, increased chance of infection, and bleeding tendency. Low blood counts are described in the COG Family Handbook for Children with Cancer.

There is a risk that the treatment will not cure the cancer or that the cancer can go away after the treatment and then come back at a later date.

We will be checking your child closely to see if any side effects are happening. Side effects of chemotherapy drugs usually get better if the treatment is stopped, but in some cases the side effects can be serious or long lasting. If your child does have side effects, we may recommend medicine or treatments to try to control them and make your child more comfortable.

**Risks of Study**

In addition to the risks described above, participation in this study may result in additional risks. For instance, we do not know how the changes made to the standard chemotherapy regimen might affect the subject’s response to therapy. Adding the high dose cytarabine earlier in treatment might cause more side effects. Using less daunorubicin and IT cytarabine might make the treatment less effective in killing all of the cancer cells. In addition to the risks discussed above, there may be unknown risks, or risks that we did not anticipate, associated with being in this study.

**ARE THERE BENEFITS TO TAKING PART IN THE STUDY?**

We hope that you will get personal medical benefit from participation in this clinical trial, but we cannot be certain. These potential benefits could include a better chance of a cure or fewer side effects. While doctors hope that the treatments used on this study will be more useful against cancer compared to the usual treatment, there is no proof of this yet.

Taking part in this study may or may not make your child’s health better. While doctors hope that the treatments used on this study will be more useful against cancer compared to the usual treatment, there is no proof of this yet. We do know that the information from this study will help doctors learn more about the drugs used on this study as a treatment for cancer. This information could help future cancer patients.

**WHAT OTHER OPTIONS ARE THERE?**

Your other choices may include:

- Getting treatment or care for your child’s cancer without being in a study.
- Taking part in another study.

Talk to your child’s doctor about your child’s choices before you decide if you will allow your child to take part in this study.
WHAT ABOUT CONFIDENTIALITY?

We will do our best to make sure that the personal information in your child’s medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your child’s name and other personal information will not be used.

It is very unlikely that the research testing might uncover important information about your child’s current or future health. If this unlikely event occurs, the researchers may contact your child’s doctor through COG’s Data Center about what the research test results might mean. Only the doctor will be notified and the information will remain confidential. Your child’s doctor may discuss this unexpected finding with you, and may recommend consultation with a genetic counselor and/or repeat testing in a clinical (not research) laboratory if necessary. It is possible that your child’s doctor may recommend that no additional action is necessary.

The Children’s Oncology Group has a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. Information about the certificate is attached at the end of this consent.

You may read your child’s medical record. The records are available to those caring for your child at this hospital.

Organizations that may look at and/or copy your child’s medical records for research, quality assurance, and data analysis include:

- The Children’s Oncology Group,
- The Institutional Review Board of this hospital,
- Representatives of the National Cancer Institute (NCI), Food and Drug Administration (FDA),
- Other U.S. and international governmental regulatory agencies involved in overseeing research, and
- The Pediatric Central Institutional Review Board (PedCIRB) of the National Cancer Institute.

WHAT ARE THE COSTS?

Taking part in this study may lead to added costs to you or your insurance company. Please ask about any expected added costs or insurance problems. Staff will be able to assist you with this.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury. However by signing this form, you are not giving up any legal rights to seek to obtain compensation for injury.

You or your insurance company will be charged for continuing medical care and/or hospitalization.
For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute’s Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the “Clinical Trials and Insurance Coverage” information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

If you choose to enroll on this study, this institution will receive some money from the Children’s Oncology Group to perform the research. There are no plans to pay you for participation in this study.

WHAT ARE MY CHILD’S RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to participate in this study. If you decide not to participate, you will not be penalized and you will not lose any benefits to which you are entitled. You will still receive medical care. You may discontinue your participation in the study at any time. If you discontinue participation in the study, you will not be penalized and you will not lose any benefits to which you are entitled. If you decide to stop being in the study, please talk with the researcher or your doctor so medicines can be stopped in the safest possible way. Physicians and hospital personnel will still take care of you.

We will tell you about new information that may affect your child’s health, welfare, or willingness to stay in this study. A committee outside of COG closely monitors study reports and notifies institutions if changes must be made to the study. Members of COG meet twice a year to evaluate results of treatment and to plan new treatments.

During your follow-up visits after treatment, you may ask to be given a summary of the study results after they are written up. This may be several years after all subjects have completed the study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or if you have a research-related problem or if you think you have been injured in this study, you may contact Dr. __________ or your doctor at __________.

If you have any questions about your rights as a research participant or any problems that you feel you cannot discuss with the investigators, you may call __________ IRB Administrator at __________.

If you have any questions or concerns that you feel you would like to discuss with someone who is not on the research team, you may also call the Patient Advocate at __________.

WHERE CAN I GET MORE INFORMATION?

The COG Family Handbook for Children with Cancer has information about specific cancers, tests, treatment side effects and their management, adjusting to cancer, and resources. This can be found on the COG website. Visit the COG Web site at http://www.curesearch.org.

If you are in the United States, you may call the NCI’s Cancer Information Service at 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615.
Visit the NCI’s Web site at http://www.nci.nih.gov/cancerinfo/

Visit the COG Web site at http://www.curesearch.org

Information about long term follow-up after cancer treatment can be found at http://www.survivorshipguidelines.org/

You will get a copy of this form. You may also ask for a copy of the protocol (full study plan).

**OPTIONAL RESEARCH STUDIES**

This section is about the additional research studies that are being done with children who are taking part in the main study. Your child may only take part in these additional studies if you agree. Your child can still be a part of the main study even if you say ‘no’ to taking part in any of these additional studies.

If you participate in the optional biology studies, you will be providing specimens to researchers. There are no plans for you to profit from any new products developed from research done on your child’s specimens.

You can say “yes” or “no” to each of the following studies. Please circle and initial your choice for each study.

**Genetic Testing (Optional)**

Study doctors would like to find out more about how genetic changes are related to Down syndrome children and cancer. With your permission, we would like to study the genetic makeup in your child’s cells. One of the tests involves finding changes in certain genes (called GATA1 mutations), which we think help cause the cancer. We would also like to find out if there is a relationship between changes in other genes and the risk of developing leukemia, and if these genetic changes affect how well your child responds to the treatment on this study.

In order to allow us to do these tests, we will need an extra ½ teaspoon to 1 teaspoon of your child’s bone marrow and the same amount of blood. The specimens are taken when the required blood and bone marrow tests are being done at study entry. The required tests use about ½ teaspoon of blood and bone marrow, so if you agree to have your child participate in this portion of the study, a total of about 1 teaspoon or 1½ teaspoon of blood and bone marrow will be taken at study entry. This amount of blood is safe even for small children.

The extra marrow can usually be drawn through the same needle stick as the sample for the required test. However, there may be additional pain associated with obtaining the extra marrow and sometimes a second needle stick may be required. The marrow and blood are sent to outside research laboratories, and the results of these tests will not be made known to you or your doctor. The results from this biology study will not become a part of your child’s medical record.

Please take your time to make your decision and then let us know by answering the question below.
My child’s bone marrow and blood may be used for genetic testing.

YES    NO     Initials ___________

**Tests on Bone Marrow for Minimal Residual Disease (MRD) – (Optional)**

We would like to perform minimal residual disease (MRD) tests in order to detect small amounts of leukemic cells in the blood and bone marrow. We want to study MRD tests to see if they can be used in future studies to tell us how well patients will do with the treatment.

If you agree, about an extra ½ teaspoon of bone marrow will be taken at the same times that the required bone marrow is being taken. The bone marrow for the MRD tests will only be taken at the times when bone marrow is taken as part of the treatment on this study. Bone marrow aspirations just to obtain research samples will not be performed.

The extra marrow can usually be drawn through the same needle stick as the samples for the required tests. However, there may be additional pain associated with obtaining the extra marrow and sometimes a second needle stick may be required.

We would also like to draw about 1 teaspoon of blood for MRD at the same times we collect bone marrow.

The bone marrow and blood is sent to an outside research laboratory for testing, and the results will not be made known to you or your doctor. Please take your time to make your decision and then let us know by answering the question below.

I agree to have my child participate in the minimal residual disease tests.

YES    NO     Initials ___________

**Pharmacokinetic (PK) Tests (Optional)**

Your child will get “high dose” cytarabine during Induction II therapy. Each dose of cytarabine is given through an IV over a 3-hour period. Doctors would like to perform PK tests to see what happens to the drug in your child’s body. If you agree to these tests, you child will have about ½ teaspoon of blood withdrawn before the start of the drug. Your child will then have about ¼ teaspoon of blood withdrawn at the following times: 30 minutes before stopping the drug; at 5, 10 and 30 minutes after the drug is stopped; and at 1, 4 and 8 hours after getting the drug. The blood tests to measure cytarabine levels will be drawn from a vein.

The blood is sent to an outside research laboratory for testing, and the results will not be made known to you or your child’s study doctor. Please take your time to make your decision and then let us know by answering the question below.

I agree to have my child participate in the PK tests.

YES    NO     Initials ___________
Consent Form for Use of Tissue for Research (Banking of Specimens) (Optional)

About Using Tissue for Research
Your child is going to have bone marrow and blood specimens taken as part of this study to see if your child has cancer and to see what effect the study is having on the cancer. The results of these tests will be given to you by your study doctor and will be used to plan your child’s care.

We would like to keep some of the tissue that is leftover for future research. If you agree, this tissue will be kept and may be used in research to learn more about cancer and other diseases. Please read the information sheet called "Providing Your Tissue for Research: What You Need to Know" to learn more about tissue research.

[Note to Local Investigator: This information sheet is available on the COG web site at: https://members.childrensoncologygroup.org/prot/reference_materials.asp under CONSENTS AND IRB FORMS]

Your child’s specimens may be helpful for research whether your child does or does not have cancer. The research that may be done with your child’s specimens is not designed specifically to help your child. It might help people who have cancer and other diseases in the future.

Reports about research done with your child’s tissue will not be given to you or your study doctor. These reports will not be put in your child’s health record. The research will not have an effect on your child’s care.

Things to Think About
The choice to let us keep the leftover tissue for future research is up to you. No matter what you decide to do, it will not affect your child’s care.

If you decide now that your child’s tissue can be kept for research, you can change your mind at any time. Just contact us and let us know that you do not want us to use your child’s tissue. Then any tissue that remains will no longer be used for research.

In the future, people who do research may need to know more about your child’s health. While this institution may give them reports about your child’s health, it will not give them your child’s name, address, phone number, or any other information that will let the researchers know who your child is.

Sometimes tissue is used for genetic research (about diseases that are passed on in families). Even if your child’s tissue is used for this kind of research, the results will not be put in your child’s health records.

Your child’s tissue will be used only for research and will not be sold. The research done with your child’s tissue may help to develop new products in the future.

Benefits
The benefits of research using tissue include learning more about what causes cancer and other diseases, how to prevent them, and how to treat them.

Risks
The greatest risk to your child is the release of information from your child’s health records. We...
will do our best to make sure that your child’s personal information will be kept private. The chance that this information will be given to someone else is very small.

Making Your Choice
Please read each sentence below and think about your choice. After reading each sentence, circle "Yes" or "No" and write your initials in the space provided.

If you have any questions, please talk to your study doctor or nurse, or call the research review board at phone number ________________________.

No matter what you decide to do, it will not affect your child’s care.

1. My child’s specimens may be kept for use in research to learn about, prevent, or treat cancer.

   YES    NO     Initials ____________

2. My child’s specimens may be kept for use in research to learn about, prevent or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease).

   YES    NO     Initials ____________

SIGNATURE

I have been given a copy of all _____ [insert total of number of pages] pages of this form. This form includes 3 attachments.

I have read it or it has been read to me.

I have reviewed the information and have had my questions answered. I agree to have my child take part in this study.

Parent (or Guardian) ____________________________________________________________

Date ________________

Physician/PNP obtaining consent_________________________________________Date ____________
Central Line
For drugs to be given by vein, your doctor will likely recommend that your child have a central venous line placed. A description of the types of central lines is in your COG Oncology Family Handbook for Children with Cancer.

CNS Disease
If cancer cells are found in the spinal fluid after IT cytarabine is given, the subject will be considered as having Central Nervous System (CNS) disease, and IT cytarabine will be given twice a week until the spinal fluid is clear plus two additional IT treatments. Your child will get sedation or general anesthesia when spinal fluid is taken out for testing and when medication is given directly into the spinal fluid. It is very rare for Down syndrome patients to have CNS disease, which is why the IT cytarabine treatments are being reduced from standard therapy on this study. Subjects with chloromas, the solid tumor of AML, will also be considered as having CNS disease.

Medical Tests and Evaluations: Before your child begins the study
Your child will have a bone marrow aspiration and a lumbar puncture (spinal tap) prior to study entry in order to make the proper diagnosis. These procedures are part of regular cancer care and may be done even if you do not enroll your child on this study. Your child is eligible for this study depending on the results of these tests. More information about bone marrow aspiration and lumbar puncture can be found in the COG Family Handbook for Children with Cancer.

Medical Tests and Evaluations: During the study
If the results of the diagnostic bone marrow aspiration and lumbar puncture show that your child can be in the study, and you choose to have your child take part, then your child will need the following tests and procedures. These medical tests are part of regular cancer care and would be given even if your child did not participate in this study. These tests include:

- Physical exams (including height, weight, body surface area, blood pressure, pulse, temperature, and respiration).
- Blood tests
- Tests of kidney function
- Chest X-rays
- Tests of heart function (Echo/EKG or MUGA).
- Tests of liver function.
- Tests of lung function.
- Immunophenotyping (tests on the cancer cells to determine the types of molecules that make up the cancer cells. These molecules are found on the outside of the cells).
- Histocytochemistry (tests on the chemicals and other materials inside the cells by means of staining reactions or other methods).
- Cytogenetic Tests (a portion of bone marrow will be used to study the genetic material within the cells [i.e. chromosomes]. The tests that study chromosomes and chromosomal changes are called cytogenetic tests. The results of these cytogenetic tests will be evaluated by members of the Children’s Oncology Group Cytogenetics Committee).
Bone marrow aspirations:
Subjects will have about ½ teaspoon of their bone marrow taken with a needle (aspirate). The test may be painful and has some small risk of infection or bleeding. The pain normally lessens within seconds to hours. In many cases, children will get medications by vein to numb the pain and blur the memory. Sometimes they may be given general anesthesia. These tests are necessary to see how well the therapy is working for your child.

Bone marrow aspirations will be done according to the following timetable:

<table>
<thead>
<tr>
<th>Study Entry</th>
<th>Day 14, Induction I*</th>
<th>Day 28 Induction I</th>
<th>Day 14 Induction II**</th>
<th>Day 28 Induction I**</th>
<th>Day 28 Induction IV</th>
<th>End of Therapy***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* If the Day 14 bone marrow test shows that you still have a high number of blasts present, and the non-blast cells in your bone marrow appear normal, you will not receive a Day 28 bone marrow test.

** If the bone marrow looks free of leukemic cells after Induction I, no further marrow will be collected during Induction II. If leukemic cells are present in the marrow after Induction I, then patients will have bone marrow aspirations at these times.

*** End of therapy is after Intensification II.

Attachment #2
Following are the risks and side effects related to the treatment drugs used in this study.

Risks and side effects related to asparaginase include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain at the injection site</td>
<td>Rashes</td>
<td>Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever</td>
</tr>
<tr>
<td>Some sort of allergic reaction such as a rash, hives or fever that may require pretreatment with antihistamines prior to the injection</td>
<td>Puffiness around the eyes</td>
<td>Convulsions</td>
</tr>
<tr>
<td>An increase in the level of ammonia that is found in the blood</td>
<td>Fluid retention</td>
<td>Coma</td>
</tr>
<tr>
<td>A decrease in levels of factors in the blood that help your blood to clot normally</td>
<td>High blood sugar which may require treatment</td>
<td>Disorders of blood clotting that can lead to bleeding or to excessive clotting in blood vessels including those that lead to the brain</td>
</tr>
<tr>
<td></td>
<td>Elevation in the blood of certain enzymes found in the liver</td>
<td>High levels of nitrogen in the blood which may indicate that the kidneys are not working as well as normal</td>
</tr>
<tr>
<td></td>
<td>High levels of uric acid in the blood which could damage the kidneys</td>
<td>Severe kidney damage</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>Inflammation of the pancreas which can cause severe abdominal pain</td>
</tr>
<tr>
<td></td>
<td>Loss of appetite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild nausea and/or vomiting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muscle aches and pains</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chills and fever</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A feeling of extreme tiredness, inability to stay awake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Changes in your mood such that you feel depressed, irritable, confused or have hallucinations (see or hear things that are not</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• An increase in the levels of lipids (fats) in the blood
• Shakiness or tremor which may cause jerky movements
• Pain in the abdomen
• Fewer white blood cells and platelets in the blood
  o a low number of white blood cells can make it easier to get infections
  o a low number of platelets causes you to bruise and bleed more easily

Risks and side effects related to cytarabine include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea and vomiting</td>
<td>Rash</td>
<td>Allergic reactions (can be severe and life-threatening causing difficulty in breathing and or a drop in blood pressure)</td>
</tr>
<tr>
<td>Hair loss</td>
<td>Severe rash with redness and pain on the palms of the hand and soles of the feet</td>
<td>A syndrome called Ara-C syndrome where there is fever, aches, pains, sometimes chest pain, a rash and inflammation of the eye</td>
</tr>
<tr>
<td>Mouth sores</td>
<td>Flu type symptoms with fever, tiredness, aches and pains</td>
<td>With higher doses of cytarabine there can be effects on the brain which can lead to headaches, incoordination of the muscles when walking, rapid jerky eye movements, difficulty with speech, sleepiness, personality changes, coma</td>
</tr>
<tr>
<td>Loss of desire to eat</td>
<td>Diarrhea</td>
<td>With higher doses of cytarabine there can be effects on the heart which can lead to chest pain and damage to the heart muscle</td>
</tr>
<tr>
<td>Fewer red and white blood cells and platelets in the blood</td>
<td>Low levels of certain salts in the body like potassium and calcium</td>
<td>Inflammation or damage to the liver which can be severe and life-threatening and which may lead to an enlarged liver and spleen,</td>
</tr>
</tbody>
</table>
  o a low number of red blood cells can make you feel tired and weak | Difficulty emptying the bladder |                                                                                   |
  o a low number of white blood cells can make it easier to get infections | High levels of uric acid in the blood which could damage the kidneys |                                                                                   |
  o a low number of platelets causes you to bruise and bleed more easily | With higher doses of cytarabine fluid may accumulate in the lungs making it difficult to breathe |                                                                                   |
bleeding from the veins in the esophagus (the passage that leads from the throat to the stomach), a yellow appearing skin, and fluid collection in the abdomen which makes it look larger.

- Kidney Damage
- Muscle breakdown which can lead to injury to the kidneys and other organs

### Risks and side effects related to intrathecal cytarabine include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Nausea and vomiting</td>
<td>Sleepiness</td>
<td>- Convulsions</td>
</tr>
<tr>
<td>- Fever</td>
<td>Inflammation of the lining of the brain that can lead to headache, numbness and tingling</td>
<td>- Partial paralysis</td>
</tr>
<tr>
<td>- Headache</td>
<td>Dizziness and incoordination of the muscles when walking</td>
<td>- Blindness</td>
</tr>
<tr>
<td></td>
<td>Fewer red and white blood cells and platelets in the blood</td>
<td>- Damage to the brain that may result in a decrease in the ability to learn</td>
</tr>
<tr>
<td></td>
<td>o a low number of red blood cells can make you feel tired and weak</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o a low number of white blood cells can make it easier to get infections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o a low number of platelets causes you to bruise and bleed more easily</td>
<td></td>
</tr>
</tbody>
</table>
Risks and side effects related to daunorubicin include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Nausea</td>
<td>• Inflammation and/or sores in the mouth, throat and/or esophagus</td>
<td>• Severe allergic reaction which can be life threatening with</td>
</tr>
<tr>
<td>• Vomiting</td>
<td>• Fever and chills</td>
<td>shortness of breath, low blood pressure and a rapid heart rate</td>
</tr>
<tr>
<td>• Temporary hair loss</td>
<td>• Diarrhea and/or abdominal pain</td>
<td>• An irregular heart beat which can be life-threatening</td>
</tr>
<tr>
<td>• Pink or red color to urine, sweat, tears, saliva</td>
<td>• Damage to the heart muscle which may not be noticeable or may make you feel tired, weak, feel short of breath, and retain fluid</td>
<td>• Severe damage to the heart muscle which may lead to severe heart failure</td>
</tr>
<tr>
<td>• Fewer white blood cells, red blood cells and platelets in the blood.</td>
<td>• High levels of uric acid in the blood which could damage the kidneys</td>
<td>• A new cancer or leukemia resulting from this treatment.</td>
</tr>
<tr>
<td>○ A low number of red blood cells can make you feel tired and weak</td>
<td>• Dark discoloration under the fingernails</td>
<td></td>
</tr>
<tr>
<td>○ A low number of white blood cells can make it easier to get infections</td>
<td>• Damage to the skin if the medication leaks from a vein</td>
<td></td>
</tr>
<tr>
<td>○ A low number of platelets causes you to bruise and bleed more easily</td>
<td>• Thickening and hardening of the veins through which the medication is given</td>
<td></td>
</tr>
<tr>
<td>• Inflammation and/or sores in the mouth, throat and/or esophagus</td>
<td>• Elevation in the blood of certain enzymes found in the liver which may mean liver irritation or damage</td>
<td></td>
</tr>
<tr>
<td>• Fever and chills</td>
<td>• Tearing and inflammation of the eyes</td>
<td></td>
</tr>
<tr>
<td>• Diarrhea and/or abdominal pain</td>
<td>• Rashes and itching of the skin</td>
<td></td>
</tr>
<tr>
<td>• Damage to the heart muscle which may not be noticeable or may make you feel tired, weak, feel short of breath, and retain fluid</td>
<td>• Loss of Appetite</td>
<td></td>
</tr>
<tr>
<td>• High levels of uric acid in the blood which could damage the kidneys</td>
<td>• Redness and burning at sites which have received radiation in the past</td>
<td></td>
</tr>
</tbody>
</table>

The risk of heart damage may be greater in very young children than in older ones.

Risks and side effects related to etoposide include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Nausea and vomiting</td>
<td>• Loss of appetite</td>
<td>• Damage to the liver</td>
</tr>
<tr>
<td>• Hair Loss</td>
<td>• Decreased blood pressure during the infusion which may require treatment</td>
<td>• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever</td>
</tr>
<tr>
<td>• A feeling of weakness or tiredness</td>
<td>• Rashes</td>
<td>• A new cancer or leukemia resulting from this treatment</td>
</tr>
<tr>
<td>• fewer red and white blood cells and platelets in the blood</td>
<td>• Diarrhea</td>
<td>• Severe rashes which can result in loss of skin and damage to mucous</td>
</tr>
<tr>
<td>○ a low number of red blood cells can make you feel tired and weak</td>
<td>• Pain in the abdomen</td>
<td></td>
</tr>
<tr>
<td>○ a low number of white blood cells can</td>
<td>• Mouth sores</td>
<td></td>
</tr>
<tr>
<td>• Inflammation and/or sores in the mouth, throat and/or esophagus</td>
<td>• Tingling sensation or loss of sensation in fingers or toes</td>
<td></td>
</tr>
<tr>
<td>• Fever and chills</td>
<td>• A feeling of extreme</td>
<td></td>
</tr>
</tbody>
</table>
make it easier to get infections
  - A low number of platelets causes you to bruise and bleed more easily

tiredness or weakness
  - The finger or toe nails may loosen from their nail beds
  - Inflammation of the vein through which the medication was given
  - Chest pain

membranes
  - Absence or decrease of monthly periods which may be temporary or permanent and which may decrease the ability to have children
  - Damage to the heart muscle which may make you feel tired, weak, feel short of breath, and retain fluid

Risks and side effects related to thioguanine include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare But Serious</th>
</tr>
</thead>
</table>
| • Fewer white blood cells, red blood cells and platelets in the blood.  
  - A low number of red blood cells can make you feel tired and weak  
  - A low number of white blood cells can make it easier to get infections  
  - A low number of platelets cause you to bruise and bleed more easily | • Loss of appetite  
• Nausea  
• Vomiting  
• Diarrhea  
• Inflammation and/or sores in the mouth  
• Unsteadiness when walking  
• High levels of uric acid in the blood which could damage the kidneys  
• Rash or hives  
• Elevation in the blood of certain enzymes or bilirubin found in the liver  
• A feeling of extreme tiredness, weakness or not feeling well | • The rapid death of large numbers of tumor cells which can cause the potassium and phosphate salts and the uric acid in the blood to rise quickly and this could lead to a life-threatening irregular heart beat or damage to the kidneys.  
• Inflammation or damage to the liver which can be severe and life-threatening and which may lead to an enlarged liver and spleen, bleeding from the veins in the esophagus (the passage that leads from the throat to the stomach), a yellow appearing skin, and fluid collection in the abdomen which makes it look larger. |
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 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.