Activated: 11/23/2015
Closed: 04/15/2022
Version Date: 09-18-2020
Amendment: 6

CHILDREN'S ONCOLOGY GROUP

AAML1531

Risk-stratified Therapy for Acute Myeloid Leukemia in Down Syndrome

A COG Groupwide Phase 3 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.*

CONTACT INFORMATION		
For Regulatory Requirements	**For patient enrollments:**	**For Data Submission**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. (Sign in at www.ctsu.org, and select the Regulatory > Regulatory Submission.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN). OPEN is accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions by phone or email: 1-888-823-5923, or ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the Data Submission Schedule in the CRF packet for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific page located on the CTSU members' website (https://www.ctsu.org). Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires log in with a CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU Regulatory Support System (RSS).		
For clinical questions (i.e. patient eligibility or treatment-related) Contact the Study PI of the Lead Protocol Organization.		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission) Contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Website is located at https://www.ctsu.org.		

TABLE OF CONTENTS

[redacted]

[redacted]

[redacted]

AGENT	NSC#	IND#
Cytarabine	# 063878	Commercial
Daunorubicin	# 82151	Commercial
Asparaginase (Erwinia)	#106977	Commercial
Etoposide	# 141540	Commercial
Asparaginase (E.coli)	# 109229	Commercial
Mitoxantrone	# 301739	Commercial
Thioguanine	# 000752	Commercial

SEE SECTIONS 13 and 14 FOR SPECIMEN SHIPPING ADDRESSES.

ABSTRACT

Acute Myeloid Leukemia in children with Down syndrome (DS) is a distinct form of myeloid leukemia (DS AML) that is characterized by a young age of onset (< 4 years of age), a frequently megakaryoblastic blast morphology and immunophenotype, a common prodrome of myelodysplastic syndrome, and a high probability of event-free survival (EFS) for the majority of children compared to AML in children without DS. DS AML blasts typically contain somatic mutations of the gene encoding the hematopoietic transcription factor *GATA1* and are hypersensitive to selected chemotherapeutic agents such as cytarabine, which has historically been used at high doses for the treatment of AML. Recent studies, therefore, have pursued reduced treatment intensity for children with DS AML to reduce the morbidity associated with its treatment.

COG study A2971 used therapy based on CCG 2891 but eliminated dexamethasone as well as etoposide and, despite this reduction in treatment, maintained favorable survival outcomes (5-year EFS 79%). AAML0431, building on A2971, focused on the reduction of the cumulative dose of daunorubicin (by 25%) and of intrathecal chemotherapy (from 7 to 2 doses) and achieved a 3-year EFS of 90%. These results demonstrate that the 85-90% of children with DS AML have a highly favorable prognosis and may therefore benefit from further reduction of treatment intensity. Therefore, given the hypersensitivity of DS AML blasts to cytarabine and the profound neutropenia and infections associated with its use, the elimination of high-dose cytarabine therapy (HD Ara-C) is the next logical step in reducing treatment intensity for the majority of children with DS and AML. As described below, children with DS AML will be stratified based on the level of MRD at the end of induction to receive reduced intensity therapy versus standard therapy. Using this approach, we aim to maintain the overall excellent outcome for children with DS AML, yet reduce morbidity in the 85-90% with a favorable prognosis. AAML0431, the level of minimal residual disease (MRD) measured by multi-parameter flow cytometry in the bone marrow at the end of this first cycle of induction therapy, predicted outcome. Therefore, as described below, MRD will be used to stratify patients with DS AML to reduced intensity versus standard therapy.

All patients will first receive a common cycle of induction therapy that includes a continuous infusion of standard-dose cytarabine, bolus infusion of daunorubicin and oral 6-thioguanine (TAD). Following interim analysis, patients in the standard risk group (MRD ≤ 0.05% at the end of Induction I) who received reduced intensity therapy consisting of two additional induction cycles of TAD and two cycles of intensification therapy with standard-dose cytarabine and etoposide with the elimination of HD Ara-C were found to have an inferior 2 year event-free survival (EFS) compared to patients who received HD Ara-C on AAML0431. Thus, following amendment 4A, the standard risk arm of this study is permanently closed. Patients enrolled after amendment 4A who are assigned to the standard risk arm after Induction I will be removed from study.

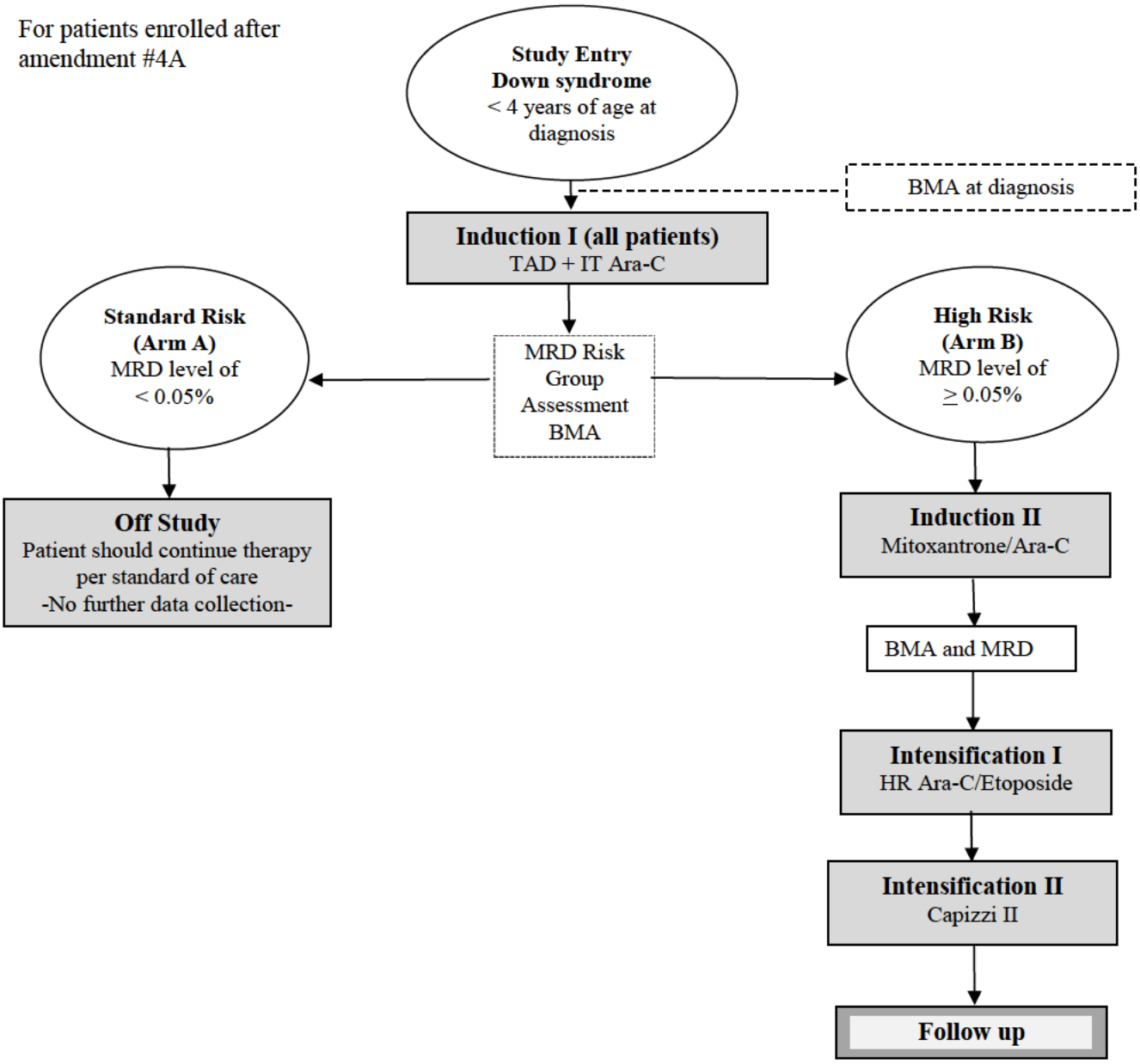
Patients in the high risk group (MRD ≥ 0.05% at the end of Induction I) will remain on protocol therapy and be treated with intensified therapy consisting of cycles of mitoxantrone and cytarabine (MA), etoposide and cytarabine (AE) and Capizzi II (HD Ara-C and asparaginase) as used for high risk AML in children without DS.

The exploratory aims of this study include the analysis of a comparison of different methods to detect MRD in DS AML (flow cytometry, PCR-based and sequencing-based detection of clone-specific *GATA1* mutations) with regard to feasibility and analytical characteristics. Finally, a bank of viably cryopreserved DS AML bone marrow samples collected at end of induction and relapse, and corresponding non-tumor DNA collected at end of Induction I, will be established.

In sum, study AAML1531 will tailor the intensity of AML treatment for children with DS (under 4 years of age) to the risk of relapse as measured by MRD after the first cycle of treatment. The study aims to maintain the excellent survival outcome, to reduce toxicity of therapy for the majority of patients with favorable disease and to improve survival for the minority of high risk patients who may benefit from therapy intensification.

EXPERIMENTAL DESIGN SCHEMA

For patients enrolled after amendment #4A



BMA = Bone Marrow Aspirate. MRD = Minimal Residual Disease

Induction I (All patients)
TAD (continuous infusion Cytarabine (Ara-C) ; short infusion Daunorubicin ; oral 6-Thioguanine) and intrathecal Cytarabine

STANDARD ARM (Arm A)
Patients will be removed from study.

HIGH RISK ARM (Arm B)
Induction II: Mitoxantrone/ Cytarabine (Ara-C)
Intensification I: High dose Cytarabine (Ara-C)/Etoposide
Intensification II: Capizzi II (High Dose Cytarabine (Ara-C)/Asparaginase)

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To determine the 2-year event-free-survival (EFS) for children with standard risk DS AML (MRD-negative after one cycle of induction therapy) after elimination of HD Ara-C from the treatment regimen.

1.1.2 To determine the 2-year EFS for children with high risk DS AML (MRD-positive after one cycle of induction therapy) after intensification of treatment equivalent to that used for high risk AML in children without DS.

1.2 Exploratory Aims

1.2.1 To compare the feasibility and analytical characteristics of flow cytometry, PCR and targeted error-corrected sequencing of *GATA1* mutations as methods to detect MRD in DS AML.

1.2.2 To establish a DS AML cell bank of viably frozen bone marrow samples collected at the end of induction and corresponding non-tumor DNA samples collected at end of Induction 1.

2.0 BACKGROUND

2.1 Outcomes of AML in Children with Down Syndrome

During the early 1990s, it was recognized that children with Down syndrome (DS) and AML (DS AML) have a significantly higher EFS and lower relapse rate compared to children without DS. The landmark study by the Pediatric Oncology Group POG 8498 demonstrated both the feasibility of clinical trials in children with DS AML and unexpectedly superior outcomes (EFS 100% at 4 years).[1] DS AML is now recognized as a clinically and biologically distinct form of leukemia. It is characterized by a 150-fold increased incidence,[2] younger age of onset (under 4 years of age)[2,3], predominant megakaryoblastic blast phenotype, high prevalence of antecedent myelodysplastic syndrome[4] and significantly increased sensitivity to cytotoxic drugs such cytarabine and anthracyclines (see Section 2.3). Somatic mutations of the gene encoding the hematopoietic transcription factor *GATA1* were identified as a specific disease mechanism underlying DS AML[5-7] as well as the observed hypersensitivity of DS AML blasts to selected chemotherapeutic agents.[8] This biologically distinct character of DS AML is further supported by the observation that DS AML evolves from a fetal accumulation of megakaryoblasts - termed transient myeloproliferative disorder (TMD), transient leukemia or transient abnormal myelopoiesis. TMD is detectable in 10-30% of DS newborns and spontaneously resolves in the vast majority of cases. In approximately 20% of cases, TMD is followed within the first 4 years of life by the onset of DS AML, which typically harbors a concordant, clone-specific mutation of *GATA1*.[9]

Based on these observations, the extrapolation to children with DS of AML treatment approaches that were optimized in the overall pediatric population has been replaced by the design of specific treatment protocols for DS AML.[10-12] In view of the excellent survival of patients with DS AML, early attempts at reducing the intensity of treatment were initiated and proved successful.[10-12] However, despite the hypersensitivity of DS AML blasts to cytarabine, the use of HD Ara-C continues to be carried over from the treatment of AML in the general population to treatment protocols for DS AML.[1,3,4,13-15] The excellent survival of children with DS AML, now approaching 90%, suggests the feasibility of reducing the intensity of treatment for the majority of patients. In contrast, a small proportion of patients with DS AML do not benefit from current treatment approaches and experience poor survival due to refractory or relapsed disease.[16-18] For this small, but important group, intensification of treatment may be necessary.

2.2 Rationale for Study Design - Introduction of Risk-Adapted Therapy for DS AML

In contrast to other contemporary treatment approaches to pediatric leukemia, current protocols for DS AML lack a risk-adapted stratification of treatment intensity. As a result, cycles of high-dose chemotherapy such as HD Ara-C continue to be administered to all patients with DS AML.

The majority of patients with DS AML have prognostically very favorable disease and do not benefit from high-intensity chemotherapy. Maximizing dose intensity has proved a successful strategy for the treatment of AML in the general pediatric population.[4,19] In contrast, a highly dose-intense AML chemotherapy protocol resulted in a 32% induction death rate and decreased EFS in children with DS.[4] Given both the decreased survival of children with DS treated with intensive AML therapy protocols, which were optimized in the non-DS pediatric population, and the hypersensitivity of DS AML blasts to cytarabine and anthracyclines, COG has pursued the rational development of treatment protocols

specific for DS AML. Initial efforts successfully reduced anthracycline exposure while maintaining high EFS. Prompted by the hypersensitivity of DS AML blasts to cytarabine, we hypothesize that use of HD Ara-C, a treatment element which was developed for AML in the overall pediatric population but has remained part of treatment protocols for DS AML, substantially contributes to adverse events of AML therapy in children with DS without improving EFS for these patients.

Therefore, we propose to eliminate HD Ara-C from the treatment of AML in the majority of children with DS who have standard risk disease. To guard against any decrease of the current favorable EFS, the proposed reduction of treatment intensity will be limited to children with DS AML lacking known risk factors for an adverse outcome (DS AML standard risk group, SR). Age under 4 years and negative minimal residual disease (MRD) in the bone marrow as determined by multi-parameter flow cytometry at the end of the first induction cycle identified a prognostically highly favorable subset of DS AML patients in study AAML0431.[20] Approximately 85% of DS- AML patients are expected to fall into this SR group and to have a highly favorable outcome.[10-12] For these patients, treatment intensity will be reduced by discontinuing the administration of HD Ara-C (See Table 1 below).

At the same time, treatment studies consistently identify a small subset of patients who do not achieve long term survival with current treatment protocols for DS AML due to relapse or refractory disease.[3,11,16-18,21] Survival of patients with relapsed or refractory DS AML is poor even following treatment with hematopoietic stem cell transplantation (HCT; EFS 26%[17]; overall survival, OS, 21%).[16] Subsequent relapse rather than toxicity of HCT was a major barrier to successful outcomes.[16,18] This suggests treatment intensification in this group may provide a benefit of improved disease control that counterbalances the risk associated with intensive treatment. Early response to treatment (as measured by MRD in the bone marrow after the first cycle of induction therapy) will be used to identify this high risk group.

In sum, patients will be stratified according to relapse risk into one of two arms. Arm A will include patients with standard risk DS AML (approximately 85% of all patients) and administer treatment of reduced intensity (without HD Ara-C) compared to predecessor study AAML0431. Arm B will include the approximately 15% of patients with high risk DS AML who will receive intensified treatment compared to predecessor study AAML0431 (equivalent to the high risk arm of AAML1031).

Outcomes of this study will be compared to AAML0431 from the start of Induction II since both studies include the same treatment during Induction I.

Table 1: Comparison of Cumulative Drug Doses and Number of Treatment Cycles in study AAML1531 with predecessor study AAML0431

	AAML0431	AAML1531 Arm A Standard Risk	AAML1531 Arm B High Risk
Ara-C (g/m^2)	**27.8**	**3.8**	**40**
Daunorubicin equivalents (mg/m^2)	**240**	**240**	**224***
Etoposide (mg/m^2)	**750**	**750**	**750**
Total number of cycles (IT doses)	**6(2)**	**5(1)**	**4(1)**

*Based on a 3:1 conversion of mitoxantrone to daunorubicin equivalents

2.3 Biological Rationale for a Reduction of Treatment Intensity in DS AML: Hypersensitivity of DS AML Blasts to Cytarabine

Compared to non-DS AML, the leukemic blasts of DS AML are 4.5-fold and 12-fold more sensitive *in vitro* to cytarabine (median IC50 77.5 vs. 350.9 nM) and daunorubicin (median IC50 5.8 vs. 71.2 nM), respectively.[22,23] This increased sensitivity of DS AML blasts to cytarabine is explained by a 5.2-fold greater accumulation of the active drug metabolite Ara-CTP compared to non-DS AML blasts.[24] In addition to the modulation of drug metabolism by constitutionally expressed genes encoded on chromosome 21, such as cystathionine-ß-synthase (CBS),[23] mutant *GATA1* protein itself enhances sensitivity to cytarabine.[25,26] These observations of increased sensitivity of DS AML blasts to cytotoxic drugs *in vitro* are complemented by pioneering clinical studies in which very low doses of cytarabine administered over a prolonged period of time (10 mg/m^2 s.c. q12 hours for 7 days every two weeks for 2 years),[27,28] resulted in durable remissions in as many as 67% of cases (intention to treat analysis).[27] Although outcomes of the very low dose cytarabine regimen eventually did not match those of standard dose cytarabine,[28] they highlight the unique hypersensitivity of DS AML to cytarabine, which is not encountered in pediatric non-DS AML. In 2007, Japanese investigators showed that HD Ara-C could successfully be eliminated from a treatment protocol for DS AML without compromising EFS (4 year EFS 83.3%).[11]

2.4 Clinical Rationale for a Reduction of Treatment Intensity in DS AML: Adverse Events after HD Ara-C in Predecessor Study AAML0431

The largest number of adverse events, which fortunately did not include Grade 5 events, occurred during Induction cycle II of the recently completed treatment study AAML0431. This cycle included administration of HD Ara-C (Capizzi II) and with a median duration of 42 days (Table 2) was the longest of all 6 cycles of therapy (Induction I, 34 days; Induction III, 32 days; Induction IV, 34 days; Intensification I, 35 days; Intensification II, 35 days). This cycle was also associated with the highest number of adverse events (27.5%

of the total reported; 65% classified as Grade 3 or greater, 6.5% as Grade 4)[20] and the highest incidence of Grade 3 or higher febrile neutropenia and bacterial infections at sterile sites (See Table 2 below). In addition to this direct health-related burden on these vulnerable patients, the administration of the HD Ara-C-containing cycle Induction II resulted in substantial resource utilization that is consistent with the observation that sepsis is the single most important factor determining length of stay and hospital costs in the treatment of children with leukemia.[29]

Table 2: Adverse events per cycle in predecessor study AAML0431

Cycle	Median number of days on cycle (range)	Time to ANC recovery (median, days)	ICU admission (% of patients)	Febrile neutropenia Grade ≥3 (% patients)	Sterile site bacterial infection Grade > 3 (% patients)
Induction I TAD	34 (15-66)	30	7	27	19.2
Induction II HD Ara-C	42 (27-69)	37	7	29.7	22.6
Induction III TAD	32 (20-59)	28	2	6.2	11.3
Induction IV TAD	34 (22-63)	28	2	5.6	8.8
Intensification I Ara-C/Etoposide	35 (27-58)	33	4	11.5	12.7
Intensification II Ara-C/Etoposide	35 (22-124)	32	1	12.4	8.4

2.5 Safety and Feasibility of Lower Intensity Treatment Protocols for Standard Risk DS AML

A series of observations supports the feasibility of reducing treatment intensity for the majority of patients with DS AML. Chemotherapy of standard intensity resulted in superior survival for children with DS compared to the general pediatric population.[1] In contrast to patients with non-DS AML, DS AML patients enrolled in study CCG 2891 had superior outcomes with standard timing of induction elements (DCTER-DCTER) compared with intensive timing.[4] Study A2971, the first DS-specific AML trial conducted by COG and legacy groups, used CCG 2891-based therapy (with standard timing of DCTER-DCTER induction) but eliminated dexamethasone and etoposide. Despite this reduction, outcomes remained comparable (A2971: OS 84±6%, EFS 79±7%, disease-free survival, DFS 89±6%; CCG 2891: OS 79±7%, EFS 77+7%, DFS 85±6%). Finally, Japanese investigators using pirarubicin (25 mg/m^2/d for 2 days), cytarabine (100 mg/m^2/d for 7 days), and etoposide (150 mg/m^2/d for 3 days achieved an excellent survival outcome (4 year EFS 83.3%)[11] using a treatment protocol for DS AML that did not contain cycles of HD Ara-C. Based on these observations, HD Ara-C will be eliminated in study AAML1531 from the treatment of patients with standard risk DS AML (approximately 85%).

The administration of daunorubicin as a continuous infusion (over 96 hours) was successfully used in both predecessor studies of DS AML, A2971 (n = 132)[10] and COG AAML0431 (n = 205).[20] There have been no reports of excessive mucosal toxicity, adverse events related to drug administration or significant acute cardiac toxicity. At the same time,

continuous infusion of anthracyclines could not be shown to provide a benefit of lower cardiotoxicity compared to bolus infusion.[30-33] Therefore, in study AAML1531 daunorubicin will be administered as short minute infusion, rather than the previously used continuous infusion over 96 hours. The cumulative dose of anthracycline in the standard risk group (240 mg/m^2) remains identical to that of AAML0431, while patients in the high risk group will receive 272 mg/m^2. The use of dexrazoxane will be allowed in the current study as per institutional preference (refer to Section 4.1.7 for additional information).

2.6 Poor Outcome of Patients with Relapsed/Refractory DS AML

While the majority of DS AML patients (approx. 85%) have a highly favorable outcome across a range of treatment regimens developed by the COG, Berlin-Frankfurt-Münster (BFM), and Japanese study groups,[10-12] a lack of response to DS-specific AML therapy is associated with a low probability of long-term survival. Three-year OS was 25.9% among 29 children with relapsed and refractory DS AML.[17] Allogeneic HCT was successful only in 2 of 8 recipients.[17] Similar outcomes were reported by a registry-based study with a 3-year OS of 21% among 21 children with DS AML undergoing HCT.[16] Of note, subsequent relapse (cumulative incidence of relapse, CIR, 62%) rather than treatment-related toxicity (CI TRM 25%) was the predominant cause of treatment failure.[16] These observations support the clinical study of an intensification of treatment compared to current DS AML-specific protocols for the high risk group of DS AML.

2.7 Justification and Safety of Intensified Treatment for High Risk DS AML

Several study groups have gained experience with more intensive therapy for DS AML as proposed by this study for the 10-15% of patients in the high risk group. BFM study AML-BFM 93 used mitoxantrone and Ara-C (HAM, Ara-C 3g/m^2 q12h on day 1-3; mitoxantrone 10 mg/m^2 on day 4, 5) for the treatment of DS AML and reported five early deaths among 51 children with DS AML, one prior to start of chemotherapy, two during induction, one after HAM and one on day 40 due to intracranial hemorrhage.[12]

Intensification of treatment using the proposed cycles AE and MA was also used for older patients with DS AML (> 4 years of age) on AAML0531. Of 6 eligible patients, one patient without reported adverse events during the first two cycles subsequently relapsed and went off study. A total of 4 patients received AE (Intensification I) without Grade 4 or 5 adverse events. During the subsequent MA cycle, one each among these 4 patients developed Grade 4 infection/Grade 3 hypotension, Grade 3 infection/Grade 3 mucositis and Grade 3 infection and one had no reported adverse events (Alan Gamis, Todd Alonzo, personal communication). One patient subsequently died after the fifth cycle of therapy (Intensification III/Capizzi II HD Ara-C) due to colitis and systemic inflammatory response syndrome in the setting of relapse and pancytopenia.

Administration of reduced doses of mitoxantrone and cytarabine (hAM; Ara-C 1g/m^2 q12 on Day 1-3 and mitoxantrone 7 mg/m^2 on Day 3, 4) is being used in study BFM DS-ML 2006 by the BFM study group for all patients with DS AML. This treatment element, however, is equivalent in intensity to predecessor study AAML0431 (cumulative drug doses BFM DS-ML 2006 v. AAML0431: Cytarabine 27.4 v. 27.8 g/m^2; doxorubicin equivalents 226 v. 240 mg/m^2; etoposide 450 v. 740 mg /m^2) and, therefore, appears more appropriate for patients with standard risk DS AML rather than the group of high risk patients selected for an intensified approach.

Based on these reports, MA as currently used for high risk non-DS AML in study AAML1031, will be used for intensification of treatment for DS-AML. The intensive therapy proposed for the high risk group of DS AML in this study is expected to be associated with increased morbidity, but not with excessive mortality. Given the overall poor prognosis of relapsed DS AML, the risks associated with intensification of treatment for high risk DS AML are expected to be balanced by the benefit of improved disease control. Since intermediate steps short of introduction of MA (and AE) will not achieve the intended net intensification of treatment for high risk DS AML, we will adopt cycles MA (Ara-C 1 g/m^2 q12 h on day 1-4, mitoxantrone 12 mg/m^2 on day 3-6) and AE (Ara-C 1g/m^2 q12h on day 1-5, etoposide 150mg/m^2 on day 1-5) from the treatment protocol for high risk non-DS AML, which was associated with a 22.5% rate of febrile neutropenia and 1.6% rate of non-leukemic death in study AAML0531 in children without DS[34] and retain a cycle of Capizzi II from the treatment of DS AML used in predecessor study AAML0431, which was associated with a 29.7% rate of > Grade 3 febrile neutropenia (see Section 2.4, Table 2) but no treatment-related mortality.[35]

To guard against excessive toxicity, the proposed approach will take advantage of the experience of studies CCG 2891, NOPHO-93[36] and BFM DS-ML 2006 and allow recovery from myelosuppression prior to and after cycles including HD Ara-C and mitoxantrone. Monitoring rules for excessive toxicity and an interim safety analysis after the first 6 patients on the high risk arm have completed treatment with MA will be included to meet the objective of safety while pursuing adequate intensification of treatment for high risk disease.

The favorable outcomes of DS AML (EFS 80% or greater) have been achieved by different study groups with a variety of treatment approaches. Studies by COG and legacy groups, for example, have used 6 cycles in A2971 and AAML0431. A Japanese study group[11] achieved similar results with only 5 cycles. Interim data for the ongoing DS AML study DS-ML 2006 by the BFM study group presented at the COG Fall Meeting in 2014 showed an OS of 90% with treatment using 4 cycles of chemotherapy (D. Reinhardt, pers. communication). Outcomes appear to be a function of treatment intensity (in the context of increased sensitivity of DS AML blasts to cytarabine, anthracyclines, and etoposide) rather than of the number of treatment cycles. The same conclusion appears to follow from the successful reduction of treatment cycles for non-DS AML from 5 in AAML0531 to 4 on AAML1031 in keeping with the approach of the MRC in the UK.[37] Thus, on AAML1531, standard risk DS AML patients will receive 5 cycles of less intensive therapy and high risk DS AML patients will receive 4 cycles of more intensive therapy.

2.8 Impact of This Study on Trial Design for DS AML

Children with DS AML represent an ideal group to pursue a strategy of dose reduction, particularly of cytarabine, based on a strong biological, pharmacological rationale and feasibility. Two previous COG studies initiated this effort. A2971 broke ground by using induction therapy cycles of lower intensity (TAD) and AAML0431 reduced the dose of anthracycline (to 240 mg/m^2), eliminated CNS-directed consolidation therapy and collected first data on the prognostic value of MRD in DS AML in a blinded fashion. Both studies, however, retained cycles of HD Ara-C, which originate from the optimization of AML treatment approaches in the general pediatric population and continue to be administered to children with DS AML. The hypothesis that HD Ara-C is not required for the majority of patients with DS AML to maintain the high survival but contributes to avoidable infectious toxicity, prolonged hospitalization and increased resource utilization

needs to be tested. AAML0431 retrospectively demonstrated the prognostic value of MRD in DS AML. AAML1531 now introduces the prospective use of MRD for the stratification of treatment intensity for DS AML according to the risk for relapse.

In summary, the proposed study AAML1531 builds on the success of the initial steps taken by two previous COG studies, A2971 and AAML0431; implements MRD-based risk stratification of DS AML; and by eliminating HD Ara-C advances AML therapy for children with DS in the direction of treatment reduction for the majority of patients. In addition, for the first time, this protocol provides an intensified therapeutic strategy targeted to high risk patients with DS AML, for whom there are currently neither specific treatment studies nor treatment recommendations. Thus, AAML1531 achieves risk-adapted stratification of treatment for DS AML as a rational and necessary next step of trial design for this vulnerable population.

2.9 Interim Analysis of Patients Treated on the Standard Risk Arm

According to the study analysis plan, an interim analysis of treatment outcomes for lack of efficacy on the Standard Risk (SR) arm (Arm A) was performed after 50% of the expected number of EFS events had been observed (after 8 of 16 events). Based on a model generated using data of SR patients in the historical control (predecessor study AAML0431) and the amount of follow-up available at the time of data cut off (30 June 2018), 2.4 EFS events were expected in SR patients compared to 8 EFS events observed. The projected 2-year EFS for SR patients on AAML1531 is significantly lower than on AAML0431 (85.6% compared to 93.5%, p 0.0002). As of October 5, 2018, 9 of 109 SR patients on AAML1531 have experienced a relapse at 136 to 327 days after study entry. Four of these relapses occurred on therapy (1 during Intensification I, 3 during Intensification II) and 5 during the first 6 months of follow up after completion of protocol therapy. Of the 9 relapsed SR patients, 8 have died.

These data show lack of sufficient efficacy of AAML1531 protocol therapy for SR patients. Based on this information, post-Induction I treatment on the SR Arm A of AAML1531 was permanently closed.

2.10 Risk Stratification in DS AML

2.10.1 Prognostic Value of Minimal Residual Disease in Pediatric AML

Early response to treatment as assessed by flow cytometric measurement of MRD in the bone marrow after induction therapy has been shown to be an independent and powerful prognostic factor in pediatric leukemia. In contemporary trials for pediatric acute lymphoblastic leukemia for example, MRD has become an indispensable part of risk stratification.[38] Similarly, MRD has been found to predict relapse in pediatric non-DS AML.[39,40] Particularly in the large subset of intermediate risk AML, MRD allowed the distinction of standard and high risk groups whereas the use of morphology alone proved insufficient[41] (and S. Meshinchi, personal communication). The addition of flow cytometric MRD measurement to standard morphological bone marrow evaluation of early treatment response improved prognostication in study CCG 2961. The 3-year OS of patients in morphological remission after Induction I was 41 ± 26% if MRD was positive (> 0.1%), compared to 69 ± 10% for those who were MRD-negative.[42] A strong prognostic impact of MRD was also apparent in the recent study AAML03P1. Among patients in morphological remission at the end of Induction I, 46 (24%) of 188 were MRD-positive and experienced an increased relapse risk (RR) at 3 years

(60 ± 16%, P < 0.001) and lower OS (56 ± 16%, P = 0.002) compared with those who were MRD-negative (RR 29 ± 8%; OS 80 ± 8%).[41] This study not only demonstrated the prognostic value of MRD in non-DS AML over morphology alone but also showed that 30% of patients deemed not to be in morphological remission were in fact MRD-negative and had a good outcome.[41] MRD was analyzed retrospectively in the recently closed successor study AAML0531. The positive predictive value of MRD for relapse was 52.5% and the negative predictive value was 86.2% demonstrating that MRD is an important prognostic marker in pediatric AML.[34]

2.10.2 Prognostic Value of Early Response to Treatment in DS AML

2.10.2.1 Evaluation of early response of DS AML to treatment by morphology

A2971 indicated a prognostic role for early response to induction chemotherapy. Patients with < 5% blasts in the bone marrow (by morphology) on day 14 of induction therapy (n = 77) showed a trend to increased 5-year DFS compared to those (n = 27) with > 5% blasts (86% vs. 72%, P = 0.12).[43] Both 5-year EFS and DFS were significantly higher for patients in morphological remission at the end of Induction I. The morphological blast percentage proved prognostic. EFS was 88 ± 9% for patients with 0-1% blasts (n = 53) compared with 73 ± 11% for those with > 1% blasts (n = 63; P = 0.032). Similarly, patients with 0-5% blasts (n = 107) at the end of Induction I had an EFS of 83 ± 7% compared to only 44 ± 33% (P < .001) for those with > 5% blasts (n = 9). DFS was 90 ± 9% for patients with 0-1% blasts (n = 52) vs. 73 ± 11% for those with > 1% blasts (n = 63, P = 0.015). The corresponding DFS using 5% blasts at the end of induction as a cutoff was 83 ± 7% (0-5% blasts, n = 106) and 44% ± 33% (> 5% blasts, n = 9; P < 0.001, T. Alonzo, personal communication). These data suggest that, as in non-DS AML, early response to therapy is prognostic in DS AML, although morphology alone may not be sufficiently sensitive.

2.10.2.2 Evaluation of Early Response of DS AML to Treatment by MRD

Data collected from 146 patients in the recently completed study AAML0431 provide direct evidence for the prognostic value of MRD measured by flow cytometry after Induction I in DS AML. The unique immunophenotype of DS AML blasts permitted their detection by multi-parameter flow cytometry at a level as low as 0.01% (D. Campana, personal communication). EFS among 125 patients who were MRD-negative (MRD < 0.05%) was significantly higher than in 21 patients (14%) who were MRD-positive (93% v. 76% at 2 years; log-rank $P = .007$). This difference extended to OS (2-yr OS 96% v. 76%, log-rank $P < 0.001$), indicating that the prognostically unfavorable group of DS AML patients can be identified by MRD and that this cohort may benefit from intensification of therapy. Complying with the FDA requirement that the MRD cut-off be above the threshold of sensitivity of the assay, AAML1531 will use a cutoff of 0.05%. Based on the data of AAML0431, this cut-off safely captures the large majority of MRD-negative patients for the proposed reduction in therapy (only 5 patients had MRD levels between 0.01 and 0.1%, with 2 of these patients relapsing, 1 electively

withdrawing at the end of induction, and 2 patients remaining in remission).

While MRD measured in the bone marrow by flow cytometry at the end of Induction I is prognostic and provides superior detection of persistent disease compared to morphology alone, this approach does have limitations. On AAML0431, 14 patients relapsed and of the 12 patients for whom MRD data was available, only 7/12 (58%) were MRD positive at the end of Induction I, suggesting that MRD by flow cytometry is unable to detect all high risk patients. The reasons for this may be related to the flow cytometry method used to identify residual clones, namely the leukemia-associated immunophenotype (LAIP) at diagnosis, which is susceptible to immunophenotypic gains or losses in the major clone as well as the emergence of initially undetected minor clones.[41,44] A newer flow cytometry methodology based on the detection of "different from normal", which is expected to have improved accuracy and sensitivity, will be employed in the current study. The same methodology is currently used on AAML1031 to detect MRD in non-DS AML. In addition, the proposed study will evaluate alternative DNA-based methods to measure MRD such as digital PCR and targeted sequencing. The presence of clone-specific somatic mutations of the *GATA1* gene in DS AML blasts provides a particularly suitable target for the detection and quantification of MRD for response assessment in clinical trials. Currently there is no validated PCR or sequencing assay that could serve as the gold standard for the quantitative detection of cells harboring clone-specific *GATA1* mutations. The value of these molecular methods to detect MRD in DS AML will be determined in Exploratory Aim 1.2.2.

2.10.3 Age

Age at diagnosis was prognostic in a multivariate analysis of outcomes for 161 DS AML patients treated on CCG 2891 with standard timed induction. Age 2 years or older was associated with an odds ratio of 4.9 ($P = 0.006$) for relapse. Children older than 4 years (n = 9), in particular, had a significantly lower 6-year-EFS of 33 ± 31%.[3] This finding was confirmed by study A2971, in which patients younger than 4 years of age has a significantly higher 5-year-EFS than older children (81 ± 7% v. 33 ± 38%) although there were only six children older than 4 years of age.[10] Consistent with a prognostic impact of age in DS AML, a relapse rate of 40% was observed in a cohort of 10 children with DS AML 4 years of age or older at diagnosis.[45] In addition, blasts in 8 of these 10 older children with DS and AML lacked a *GATA1* mutation. Taken together, the poor outcome and lower prevalence of somatic *GATA1* mutations suggest that older children have a disease more in keeping with pediatric non-DS AML. Children with DS and AML aged 4 years and older, therefore, are not eligible for enrollment on the current study.

2.10.4 Blast Cytogenetics

The impact of cytogenetic abnormalities on outcome in DS AML is less well defined than in non-DS AML. Monosomy 7, an accepted marker of high risk pediatric and adult AML in the absence of DS, also occurs in DS AML (3.5 - 10%).[3,11,46,47] An international retrospective study of 451 DS AML patients found monosomy 7 was associated with a moderately worse outcome (7-year EFS 67%),

but this was not statistically significant.[47] Combining data reported for CCG 2891 and A2971 with a Japanese cohort,[11] only 11 of 16 patients (69%) with DS AML and monosomy 7 were in continuous remission. These data suggests a potentially inferior outcome for patients with DS AML and monosomy 7 compared with overall outcomes for DS AML after treatment with CCG-based (EFS 79%)[10] and MRC-based protocols (DFS 83%).[21] However, as these data are limited and involve a very small number of patients, DS AML patients enrolled on AAML1531, whose blasts are found to have monosomy 7 will not be assigned to the high risk arm based on this cytogenetic finding alone. Instead they will be stratified like patients without this cytogenetic abnormality according to the MRD-based assessment of their early response to treatment.

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the Patient Registry module in Open 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. For additional help or information, please contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

In order for an institution to maintain COG membership requirements, every patient with a known or suspected neoplasm needs to be offered participation in APEC14B1, *Project:EveryChild A Registry, Eligibility Screening, Biology and Outcome Study*. At this time, however, enrollment onto APEC14B1 is NOT a prerequisite to enrollment on AAML1531.

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

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

3.1.2 IRB Approval

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. For CTEP and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases after March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB). In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating with the NCI CIRB must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site

preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB, must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria in order for the processing of the IRB/REB approval record to be completed:

- Holds an active CTEP status;
- Rostered at the site on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating roster;
- If using NCI CIRB, rostered on the NCI CIRB Signatory record;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO); and
- Compliance with all protocol-specific requirements (PSRs).

For information about the submission of IRB/REB approval documents and other regulatory documents as well as checking the status of study center registration packets, please see Appendix I.

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

Note: Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review.

3.1.3 Study Enrollment

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account;
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrars must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

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

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

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

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

3.1.4 Timing

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.

NOTE: the above timing does not apply to the diagnosis of Down syndrome, only the clinical and laboratory studies.

3.1.5 Inclusion of Women and Minorities

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

3.2 Patient Eligibility Criteria

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

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment (except studies confirming the diagnosis of Down syndrome) unless otherwise indicated. Bone marrow evaluations must be completed within 14 days prior to enrollment. Laboratory values used to assess eligibility must be no older than seven (7) days at the start of therapy. Laboratory tests need not be repeated if therapy starts within seven (7) days of obtaining labs to assess eligibility. If laboratory values are > 7 days old, then the following laboratory evaluations must be re-checked within 48 hours prior to initiating therapy: CBC with differential, bilirubin, ALT and serum creatinine. Imaging studies, if applicable, must be obtained within 2 weeks prior to start of protocol therapy (repeat the tumor imaging if necessary).

3.2.1 Age at Diagnosis

Children with Down syndrome > 90 days and < 4 years of age at diagnosis of AML or Myelodysplastic Syndrome (MDS) (see Appendix III).

NOTE: The presence of myeloblasts in infants with DS during the first 90 days of life is consistent with transient myeloproliferative disease (TMD) rather than DS AML.

3.2.2 Diagnosis

3.2.2.1 Patients must have constitutional trisomy 21 (Down syndrome) or trisomy 21 mosaicism (by karyotype or FISH).

3.2.2.2 Patient has one of the following:

- Patient has previously untreated *de novo* AML and meets the criteria for AML with ≥ 20% bone marrow blasts as set out in the WHO Myeloid Neoplasm classification (see Appendix II).
    - Attempts to obtain bone marrow either by aspirate or biopsy must be made unless clinically prohibitive. In cases where it is clinically prohibitive, peripheral blood with an excess of 20% blasts and in which adequate flow cytometric and cytogenetics/FISH testing is feasible can be substituted for the marrow exam at diagnosis.

- Patient has cytopenias and/or bone marrow blasts but does not meet the criteria for the diagnosis of AML (WHO Myeloid Neoplasm classification,

see Appendix II) because of < 20% marrow blasts) and meets the criteria for a diagnosis of MDS (see Appendix III).

- For patients who do not meet criteria for AML or MDS as outlined above. Patient has a history of Transient Myeloproliferative Disorder (which may or may not have required chemotherapy intervention) and:
  i) is > 8 weeks since resolution of TMD with ≥ 5% blasts in the peripheral blood, OR
  ii) has an increasing blast count (≥ 5%) in serial bone marrow aspirates performed at least 4 weeks apart.

3.2.3 Prior Therapy

Children who have previously received chemotherapy, radiation therapy or any anti-leukemic therapy are not eligible for this protocol, with the exception of cytarabine for the treatment of TMD (see Section 3.2.5.2 for timing restriction).

3.2.4 Organ Function Requirements

There are no minimal organ function requirements for enrollment on this study.

Note: See Section 5.0 for dose adjustment in case of significant organ dysfunction. Previous cardiac repair with sufficient cardiac function as outlined in Section 5.0 is not an exclusion criteria.

3.2.5 Exclusion Criteria

3.2.5.1 Patients with promyelocytic leukemia (FAB M3)

3.2.5.2 Prior therapy

Patients ≤ 30 days from the last dose of cytarabine used for treatment of TMD.

3.2.6 Regulatory Requirements

3.2.6.1 Each patient's parents or legal guardians must sign a written informed consent.

3.2.6.2 All institutional, FDA, and NCI requirements for human subjects research must be met.

3.3 Definitions

3.3.1 Transient myeloproliferative disease (TMD)

A disorder of fetal hematopoiesis defined by the presence of circulating non-lymphoid, non-erythroid blasts in the blood of infants (< 90 days of age) with constitutional trisomy 21 or trisomy 21 mosaicism.
Note: the resolution date of TMD is defined as the date when blasts, abnormal blood counts and clinical signs of TMD were first documented as resolved.

3.3.2 CNS Leukemia

3.3.2.1 CNS disease at diagnosis is defined as:

- Any number of blasts on a cytospin prep in an atraumatic (< 100 RBCs) lumbar puncture.
- Blasts in a traumatic tap in which the WBC/RBC ratio in the CSF is twice that in the peripheral blood.
- Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome). Extra-ocular orbital masses are not considered CNS leukemia.
- Radiographic evidence of an intracranial, intradural mass consistent with a chloroma.

See Appendix V for more details.

3.3.2.2 Method of Evaluating Initial Traumatic Lumbar Punctures:
If the patient has leukemic cells in the peripheral blood and the lumbar puncture is traumatic and contains blasts, the following algorithm should be used to diagnose CNS disease:

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

A patient with CSF blasts, whose CSF WBC/RBC is 2X greater than the blood WBC/RBC ratio, has CNS disease at diagnosis. For example: CSF WBC = 60/µL; CSF RBC = 1,500/µL; blood WBC = 46,000/µL; blood RBC = 3 X 10^6/µL:

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

3.3.3 Initial CBC

The first WBC at the treating COG institution.

3.3.4 High Risk MDS/AML of Down syndrome

Minimal residual disease (MRD) ≥ 0.05% in the bone marrow at the end of Induction I.

3.3.5 Standard Risk MDS/AML of Down syndrome

Minimal residual disease (MRD) < 0.05% in the bone marrow at the end of Induction I.

Note: For definitions of response criteria, please see Section 10.2

4.0 TREATMENT PLAN

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

4.1 Overview of Treatment Plan

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

For COG Supportive Care Guidelines see:
https://childrensoncologygroup.org/index.php/cog-supportive-care-guidelines under Standard Sections for Protocols.

IMPORTANT: An aliquot of the diagnostic bone marrow sample must be sent to the laboratory of Dr. M. Loken at Hematologics, Inc. to facilitate later MRD measurement (for shipping information see Section 14.2). If a bone marrow sample cannot be obtained, please send a blood sample (see Section 14.1).

4.1.1 Induction I (All Patients)

All patients enrolled on AAML1531 receive Induction I as the first treatment cycle with intrathecal cytarabine, continuous infusion of cytarabine (96 hours), bolus infusion of daunorubicin and oral 6-thioguanine (6-TG) twice daily for 4 days (TAD). This cycle has a minimum duration of 28 days.

4.1.2 End of Induction I Bone Marrow Aspirate (BMA)

1) End of Induction I BMA should be obtained between Induction Day 28 to Day 49 as guided by count recovery.

2) If the marrow is hypoplastic and/or there is little or no evidence of normal hematopoiesis, a repeat marrow should be performed after a further 7–21 days (based upon the peripheral blood count recovery and the clinician's judgment) and remission status assessed at this later time point. If the bone marrow remains hypoplastic, then marrow studies should be repeated every 1-3 weeks (based upon the peripheral blood count recovery and the clinician's judgment) until an accurate bone marrow status is ascertained.

3) Patients with an MRD value < 0.05% (MRD-negative) will be removed from study and should receive the remainder of therapy per standard of care. Patients with an MRD value ≥ 0.05% (MRD-positive) will continue treatment on the High Risk Arm (Arm B). All patients will need to wait for the MRD

assessment prior to proceeding to Induction II (partial response and refractory disease are described below).

4.1.3 Risk Group Assignment

After recovery from the Induction I, patients are assigned to either the Standard or High Risk Group based on the level of MRD in the bone marrow aspirate (approximately Day 28). If there is no sign of count recovery a bone marrow aspirate should be performed no later than Day 49 from the start of Induction I to rule out the presence of refractory MDS/AML.

4.1.4 Standard Risk Patients

Standard risk patients are those with an MRD level of < 0.05% at the end of Induction I. **Arm A for Standard Risk patients closed to accrual and treatment with amendment #4A.** Patients enrolled after amendment #4A who are Standard Risk after Induction I will be removed from study and will continue treatment off protocol per standard of care. No further data will be collected on these patients.

4.1.5 High Risk Patients

High risk patients are those with a MRD level of ≥ 0.05% in the bone marrow at the end of Induction I. They are treated according to Arm B. Following Induction I they receive a cycle of mitoxantrone and cytarabine (MA) as Induction II; a cycle of cytarabine and etoposide (HR-AE) as Intensification I; a cycle of Capizzi II (HD Ara-C and Asparaginase) as Intensification II for a total of 4 cycles of intensive therapy. No additional intrathecal therapy will be given after Induction I.

Patients in the High Risk Group (Arm B) will have a repeat bone marrow aspirate and MRD measurement performed at the end of Induction II (MA) as part of standard clinical care for patients with previously positive MRD. The MRD result at the end of Induction II will be reported. However, MRD data obtained at the end of Induction II will not be used for treatment decisions but will aid in evaluating the performance of the MRD test. HR patients in a morphologic remission but MRD-positive should remain on protocol therapy.

4.1.6 Peripheral Blood Count Recovery

1) If the bone marrow after Induction I shows a complete response (CR, see Section 10.2), Induction II will be administered when the ANC ≥ 1000/µL and platelets ≥ 100,000/µL. A CBC should be repeated every 4 days until counts are adequate.

2) For all cycles, if the counts have not recovered by Day 49, repeat the bone marrow studies. A bone marrow biopsy is encouraged.

3) 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 cycle of therapy.

4) Note: Patients with a Partial Response (PR) or Refractory Disease (RD) (> 5% leukemic blasts confirmed by MRD) should start Induction II (MA) on the High risk Arm (Arm B) regardless of blood counts. Patients who have a

positive MRD (≥ 0.05% leukemic blasts) at the end of Induction I should start Induction II (MA) on the High risk Arm (Arm B) no later than Day 56 even if they have not met criteria for count recovery.

4.1.7 Use of Cardioprotectant (Dexrazoxane)

The use of dexrazoxane in association with anthracyclines will be left to institutional preference and per institutional guidelines. Therefore, is neither mandated nor prohibited by the AAML1531 protocol. Data on dexrazoxane use and cardiac outcomes (shortening fraction and ejection fraction) will be prospectively collected during chemotherapy and follow-up.

NOTE: As per the product monograph ZINECARD® (dexrazoxane) is indicated for reducing (preventing) the incidence and severity of cardiotoxicity associated with doxorubicin administration for the treatment of metastatic breast cancer in patients who have already experienced a partial response or at least maintained stable disease.[48] This is based on efficacy for cardioprotection in metastatic breast cancer patients who had already received at least 300 mg/m^2 of doxorubicin.[49]

4.2 Induction I (All Patients)

4.2.1 Therapy Delivery Map – Induction I (All Patients)

Induction I is a minimum of 28 days.

_____ Patient COG ID

_____ DOB

Criteria to start Induction I are the patient eligibility criteria described in Section 3.2. This Therapy Delivery Map is on 2 pages.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Intrathecal Cytarabine (IT ARAC)	IT	Age (months) / Dose 0 to < 13 mo. 20 mg 13 to < 25 mo. 30 mg 25 to < 36 mo. 50 mg ≥ 36 mo. 70 mg	1*	*If not given at time of diagnostic LP (see Section 4.2.3). **CNS positive patients** receive additional doses of IT ARAC as detailed in Section 4.2.3.
Cytarabine (ARAC IV)	Continuous IV infusion for 96 hrs	Age (months) / Dose < 36 mo. 6.67 mg/kg/24 hrs ≥ 36 mo. 200 mg/m²/24 hrs	1-4	
DAUNOrubicin (DAUN)	Slow IV push or infusion over 1-15 minutes	Age (months) / Dose < 36 mo. 0.67 mg/kg/day ≥ 36 mo. 20 mg/m²/day	1-4	See Section 4.2.3 for additional administration guidelines. Dexrazoxane may be administered at the discretion of the treating team. Please record below. See Section 4.1.7.
Thioguanine (TG)	PO	Age (months) / Dose < 36 mo. 1.65 mg/kg/dose, BID ≥ 36 mo. 50 mg/m²/dose, BID	1-4	Administer dose in AM & at bedtime. Round doses to nearest 10 mg if using the tablet formulation.

Ht cm **Wt** kg **BSA** m²

Date Due	Date Given	Day	IT ARAC _____mg	ARAC IV _____mg	DAUN _____mg	TG _____mg _____mg	Studies	Dexrazoxane Yes/No
			Enter calculated dose above and actual dose administered below					
		1	_____mg*	_____mg	_____mg	_____mg _____mg	a – l[#]	
		2		_____mg	_____mg	_____mg _____mg		
		3		_____mg	_____mg	_____mg _____mg		
		4		_____mg	_____mg	_____mg _____mg	a	
		5						
		6						
		7						
		8					a, b, c, d	
		9						
		10						
		11					a	
		12						
		13						
		14						
		15					a, b, c, d	
		16						
		17						
		18					a	
		19						
		20						
		21						
		22					a, b, c, d	
		23						
		24						
		25					a	
		26						
		27						
		28	Standard risk patients (MRD < 0.05%) will be removed from study and High risk patients (MRD ≥ 0.05%) continue with Induction II of Arm B (see Sect 4.3) when ANC ≥ 1000/μL and a platelet count ≥ 100, 000/μL. NOTE: exceptions exist, as detailed in Section 4.16.				a, k, l, m	

See Section 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

[#] PRIOR TO THERAPY

4.2.2 Required Observations in Induction I

All baseline studies (a-m below) must be performed prior to starting protocol therapy unless otherwise indicated below.

a. Physical exam, CBC with differential & platelets, twice weekly while in the hospital.
b. Creatinine, BUN, weekly while in the hospital.
c. Electrolytes (Ca^{++}, Mg^{++}, PO_4), weekly while in the hospital.
d. AST, ALT, bilirubin (unconjugated and conjugated), weekly while in the hospital.
e. Height, weight.
f. ECG.
g. ECHO or MUGA.
h. Unilateral bone marrow aspirate (BMA); biopsy if unable to obtain a BMA
i. Bone marrow baseline immunophenotype (Immunophenotyping including CD41 and/or CD61, CD33, CD34, CD14, CD7 and Gly-A is strongly recommended).
j. CSF cell count and cytospin.
k. BMA for MRD, submit sample to Hematologics (peripheral blood may be used for baseline sample, see Section 14.1).
l. Optional bone marrow sample for *GATA1* mutation analysis (see Section 14.1).
m. Optional bone marrow sample for banking (see Section 14.1).

OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

COMMENTS

4.2.3 Induction I Treatment Details (All Patients)

Criteria to start Induction I are the patient eligibility criteria described in Section 3.2.

Intrathecal Cytarabine:
The dose of intrathecal (IT) cytarabine may be given at the time of the diagnostic lumbar puncture. If IT cytarabine 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:

Age (months):		Dose:
0 to	< 13 months	20 mg
13 to	< 25 months	30 mg
25 to	< 36 months	50 mg
≥ 36 to	< 48 months	70 mg

***For CNS positive patients*:** IT cytarabine will be given twice weekly until the CSF is clear plus two additional intrathecal treatments (for a minimum of 4 and maximum of 6 doses). Patients with persistent CNS leukemia after 6 doses of IT cytarabine will be taken off protocol therapy.

Cytarabine: Continuous IV infusion for 96 hours
Days: 1-4.
Age-based dosing:

Age (months):	Dose:
< 36 months	6.67 mg/**kg**/24 hrs
≥ 36 months	200 mg/**m²**/24 hrs

DAUNOrubicin: Slow IV push or infusion over 1-15 minutes
Days: 1-4.
Age-based dosing:

Age (months):	Dose:
< 36 months	0.67 mg/**kg**/ day
≥ 36 months	20 mg/**m²**/ day

It is suggested that DAUNOrubicin be administered through the tubing of rapidly infusing solution of D5W or 0.9% NaCl and that it is infused into a large vein. Protect from sunlight.

Thioguanine: **PO BID**
Days: 1-4.
Age-based dosing:

Age (months):	Dose:
< 36 months	1.65 mg/**kg**/dose BID
≥ 36 months	50 mg/**m²**/dose BID

Administer dose in AM and at bedtime. Round thioguanine doses to nearest 10 mg by quartering tablets. The totally daily dose may be divided unequally in the morning and evening to minimize rounding.

See Section 5.0 for Dose Modifications based on Toxicities.

Duration Induction I

The duration of Induction I is a minimum of 28 days. Patients enrolled after amendment 4A who are Standard Risk (MRD < 0.05%) after Induction I will be removed from study (See Section 8.2). High risk patients (MRD ≥ 0.05%) continue with Induction II of Arm B when ANC ≥ 1000/μL and a platelet count ≥ 100,000/μL (see Section 4.1.6 for exceptions).

4.3 Induction II (High Risk-Arm B)

4.3.1 Therapy Delivery Map - Induction II (High Risk-Arm B) **Induction II lasts 28 days or longer.** This Therapy Delivery Map is on 2 pages.	_____ Patient name or initials _____ DOB

MRD results at end of Induction I are required for risk assessment prior to initiating Induction II. It is required that high risk DS patients (MRD ≥ 0.05%) have an ANC ≥ 1000/µL and a platelet count ≥ 100,000/µL before proceeding with therapy. Exception: if the platelet count is rising progressively to 75,000/µL but has not reached 100,000/µL by 3 weeks after the ANC has reached 1,000/µL, patients may proceed to Induction II. Note: patients with a PR or RD should start Induction II any time after Day 28 of Induction I, regardless of blood counts. Hospitalization is mandatory for the duration of Induction II until count recovery.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Cytarabine (HD ARAC1)	IV over 1-3 hours	All patients 33 mg/**kg**/dose every 12 hrs	1-4	Total dose: 66 mg/**kg**/day, divided. Use steroid eye drops (see Section 4.3.3).
MitoXANTRONE (MITOX)	IV over 15-30 minutes	All patients 0.4 mg/**kg**/day	3-6	Obtain ECG and ECHO/MUGA prior to Induction II. See Section 4.3.3 for administration guidelines. On Days 3 and 4, administer 8 hours after the 5th and 7th cytarabine infusions are completed. Dexrazoxane may be administered prior to Mitoxantrone at the discretion of the treating team. Record below.

Ht_____cm **Wt_____kg** **BSA____m^2**

Date Due	Date Given	Day	HD ARAC ____mg ____mg	MITOX ____mg	Studies	Dexrazoxane
		Enter calculated dose above and actual dose administered below				**Yes / No**
		1	_____mg _____mg		a-h	
		2	_____mg _____mg			
		3	_____mg _____mg	____mg		
		4	_____mg _____mg	____mg	a	
		5		____mg		
		6		____mg		
		8			a, b, c, d	
		11			a	
		15			a, b, c, d	
		18			a	
		22			a, b, c, d	
		25			a,	
		28			i%	
		Patients with a morphologic CR following Induction II will begin Intensification I when ANC ≥ 1000/µL and platelets ≥ 100,000/µL. See Section 4.1.6 for exceptions. Patients who do not achieve a morphological remission will be off protocol therapy.				

% MRD test will be performed upon count recovery. See Section 4.3.3.

See Section 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

Induction II (High Risk - Arm B)

4.3.2 Required Observations in Induction II (High Risk-Arm B)

a. Physical exam, CBC with differential & platelets, twice weekly while in the hospital.
b. Creatinine, BUN, weekly while in the hospital.
c. Electrolytes (Ca^{++}, Mg^{++}, PO_4), weekly while in the hospital.
d. AST, ALT, bilirubin (unconjugated and conjugated), weekly while in the hospital.
e. Height, weight.
f. Creatinine clearance or GFR if serum creatinine >2 mg/dL (177 µmol/L) or > 2x normal for age.
g. ECG prior to Induction II.
h. ECHO or MUGA prior to Induction II.
i. BMA and MRD sample for all patients. Note: MRD results will be reported to the study site but not be used for treatment stratification at this time point. See Section 14.2 for details.

OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

COMMENTS

Induction II (High Risk - Arm B)

4.3.3 Induction II Treatment Details (High Risk-Arm B)

Induction II will be administered to high risk DS AML patients when ANC ≥ 1000/µL and platelets ≥ 100,000/µL, or in the setting of a PR or RD any time after Day 28 of Induction I. Exception: if the platelet count is rising progressively to 75,000/µL but has not reached 100,000/µL by 3 weeks after the ANC has reached 1,000/µL, patients may proceed to the next cycle of therapy.

Hospitalization is **mandatory** for the duration of Induction II until count recovery. Dexrazoxane may be administered at the discretion of the treating institution prior to Mitoxantrone. Information whether or not dexrazoxane was used must be recorded.

The BMA and MRD test will be administered upon count recovery from Induction II, and no later than 49 days from the start of Induction II if there is no sign of count recovery.

The duration of Induction II is a minimum of 28 days.

High Dose Cytarabine: IV infusion over 1-3 hours, twice a day
Days: 1-4.
All patients: 33 mg/**kg**/dose q12 hours (i.e. 66 mg/**kg**/day, divided.)

Note: Administer the diluted solution at Hours 0-3 and Hours 12-15.

Administer steroid eye drops such as 0.1% dexamethasone or 1% prednisolone ophthalmic solution, 2 drops in each eye 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.

MitoXANTRONE: IV over 15 to 30 minutes
Days: 3-6.
All patients: 0.4 mg/**kg**/day

Administer through the tubing of a rapidly infusing solution of D5W or 0.9% NaCl. Avoid extravasation; the use of a central line is suggested.

Note: On the Days 3 and 4, mitoXANTRONE should be given 8 hours after the 5th and 7th high dose cytarabine infusions are completed.

See Section 5.0 for Dose Modifications based on Toxicities.

Patients who do not achieve a complete morphological remission after the end of Induction II will go off protocol therapy.

Intensification I will be administered to high risk DS AML patients with a morphologic CR (see Section 10.2) when ANC ≥ 1000/µL and platelets ≥ 100,000/µL. See Section 4.1.6 for exceptions.

Induction II (High Risk - Arm B)

4.4 Intensification I (High Risk-Arm B)

4.4.1 Therapy Delivery Map - Intensification I (High Risk-Arm B) **Intensification I lasts 28 days or longer.** Therapy Delivery Map is on 2 pages.	_____ Patient name or initials _____ DOB

It is required that patients have an ANC ≥ 1000/μL and a platelet count ≥ 100,000/μL before proceeding with therapy. Exception: if the platelet count is rising progressively to 75,000/μL but has not reached 100,000/μL by 3 weeks after the ANC has reached 1,000/μL, patients may proceed to Intensification I. Hospitalization is mandatory for the duration of Intensification I until count recovery.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Cytarabine (HD ARAC1)	IV over 1-3 hours	All patients 33 mg/**kg**/dose every 12 hrs	1-5	Total dose: 66 mg/**kg**/day, divided. Use steroid eye drops as described in Section 4.4.3.
Etoposide (ETOP)	IV over 90 - 120 minutes	All patients 5 mg/**kg**/day	1-5	Slow rate of administration if hypotension occurs. See Section 5.1.2 on allergy to etoposide. Etoposide doses should immediately follow the 1st, 3rd, 5th, 7th, and 9th dose of cytarabine.

Ht____cm Wt____kg BSA____m^2

Date Due	Date Given	Day	HD ARAC ____mg ____mg	ETOP ____mg	Studies
		Enter calculated dose above and actual dose administered below			
		1	____mg ____mg	____mg	a-f
		2	____mg ____mg	____mg	
		3	____mg ____mg	____mg	
		4	____mg ____mg	____mg	a
		5	____mg ____mg	____mg	
		8			a, b, c, d
		11			a
		15			a, b, c, d
		18			a
		22			a, b, c, d
		25			a
		28	Intensification II will start for high risk patients when the ANC ≥1000/μL and platelet count ≥100,000/μL. See Section 4.1.6 for exceptions.		

See Section 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines

Intensification I (High Risk -Arm B)

4.4.2 Required Observations in Intensification I (High Risk-Arm B)

a. Physical Exam, CBC with differential & platelets, twice weekly while in the hospital
b. Creatinine, BUN, weekly while in the hospital
c. Electrolytes (Ca^{++}, Mg^{++}, PO_4), weekly while in the hospital
d. AST, ALT, bilirubin (unconjugated and conjugated), weekly while in the hospital
e. Height, weight
f. Creatinine clearance or GFR if serum creatinine >2 mg/dL (177 µmol/L) or >2x normal for age

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

COMMENTS

4.4.3 Intensification I (High Risk-Arm B)

Patients who do not achieve a complete morphological remission by the end of Induction II will be off protocol therapy

Intensification I will be administered to high risk DS AML patients when ANC ≥ 1000/µL and platelets ≥ 100,000/µL. Exception: if the platelet count is rising progressively to 75,000/µL but has not reached 100,000/µL by 3 weeks after the ANC has reached 1,000/µL, patients may proceed to Intensification I. Intensification I is a minimum of 28 days. Hospitalization is **mandatory** for the duration of Intensification I until count recovery.

High Dose Cytarabine: IV over 1-3 hours, twice a day
Days: 1-5.
All patients: 33 mg/**kg**/dose every 12 hrs (i.e. 66 mg/**kg**/day, divided)

Administer steroid eye drops such as 0.1% dexamethasone or 1% prednisolone ophthalmic solution, 2 drops in each eye 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.

Etoposide (ETOP): IV infusion over 90-120 minutes
Days: 1-5.
All patients : 5 mg/**kg**/day

Etoposide doses should immediately follow the 1st, 3rd, 5th, 7th, and 9th dose of cytarabine.

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

See Section 5.1.2 on Allergy to Etoposide.

See Section 5.0 for Dose Modifications based on Toxicities.

It is required that patients have an ANC ≥ 1000/µL and a platelet count ≥ 100,000/µL before proceeding with Intensification II. See Section 4.1.6 for exceptions.

Intensification I (High Risk -Arm B)

4.5 Intensification II (High Risk-Arm B)

4.5.1 Therapy Delivery Map – Intensification II (High Risk – Arm B) **Intensification II is a minimum of 28 days.**	Patient name or initials DOB

Patients must have an ANC ≥ 1000/µL and a platelet count ≥ 100,000/µL before proceeding with therapy. Exception: if the platelet count is rising progressively to 75,000/µL but has not reached 100,000/µL by 3 weeks after the ANC has reached 1,000/µL, patients may proceed to Intensification II. This Therapy Delivery Map is on 2 pages. Treatment details are provided in Section 4.5.3.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Cytarabine (HD ARAC3)	IV over 3 hours	All patients 100 mg/**kg**/dose every 12 hours	1, 2, 8, 9	Total dose: 200 mg/**kg**/day, divided. Use eye drops as described in Section 4.5.3.
Asparaginase (*E. coli*) **OR** Asparaginase (*Erwinia*)	IM or IV	Asparaginase (*E. coli*) All patients : 200 international units/**kg**/dose **OR** Asparaginase (*Erwinia*) All patients: 830 international units/**kg**/dose	2, 9	To be given at hour 18 on Days 2 and 9 (6 hours after the start of the 4th and 8th dose of cytarabine)

Ht_____cm Wt_____kg BSA_____m^2

Date Due	Date Given	Day	HD ARAC ____mg ____mg	*Asparaginase E. coli* or *Erwinia* international units	Studies
		Enter calculated dose above and actual dose administered below			
		1	_____mg _____mg		a-f
		2	_____mg _____mg	_____international units	
		3			
		4			a
		8	_____mg _____mg		a, b, c, d
		9	_____mg _____mg	_____international units	
		10			
		11			a
		15			a, b, c, d
		18			a
		22			a, b, c, d
		25			a
		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). **See End of Therapy observations in Section 7.1.**		a-e

See Section 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

Intensification II (High Risk -Arm B)

4.5.2 Required Observations in Intensification II (High Risk-Arm B)

a. Physical exam, CBC with differential & platelets, twice weekly while in the hospital.
b. Creatinine, BUN, weekly while in the hospital.
c. Electrolytes (Ca^{++}, Mg^{++}, PO_4), weekly while in the hospital.
d. AST, ALT, bilirubin (unconjugated and conjugated), weekly while in the hospital.
e. Height, weight.
f. Creatinine clearance or GFR if serum creatinine >2 mg/dL (177 µmol/L) or > 2x normal for age.

OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

COMMENTS

Intensification II (High Risk -Arm B)

4.5.3 Intensification II Treatment Details (High Risk-Arm B)

Intensification II will be administered to high risk DS AML patients when ANC ≥ 1000/µL and platelets ≥ 100,000/µL. Exception: if the platelet count is rising progressively to 75,000/µL but has not reached 100,000/µL by 3 weeks after the ANC has reached 1,000/µL, patients may proceed to Intensification II Intensification II is a minimum of 28 days. Hospitalization is **mandatory** for the duration of Intensification II until count recovery.

High Dose Cytarabine: IV infusion over 3 hours, **every 12 hours** Days: 1, 2, 8 and 9.
All patients: 100 mg/**kg**/dose, q12 hours (i.e. 200 mg/**kg**/day, divided).

Note: Administer the diluted solution at Hours 0-3 and Hours 12-15.

Administer steroid eye drops such as 0.1% dexamethasone or 1% prednisolone ophthalmic solution, 2 drops in each eye 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.

Asparaginase (*E. coli*): IM (preferred) or IV over at least 30 minutes through the tubing of a freely running IV of a normal saline or D5W infusion
Days : 2 and 9.
All patients : 200 international units/**kg**/dose

Note: Administer at hour 18 on Days 2 and 9 (i.e. 6 hours after the start of the 4th and 8th dose of cytarabine).

OR

***Asparaginase (Erwinia)*: IM or IV in 100 mL of normal saline over 1-2 hours**
Days: 2 and 9.
All patients: 830 international units/**kg**/dose

Notes:

- Administer at hour 18 on Days 2 and 9 (i.e. 6 hours after the start of the 4th and 8th dose of cytarabine).
- Some product lots are **not** for IV administration; refer to manufacturer for batch-specific information.

If Asparaginase (*E.coli* or *Erwinia)* is not available, pegaspargase should **not** be given. In this case asparaginase should be omitted.

See Section 5.0 for Dose Modifications based on Toxicities.

Protocol therapy is completed after blood count recovery (ANC ≥ 1000/µL and platelets ≥ 100,000/µL and ≥ 7 days from the last platelet transfusion) after Intensification II.

Intensification II (High Risk -Arm B)

5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 Allergy

5.1.1 Allergy to Asparaginase

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

Continue asparaginase *(E.coli* or *Erwinia*) administration in the presence of Grade 1 allergic reaction (transient flushing or rash; drug fever < 38°C). Premedication with antihistamines in the absence of prior hypersensitivity has been discouraged in the past since antihistamine use may mask the appearance of systemic allergy and fail to alert the provider of the presence of asparaginase neutralizing antibodies. Asparaginase activity assays are now commercially available and may help determine if neutralizing antibodies are present, thus the use of premedication is left to the discretion of the provider.

5.1.1.2 Anaphylaxis/Systemic Allergic Reactions:

Discontinue asparaginase (*E.coli* or *Erwinia*) if the patient develops a systemic allergic reaction (urticaria, wheezing, laryngospasm, hypotension, etc.). Should an allergy be diagnosed after the first dose given on Day 2 during Intensification II (for high risk patients), then the dose due on Day 9 should not be administered (unless the patient previously received *E. Co*li and *Erwinia* is available, in this case *Erwinia* dosing can be substituted for Day 9 asparaginase).

5.1.2 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 (maximum dose 50 mg), hydrocortisone 1-4 mg/kg IV, and by slowing the rate of the infusion. Etoposide phosphate may be substituted for etoposide for reactions that cannot be controlled with pre-medication and the slowing of the etoposide infusion rate.

Etoposide phosphate is rapidly converted to etoposide *in vivo* and provides total drug exposure, as represented by AUC (0-infinity) that is statistically indistinguishable from that measured for etoposide at equimolar doses. As such etoposide phosphate may be substituted at the same dose and at the same rate.

5.1.3 ARAC syndrome:

ARAC Syndrome: Do not withhold cytarabine for fever if it is likely to have been caused by the cytarabine. Obtain blood cultures if a central line is present. For Grade 3-4 rash or conjunctivitis, withhold cytarabine until toxicity resolved.

5.2 Cardiac Toxicity

5.2.1 Left Ventricular Systolic Dysfunction

Daunorubicin and mitoxantrone will be held if there is evidence of cardiac disease by echocardiogram or MUGA (shortening fraction < 28% or EF < 55%). Cardiac examination with echocardiogram (or MUGA) is required prior to the start of all chemotherapy cycles containing daunorubicin or mitoxantrone, at the end of protocol therapy, and in follow up. Please see Section 7.1 for long-term follow-up monitoring.

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

5.3 CNS Events (Bleed, Thrombosis or Infarction)

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

5.4 Coagulopathy

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

5.5 Hepatic Toxicity

5.5.1 Transaminases

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

5.5.2 Hyperbilirubinemia

If the conjugated/direct bilirubin is > 3 mg/dL and is not a result of the leukemia, modify the doses of daunorubicin, etoposide, and mitoxantrone as follows below. For all cases in which the direct bilirubin is elevated at the point in time that the next cycle is to begin, consider delaying the cycle for 1 week to determine whether the direct bilirubin falls to an acceptable level.

Asparaginase
Asparaginase has been associated with hepatic toxicity but dosing guidelines for hepatic toxicity are not available. Thus, asparaginase administration (*E.coli* or *Erwinia*) in the setting of hepatic toxicity is at the clinician's discretion.

Daunorubicin, Etoposide and Mitoxantrone
Dosage adjustments should be made as indicated in the table below:

Direct Bilirubin	Daunorubicin	Etoposide	Mitoxantrone
≥ 2 and < 3 mg/dL	50% of the calculated dose	50% of the calculated dose	50% of the calculated dose
≥ 3 and < 5 mg/dL	25% of the calculated dose	25% of the calculated dose	25% of the calculated dose
≥ 5 mg/dL	Hold dose	Hold dose	Hold dose

5.6 Hyperglycemia

Do not modify the dose of asparaginase. Treat hyperglycemia as medically indicated.

5.7 Hyperlipidemia

Do not modify the dose of asparaginase.

5.8 Hypotension

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

5.9 Ketoacidosis

Hold asparaginase until blood glucose can be regulated with insulin.

5.10 Neurologic Toxicity

The most common nervous system adverse event is an acute cerebellar syndrome that may manifest itself as ataxia, nystagmus, dysarthria, or dysmetria. However, seizures and encephalopathy have also occurred following therapy with high dose cytarabine.

Patients with ≥ Grade 3 CTCAE version 5.0 nervous system disorders from high dose cytarabine should not receive further high dose cytarabine**. Grade 3 or higher neurotoxicity from high-dose cytarabine (HR group, Arm B only) is a criterion for removal from protocol therapy.**

5.11 Pancreatitis

Discontinue asparaginase (*E.coli* or *Erwinia*) in the presence of hemorrhagic pancreatitis or severe pancreatitis (Grade 3-4). In the case of mild pancreatitis after Day 2 (< Grade 3 using CTCAE version 5.0 criteria), Day 9 asparaginase (*E.coli* or *Erwinia*) may be given only if symptoms and signs subside.

5.12 Renal Toxicity

5.12.1 Cytarabine

Patients with nephrotoxicity secondary to antibiotics, or antifungals, may have prolonged excretion of cytarabine leading to increased bone marrow and extramedullary toxicity. Patients with a serum creatinine > 2 mg/dL or > 2x 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[50] before proceeding with HD cytarabine therapy (doses of > 1 g/m^2). If the CrCl is abnormal (< 60 mL/min/1.73m^2) 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 prevented subsequent neurotoxicity in recipients of high dose cytarabine in the face of renal insufficiency.[51]

5.12.2 Etoposide

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/1.73m^2 give full dose, for CrCl of 15-60 mL/min/1.73m^2 give 75% of the dose (25% dose reduction). **Subsequent doses** should be based on patient tolerance and clinical effect.

5.13 Thrombosis

Discontinue asparaginase and treat with appropriate antithrombotic therapy, as indicated.

6.0 DRUG INFORMATION

Please see Appendix IV for drug interactions associated with the drugs used in this study.

See the consent document for toxicities. All other information is available on the COG website in the Commercial Agent Monographs (https://www.cogmembers.org/_files/disc/pharmacy/CommercialAgentsMonographs.pdf) and is provided under Standard Sections for Protocols at: https://cogmembers.org/site/pages/default.aspx?page=Prot_reference_materials.

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 (except where explicitly prohibited within the protocol).

7.1 End of Therapy & Follow-up

Observation	End of Therapy	1 month off therapy	Monthly for first 12 months from completion of therapy	Every 3 months for month 12 to 24 from completion of therapy	Every 6 months from month 24 until 5 years following completion of therapy	Annually for 10 years following completion of therapy	At relapse
Physical exam	■	■	■	■	■		■
Height, weight	■	■	■	■	■		■
CBC with diff/platelets	■	■	■	■	■		■
Creatinine, BUN	■	■					■
Electrolytes, Ca^{++}, PO_4, Mg^{++}	■	■					■
AST, ALT, Bilirubin unconjugated and conjugated	■	■					■
ECG	■					■	■
MUGA or ECHO	■					■	■
Unilateral BM Asp/Biopsy*							■
Bone marrow for banking							■

* If unable to obtain a bone marrow aspirate

See COG Late Effects Guidelines for recommended post treatment follow-up:
http://www.survivorshipguidelines.org/

Note: Follow-up data are submitted per the Case Report Forms (CRFs) schedule.

7.2 Research Studies

Study	Specimen	Pre-treatment	End of Induction I	End of Induction II (High Risk Patients ONLY)
MRD	Bone marrow	X[#]	X	X
GATA-1 mutation analysis	Bone marrow	O	O	
Banking	Bone marrow	O	O	

X: Required
O: Optional requires patient consent.
[#]: If a bone marrow sample cannot be obtained, please send a blood sample (see Section 14.1).
For additional details on research studies, see Section 14.0.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) PR, RD, or relapse following Induction II.
b) Persistent CNS leukemia after 6 doses of IT Ara-C after Induction I.
c) Grade 3 or greater neuropathy, myelitis and nervous system disorder, and other, including cerebellar toxicity from HD Ara-C (HR group, Arm B only).
d) Refusal of further protocol therapy by patient/parent/guardian.
e) Completion of planned therapy.
f) Physician determines it is in patient's best interest.
g) Development of a second malignancy.
h) Repeat eligibility studies (if required) prior to the initiation of protocol therapy are outside the parameters required for eligibility (see Section 3.2).
i) Failure to obtain a result for the MRD test at the end of Induction I.

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 patient is taken off study.

8.2 Off Study Criteria

a) Death.
b) Lost to follow-up.
c) Patient enrollment onto another COG study with tumor therapeutic intent (e.g., at recurrence).
d) Withdrawal of consent for any further data submission.
e) Tenth anniversary of the date the patient was enrolled on this study.
f) For patients enrolled after the implementation of Amendment #4A: Patient is Standard Risk (MRD < 0.05% in the bone marrow) after Induction I.

9.0 STATISTICAL CONSIDERATIONS

9.1 Statistical Design

This study is a non-randomized study of children with DS and AML. The primary endpoint of interest will be the event-free survival (EFS) from the end of Induction I to failure to achieve remission at the end of Induction II, relapse, occurrence of a second malignancy or death.

Other outcome measures will include average total number of days per patient spent on protocol therapy, early death rates, overall survival from on study, treatment related mortality, relapse risk, percentage of patients experiencing Grade 3 or higher toxicity, time to count recovery, duration of hospitalization, and infection rates.

9.2 Patient Accrual and Expected Duration of Trial

AAML1531 will be compared against a fixed 2-year EFS of 93.5% for SR patients and 76% for HR patients which were observed for comparable patients treated on the prior COG study of DS AML patients (AAML0431). For SR patients, assuming a null EFS of 93.5% at 2 years, there is 95% power to detect an alternative EFS of 87% at 2 years with 1-sided testing at the 10% level of statistical significance if there are 200 SR patients who continue to Induction II. For HR patients, assuming a null EFS of 76% at 2 years, there is 80% power to detect an alternative EFS of 88% at 2 years with 1-sided testing at the 10% level of statistical significance if there are 41 high risk patients who continue to Induction II. The necessary sample size was computed using a binomial test. The final analysis of EFS will occur after a minimum of 2 years after the last patient is enrolled.

* Replaced with Amendment #6
Up to 3% of patients enrolled are expected to be found ineligible, up to 3% are expected to go off protocol therapy at the end of Induction I, and approximately 83% of patients are expected to be SR. Therefore, enrollment of up to 256 patients will be required to assure that there are 200 SR patients who continue to Induction II and there are 41 HR patients continue to Induction II. Based on an accrual estimate of 60 patients per year, accrual of 256 patients is expected to be completed in approximately 4.3 years.

* Updated with Amendment #4A
As of October 22, 2018, 26 of the 143 patients enrolled are HR patients who continued to Induction II. Up to 3% of patients enrolled are expected to be found ineligible, up to 3% are expected to go off protocol therapy at the end of Induction I, and approximately 83% of patients are expected to be SR. Therefore, enrollment of up to 256 patients will be required to assure that there are 41 HR patients who continue to Induction II. Based on an accrual estimate of 60 patients per year, accrual of an additional 113 patients is expected to be completed in approximately 2 years.

* Updated with Amendment #6
As of August 25, 2020, 32 of the 222 patients enrolled are HR patients who continued to Induction II and 5 patients have not been risk classified yet. Assuming 10% of enrolled patients will be HR, enrollment of up to 312 patients will be required to assure that there are 41 HR patients who continue to Induction II. Based on an accrual estimate of 60 patients per year, accrual of an additional 90 patients is expected to be completed in approximately 1.5 years.

9.3 Statistical Analysis Methods

9.3.1 Analysis Plan

The Kaplan-Meier method will be used to estimate 2-year EFS from the end of Induction I along with 95% log-minus-log transformed confidence limits separately for HR and SR patients at end of Induction I. To compare the Kaplan Meier estimate of 2-year EFS (KM2) with fixed values, we will use the test statistic $\frac{\ln(-\ln(\text{KM2}))-\ln(-\ln(0.935))}{Estimated\ SD\ [\ln(-\ln(KM2))]}$ for SR and $\frac{\ln(-\ln(\text{KM2}))-\ln(-\ln(0.76))}{Estimated\ SD\ [\ln(-\ln(KM2))]}$ for HR patients and compare these with a standard normal distribution.[52]

* Updated with Amendment #4A
AAML1531 was temporarily closed to accrual on October 1, 2018, due to results of an interim analysis identifying a significantly lower EFS for SR patients than that expected based on comparative data from AAML0431. Post-Induction I treatment on the SR Arm A was permanently closed on October 18, 2018. All patients enrolled prior to amendment #4A who were assigned to the SR arm were to be taken off protocol therapy but may stay on study. These SR patients enrolled prior to amendment #4A who are taken off protocol therapy due to the closure of treatment on the SR arm will be censored in future EFS analysis at the time they were taken off protocol therapy. Patients enrolled after amendment #4A who are assigned to the SR arm will be removed from study and continue treatment off protocol per standard of care. No further data will be collected on these patients.

9.3.2 Interim Monitoring

Standard Risk Patients - lack of efficacy
Monitoring for insufficient EFS from the end of Induction I of the treatment for SR patients on this study will utilize monitoring based on the Lan-DeMets criterion with an α-spending function αt^2 (truncated at 3 standard deviations) and 10% type I error. Formal monitoring analyses of differences in the number of EFS 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 after approximately 50% of the expected number of EFS events have been observed (after 8 of 16 total events for SR) which is anticipated to occur after approximately 156 patients are assigned to the SR arm. For interim monitoring, we will conduct a Woolson one-sample log rank test for SR to compare the observed EFS with AAML0431 EFS for patients who continue to Induction II treated as fixed and known. EFS for AAML0431 will be characterized by the following separate cure model for SR patients:

$S(t) = 0.905 + (0.095)*\exp(-0.00127*t)$ t measured in days

A p-value that is less than the boundary value of 0.025 for 50% information time will result in rejection of the hypothesis and the possible suspension of enrollment to the stratum will be identified to the COG DSMC.

* Updated with Amendment #4A
Interim monitoring has been completed for standard risk patients.

High Risk Patients - efficacy

Monitoring for improvement in EFS from the end of Induction I of the treatment for HR patients on this study will utilize monitoring based on the Lan-DeMets criterion with an α-spending function αt^2 (truncated at 3 standard deviations) and 10% type I error. Formal monitoring analyses of differences in the number of EFS 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 after approximately 50% of the expected number of EFS events have been observed (5 to 6 of 11 total events for HR) which is anticipated to occur after approximately 16-21 patients are assigned to the HR arm. For interim monitoring, we will conduct a Woolson one-sample log rank test for HR patients to compare the observed EFS with AAML0431 EFS for patients who continue to Induction II treated as fixed and known. EFS for AAML0431 will be characterized by the following cure model for HR patients:

$S(t) = 0.75 + (0.25)*\exp(-0.0037*t)$ t measured in days.

A p-value that is less than the boundary value of 0.025 for 50% information time will result in rejection of the hypothesis and the possible suspension of enrollment to the stratum will be identified to the COG DSMC.

High Risk Patients – toxic deaths

No toxic deaths were observed on AAML0431. Possible suspension of enrollment to the High Risk stratum will be considered if 3 or more toxic deaths, i.e. deaths as first event while on protocol therapy or within 30 days of going off protocol therapy, are observed on the High Risk arm. For a true toxic death rate of 2%, there is a 4.9% chance of observing 3 or more toxic deaths. For a true toxic death rate of 10%, there is a 79.1% chance of observing 3 or more toxic deaths.

9.4 Gender and Minority Accrual Estimates

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

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	12	9	0	0	**21**
Native Hawaiian or Other Pacific Islander	0	3	0	0	**3**
Black or African American	21	27	0	0	**48**
White	105	77	29	29	**240**
More Than One Race	0	0	0	0	**0**
Total	**138**	**116**	**29**	**29**	**312**

This distribution was derived from AAML0431

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 5.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v5.0' is understood to represent the most current version of CTCAE v5.0 as referenced on the CTEP website (i.e., v5.02 and all subsequent iterations prior to version 6.0).

10.2 Response Criteria for Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome of DS

If the bone marrow sample used for the assessment of response is aplastic or severely hypocellular, the bone marrow aspirate and complete response assessment (including MRD) should be repeated every 7 to 21 days until a response evaluation can be made.

10.2.1 Complete Response (CR)

The bone marrow is cellular with regenerating normal hematopoietic cells. There are < 5% blast cells by morphology and there is no evidence of extramedullary disease (EMD).

10.2.2 Partial Response (PR)

If there is neither a Complete Response (CR) nor Refractory Disease (RD).

10.2.3 Refractory Disease (RD)

Two consecutive bone marrow evaluations (separated by at least 2 weeks) that are at least moderately cellular and contain ≥ 5% leukemic blasts by morphology and MRD **or** evidence of extra-medullary disease at the end of Induction II. In patients with myelodysplastic syndrome, there are > 5% malignant blasts.

10.2.4 Relapse

Morphologic relapse after CR is defined as a reappearance of leukemic blasts in the peripheral blood or ≥ 5% blasts in the bone marrow not attributable to any other cause (e.g. bone marrow regeneration) after documented CR. In the setting of recent treatment, if there are no circulating blasts and the bone marrow contains 5% to 20% blasts, **a repeat bone marrow performed at least a week later is necessary** to distinguish relapse from bone marrow regeneration. Should local flow cytometric analyses suggest relapse (by the reappearance of a similar immunophenotype to the original leukemia) in the presence of <5% blasts, or ≥ 5% blasts in a regenerating marrow, **a repeat bone marrow(s) performed at least a week later is necessary** to confirm relapse by morphologic methods. In such instances the date of recurrence is defined as the first date that more than 5% leukemic blasts were observed in the marrow.

Cytogenetic relapse is characterized by reappearance of a cytogenetic or molecular abnormality after documented CR. A bone marrow examination performed at least a week later is necessary to confirm cytogenetic relapse.

Extramedullary disease relapse is defined as appearance of cytologically proven extramedullary disease after documented CR.

10.2.5 Unevaluable

The bone marrow is aplastic or severely hypocellular (with any blast percentage). In this instance, bone marrow evaluation should be repeated every 7 to 21 days until a response determination can be made.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 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.

11.2 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.***

Secondary Malignancy

A ***secondary*** malignancy is a cancer caused by treatment for a previous malignancy (eg, treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

All secondary malignancies that occur following treatment need to be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

Second Malignancy
A ***second*** malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

11.3 Reporting of Adverse Events for Commercial Agents – via CTEP-AERS

Expedited AE reporting must use CTEP-AERS (Adverse Event Expedited Reporting System), accessed via https://eapps-ctep.nci.nih.gov/ctepaers

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 CTEP-AERS report to be submitted within 7 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.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Table B
Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

CTEP-AERS Reporting Requirements for Adverse Events That Occur During Therapy with a Commercial Agent or Within 30 Days[1]

Attribution	Grade 4		Grade 5
	Unexpected	Expected	
Unrelated or Unlikely			CTEP-AERS
Possible, Probable, Definite	CTEP-AERS		CTEP-AERS

[1]This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent that can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via CTEP-AERS.

11.4 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 CTEP-AERS 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 toxicities reported via CTEP-AERS and all Grade 3 and higher non-hematologic Adverse Events, and all grades of the following cardiac Adverse Events: prolonged QTc interval and left ventricular systolic dysfunction.

11.5 Syndrome Reporting

Unless otherwise specified in this protocol, syndromes should be reported as a single event using the CTCAE term for the composite syndrome, and not as the individual events that make up the syndrome. For example, Tumor Lysis Syndrome should be reported under the composite definition rather than reporting the component events (hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia) separately.

12.0 RECORDS AND REPORTING

See the Case Report Forms posted on the COG web site with each protocol under "*Data Collection/Specimens*". A submission schedule is included.

12.1 Clinical Data Update System (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.

13.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS

13.1 Diagnostic Specimen Requirements for Central Review

1. One Wright & Giemsa stained and 6 unstained bone marrow aspirate smears.
2. Flow cytometry report.
3. Bone marrow pathology report.
4. Cytogenetic report and reports for any molecular testing for chromosomal translocations
5. Results of a complete blood and differential count (manual or automated) obtained on the same day as the bone marrow aspirate
6. One H&E stained and 6 unstained sections of bone marrow core biopsy (if obtained).
7. One H&E stained and 6 unstained sections of bone marrow clot section (if available).
8. One Wright & Giemsa stained and 2 unstained peripheral blood smears (if available).
9. Specimen Transmittal Form.

For patients with myeloid sarcoma (chloroma), the following materials must also be submitted for central pathology review:

1) One H&E stained slide and 6 unstained recuts from the biopsy material of the myeloid sarcoma, if obtained. Slides must be sent from a representative block with remaining diagnostic material.
2) Immunohistochemistry stained slides that were used to establish the diagnosis (this is strongly recommended, but not required; these special stained slides will be sent back at the conclusion of review, if requested by contributor).
3) A copy of pathology report (including results of special stains, flow cytometry and cytogenetics, if performed).

Please label all materials for Central Review with the subject's COG identification number, the institutional surgical pathology identification number (SPID) and the block number from the corresponding report.

13.1.1 Specimen Shipment for Central Review

Please send all the above mentioned materials by mail or using your institution's courier account to:

Biopathology Center – AAML1531
Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, OH 43205
Phone: (614) 722-2865
Fax: (614) 722-2897
Email: BPCParaffinTeam@nationwidechildrens.org

The Biopathology Center will forward the materials to:

[redacted]

13.2 Local Cytogenetic Analysis and Data Submission to Central Laboratory

13.2.1 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 your institutional cytogenetics laboratory 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.

13.2.2 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	EAST OF MISSISSIPPI RIVER (EXCLUDE MINNESOTA AND WISCONSIN), EUROPE, EAST CANADA
SEND TO:	SEND TO:
[redacted]	[redacted]

14.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

14.1 Summary of Specimens

Study	Time points	Collection Details	Additional Details	Mailing Destination
MRD Required from all patients	→ Pre-treatment → End of Induction I → End of Induction II (High Risk Patients only)	2-4 mL of anticoagulated bone marrow in sodium heparin (green top tube). **For the Pre-Treatment sample only**: If BM sample cannot be obtained, and the peripheral blood has at least 20% blasts, send 5 mL of blood in sodium heparin (green top tube).	Store at room temperature. Ship on the day of collection. If sample is stored for longer than 24 hours, add **equal volume** of RPMI medium. **Do not freeze MRD samples**. (See Section 14.2)	1 (Loken's lab) Section 14.2.3
Banking of viable cells (Optional: Patient Consent Required)	→ Pre-treatment → End of Induction I → Relapse	3-10 mL bone marrow into a sterile syringe containing a sterile solution of preservative-free heparin.	Place in a 15 mL sterile conical centrifuge tube and add 5 mL of sterile RPMI-1640 with 20% fetal calf serum. If specimen is not shipped on the day of collection, store in a refrigerator until shipment. (See Section 14.3)	2 (Leukemia Biospecimen Bank at BPC) Section 14.3.3
GATA-1 mutation (Optional: Patient Consent Required)	→ Pre-treatment → End of Induction I	2-4 mL bone marrow into an EDTA containing tube (purple top).	If specimen is not shipped on the day of collection, store in a refrigerator until shipment. (See Section 14.3)	2 (Leukemia Biospecimen Bank at BPC) Section 14.3.3

14.2 Destination 1 (MRD for Michael Loken's Laboratory)

14.2.1 Forms

Utilize AML Specimen Transmittal forms specific for AAML1531.

14.2.2 Labeling and Packaging

- Label the tubes with COG ID number, date and time of collection, time point (include treatment cycle and day of cycle), and source of material (e.g., bone marrow).
- Specimens should be placed inside a leak proof biohazard envelope with absorbent material and then a pressure resistance Tyvek envelope.

14.2.3 Shipping

MRD samples should be mailed by FEDERAL EXPRESS PRIORITY OVERNIGHT. COG sites may use the COG Federal Express account number available at:
https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf

Include a COG Specimen Transmittal form and ship at room temperature to:

[redacted]

Lab contact information:
Phone: (800) 860-0934 or (206) 223-2700
Fax: (206) 223-5550
Weekends and After Hours: (206) 264-4459
Email: clientservices@hematologics.com

Weekend Specimens: The lab is staffed 6 days a week. For Saturday delivery, please use a *Saturday delivery* sticker and check the *Saturday delivery* box on the address label. Both sticker and checked box are necessary to insure proper handling.

Please allow 2-4 business days for samples to be processed. Results from end of Induction 1 will be communicated by fax and entered into RAVE.

14.3 Destination 2 (COG Leukemia Biospecimen Bank, BPC)

14.3.1 Forms

Utilize AML Specimen Transmittal forms specific for AAML1531.

14.3.2 Labeling and Packaging

- Ship at room temperature.
- Label the tubes with COG ID number, patient name, date of birth, date and time of collection, time point (include treatment cycle and day of cycle), and source of material (i.e., bone marrow).
- Specimens should be placed inside a leak proof biohazard envelope with absorbent material and then a pressure resistance Tyvek envelope as per IATA regulations.

14.3.3 Shipping

COG Leukemia Biospecimen Bank
Nationwide Children's Hospital
700 Children's Drive, C0825
Columbus, OH 43205

Leukemia Bank contact information:
Phone: (614) 722-3270
Fax: (614) 722-2856
Email: MGLab@nationwidechildrens.org

Call or email the Leukemia Bank only when shipping a sample to be delivered on Saturday.

Samples should be mailed by FEDERAL EXPRESS PRIORITY OVERNIGHT. COG sites may use the COG Federal Express account number available at: https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf

Samples should be sent as soon as possible and preferably less than 24 hours from sample collection; except samples collected on weekends or holidays should be shipped the first working day following collection.

Samples may be shipped on Monday through Friday for Tuesday through Saturday delivery. If specimen is not shipped on the day of collection, **please store in a refrigerator until shipment**.

14.4 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, as detailed in Section 14.1. Samples should be labeled and shipped as detailed in Section 14.3.

15.0 DESCRIPTION AND ANALYSIS OF EXPLORATORY STUDIES

The exploratory studies detailed in this section are for research purposes only, and results will not be used to make clinical treatment decisions. **With the exception of the MRD results after Induction I,** all other results will not be obtained in real-time (but rather batched for retrospective review,) and results will not be returned to the treating physician.

Please note, if there is an inadequate amount of bone marrow for all studies, the studies should be prioritized in the following order:

1. Bone marrow for Minimal Residual Disease Testing (See Section 14.2)
2. Bone marrow for *GATA1* (See Section 14.3)
3. Bone marrow for viable cell banking (See Section 14.3)

15.1 Exploratory Aims

15.1.1 To determine the extent to which elimination of HD Ara-C from treatment of standard risk DS AML decreases adverse events and resource utilization in children with DS. With amendment #4A, this exploratory aim is complete.

Comparison will be made to predecessor study AAML0431, which included treatment with HD Ara-C. Since the first cycle of induction therapy is identical in both studies, comparison will be made for the time interval from end of Induction I to the completion of protocol therapy. Specifically, we will determine if elimination of HD Ara-C from treatment of standard risk DS AML results in:

15.1.1.1 A significant decrease of the number of days per patient spent on protocol therapy compared to predecessor study AAML0431.

Subjects who are MRD negative following Induction I, will continue on Arm A for SR patients with the elimination of HD Ara-C. We hypothesize that the elimination of the cycle of HD Ara-C will result in overall shorter treatment duration by reducing the total number of cycles of therapy as well as less cumulative myelotoxicity that might contribute to delays in starting subsequent cycles. A two-sample t-test will be used to compare average number of days per patient spent on protocol therapy for standard risk patients on AAML1531 compared to patients treated on AAML0431.

15.1.1.2 A significant decrease of the average number of days of hospitalization per patient compared to predecessor studies AAML0431 and A2971.

We similarly anticipate that the duration of hospitalization throughout protocol therapy will be reduced. The number of days in hospital will be determined for patients treated on AAML1531 Arm A and compared with the predecessor study A2971. A2971 patients with DS AML less than 4 years of age stayed a mean of 35.1 days in hospital (standard deviation 18.9) after the start of Induction II through the remainder of protocol therapy.[10] Data regarding days of hospitalization during protocol treatment were not collected for study AAML0431. These data, however, are available for a subset of patients who were

treated at centers contributing data to the Pediatric Health Information Systems (PHIS) database. Data from AAML0431 will be merged with PHIS resource utilization data as previously described.[53] Based on this prior experience, approximately 40% of patients enrolled on AAML0431 will have PHIS data available. Two-sample t-test will be used to compare average number of days of hospitalization per patient for standard risk patients on AAML1531 compared to patients treated on AAML0431 and A2971.

15.1.1.3 A significant decrease of the number (per patient) and rate (per duration of treatment) of sterile site infections compared to the predecessor study AAML0431.

In addition, we hypothesize that the elimination of HD Ara-C will lead to less morbidity compared to patients whose treatment historically included this cycle. On the predecessor study, AAML0431, the greatest number of adverse events occurred during the administration of HD Ara-C (27.5% of the total reported), with 65% of adverse events classified as Grade 3 or greater (6.5% were Grade 4). These included Grade 3 or greater sterile site bacterial infection in 23% of patients and febrile neutropenia in 30% of patients, resulting in the need for continuous hospitalization.[35] We anticipate fewer sterile site infections for patients with DS AML being treated on AAML1531 without HD Ara-C compared with the predecessor study, AAML0431, which included the use of HD Ara-C. The number (per patient) and rate (per duration of treatment) of sterile site infections of patients treated for DS AML on Arm A will be compared to AAML0431 after the start of Induction II. Poisson regression will be used to compare rate (per duration of treatment) of sterile site infections for standard risk AAML1531 patients with AAML0431 patients.

15.1.1.4 A significant decrease of resource utilization by AML treatment compared to the predecessor study AAML0431.

Given our anticipation of fewer sterile site infections and shorter hospitalization, we hypothesize that the elimination of HD Ara-C on AAML1531 will ultimately result in decreased resource utilization (after the start of Induction II on AAML1531 Arm A), as sepsis is the single most important factor determining length of stay and hospital costs in the treatment of children with leukemia.[29] Resource utilization will be compared between patients on AAML0431 and AAML1531 at PHIS sites by comparing rates of inpatient resource utilization including antimicrobial usage, ICU level care resources, standard pharmaceutical supportive care measures, and blood products. PHIS adjusted inpatient costs will also be compared between patients enrolled on AAML0431 and AAML1531.[54,55] These measurements of resource utilization and standardized costs will be compared between the current treatment protocol (Arm A, no HD Ara-C cycle) and predecessor study AAML0431, which included the use of HD Ara-C.

These data on duration of therapy, length of hospital admission and sterile site infections are routinely submitted for patients with AML. Analysis of these exploratory objectives is expected to measure potential gains made for SR DS AML patients by decreasing the numbers of adverse events through a reduction in treatment intensity.

15.1.2 To compare feasibility and analytical characteristics of flow cytometry, PCR and targeted error-corrected sequencing of GATA1 mutations as methods to detect MRD in DS AML (Exploratory aim 1.2.1)

Background. *MRD.* The assessment of early response to treatment by measuring minimal residual leukemic disease (MRD) in the bone marrow has provided a new, independent and powerful prognostic marker for children with leukemia.[38,41] In clinical practice, the blasts of acute lymphoblastic leukemia (ALL) can be detected by multi-dimensional flow cytometry based on patterns of surface and cytoplasmic proteins that are either different from normal hematopoietic cells[41] or associated with a patient's specific leukemia.[56] Alternatively, malignant lymphoblasts can be quantified at submicroscopic levels by DNA-based polymerase chain reactions designed to amplify an individual patient's clone-specific rearrangement of antigen receptor (immunoglobulin and T cell receptor) genes.[57] In contrast, detection of MRD in acute myeloid leukemia (AML) relies predominantly on multi-dimensional flow cytometry[58] due to the absence of widely prevalent, clone-specific molecular lesions.[59] The blasts of AML in young children with Down syndrome (DS), however, typically contain somatic, clone-specific mutations of the gene encoding the hematopoietic transcription factor *GATA1* on the X chromosome.[7] These mutations consist of short insertions, deletions and point mutations clustering within and adjacent to exon 2.[6,7,60] As a result, measurement of MRD in DS AML is uniquely feasible both by multi-dimensional flow cytometry and DNA-based methods.

Methods. Multi-dimensional flow cytometry has the advantage that it can be performed rapidly, but its sensitivity and specificity is limited in DS AML.[35] Of note, interpretation of flow cytometric MRD data in DS AML is complicated by the observation that normal bone marrow cells in DS may express patterns of surface markers that are absent from normal non-DS bone marrow (M. Loken, personal communication). These patterns must not be confused with evidence of residual leukemia (when defined as marker pattern that is different from normal). DNA-based methods, such as quantitative real time PCR can detect individual patients' clone-specific *GATA1* mutations in exon 2 using either mutation-specific primers (and a shared probe) or a mutation-specific probes (and shared primers) with high sensitivity and specificity but can encounter limitations to feasibility due to constraints on the design of primers and probes in a short exon. Digital PCR technology, with its increased options for primer design, and massively parallel sequencing, which circumvents the constraints of designing mutation-specific oligonucleotide primers and probes, provide promising new approaches to the detect *GATA1* mutations and measure MRD in DS AML.

Hypotheses. We hypothesize that measurement of MRD in DS AML by digital PCR to detect clone-specific *GATA1* mutations is feasible and both more sensitive and specific than multi-dimensional flow cytometry. We hypothesize further that

measurement of MRD in DS AML by error-corrected sequencing (ECS) of exon 2 of *GATA1* to detect clone-specific mutations is as sensitive and specific as digital PCR but more cost-effective (since there is no need for mutation-specific primer and probe design). ECS has shown sensitivity for single base substitutions to 1:10,000, validated by digital PCR[61] and comparable to flow cytometry or RT-PCR.[61]

Aims

1. To determine the feasibility, sensitivity and specificity of digital PCR as a method to detect MRD in DS AML (by amplifying each patient's clone-specific *GATA1* mutation) compared to flow cytometry.

2. To determine the feasibility, sensitivity, specificity and cost of targeted, error-corrected sequencing (of a region encompassing exon 2 of *GATA1*) as a method to detect MRD in DS AML compared to digital PCR and flow cytometry.

Experimental Plan. DNA will be extracted from the diagnostic bone marrow samples of the patients with DS AML accrued by AAML1531. Of the 240 evaluable patients expected to enroll on AAML1531, 228 (95%) are anticipated to have diagnostic bone marrow available for analysis. Somatic *GATA1* mutations will be determined for all samples. MRD will be measured on Day 28 after the start of the first cycle of induction chemotherapy (Induction I). During this interval, a patient-specific *GATA1* mutation probe will be designed and tested for sensitivity and specificity in serial dilutions of diagnostic bone marrow DNA. Suitable probes will achieve $>10^{-4}$ *GATA1* mutation-positive blasts in a background of normal bone marrow DNA. In parallel to MRD measurement by multi-dimensional flow cytometry, for patients who consent to this optional study, bone marrow samples obtained on Day 28 will be assessed for MRD by digital PCR (using the prepared probe and standard primer combinations) as well as ECS of exon 2 of *GATA1* (using high-fidelity PCR amplification across the 240 base pair exon, which can be individually barcoded and multiplexed for simultaneous amplification from multiple patients).

Analysis Plan. To determine feasibility, the proportion of cases will be recorded for which the design of a sufficiently sensitive primer and probe combination for digital PCR was successful by Day 28. Similarly, the proportion of cases will be determined for which the requirements for DNA quality and multiplex amplification were met. Library preparation, sequencing and bioinformatic analysis will follow previously established protocols.[62] Positive and negative percent agreement of MRD results obtained on Day 28 will be compared between flow cytometry, digital PCR and ECS for each pair of detection methods. Corresponding 95% confidence intervals will be calculated for the percent positive and negative agreement for each pair of detection methods. McNemar's test will be used to compare percent positive and negative agreement for two detection methods relative to the third detection method. MRD results obtained with each method will be correlated with the probability of disease-free survival and the cumulative incidence of relapse.

Relevance. This direct comparison of methods is expected to determine the optimal method to measure treatment response by MRD and predict relapse risk in DS AML in the context of risk-based treatment assignment by flow cytometry based MRD. Results, therefore, will establish the foundation of accurate risk-based stratification of treatment intensity for future therapeutic trials in DS AML.

15.1.3 To establish a DS AML cell bank of viably frozen bone marrow samples and corresponding non-tumor DNA samples collected at end of induction.

Background. The exploratory aim 1.2.2 is to establish a biobank of viably frozen bone marrow samples from DS AML patients collected at the end of Induction I for future research.

Experimental Plan. Bone marrow cells will be collected at end of Induction 1 and viably cryopreserved for those patients who consent to this optional study. Bone marrow samples collected at the time point of MRD analysis, which in the majority of cases will be obtained at the time of morphological remission, will provide non-tumor DNA for genomic analyses of DS AML blasts.

Relevance. The repository of viably cryopreserved bone marrow samples of DS AML, remission samples and other sources non-tumor DNA will provide essential support for the objectives of this study and serve as a vital resource for future mechanistic studies in DS AML.

APPENDIX I: CTEP AND CTSU REGISTRATION PROCEDURES

INVESTIGATOR AND RESEARCH ASSOCIATE REGISTRATION WITH CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR — MD, DO, or international equivalent;
- NPIVR — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- AP — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges;
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
Agent Shipment Form (if applicable)	✔				
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol Principal Investigator (PI) on the IRB approval
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the CI on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

CTSU REGISTRATION PROCEDURES

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Requirements For AAML1531 **Site Registration:**

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

Submitting Regulatory Documents:
Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal log in to the CTSU members' website, go to the Regulatory section and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Checking Your Site's Registration Status

Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration; and*
- Enter the site's 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

Data Submission / Data Reporting

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

- A valid CTEP-IAM account; and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

Rave role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR); and
- Rave Read Only role must have at a minimum an Associates (A) registration type.

Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, and DQP Delinquent Forms modules.

Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

APPENDIX II: WHO CLASSIFICATION OF AML[63]

Acute myeloid leukemia and related neoplasms

- Acute myeloid leukemia with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
  - APL with t(15;17)(q22;q12); *PML-RARA*
  - AML with t(9;11)(p22;q23); *MLLT3-MLL*
  - AML with t(6;9)(p23;q34); *DEK-NUP214*
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
  - AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
  - *Provisional entity: AML with mutated NPM1*
  - *Provisional entity: AML with mutated CEBPA*
- Acute myeloid leukemia with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Acute myeloid leukemia, not otherwise specified
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Acute erythroid leukemia
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

APPENDIX III: WHO DEFINITION OF MYELODYSPLASTIC SYNDROME (MDS)[63]

In cases where the WHO criteria do not appear clear, please discuss with study chairs or study pathologist (karen.chisholm@seattlechildrens.org).

Disease	Peripheral Blood Findings	BM findings
Refractory cytopenia with unilineage dysplasia (RCUD): (refractory anemia [RA]; refractory neutropenia [RN]; refractory thrombocytopenia [RT])	Unicytopenia or bicytopenia* No or rare blasts (< 1%)†	Unilineage dysplasia: ≥ 10% of the cells in one myeloid lineage < 5% blasts < 15% of erythroid precursors are ring sideroblasts
Refractory anemia with ring sideroblasts (RARS)	Anemia No blasts	≥ 15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (< 1%)† No Auer rods< 1 × 10^9/L monocytes	Dysplasia in ≥ 10% of the cells in ≥ 2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) < 5% blasts in marrow No Auer rods ± 15% ring sideroblasts
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s)< 5% blasts† No Auer rods< 1 × 10^9/L monocytes	Unilineage or multilineage dysplasia 5%-9% blasts† No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s)5%-19% blasts† Auer rods ±†< 1 × 10^9/L monocytes	Unilineage or multilineage dysplasia 10%-19% blasts† Auer rods ±†
Myelodysplastic syndrome—unclassified (MDS-U)	Cytopenias< 1% blasts†	Unequivocal dysplasia in < 10% of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS (see Table 6) < 5% blasts
MDS associated with isolated del(5q)	Anemia Usually normal or increased platelet count No or rare blasts (< 1%)	Normal to increased megakaryocytes with hypolobated nuclei < 5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

- * Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U.
- † If the marrow myeloblast percentage is < 5% but there are 2% to 4% myeloblasts in the blood, the diagnostic classification is RAEB-1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS-U.
- ‡ Cases with Auer rods and < 5% myeloblasts in the blood and less than 10% in the marrow should be classified as RAEB-2. Although the finding of 5% to 19% blasts in the blood is, in itself, diagnostic of RAEB-2, cases of RAEB-2 may have < 5% blasts in the blood if they have Auer rods or 10% to 19% blasts in the marrow or both. Similarly, cases of RAEB-2 may have < 10% blasts in the marrow but may be diagnosed by the other 2 findings, Auer rod+ and/or 5% to 19% blasts in the blood.

APPENDIX IV: POSSIBLE DRUG INTERACTIONS

The lists below do not include everything that may interact with chemotherapy. You should be encouraged to talk to your child's doctors before starting any new medications, using over-the-counter medicines, or herbal supplements and before making a significant change in the diet.

Cytarabine (IV)

Some drugs, food, and supplements may interact with cytarabine (be vein). Examples include:

Drugs that may interact with cytarabine
• Clozapine, natalizumab, flucytosine, leflunomide

Food and supplements that may interact with cytarabine
Echinacea

Daunorubicin

Some drugs, food, and supplements may interact with daunorubicin. Examples include:

Drugs that may interact with daunorubicin
• Some antibiotics and antifungals (clarithromycin, erythromycin, itraconazole, ketoconazole, rifampin) • Some antiepileptics (carbamazepine, phenobarbital, phenytoin, fosphenytoin) • Some antiretrovirals (lapatinib, lopinavir; nelfinavir, ritonavir, saquinavir, telaprevir, tipranavir) • Some heart medications (amiodarone, carvedilol, digoxin, dronedarone, quinidine, propafenone, verapamil) • Some chemotherapy (be sure to talk to your doctor about this)   • Ado-trastuzumab emtansine, bevacizumab, trastuzumab, taxane derivatives) • Other agents, such as atorvastatin, clozapine, cyclosporine, dexamethasone, ivacaftor, leflunomide, lumacaftor, natalizumab, nefazodone, progesterone, ranolazine, rifampin, tacrolimus, tofacitinib, and trazodon

Food and supplements that may interact with daunorubicin
• Echinacea • Grapefruit, grapefruit juice, Seville oranges, star fruit • St. John's Wort • Drinks, food, supplements, or vitamins containing "flavonoids" or other "antioxidants"

Etoposide

Some drugs, food, and supplements may interact with etoposide. Examples include:

Drugs that may interact with etoposide

• Antibiotics
  ○ Clarithromycin, erythromycin, nafcillin, rifapentin, rifampin, telithromycin
• Antidepressants and antipsychotics
  ○ Clozapine, nefazodone
• Antifungals
  ○ Fluconazole, itraconazole, ketoconazole, posaconazole, voriconazole
• Arthritis medications
  ○ Leflunomide, tofacitinib
• Anti-rejection medications
  ○ Cyclosporine
• Antiretrovirals and antivirals
  ○ Atazanavir, darunavir, delaviridine, efavirenz, etravirine, fosamprenavir, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, Stribild®, telaprevir,
• Anti-seizure medications
  ○ Carbamazepine, fosphenytoin, phenobarbital, phenytoin, primidone
• Heart medications
  ○ Amiodarone, dronenarone, verapamil
• Some chemotherapy (be sure to talk to your doctor about this)
• Many other drugs, including the following:
  ○ Aprepitant, atovaquone, bosentan, deferasirox, ivacaftor, lomitapide, mifepristone, modafinil, natalizumab, pimozide

Food and supplements that may interact with etoposide
• Echinacea • Glucosamine • St. John's Wort • Grapefruit, grapefruit juice, Seville oranges, star fruit

Mitoxantrone

Some drugs, food, and supplements may interact with mitoxantrone. Examples include:

Drugs that may interact with mitoxantrone
• Aripiprazole • Clozapine • Cyclosporine • Eltrombopag • Leflunomide • Natalizumab • Tofacitinib

Food and supplements that may interact with mitoxantrone
• Echinacea

Thioguanine

Some drugs, food, and supplements may interact with thioguanine. Examples include:

Drugs that may interact with thioguanine
• Arthritis medications: leflunomide, tofacitinib • Other medications, such as adalimumab, allopurinol, azathioprine, certolizumab pegol, clozapine, etanercept, golimumab, infliximab, natalizumab, olsalazine, sulfasalazine

Food and supplements that may interact with thioguanine
• Echinacea

APPENDIX V: DEFINITION OF CNS LEUKEMIA

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

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

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

REFERENCES

1. Ravindranath Y, Abella E, Krischer JP, et al: Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. Blood 80:2210-4, 1992
2. Hasle H, Clemmensen IH, Mikkelsen M: Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet 355:165-9, 2000
3. Gamis AS, Woods WG, Alonzo TA, et al: Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. J Clin Oncol 21:3415-22, 2003
4. Lange BJ, Kobrinsky N, Barnard DR, et al: Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood 91:608-15, 1998
5. Hitzler JK, Cheung J, Li Y, et al: GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood 101:4301-4, 2003
6. Hitzler JK, Zipursky A: Origins of leukaemia in children with Down syndrome. Nat Rev Cancer 5:11-20, 2005
7. Wechsler J, Greene M, McDevitt MA, et al: Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet 32:148-52, 2002
8. Ge Y, Stout ML, Tatman DA, et al: GATA1, cytidine deaminase, and the high cure rate of Down syndrome children with acute megakaryocytic leukemia. J Natl Cancer Inst 97:226-31, 2005
9. Gamis AS, Alonzo TA, Gerbing RB, et al: Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. Blood 118:6752-9; quiz 6996, 2011
10. Sorrell AD, Alonzo TA, Hilden JM, et al: Favorable survival maintained in children who have myeloid leukemia associated with Down syndrome using reduced-dose chemotherapy on Children's Oncology Group trial A2971: a report from the Children's Oncology Group. Cancer 118:4806-14, 2012
11. Kudo K, Kojima S, Tabuchi K, et al: Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol 25:5442-7, 2007
12. Creutzig U, Reinhardt D, Diekamp S, et al: AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia 19:1355-60, 2005
13. Ravindranath Y, Chang M, Steuber CP, et al: Pediatric Oncology Group (POG) studies of acute myeloid leukemia (AML): a review of four consecutive childhood AML trials conducted between 1981 and 2000. Leukemia 19:2101-16, 2005
14. Ravindranath Y, Steuber CP, Krischer J, et al: High-dose cytarabine for intensification of early therapy of childhood acute myeloid leukemia: a Pediatric Oncology Group study. J Clin Oncol 9:572-80, 1991
15. Creutzig U, Zimmermann M, Lehrnbecher T, et al: Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol 24:4499-506, 2006
16. Hitzler JK, He W, Doyle J, et al: Outcome of transplantation for acute myelogenous leukemia in children with Down syndrome. Biol Blood Marrow Transplant 19:893-7, 2013
17. Taga T, Saito AM, Kudo K, et al: Clinical characteristics and outcome of refractory/relapsed myeloid leukemia in children with Down syndrome. Blood 120:1810-5, 2012
18. Meissner B, Borkhardt A, Dilloo D, et al: Relapse, not regimen-related toxicity, was the major cause of treatment failure in 11 children with Down syndrome undergoing haematopoietic stem cell transplantation for acute leukaemia. Bone Marrow Transplant 40:945-9, 2007

19. Gibson BE, Webb DK, Howman AJ, et al: Results of a randomized trial in children with Acute Myeloid Leukaemia: medical research council AML12 trial. Br J Haematol 155:366-76, 2011
20. Jeffrey W Taub, Jason N Berman, Johann K. Hitzler, et al: Improvement in Treatment Outcome and Identification of a New Prognostic Parameter in Down Syndrome Acute Myeloid Leukemia (DS-AML): Results of the Children's Oncology Group (COG) Phase III AAML0431 Trial. Abstract #278, 56th American Society of Hematology Annual Meeting, San Francisco, CA, Dec 6-9, 2014
21. Rao A, Hills RK, Stiller C, et al: Treatment for myeloid leukaemia of Down syndrome: population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. Br J Haematol 132:576-83, 2006
22. Zwaan CM, Kaspers GJ, Pieters R, et al: Cellular drug resistance in childhood acute myeloid leukemia is related to chromosomal abnormalities. Blood 100:3352-60, 2002
23. Taub JW, Huang X, Matherly LH, et al: Expression of chromosome 21-localized genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. Blood 94:1393-400, 1999
24. Taub JW, Ge Y: Down syndrome, drug metabolism and chromosome 21. Pediatr Blood Cancer 44:33-9, 2005
25. Ge Y, Dombkowski AA, LaFiura KM, et al: Differential gene expression, GATA1 target genes, and the chemotherapy sensitivity of Down syndrome megakaryocytic leukemia. Blood 107:1570-81, 2006
26. Xavier AC, Edwards H, Dombkowski AA, et al: A unique role of GATA1s in Down syndrome acute megakaryocytic leukemia biology and therapy. PLoS One 6:e27486, 2011
27. Al-Ahmari A, Shah N, Sung L, et al: Long-term results of an ultra low-dose cytarabine-based regimen for the treatment of acute megakaryoblastic leukaemia in children with Down syndrome. Br J Haematol 133:646-8, 2006
28. Tandonnet J, Clavel J, Baruchel A, et al: Myeloid leukaemia in children with Down syndrome: report of the registry-based French experience between 1990 and 2003. Pediatr Blood Cancer 54:927-33, 2010
29. Allareddy V, Rampa S, Allareddy V: Hospital charges and length of stay associated with septicemia among children hospitalized for leukemia treatment in the United States. World J Pediatr 8:222-8, 2012
30. Lipshultz SE, Giantris AL, Lipsitz SR, et al: Doxorubicin administration by continuous infusion is not cardioprotective: the Dana-Farber 91-01 Acute Lymphoblastic Leukemia protocol. J Clin Oncol 20:1677-82, 2002
31. Lipshultz SE, Miller TL, Lipsitz SR, et al: Continuous Versus Bolus Infusion of Doxorubicin in Children With ALL: Long-term Cardiac Outcomes. Pediatrics 130:1003-11, 2012
32. Levitt GA, Dorup I, Sorensen K, et al: Does anthracycline administration by infusion in children affect late cardiotoxicity? Br J Haematol 124:463-8, 2004
33. Gupta M, Steinherz PG, Cheung NK, et al: Late cardiotoxicity after bolus versus infusion anthracycline therapy for childhood cancers. Med Pediatr Oncol 40:343-7, 2003
34. Gamis AS, Alonzo TA, Meshinchi S, et al: Gemtuzumab Ozogamicin in Children and Adolescents With De Novo Acute Myeloid Leukemia Improves Event-Free Survival by Reducing Relapse Risk: Results From the Randomized Phase III Children's Oncology Group Trial AAML0531. J Clin Oncol, 2014
35. Taub J, Berman J, Hitzler J, et al: Minimal Residual Disease (MRD) Identifies Down Syndrome Acute Myeloid Leukemia (DS-AML) Patients with Extremely High Event Free Survival (EFS) Rates: Results of the Children's Oncology Group (COG) Phase III AAML0431 Trial. ASH Abstract 2014.

36. Abildgaard L, Ellebaek E, Gustafsson G, et al: Optimal treatment intensity in children with Down syndrome and myeloid leukaemia: data from 56 children treated on NOPHO-AML protocols and a review of the literature. Ann Hematol 85:275-80, 2006
37. Gibson BE, Wheatley K, Hann IM, et al: Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. Leukemia 19:2130-8, 2005
38. Borowitz MJ, Devidas M, Hunger SP, et al: Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 111:5477-85, 2008
39. Rubnitz JE, Inaba H, Dahl G, et al: Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. Lancet Oncol 11:543-52, 2010
40. van der Velden VH, van der Sluijs-Geling A, Gibson BE, et al: Clinical significance of flowcytometric minimal residual disease detection in pediatric acute myeloid leukemia patients treated according to the DCOG ANLL97/MRC AML12 protocol. Leukemia 24:1599-606, 2010
41. Loken MR, Alonzo TA, Pardo L, et al: Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. Blood 120:1581-8, 2012
42. Sievers EL, Lange BJ, Alonzo TA, et al: Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 patients with acute myeloid leukemia. Blood 101:3398-406, 2003
43. Sorrell AD, Taub J, Alonzo TA, et al: Remission Marrow Blast Percentage Predicts Relapse Risk In Children With Myeloid Leukemia Associated With Down Syndrome. Pediatr Blood Cancer. 58:1039, 2012
44. Zeijlemaker W, Gratama JW, Schuurhuis GJ: Tumor heterogeneity makes AML a "moving target" for detection of residual disease. Cytometry B Clin Cytom 86:3-14, 2014
45. Hasle H, Abrahamsson J, Arola M, et al: Myeloid leukemia in children 4 years or older with Down syndrome often lacks GATA1 mutation and cytogenetics and risk of relapse are more akin to sporadic AML. Leukemia 22:1428-30, 2008
46. Blink M, van den Heuvel-Eibrink MM, de Haas V, et al: Myeloid Leukemia of Down Syndrome: The Results of an International Retrospective Study. Blood. 116:1120, 2010
47. Blink M, Zimmermann M, von Neuhoff C, et al: Normal karyotype is a poor prognostic factor in myeloid leukemia of Down syndrome: a retrospective, international study. Haematologica 99:299-307, 2014
48. ZINECARD® (dexrazoxane) product monograph. Pfizer Canada Inc. Date of Preparation: July 2012 www.pfizer.ca (last accessed March 12, 2015).
49. Marty M, Espie M, Llombart A, et al: Multicenter randomized phase III study of the cardioprotective effect of dexrazoxane (Cardioxane) in advanced/metastatic breast cancer patients treated with anthracycline-based chemotherapy. Ann Oncol 17:614-22, 2006
50. Schwartz GJ, Gauthier B: A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr 106:522-6, 1985
51. Smith GA, Damon LE, Rugo HS, et al: High-dose cytarabine dose modification reduces the incidence of neurotoxicity in patients with renal insufficiency. J Clin Oncol 15(2):833-839, 1997
52. Kalbfleisch JD, Prentice RL: The statistical analysis of failure time data (ed 2nd ed.). Hoboken, N.J, J. Wiley, 2002, xiii
53. Aplenc R, Fisher BT, Huang YS, et al: Merging of the National Cancer Institute-funded cooperative oncology group data with an administrative data source to develop a more effective platform for clinical trial analysis and comparative effectiveness research: a report from the Children's Oncology Group. Pharmacoepidemiol Drug Saf 21 Suppl 2:37-43, 2012
54. Aplenc R, Fisher B T, Li Y, et al: Standardized costs and outcome in children treated with gemtuzumab on the AAML0531 trial: a report from the Children's Oncology Group. J Clin Oncol 32(5s): Suppl; abstr 7086, 2014

55. Kavcic M, Fisher BT, Li Y, et al: Induction mortality and resource utilization in children treated for acute myeloid leukemia at free-standing pediatric hospitals in the United States. Cancer 119:1916-23, 2013
56. Campana D: Minimal residual disease in acute lymphoblastic leukemia. Semin Hematol 46:100-6, 2009
57. Conter V, Bartram CR, Valsecchi MG, et al: Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood 115:3206-14, 2010
58. Loken MR: Residual disease in AML, a target that can move in more than one direction. Cytometry B Clin Cytom 86:15-7, 2014
59. Tarlock K, Meshinchi S: Pediatric acute myeloid leukemia: biology and therapeutic implications of genomic variants. Pediatr Clin North Am 62:75-93, 2015
60. Klusmann JH, Creutzig U, Zimmermann M, et al: Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. Blood 111:2991-8, 2008
61. Young A, Wong T, Hughes A, et al: Quantifying ultra-rare pre-leukemic clones via targeted error-corrected sequencing. Leukemia 2015 (in press)
62. Wong TN, Ramsingh G, Young AL, et al: Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature, 2014
63. Vardiman JW, Thiele J, Arber DA, et al: The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 114:937-51, 2009