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AML08: A PHASE III RANDOMIZED TRIAL OF CLOFARABINE PLUS CYTARABINE VERSUS CONVENTIONAL INDUCTION THERAPY AND A PHASE II STUDY OF NATURAL KILLER CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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AML08: A PHASE III RANDOMIZED TRIAL OF CLOFARABINE PLUS CYTARABINE VERSUS CONVENTIONAL INDUCTION THERAPY AND A PHASE II STUDY OF NATURAL KILLER CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

Letter of Amendment

January 9, 2014

This letter is being sent as clarification that collaborating sites may choose to not participate in the vorinostat exploratory objective of this study, as proposed with amendment 9.0.

If the decision is made that a site will opt out, then all applicable patients at that site will receive standard therapy for Induction II with LD-ADE alone.

Since this objective is exploratory and does not require a specific number of patients, this will not adversely affect the study analyses.

This information will be forthcoming with the next protocol amendment; however, sites may choose to opt out at any time.

AML08: A PHASE III RANDOMIZED TRIAL OF CLOFARABINE PLUS CYTARABINE VERSUS CONVENTIONAL INDUCTION THERAPY AND A PHASE II STUDY OF NATURAL KILLER CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

Letter of Amendment

November 20, 2013

Rationale

This letter is being sent as clarification that if Erwinia asparaginase is given (see Section 4.5 of protocol), it may be administered according to local institutional guidelines, either IV or IM.

Currently, the protocol and consent state IV administration. These will be revised to IV or IM with the next protocol amendment.

Collaborating sites may follow local administration guidelines for the administration of this commercially available agent. The following St. Jude guidelines may be used, but are not required:

Administration

For IV administration, *Erwinia* asparaginase will be diluted in 50 mL NS to infuse over 30 minutes to 1 hour via syringe. *Erwinia* asparaginase should be administered to run concurrently with IVF. (NS at TKO is sufficient.)

Erwinia asparaginase should be administered at least 2 hours after an LP/IT since some cases of over sedation were reported when given "simultaneously" with sedation and/or an LP/IT at other institutions. Further, it is desirable to avoid asparaginase directly before an IT because asparaginase could possibly interfere with the efficacy of methotrexate.

Erwinia asparaginase may also be administered IM.

Monitoring and concerns for anaphylaxis

Obtain vital signs pre infusion, and then remain at the patient's bedside for the first 5 minutes. Visually observe the patient at 10 minutes, and directly observe and obtain vital signs at 15 minutes and at the end of the infusion. Repeat vital signs at discharge. Patients must remain in

the Medicine Room for 1 hour after administration to be observed for adverse effects. Keep NS at TKO during the 1 hour post watch time unless otherwise ordered. To prepare for anaphylaxis:

- Have oxygen, suction and pulse oximetry at bedside during and after infusion
- Have readily available* the following medications:

    - Diphenhydramine 1 mg/kg (max 50 mg) for IV administration
    - Epinephrine (1:1000) 0.01 mL/kg (max 0.3 mL) for **SQ** administration
    - Hydrocortisone 100 mg/m^2 for IV administration
    - NS for IV administration

*Readily available means in the general area – such as the emergency medication box in the medicine room or inpatient areas.

St. Jude Children's Research Hospital
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Finding cures. Saving children.

MEMORANDUM
Department of Oncology

TO: IRB/CPSRMC

FROM: Jeffrey E. Rubnitz, MD, PhD

DATE: June 22, 2010

RE: AML08: A PHASE III RANDOMIZED TRIAL OF CLOFARABINE PLUS CYTARABINE VERSUS CONVENTIONAL INDUCTION THERAPY AND A PHASE II STUDY OF NATURAL KILLER CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

LETTER OF AMENDMENT: DELETION OF MYLOTARG

This letter serves as notification to the St. Jude IRB, collaborating sites, and the FDA that **effective immediately, patients will NOT receive mylotarg (gemtuzumab ozogamicin) on any treatment arm on AML08**. Amendment 5.0 will be forthcoming shortly to address this, as well as to provide further guidance on notifying patients enrolled on the trial. However, due to the safety concerns, this information is being distributed now, so that this action can be implemented immediately at all sites participating in this trial.

This action is a result of the attached FDA Safety Notification (dated 6/21/2010) of the market withdrawal of Mylotarg due to new concerns about the product's safety and the drug's failure to demonstrate clinical benefit to patients enrolled in trials. Further information can also be found at the following website:

http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm216458.htm

Collaborating sites are requested to notify their IRBs as soon as possible, but no later than July 30, 2010 of this change and all treating investigators are instructed to discontinue the use of this drug on AML08.

Please contact the principal investigator with any questions or concerns.

cc: FDA
Investigational Pharmacists
AML08 Collaborating Institutions

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Attached supporting documents:

Therapeutic Cell Infusion
Informed Consent Statements

1.0 OBJECTIVES

The overall objective of this protocol is to improve the cure rate of acute myeloid leukemia (AML).

1.1 Primary objective

To compare the immunologic complete response rate after one course of therapy in patients who receive cytarabine + daunorubicin + etoposide (ADE) with that in patients who receive clofarabine + cytarabine (Clo/AraC).

1.2 Secondary objective

To estimate the event-free survival (EFS) of standard risk (SR) patients who receive chemotherapy alone and the EFS of SR patients who receive chemotherapy followed by natural killer (NK) cell transplantation.

1.3 Exploratory objectives

1.3.1 To genotype natural killer (NK) cell receptors and measure their expressions at diagnosis and after induction therapy, and to explore the associations of these features with treatment outcome.

1.3.2 To assess the prognostic value of levels of minimal residual disease in peripheral blood at day 8 of induction I.

1.3.3 To validate new markers and methods for minimal residual disease (MRD) detection.

1.3.4 To identify new prognostic factors by applying new technologies to study patient material.

1.3.5 To identify pharmacogenetic, pharmacokinetic and pharmacodynamic predictors for treatment-related outcomes in the context of the systemic therapy used in the protocol.

1.3.6 To describe the impact of antibiotic and antifungal prophylaxis on invasive bacterial and fungal infections, febrile neutropenia, hospitalization, and antibiotic resistance.

1.3.7 To explore the feasibility and toxicity of administering vorinostat in combination with chemotherapy in selected high-risk patients.

2.0 BACKGROUND AND RATIONALE

2.1 Background and rationale for therapy

2.1.1 Introduction

Although the treatment outcome for children with leukemia has improved dramatically during the past 20 years, the cure rate for AML continues to lag behind that achieved for children with acute lymphoblastic leukemia (ALL). Currently, greater than 80% of children with ALL are cured, compared to only about 50% of those with AML.[1-5] Most patients with AML achieve remission after 1 to 2 courses of induction therapy, but approximately half of these patients suffer relapse of their disease. In addition, despite improvements in supportive care, treatment-related morbidity and mortality remain significant problems.[6,7] Therefore, the overall goal of our AML program is to develop novel therapies that overcome drug resistance, decrease relapse rates, and reduce the short- and long-term adverse effects of treatment. The present study will assess the feasibility and efficacy of a novel form of therapy – haploidentical NK cell transplantation – in patients with standard-risk AML. In addition, we will investigate the efficacy of clofarabine + cytarabine (Clo/AraC) in newly diagnosed patients with AML and attempt to optimize outcome through the use of MRD-adapted therapy and further improvements in supportive care.

Initially, AML08 included 5 courses of chemotherapy for the following reasons: a) the data from the MRC AML12 trial had not yet been published, b) the COG trial used 5 courses, and c) five courses was, at that time, considered "standard of care." The MRC AML12 results have now been published (J Clinical Oncology 28:586-595, 2010). Briefly, the results show that among 992 patients randomized to receive 4 or 5 courses of chemotherapy, there were no differences in OS (52% vs. 53%) or relapse (54% vs. 49%). Among the 270 children randomized to 4 vs. 5 courses, there were no differences in relapse (36% vs. 36%), DFS (63% vs. 62%), or OS (74% vs. 74%). Based, in part, on these results, the upcoming COG trial will administer 4 courses of chemotherapy. Because there appears to be no benefit to course #5 (consolidation III), but some risks (mortality, morbidity, and quality of life), this amendment will eliminate consolidation III.

2.1.2 Overview of clofarabine

Clofarabine (2-chloro-9-[2'-deoxy-2'-fluoro-β-D-arabinofuranosyl]adenine; Cl-F-ara-A; CAFdA) is a rationally designed, second generation purine nucleoside analogue. Clofarabine was designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both fludarabine (F-ara-A) and cladribine (2-CdA, CdA) both of which are currently approved by various regulatory authorities for treatment of hematologic malignancies. Because clofarabine has a chloro- group at the 2-position of adenine, its chemical structure is more closely related to 2-CdA than to F-ara-A. Halogenation at the 2-position of adenine renders this class of compounds resistant to intracellular degradation by the enzyme adenosine deaminase. Substitution of a fluorine at the C-2'-position of the arabinofuranosyl moiety of clofarabine increases its stability in gastric acid and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme *Escherichia coli* purine nucleoside phosphorylase in the gastrointestinal tract, both of which may lead to enhanced oral bioavailability.[8,9] Clofarabine was approved in December 2004 by the

United States Food and Drug Administration (US FDA) for the treatment of pediatric patients with relapsed or refractory acute lymphoblastic leukemia (ALL) after at least 2 prior regimens based on the induction of complete responses.

Mechanism of action

The precise mechanism of action of clofarabine on dividing and non-dividing cells is unknown. Like the other nucleoside analogues (cytarabine, ara-A [vidarabine], cladribine, fludarabine), clofarabine must be converted to the 5'-triphosphate form by deoxycytidine kinase (dCK) to be active within cells. Clofarabine is more efficient as a substrate for purified recombinant dCK, exceeding cladribine and the natural substrate, deoxycytidine.[10] Evidence suggests that the primary cytotoxic effect of clofarabine is due to its inhibition of DNA synthesis. The triphosphate form of clofarabine is an inhibitor of both DNA polymerase α and ribonucleotide reductase.[11] These effects lead to depletion of intracellular deoxynucleotide triphosphate pools, and inhibition of elongation of DNA strands during synthesis.[12] With respect to inhibition of ribonucleotide reductase, clofarabine and cladribine are superior to fludarabine.[10] With respect to inhibition of DNA polymerase α, clofarabine and fludarabine are similar and both are superior to cladribine.[10] Thus, in comparison to cladribine and fludarabine, clofarabine more completely inhibits both ribonucleotide reductase and DNA polymerase α, versus one or the other.

Unlike fludarabine, clofarabine is active in non-dividing cells and in cells with a low proliferation rate. Clofarabine can induce the apoptotic pathway as part of its cytotoxic effect on cells.[9] Clofarabine has been shown to disrupt the integrity of mitochondria in primary chronic lymphocytic leukemia (CLL) cells. The damage leads to release of pro-apoptotic mitochondrial factors.[13] These effects are postulated to induce apoptosis in indolent, non-dividing CLL cells. This result was not seen with fludarabine and may explain, at least in part, the enhanced cytotoxicity of clofarabine though the physiologic and clinical implications of these observations remain uncertain and under continued investigation.

Pharmacokinetics of clofarabine in adult patients with AML

Pharmacokinetic data were collected from 13 adult patients with refractory or relapsed AML in an open-label study in which they were treated with clofarabine 40 mg/m^2/day IV infusion over 1 hour for 5 consecutive days (Clinical Report – Protocol CLO-221, February 13, 2004). Stationary pharmacokinetics were observed between Days 1 and 5, and plasma concentrations declined rapidly thereafter and exhibited biphasic kinetics. The estimated terminal half-life was approximately 6 hours and ranged from 4.1 to 8.6 hours. Consistent with this short half-life, pre-dose concentrations on Day 2 were about 10% or less of maximal concentrations at the end of infusion. After 4 days of dosing, pre-dose concentrations averaged 13.8 ng/mL and ranged from 4.0 to 23.1 ng/mL. Because of the short half-life of clofarabine, there was little-to-negligible accumulation with once daily dosing of clofarabine at 40 mg/m^2 by 1-hour IV infusion.

Clinical experience with clofarabine

The maximum tolerated dose (MTD) of clofarabine IV in adult patients has been determined to be 40 mg/m^2/day for 5 consecutive days, which is lower than the tolerable daily dose for pediatric patients, 52 mg/m^2/day.

Relapsed, refractory adult AML

Phase I trials were initiated in 1999 and the first study was a traditional dose-escalation study where the objective was to establish the MTD in adult patients with solid tumors or hematologic malignancies.[14] The starting dose was 15 mg/m^2 administered daily for 5 days based on an animal study in which this dose was safe with no observable toxicities. However, 2 of the first few patients on study experienced myelosuppression and required dose de-escalations before the MTD of 2 mg/m^2 was identified in patients with solid tumors. Dose escalation in patients with hematologic malignancies increased to 55 mg/m^2, at which point patients experienced dose-limiting toxicities (DLTs) of reversible hepatotoxicity, and the MTD was determined to be 40 mg/m^2/day. Among the 32 patients diagnosed with acute leukemia, 2 patients achieved a CR and 3 achieved CRp for an overall response rate of 16%. Clofarabine pharmacokinetics were dose proportional across all the doses studied, but intracellular clofarabine triphosphate (which had large interpatient variability) began to show saturation at doses greater than about 20 mg/m^2/day.

In a Phase II study reported by Kantarjian et al,[15] 62 adult patients with relapsed or refractory acute leukemia received clofarabine 40 mg/m^2 IV once daily for 5 days every 3 to 6 weeks. Twenty (20) patients achieved a CR, 9 achieved a CRp, and 1 achieved a partial response for a total response rate of 48%. The predominant toxicities were reversible liver dysfunction (as indicated by elevated ALT and AST and hyperbilirubinemia), skin rashes, palmar-plantar erythrodysesthesia syndrome, and mucositis. No correlation was observed between plasma clofarabine concentrations and intracellular clofarabine triphosphate concentrations, although it was observed that responders showed an accumulation of intracellular clofarabine triphosphate on Day 2 compared to non-responders.

In contrast, a multi-center Phase II trial (CLO221) initiated in 2002 in adult patients with relapsed or refractory acute myelogenous leukemia received clofarabine 40 mg/m^2 once daily for 5 days every 28 days for 2 cycles with subsequent cycles being dosed with 30 mg/m^2.[16,17] Only 1/40 (3%) patients achieved a CR of 20.4 weeks duration. Nausea, vomiting, headache, diarrhea, anorexia, dermatitis, and stomatitis were the most frequently drug-related reported AEs. Drug-related renal toxicities were reported for 10% of the patients; however, these patients either had a concurrent clinical condition associated with renal toxicity or at least 1 concomitant medication known to increase the potential for renal toxicity. Pharmacokinetic data available for 33% of the patients indicate clofarabine had a high tissue distribution with minimal accumulation and rapid elimination (primarily as unchanged drug) in the urine. These seemingly discrepant results must be considered in the context of the patient populations treated; in the CLO-221 experience, approximately 25% of patients were refractory to prior therapy, whereas no subgroup of patients with primary refractory disease was reported in the prior experience conducted and reported by Kantarjian and colleagues.[15]

Based upon the single-agent activity observed with clofarabine in several populations of patients with relapsed or refractory acute leukemias, efforts have been undertaken to combine clofarabine with other anti-leukemic agents. Faderl and colleagues conducted a Phase I/II study in adult patients with first relapse or first salvage of primary refractory AML or ALL.[18] Patients were treated with doses of clofarabine of 15-40 mg/m^2/day by 1-hour IV infusion daily for Days 2 through 6 followed 4 hours later by cytarabine 1 g/m^2/day by 2-hour IV infusion daily for Days 1 through 5. Cytarabine alone was administered on Day 1 at the same dose. Thirty-two patients were treated; 12 in the Phase I portion and 20 in the Phase II portion at 40 mg/m^2/day. The Phase II dose of 40 mg/m^2/day was based on the absence of DLT at this dose in combination; this dose was not exceeded since it had been determined as the MTD from single-agent studies with clofarabine. The median patient age in this study was 60 (range: 18-84 years) and the majority of patients had AML (78%). Efficacy data are available for 32 patients; of these 7/32 (22%) achieved a complete response and an additional 5/32 (16%) achieved a complete response with inadequate platelet recovery for an overall response rate of 38% (12/32 patients). In the 25 patients with AML, CR was 28% and CRp 12% for an ORR of 40%. Two of four patients with MDS had a CRp. The most frequently reported drug-related AEs (i.e., those occurring in ≥20% of patients) were nausea, diarrhea, vomiting, dermatitis, flushing, palmar-plantar erythrodysesthesia syndrome, and headache. Changes in post-baseline chemistry parameters were mild to moderate in the majority of patients and were reversible if not attributable to the disease. Bone marrow function was suppressed, resulting in neutropenia, lymphocytopenia, anemia, and thrombocytopenia. Plasma pharmacokinetic data are available for 9 patients and are consistent with those in patients with refractory or relapsed AML dose with clofarabine as a single agent, suggesting that no gross pharmacokinetic interaction was occurring between cytarabine and clofarabine.

A Phase II open label study of the same combination was conducted in patients with de novo AML, AML evolved from MDS, and relapsed AML. Patients were treated with clofarabine 40 mg/m^2/day by 1-hour IV infusion followed four hours later by cytarabine 1 g/m^2/day by 2-hour IV infusion daily for Days 1 through 5.[19] The median patient age in this study was 67 (range: 38 - 82 years) and 61% (n=14) had received at least one prior cytotoxic regimen. Two early deaths were observed due to disease progression and sepsis. Grade 4 neutropenia developed in all patients and most patients had some degree of edema or third spacing syndrome. Other AE's included reversible acral rash. Efficacy data are available for 21 evaluable patients; of these 14/21 (67%) achieved a complete response and an additional 2/21 (9%) achieved a partial response for an overall response rate of 76% (16/21 patients). Complete cytogenetic remissions occurred in 9/20 (45%) patients. Durable remissions and low toxicity allowed some patients to proceed to non-myeloablative allogeneic stem cell transplantation.

In an on-going open-label Phase I/II study, patients with previously untreated and refractory AML received therapy with a combination of clofarabine and daunorubicin.[20] Patients were enrolled into five treatment cohorts containing 6 patients each which were expanded based on toxicity scoring. All patients received 50 mg/m^2/day daunorubicin on days 1, 3 and 5. Cohorts 1, 2, 3, and 4 received clofarabine doses 15 mg/m^2/day, 20 mg/m^2/day, 25 mg/m^2/day, and 30 mg/m^2/day, respectively on days 1 – 5 of the dosing regimen. Cohort 5 received the feasible dose of clofarabine derived from cohorts 1 to 4 in addition to 3 mg/m^2/day gemtuzumab ozogamicin (GO) on day 1 of the dosing regimen.

Three induction deaths were reported in the third cohort, and as a result, 20 mg/m^2/day was the dose of clofarabine chosen to combine with daunorubicin and GO. AST and ALT toxicities > grade 2 were not observed in any of the patients. Grade 4 cardiac toxicity was observed in 2 patients at the 25 mg/m^2/day cohort. One patient dosed at 30 mg/m^2 experienced grade 3 elevations in bilirubin. Another patient dosed at the same dose level had grade 4 renal toxicity. In the triplet combination, 2 patients experienced grade 3 nausea and one other patient experienced grade 4 cardiac toxicity. Efficacy data are available for 37 patients; of these 24/37 (65%).achieved a complete response. Overall survival at 1 year was 62% for the 37 patients enrolled into this study.

In an open-label Phase I study, patients with AML received salvage therapy with a combination of clofarabine and idarubicin or clofarabine, idarubicin, and cytarabine.[21] Four and three dose levels of each combination, respectively, were administered to patients. Clofarabine 22.5 mg/^{2}/day for 5 days and idarubicin 12 mg/m^2/day for 3 days resulted in grade 3 AEs of diarrhea, mucositis, esophagitis and rash which required dose de-escalation. When clofarabine 22.5 mg/m^2/day for 5 days, idarubicin 8 mg/m^2/day for 3 days, and cytarabine 1 g/m^2/day for 5 days was administered, grade 3 diarrhea, acute renal failure and elevated bilirubin was observed and required dose de-escalation. Ultimately, the MTDs were determined to be, for the two drug combination, clofarabine 22.5 mg/m²/day x 5 days plus idarubicin 10 mg/m² x 3 days, and for the three drug regimen, clofarabine 22.5 mg/m²/day x 5 days plus idarubicin 6 mg/m²/day x 3 days plus 0.75 mg/m²/day x 5 days. Dose limiting toxicities with the two drug regimen were elevated SGPT and bilirubin, and headache. For the three drug regimen, DLTs were increased bilirubin, diarrhea, mucositis, and decreased left ventricular ejection fraction. An overall response rate of 22% (3 CR and 2 CRp) was observed with the combination of clofarabine and idarubicin and an overall response rate of 48% (10 CRs) was observed with the three drug combination of clofarabine, idarubicin, and cytarabine.

Previously untreated adult AML

Faderl and colleagues conducted a Phase II study in patients ≥50 years with previously untreated AML with cytarabine 1 g/m^2 alone on Day 1, clofarabine administered by IV infusion over 1 hour followed 4 hours later by cytarabine 1 g/m^2/day administered by IV infusion over 2 hours on Days 2 through 5, and clofarabine alone administered by IV infusion over 1 hour on Day 6 repeated every 4 to 6 weeks.[22] The most frequently reported AEs reported during induction included myelosuppression, fever of unknown origin, infection, diarrhea, nausea, vomiting, headaches, skin rashes (including palmar plantar erythrodysesthesia), facial flushing, and transient abnormal liver function. During the consolidation phase, infections were reported for more than 50% of the patients and grade 3 or higher myelosuppression was reported for 98% of the patients. A total of 9 patients died during the study: 1 each from acute renal failure and bullous skin rash, Stevens-Johnson syndrome and sepsis, bacterial sepsis and multisystem organ failure, fungal sepsis, complications of neutropenia and sepsis, and 4 due to sepsis-related complications with 2 progressing to multi-organ failure. The overall response rate was 60% (52% CR and 8% CRp).

In a prospective randomized Phase II study presented at the 2005 annual meeting of the American Society of Hematology,[23] older adult patients (≥60 years with newly diagnosed

AML) were treated with clofarabine 30 mg/m^2/day IV for 5 consecutive days or clofarabine 30 mg/m^2/day IV for 5 consecutive days in combination with low dose ara-C (LDAC) 20 mg/m^2/day SC for 14 consecutive days. Based on a Bayesian randomization schema 76 patients were enrolled, of whom 16 were treated with clofarabine alone and 60 were treated with the combination of clofarabine and LDAC. The median patient age was 71 years (range: 60-83 years). Five of the 16 (31%) patients treated with clofarabine alone achieved a complete response and 38/60 (63%) patients treated with the combination therapy achieved a complete response. Both treatment regimens were tolerated in this patient population without a distinction in terms of toxicity. Interestingly, the early mortality rate was lower in the combination arm (17%) compared with the clofarabine alone arm (31%); however, conclusions regarding the early mortality rate are premature based on the limited number of patients treated with single-agent clofarabine. In an additional open-label, dose-escalation Phase I/II study reported at the 2005 annual meeting of the American Society of Hematology, elderly patients ≥60 years with de novo AML received cytarabine 100 mg/m^2/day continuous infusion for 7 days starting on Day 1 and clofarabine at a starting dose of 30 mg/m^2/day for 5 days starting on Day 2 with subsequent dose escalation based on response and toxicity.[24] Preliminary data are available for 4 patients; 2 died from drug-related infections and 2 achieved a CR. Grade 3 and 4 AEs included fever and neutropenia (2 patients), renal toxicity (2 patients), and capillary leak syndrome (1 patient). The DLTs were observed at the first dose level, therefore, the protocol was amended to allow for dose de-escalation to clofarabine 22.5 mg/m^2/day as well as to limit age to 60-75 years inclusive. As a result of the observed toxicity profile, routine use of aggressive pre-hydration and antibiotic/antifungal prophylaxis is now mandatory in this study.

The safety and efficacy of single agent clofarabine in previously untreated AML patients was reported in a single center Phase II study of 30 older adult patients (>65 years old) who were considered unfit for chemotherapy.[25] Overall response rate was 56%, with 43% CR and CRp 13%. The most common ≥ Grade 3 toxicities were transient increases in bilirubin and SGPT, hand foot syndrome, and rash. Based on these encouraging results, a multi-center study was performed in a similar patient population and results were presented at ASH 2006.[26] Patients were considered unfit for standard chemotherapy based on age, comorbidity, and performance status. Patients were treated with clofarabine at a dose of 30 mg/m^2/day for 5 days, repeated every 28 – 42 days. Sixty-two percent of patients were age 70 or older, 69% of patients had intermediate cytogenetics, 30% had adverse cytogenetics, and 31% had a prior hematologic disorder. Overall response rate was 44% (21% CR, 23 % CRi). The CR/CRi rate of 57 % and 50% in patients 70 – 74 years and > 75 years of age, respectively. The CR/CRi rate was 49% in patients with adverse cytogenetics. One year survival was 18% in non-responders as compared with a 32% observed in responders (CR + CRi).

Pediatric leukemia

In parallel to the adult program, a pediatric program was initiated in 2000. In the Phase I study in pediatric patients with hematologic malignancies, 25 patients were treated in cohorts of escalating doses up to 70 mg/m^2, a dose at which 1 patient had grade 4 hyperbilirubinemia and grade 3 elevated transaminases, and 1 had a grade 3 skin rash; the MTD was determined to be 52 mg/m^2.[27] Of the 13 patients treated at 52 mg/m^2, grade 2 to

grade 3 increases in bilirubin and liver transaminases were observed. A total of 5 patients achieved a CR and 3 achieved a PR for an overall response rate of 32%. Clofarabine plasma concentrations were generally smaller in the pediatric population than the adult population when administered the same dose, but there did not appear to be much difference in intracellular clofarabine triphosphate concentrations. Thus, the MTD was determined to be 52 mg/m^2 and the recommended Phase II dose.

Due to the results observed in the Phase I studies in pediatric patients with hematologic malignancies, interest by investigators and physicians, with support from regulatory authorities, led to the initiation of 2 parallel Phase II trials in 2002 in patients with either relapsed or refractory ALL or relapsed or refractory AML.[28,29] Both studies evaluated clofarabine 52 mg/m^2 administered once daily over a 2-hour IV infusion for 5 days every 2 to 6 weeks. In the 61 patients enrolled in the relapsed or refractory ALL study (61% males: 39% females, 1 to 20 years old), the overall remission rate (CR+CRp) was 20%; 30% (18/61) of patients showed a response (7CR, 5CRp, 6PR). Responses were noted in 15 of 50 (30%) patients with B- lineage ALL, 2 of 6 (33%) with T-cell ALL. Responders received a median of 3 prior induction regimens; 50% (9/18) had prior HSCT and 50% (9/18) were refractory to the preceding induction regimen. Response rate in refractory patients was 26% (9/35). After clofarabine treatment, 10 patients proceeded to transplant (including 8 responders). Six of 10 patients who received a transplant were alive at last follow up (survival range: 30.1+ - 145.1+ weeks). Response duration in 6 patients with CR or CRp who did not receive a transplant ranged from 4.3 to 58.6 weeks; 2 patients maintained CR for 47.9 and 58.6 weeks after clofarabine therapy. Median overall survival for the patients who achieved at least a PR was 66.6 weeks compared to 12.9 weeks for all patients.[28,29] In 42 pediatric patients with relapsed or refractory AML, the response rate was 26% (1 CRp, 10 PR). Responders had received a median of 2 prior induction regimens, 36% (4/11) had prior HSCT and 55% (6/11) patients were refractory to the preceding induction regimen. Response rate in refractory patients was 21% (6/28). One patient who had received 5 prior induction regimens achieved CRp. 13 (31%) patients (1CRp, 6PR, 3NE, 3TF) underwent HSCT after completing clofarabine therapy, 5 of whom were alive at last follow-up (survival range: 62.7+ –160.1+ weeks). Many patients proceeded to HSCT as soon as a donor was identified without waiting for the patient to go into remission, making remission difficult to assess. Median overall survival for patients who achieved at least a PR was 32.1 weeks compared to 23.4 weeks for all patients.[29]

Among the 113 pediatric patients with ALL and AML, the most frequently reported drug-related AEs were vomiting (66% ALL and 65% AML) and nausea (58% ALL and 70% AML). Other drug-related AEs reported by at least 10% of the pediatric patients overall included febrile neutropenia (31% ALL and 28% AML), pyrexia (21% ALL and 26% AML), pruritus NOS (24% ALL and 20% AML), dermatitis NOS (24% ALL and 17% AML), headache NOS (18% ALL and 35% AML), diarrhea NOS (21% ALL and 22% AML), anxiety NEC (16% ALL and 7% AML), fatigue (15% ALL and 13% AML), mucosal inflammation NOS (16% ALL and 15% AML), and flushing (12% ALL and 11% AML). Anorexia occurred in 12% ALL and 9% AML and palmar-plantar erythrodysesthesia syndrome in 12% ALL and 9% AML.

Study and dose rationale

The safety profile of clofarabine appears acceptable within the target populations studied to date in the clinical studies summarized in Section 2.4. Clofarabine has demonstrated anti-cancer activity through inhibition of DNA synthesis and repair, induction of apoptosis, and possibly through other mechanisms. Numerous responses have been observed after treatment with clofarabine in heavily pre-treated relapsed/refractory patients with ALL or AML.

The rationale for combining clofarabine with cytarabine is based on the ability of clofarabine to modulate Ara-CTP levels.[11,12] Clofarabine inhibits ribonucleotide reductase, resulting in decreased levels of deoxynucleotides, which then leads to a decrease in the feedback inhibition of deoxycytidine kinase, the rate limiting enzyme in Ara-CTP synthesis. Thus, the combination of clofarabine with cytarabine should lead to increased levels of intracellular Ara-CTP. A phase I/II study of this combination supported this strategy and demonstrated that this combination is safe and active in adults with relapsed and refractory AML.[18] Recently, the Children's Oncology Group has activated AAML0523, "A Phase I/II Study of Clofarabine in Combination with Cytarabine in Pediatric Patients with Refractory/Relapsed Leukemia."

Although three-drug induction therapy with cytarabine, an anthracycline, and etoposide is used in many contemporary clinical trials, we have demonstrated that it is safe and effective to initiate therapy with cytarabine and cladribine. In the St. Jude AML97 trial, patients initially received a 5-day course of cytarabine + cladribine, followed by induction with daunorubicin, cytarabine, and etoposide (DAV).[2] MRD-negativity was achieved in 51% of patients after the block of cytarabine + cladribine and in 61% of patients after the first course of DAV.[30] In addition, 90% of patients achieved morphologic CR after DAV #1. For the entire cohort of patients treated on AML97, the 3-yr EFS was 49%. Importantly, after an amendment to AML97 that removed the early use of autologous SCT and introduced MRC-based consolidation therapy, the 3-yr EFS estimate is 57%, similar to the preliminary results of AML02. Together, these data demonstrate that early use of cytarabine + cladribine is safe, effective at reducing tumor burden, and contributes to an overall outcome that is similar to that achieved on other contemporary trials.

2.1.3 Rationale for the use of high-dose cytarabine

Clinical trials have demonstrated that increases in the intensity of induction therapy, either through intensification of timing[3,31] or through dose intensification[32-34] lead to improvements in the outcome of AML. A Southwest Oncology Group (SWOG) study that randomly assigned adults with AML to groups that received daunorubicin with low dose cytarabine (LDAC) or with high dose cytarabine (HDAC) demonstrated a superior relapse-free survival for the HDAC arm (33% vs 21%, p=0.049).[33] Similarly, an Australian Leukemia Study Group (ALSG) trial that randomized patients to receive daunorubicin and etoposide with LDAC or HDAC showed that the estimated duration of remission was 45 months with HDAC and only 12 months with LDAC (p=0.0005). Furthermore, 49% of patients remain relapse free after achieving CR with HDAC compared to 24% after LDAC.[32]

On the recently completed St. Jude AML02 trial, pediatric patients with AML were randomly assigned to induction therapy with daunorubicin, etoposide and either low dose cytarabine (LD-ADE) or high dose cytarabine (HD-ADE). An interim analysis performed 12/5/07 revealed no statistical difference in day 22 MRD, EFS, or OS between the two arms, but there were trends toward better outcome among patients treated on the HD-ADE arm. Among patients treated on the HD-ADE arm, 31 of 86 (36%) were MRD+ at day 22, compared with 41 of 94 (44%) on the LD-ADE arm (p=0.27). The 3-year EFS and OS estimates were 59% ± 9% and 71% ± 8% for patients treated on the HD-ADE arm and 57% ± 8% and 63% ± 8% for patients treated on the LD-ADE arm (p=0.77 for EFS and 0.57 for OS). There were no significant differences in toxicity between the HD-ADE and LD-ADE arms. Therefore, the standard arm of the present trial will include the use HDAC during induction I.

2.1.4 Rationale for the addition of gentuzumab ozogamicin

Gentuzumab ozogamicin (GO) is a humanized anti-CD33–calicheamicin conjugate that was approved for use in AML by the FDA in May 2000.[35,36] In a phase I study, toxicity was primarily myelosuppression and several patients had clearing of their blood and bone marrow blasts.[37] A phase II study demonstrated a response rate of approximately 30% after the administration of two doses of GO, 9 mg/m^2 given two weeks apart.[38] In that study, 23% of patients developed grade 3 or 4 hyperbilirubinemia and 17% developed elevated liver transaminases. Hepatic veno-occlusive disease (VOD) has also been reported, primarily, but not exclusively, in patients who received GO after SCT.[39-41]

It is likely that GO will be most beneficial in combination with conventional chemotherapy. In this regard, investigators from the Medical Research Council (MRC) demonstrated that it was feasible to give GO at a dose of 3 mg/m^2 in combination with daunorubicin and cytarabine (DA) or fludarabine, cytarabine, GCSF, and idarubicin (FLAG-Ida).[42] The complete remission (CR) rate among patients who received DA + GO or FLAG-Ida + GO was 91%. At the 2006 meeting of the American Society of Hematology, Burnett et al presented preliminary results of the MRC AML15 trial. In this trial, 1115 patients were randomly assigned to chemotherapy (DA or ADE or Ida-FLAG) ± GO (3 mg/m^2) during induction. The CR rate was 85%, with no differences in induction deaths or refractory disease between the chemotherapy alone and the chemotherapy + GO arms. With a median follow up of 15 months, the 3-year DFS estimates were 51% for patients who received GO and 40% for patients who did not (p=0.008). The 3-year OS estimates were 53% and 46% respectively (p=0.4). The Children's Oncology Group (COG) recently completed a pilot study of 341 patients (AAML03P1) to test the feasibility of administering ADE + GO as Induction I to children with AML. The CR rate was 84% after one course of therapy, with a toxic mortality rate of only 1.5%. The preliminary results, with a median follow up only 226 days, indicates that the EFS (p=0.035) and OS (p=0.016) were both slightly better than that of CCG2961.

Based on the promising results from the MRC and COG, GO will be given to standard and low risk patients on the present trial. However, because of the potential increased risk of fatal VOD, high risk patients, who will undergo SCT, will not receive GO.

Update June 22, 2010: A confirmatory, post approval clinical trial was begun by Wyeth (now Pfizer) in 2004. The trial was designed to determine whether adding Mylotarg to standard chemotherapy demonstrated an improvement in clinical benefit (survival time) to AML patients. The trial was stopped early when no improvement in clinical benefit was observed, and a greater number of deaths occurred in the group of patients who received Mylotarg compared with those receiving chemotherapy alone.

As a result of the FDA Safety Report describing this trial, and the FDA's recommendation that Mylotarg not be commercially available to new patients, the AML08 trial was amended as of June 22, 2010 to delete the use of Mylotarg. This will only affect consolidation I MAG in LR and SR patients not scheduled to receive SCT.

2.1.5 Rationale for the use of NK cell transplantation

Natural killer (NK) cells can kill target cells without the need for prior sensitization or activation.[43] The ability of NK cells to perceive alterations in the expression of MHC class I on host cells was reported by Karre and colleagues more than a decade ago.[44] They observed that mouse tumors lacking MHC antigens ("missing self") were more readily killed by NK cells than tumors expressing normal levels of class I. Many human leukemia or cancer cells were found to have very low levels of MHC expression, thereby avoiding the recognition by T cells. In humans, NK cells are regulated by killer immunoglobulin-like receptors (KIRs) that are encoded by a gene family on chromosome 19q13 and recognize specific HLA class I alleles.[43,45-47] KIRs includes receptors with activating as well as inhibitory potential, receptors that have no known ligand, and receptors with specificity for MHC class I such as HLA-A, -B and -C. The receptors specific for MHC class I molecules on target cells inhibit NK effector functions such as cytotoxicity and cytokine production. Each NK cell generally expresses at least one inhibitory receptor that recognizes a self HLA molecule, thus preventing autoimmunity. Fourteen members of the KIR family have been identified on human NK cells thus far.[48] Clinical data suggest that KIR2DL2 and KIR2DL3 that recognize an epitope shared by HLA-C group 1 allotypes, KIR2DL1 that recognizes an epitope shared by HLA-C group 2 allotypes, and KIR3DL1 that recognizes an epitope shared by HLA-Bw4 allotypes are important determinants of anti-leukemic effect.[46,49-51]

In hematopoietic stem cell transplantation (SCT), the NK cells of the donor may exert potent anti-leukemia effects if the cognate MHC class I epitope is absent on the patient's leukemia cells for the donor's inhibitory KIRs. This potency has been demonstrated in both mouse models and in clinical transplantation.[46,49-51] In a study of 57 adult patients with AML, none of the 34 patients who received a KIR-ligand mismatched haploidentical SCT had a disease relapse.[52] In an analysis of 130 patients with hematologic malignancies undergoing unrelated donor SCT, those with KIR-ligand incompatibility had higher probabilities of overall survival and disease free survival and lower rates of transplant-related mortality and relapse.[50] Moreover, none of the 13 patients with myeloid malignancies who received SCT from a KIR-ligand incompatible donor relapsed; all are alive and disease free after SCT. In another study of 51 patients at the St. Jude Children's Research Hospital with direct measurement of the donor KIR repertoire, patients with a KIR matched donor had a 4-fold higher risk of relapse than those with a KIR mismatched donor.[46,53] In contrast to KIR mismatch, KIR-ligand mismatch was not a significant factor

for the prediction of relapse. The absence of beneficial effects of KIR-ligand mismatch was observed in many studies.[54-57] Taken together, these data underscore the importance of direct assessment of donor KIR repertoire rather than donor KIR-ligands.

The anti-leukemic effect of NK cells is not limited to HLA-non-identical SCT; this effect has also been demonstrated for patients undergoing SCT from HLA-identical sibling donors who, by definition, were always KIR-ligand compatible.[51] Using donor KIR genotyping, this study demonstrated that KIR mismatch was an independent predictor of relapse among patients with AML and myelodysplastic syndrome (MDS).[51]

In addition to the anti-leukemia effects, NK cells are capable of reducing the incidence of graft rejection and graft versus host disease (GVHD) in animal models; the former is in part based on the mechanism of donor NK cells against patient's T cells, and the latter on donor NK cells against patient's antigen-presenting cells.[52] Taken together, adoptive transfer of NK cells from the hematopoietic stem cell donor may have multiple advantages, including reduction in relapse, graft rejection, GVHD, and viral infections.[58]

Donor lymphocyte infusion (DLI) containing all types of lymphocytes (B, T, and NK cells) has been commonly used after SCT for the prevention or treatment of relapse, graft rejection, and viral infections. However, the potential clinical benefits of DLI are often outweighed by the risk of T cell-mediated GVHD. This is particularly problematic in haploidentical transplants, in which as few as 3×10^4 T cells may cause fatal GVHD.[59] Thus, we sought to develop a novel clinical scale isolation method to obtain highly purified NK cells for clinical use. Using the St. Jude NKCELL protocol, we performed 12 consecutive purification of NK cells from 12 normal volunteers.[58] The system uses a two-step procedure. First, mononuclear cells obtained by leukapheresis are depleted of T cells by CD3+ cell depletion using the CliniMACS. Second, the CD3-depleted product is enriched for CD56+ cells using the CliniMACS. The final products contained a median of 1.6×10^8 mononuclear cells and 91% CD3-CD56+ cells.[58] In addition, the final products had minimal contamination with T cells (at or below the lower limit of detection in 10 of the 12 products) or B cells (median 0.2%).[58] The median recovery of 160 million NK cells can provide more than 2 million NK cells per kg body weight for an adult of average size or greater than 10 million NK cells per kg body weight for a pediatric patient younger than 3 years. The number of T cells infused will be fewer than 1×10^3/kg, which is more than 10-fold below the threshold number ($3\text{-}5 \times 10^4$/kg) for the development of GVHD.[59,60] We also found that the expression of KIRs, adhesion molecules, intracellular cytokines, perforin, and granzyme B in NK cells was not significantly different before and after cell purification.[61] Extensive proliferative capacity and potent antitumor activity of the NK cells were demonstrated by using an immunodeficient mouse model.[61] In addition, GVHD developed in all mice transplanted with unpurified mononuclear cells, but in none of the 10 mice transplanted with purified NK cells.[61]

Based on these preclinical data, we initiated a clinical study of HLA-haploidentical NK cell transplantation for infants with leukemia who had received stem cell transplantation from the same donor (Protocol Mnemonic: INF-T2, FDA IDE 11533). Nine patients have been enrolled thus far. All the NK-cell grafts contained less than 5×10^4/kg T cells and no patient developed GVHD. The long-term efficacy of this treatment is not yet known because of the small number of patients with short follow-up.

Recently, Miller et al[62] demonstrated the successful transfer and expansion of haploidentical NK cells in the non-SCT setting with minimal toxicity and no GVHD. In this study, 19 adult patients with high risk AML (primary refractory disease, relapsed disease not in remission, secondary AML, or relapsed disease after SCT) received cyclophosphamide (60 mg/kg x 1 or 2 doses), fludarabine (25 mg/m^2 daily x 5 doses), IL-2 (10 million units per dose x 6 to 9 doses), and an infusion of 2 x 10^7 CD3-depleted NK cell product (NK cells enriched to approximately 40%). Eight of 15 AML patients showed at least 1% engraftment at day 7 or later after the infusion. In addition, 5 patients achieved CR, including 3 of 4 with KIR-ligand mismatched donor. Interestingly, 2 patients with refractory AML also achieved CR despite receiving NK cells from a KIR-ligand compatible donor. These data suggest that factors other than KIR are important determinants of anti-leukemic effects and patients with KIR compatible donor may also benefit from NK cell transplantation.

Based on these results, we undertook a pilot study of haplo-identical NK cell transplantation for patients with AML (Protocol Mnemonic: NKAML, FDA IDE 11533). As of December 1, 2007, we have enrolled 11 patients on the NKAML protocol, including 8 who were in CR. The median NK cell dose was 27 x 10^6/kg (range, 5 to 80 x 10^6/kg) and all patients showed evidence of engraftment (median 2% at day 2 and 6% at day 7). The procedure has been tolerated well, with only one SAE to date (prolonged pancytopenia without infection). All 8 patients who were in CR at the time of enrollment remain in CR.

2.1.6 Rationale for hematopoietic stem cell transplantation (SCT)

Studies performed more than 20 years ago demonstrated that allogeneic stem cell transplantation (SCT) is a feasible and effective alternative to chemotherapy as post remission treatment for AML.[63-65] Many subsequent studies have demonstrated that relapse-free survival rates for AML patients undergoing matched related donor SCT are better than those of patients who receive only chemotherapy.[66-70] Some studies have also shown an overall survival advantage associated with SCT; however, others have shown no such advantage, primarily because of transplant-related mortality.[71,72] On the MRC AML10 trial, SCT did reduce the risk of relapse, but neither autologous or allogeneic SCT produced an overall survival advantage.[4] Similarly, the CCG-213 trial failed to show a superiority of SCT over chemotherapy when results were analyzed by the intent to treat method.[73,74] In contrast, the largest and most recent randomized comparison of SCT versus chemotherapy, the CCG-2891 trial, demonstrated that for patients who achieved CR, survival in the allogeneic SCT group was significantly superior to that in the autologous SCT and chemotherapy arms.[3] Because of the controversies regarding the role of SCT in AML,[75,76] this protocol will include SCT only for high-risk patients.

2.1.7 Rationale for the use of sorafenib

Aberrant activation of receptor tyrosine kinases and effectors of activated signal transduction pathways involved in cellular differentiation, proliferation and survival represent therapeutic targets in AML.[77] Oncogenic mutations in *N-RAS* and *K-RAS*, leading to activation of the Ras/Raf/mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase/ERK mitogen-activated protein kinase signal transduction pathway, are observed in approximately 17% of children with AML.[78,79] Activating mutations in

FLT3 and *c-kit* are found in about 20% and 7% of pediatric patients with AML, respectively, and confer a worse prognosis.[80-82] Inhibitors of FLT3 are currently being evaluated in patients with relapsed or refractory AML containing FLT3 mutations.[83] However, therapy with highly selective tyrosine kinase inhibitors may be hampered by the activation of multiple signal transduction pathways in leukemia cells and redundancy and between the pathways.[77]

Sorafenib (BAY 43-9006) is an oral bisarylurea derivative that was initially developed as a potent inhibitor of Raf-1 kinase (IC_{50} = 6 nmol/L) but was later found to inhibit wild-type and mutant B-Raf (IC_{50} = 22 and 38 nmol/L, respectively) *in vitro* as well.[84-86] The Raf proteins are an integral component of the Ras/Raf/MAP/ERK signaling cascade with a well-established role in cellular proliferation and survival.[87] Sorafenib shows broad activity against various tumor cell lines *in vitro* and in xenograft models. In addition to its effect on Raf, sorafenib also potently inhibits receptor tyrosine kinases, including vascular endothelial growth factor receptor (VEGFR)-1/2/3, murine platelet-derived growth factor receptor (PDGFR)-ß, c-kit, and FLT3 (IC_{50} = 33 nmol/L).[84-86] Thus, the anticancer activity of sorafenib is likely due to its effects on multiple targets.

Sorafenib recently received Food and Drug Administration (FDA) approval for the treatment of adult renal cell carcinoma and hepatocellular carcinoma based on results from a phase III studies.[88] The approved adult dose is 400 mg orally BID. Diarrhea, rash, fatigue, and hand–foot skin reactions were the most common adverse events associated with sorafenib. Hypertension and cardiac ischemia were rare serious adverse events that were more common in patients receiving sorafenib than in those receiving placebo.

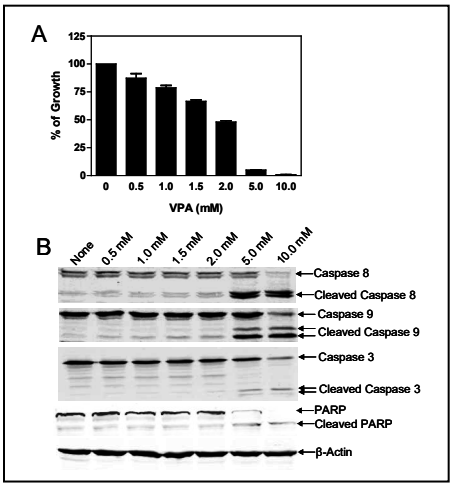
The Ras/Raf/Mek/ERK pathway has been shown to be potentially involved in AML pathophysiology.[89] In a recent study, markedly elevated phospho-ERK levels were found in 83.3% of the AML samples and treatment with a MEK inhibitor resulted in significantly decreased phospho-ERK levels.[90,91] Sorafenib also has potent activity against kinases reported to potentially be contributing to AML physiopathology. Importantly, sorafenib inhibited FLT3-ITD kinase activity and cell signaling and demonstrated potent antitumor efficacy in a FLT3-ITD leukemia tumor xenograft model.[92] Sorafenib was nearly 10 times more potent at inhibiting phosphorylation of FLT3, Stat5 and ERK1/2 in FLT3-ITD 293 cells than in FLT3-WT 293 cells. Sorafenib also potently inhibited the enzymatic activity of FLT3 activation loop mutant D835Y, suggesting that this compound may have activity against multiple mutant forms of FLT3.

Evidence of single-agent activity against AML with FLT3-ITD has been recently reported.[93] In this study, sorafenib effectively induced growth arrest and apoptosis in Ba/F3 cells containing FLT3-ITD and prolonged survival in a mouse model of FLT3-ITD-positive AML. In a phase I clinical trial in patients with refractory or relapsed AML, sorafenib reduced the blast percentages in blood and bone marrow of patients with FLT3-ITD. In this study, sorafenib was tolerated well, with no dose-limiting toxicities reported.

The Children's Oncology Group is currently conducting a Phase I study of sorafenib in children with refractory solid tumors or leukemias (ADVL0413). This trial was recently amended to exclude patients with abnormal liver function tests and to provide guidelines for the grading and treatment of hypertension related to sorafenib. The present trial will

follow the guidelines of the COG trial, as described below (section 4) and will use the current dose level of the COG trial of 200 mg/m^2/dose BID (adult equivalent dose of 360 mg BID for a BSA of 1.8 m^2).

2.1.8 Rationale for the use of valproic acid



Beginning with amendment 9.0, vorinostat will be substituted for valproic acid

In contrast to Down syndrome (DS) children with acute megakaryocytic leukemia (M7), who have EFS rates of 80-100%, non-DS children with M7 AML have much inferior EFS rates.[94-97] In a previous report from St. Jude, the EFS rate of *de novo* non-DS M7 AML patients treated with chemotherapy alone was 0%, while the EFS rate on the CCG-2861/2891 studies was 21%, significantly poorer than that for the non-DS AML group overall.[94,95] In the AML02 trial, approximately 10% of patients were classified as M7, and their 3-year EFS estimate was 44% ± 12%. Of the 18 M7 cases without the t(1;22), only 6 are alive, all after SCT. In this trial, no M7 patients without the t(1;22) have been cured by chemotherapy alone.

Histone deacetylase (HDAC) inhibitors, including the anti-epileptic agent, valproic acid, are currently being evaluated in the treatment of a variety of cancers.[98-102] The activity of valproic acid alone and in combination with chemotherapy agents has been evaluated *in vitro* against the non-DS M7 cell line, Meg-01 (Jeff Taub, unpublished observations). Meg-01 cells incubated with various concentration of valproic acid for 48h were harvested and subjected to flow cytometry analysis to determine viable cells and Western blotting to determine apoptosis. Valproic acid killed Meg-01 cells in a dose-dependent manner (Figure 1, panel A) by promoting the cells undergoing apoptosis as indicated by cleavage of caspases 8, 9, and 3 and PARP on the Western blots (Figure 1, panel B).

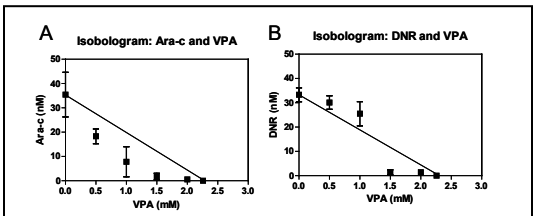


Figure 2. VPA sensitizes Meg-01 cells to ara-C and daunorubicin in vitro.

When valproic acid was incubated simultaneously with cytarabine, there was synergistic cytotoxicity against Meg-01 cells as determined by standard isobologram. Meg-01 cells with a dose range of valproic acid with up to >30-fold greater sensitivity to ara-C in the presence of 1.5 mM valproic acid than in the absence of valproic acid (Figure 2, panel A). Synergistic cytotoxicity with daunorubicin was also observed at higher valproic acid doses (1.5 mM) (Figure 2, panel B).

The incorporation of valproic acid as a new agent in the treatment of M7 AML has several potential advantages including: i) widespread use in the pediatric population with known toxicity, ii) measurement of therapeutic levels are readily available in the clinical setting,

and iii) clinically achievable trough levels (used in the treatment of pediatric patients with epilepsy[103] correspond to *in vitro* concentrations demonstrating anti-leukemia activity either as a single agent or in combination with ara-C. An oral rapid loading dose schedule of valproic acid after 3 days achieved a median trough level of 101 mg/L (equivalent to ~0.7 mM) in 35 pediatric patients.[103] Two patients with serum levels of 140 and 146 mg/L, developed elevated ammonia levels with nausea and fatigue, which resolved in both patients when the valproic acid dose was reduced.

Amendment 9.0 – vorinostat HDAC inhibitor

Although valproic acid has modest activity as a histone deacetylase inhibitor, more potent agents are now available. Vorinostat (suberoylanilide hydroxamic acid) inhibits human class 1 and 2 histone deacetylases, is orally bioavailable, and has limited single-agent activity in AML (Garcia-Manero et al, Blood 111:1060-1066, 2008). In a Phase II trial that included 75 adult patients with AML or MDS, vorinostat (500 mg po TID) was given for three days prior to idarubicin (12 mg/m^2/day x 3 days) and cytarabine (1.5 g/m^2/day x 3-4 days) (Garcia-Manero et al, J Clin Oncol 30:2204-2210, 2012). This regimen was well tolerated and resulted in an overall response rate of 85%. Vorinostat has also been safely administered for seven days prior to the combination of cytarabine and etoposide (Gojo et al, Clin Cancer Res 19:1838-1851, 2013). We have recently given vorinostat (100 mg/m^2/dose po TID x 3 days) prior to the combination of idarubicin and cytarabine in pediatric patients with relapsed AML (J Rubnitz, H Inaba, personal observations) without excessive toxicity.

Because vorinostat is a more potent HDAC inhibitor than valproic acid and can be safely combined with chemotherapy, vorinostat will be given prior to Induction II to high-risk patients with wild-type FLT3 as of amendment 9.0. Collaborating institutions may elect to opt out of treatment with vorinostat, and give standard Induction therapy with LD-ADE (without vorinostat).

2.1.9 Rationale for risk group assignment

As discussed above, several studies have demonstrated that non-Down syndrome patients with megakaryoblastic leukemia have significantly worse outcomes than patients with other subtypes of AML.[94-96] The EFS estimates for patients with megakaryoblastic leukemia treated on the CCG 2891 trial or on St. Jude trials were only 22% and 14%, respectively.[94,95] In the St. Jude study[94] and in a report from the European Group for Blood and Marrow Transplantation,[104] patients who underwent SCT in 1st CR had a better outcome than those who received chemotherapy. However, a study by French investigators suggested that children with megakaryoblastic leukemia and the t(1;22), but without Down syndrome, had a better outcome than similar children without this karyotypic abnormality, indicating that this subgroup may not need transplantation.[96] Based on these results, patients with M7 AML will be candidates for SCT only if their leukemic blasts do not carry the t(1;22).

Conventional cytogenetic studies have demonstrated that the karyotype of leukemic blasts is one of the best predictors of outcome.[105,106] An analysis of over 1600 patients enrolled on the MRC AML 10 trial revealed that the t(8;21) and inv(16) were each associated with a

favorable outcome (5-year OS estimates 69% and 61%, respectively), whereas a complex karyotype, -5, del(5q), monosomy 7 (-7), and abnormalities of 3q predicted a poor outcome.[105] Based on these observations, the MRC investigators proposed a cytogenetically-based risk classification system that is used by many cooperative groups today.[105] Among the 340 patients in the MRC study who were less than 15 years old, those with a favorable karyotype had a 3-year survival estimate of 78%, compared to 55% for the intermediate risk group and 42% for the adverse risk group. The poor results associated with -7 have been confirmed by a large international collaborative study.[107] This study, which included 172 patients with -7 (with or without other abnormalities) and 86 patients with del(7q) (also with or without other changes), demonstrated lower CR rates and worse outcome for patients with -7 compared to those with del(7q). The outcome for patients who had del(7q) and a favorable genetic alteration had a good outcome, suggesting that the presence of the del(7q) did not change the impact of the favorable feature. By contrast, patients with -7 and inv(3), -5/del(5q), or +21, had a dismal outcome (5-year OS 5%) that was not impacted by SCT.[107] On AML08, patients with core-binding factor (CBF) leukemia [t(8;21), inv(16), or t(16;16)] will be considered low risk, whereas patients whose blasts contain -5 or -7 will be candidates for SCT.

Other important cytogenetic abnormalities include rearrangements of the *MLL* gene, which are seen in up to 20% of cases of AML.[108-110] Although some studies suggest that the t(9;11) confers a favorable outcome,[109] most investigators consider patients whose leukemic cells contain 11q23 abnormalities to be at intermediate risk of relapse.[105]

During the past 10 years, molecular studies have demonstrated heterogeneity within cytogenetically defined subgroups of AML and have also identified new, prognostically important subgroups. Mutations of *c-kit*, *ras*, and *FLT3* have been detected in childhood and adult AML cases; *c-kit* mutations may be particularly important in cases of CBF leukemia.[82,111-114] Several studies of adult patients with t(8;21) demonstrated that patients with *c-kit* codon 816 mutations had significantly higher relapse rates and worse outcome than patients with t(8;21) and wild type *c-kit*.[111-113] Mutations of *c-kit* also appear to confer worse outcome among patients with inv(16) in some, but not all, studies.[115] *c-kit* mutations have been detected in 3% to 11% of pediatric AML cases, but their prognostic impact is uncertain.[114,116] In one study, 37% of CBF cases had *c-kit* mutations, but there was no difference in outcome between cases with and without mutations.[114] In contrast, investigators from the Japanese Childhood AML Cooperative Study Group found *c-kit* mutations in 8 of 46 patients with t(8;21) and demonstrated that these mutations were associated with significantly worse relapse rates, DFS, and OS.[82]

The impact of *FLT3* mutations in childhood and adult AML has been established by dozens of studies. In one of the first studies reported, the estimated 5-year OS rate was only 14% for adult patients with internal tandem duplications (ITD) of *FLT3*, whose presence was the strongest prognostic factor in multivariate analysis.[117] A subsequent study of 854 patients treated in the MRC AML trials demonstrated that a *FLT3*-ITD was present in 27% of cases and was associated with an increased relapse risk and worse probabilities of DFS, EFS, and OS.[118] Other reports have confirmed the presence of *FLT3*-ITD in 20% to 30% of adult AML cases, but some studies suggest that the negative impact of this alteration on survival may depend on the absence of the wild type allele or the ratio of the mutant to the wild type allele.[119-121]

Studies of childhood AML indicate that *FLT3*-ITD occurs in only 10%-15% of cases, but is still associated with a poor outcome.[80,81,122,123] An analysis of 91 pediatric patients with AML treated in CCG trials demonstrated an 8-year EFS estimate of only 7% for patients with *FLT3*-ITD, while a study of 234 patients treated on Dutch AML protocols showed a 5-year EFS of only 29%.[81,122] In both studies, multivariate analysis demonstrated that *FLT3*-ITD was the strongest predictor of relapse. A more recent study of 630 patients treated on contemporary CCG trials confirmed the poor outcome of patients with *FLT3*-ITD and demonstrated that survival decreased with increasing allelic ratios (AR) of *FLT*-ITD to *FLT3* wild type.[80] Patients with ITD AR greater than 0.4 had a significantly worse progression free survival than those with lower AR (16% versus 72%). Furthermore, investigators from the CCG compared the outcome of patients in whom the *FLT3-ITD* was present in CD34+/CD33- precursors to patients in whom the mutated gene was present only in the more mature CD34+/CD33+ progenitors.[124] Outcome was dramatically worse in patients in whom the mutation was present in the less mature precursors, confirming the heterogeneity within *FLT3*-ITD-positive patients and suggesting that only a subset of these patients have a poor prognosis.

Other molecular alterations implicated as prognostic factors in AML include expression of ATP-binding cassette transporters,[125-127] *CEBPA* mutations,[128,129] *DCC* expression,[130] VEGF secretion,[131] expression of apoptosis-related genes,[132-134] expression of *BAALC*,[135] expression of ERG,[136,137], *NPM1* mutations,[138-140] partial tandem duplications (PTD) of the *MLL* gene,[141,142] and global gene expression patterns.[143-147] However, data on the importance of these factors in childhood AML is not yet mature enough to allow us to use these factors in current risk group assignment.

In adults with AML, several studies have demonstrated that mutations of the *CEBPA* gene are associated with a favorable prognosis, especially among patients with normal karyotypes and wild-type *FLT3*.[128,129,148,149] Renneville et al[148] detected *CEBPA* mutations in 8% (53/638) of cases of adult AML and demonstrated that patients with mutations had better DFS (50% vs. 24%, p=0.03) and OS (47% vs. 31%, p=0.11) than patients without mutations. However, abnormal karyotype or *FLT3*-ITD predicted worse outcome among patients with *CEBPA* mutations. Outcome was particularly good for patients with normal karyotype, *CEBPA* mutations, and wild type *FLT3* (64% OS and 62% DFS).

Ho et al[150] recently reported the results of the first evaluation of the prognostic significance of *CEBPA* mutations in childhood AML. Among 847 AML cases treated on CCG/COG protocols, 4.5% (38 of 847) had *CEBPA* mutations, including 17% of cases with normal karyotypes. Patients with *CEBPA* mutations had better EFS (70% vs. 38%, p=0.015) and lower cumulative incidence of relapse (CIR, 13% vs. 44%, p=0.007) than patients without mutations. *FLT3*-ITDs were detected in only 2 cases with *CEBPA* mutations. In a multivariate analysis, *CEBPA* mutation was an independent favorable prognostic factor for EFS (HR=0.36, p=0.024), In addition, the outcome of patients with *CEBPA* was similar to that of patients with CBF leukemia (EFS, 78% vs. 59%; OS, 78% vs. 74%; CIR, 7% vs. 28%).

Like *CEBPA*, mutations of the *NPM1* gene are associated with a favorable outcome among adult cases of AML with normal karyotype and wild-type *FLT3*.[138,139,149,151,152] Brown et al[140] demonstrated that *NPM1* mutations were present in 8% (23/295) of children with

AML treated on the POG 9421 study and were associated with *FLT3*-ITD and normal karyotype. In children lacking *FLT3*-ITD, there was a favorable impact of *NPM1* mutations on outcome (EFS, 69% vs. 35%, p=0.51). The relatively small group of children with normal karyotype, wild-type *FLT3*, and *NPM1* mutation appear to have an outcome similar to that of children with CBF leukemia. Investigators from the BFM/DCOG study groups also demonstrated that *NPM1* mutations occur in about 8% of children with AML (25/297) and in 20% (20/100) of those with normal karyotypes.[153] Among patients with normal karyotypes, *NPM1* mutations were an independent prognostic factor for EFS (80% vs. 39%, p=0.01) and OS (85% vs. 60%, p=0.06).

Because the favorable impact of *CEBPA* and *NPM1* mutations previously demonstrated in adult AML studies have now been confirmed, at least retrospectively, in pediatric AML trials, all patients enrolled on AML08 will be screened for these genetic abnormalities. Patients who have normal karyotypes and mutations of *CEBPA* or *NPM1* and wild type *FLT3* will not be eligible for stem cell transplantation, even if a matched sibling donor is available.

2.1.10 Rationale for the use of minimal residual disease studies

Many studies have demonstrated the prognostic importance of early response to therapy as assessed by morphologic examination of the bone marrow[154] or by minimal residual disease (MRD) studies using flow cytometric detection of aberrant immunophenotypes.[30,155-158] In all of these studies, patients who had slow clearance of leukemic blasts had higher relapse rates and worse outcome than patients who had rapid clearance of MRD. In our ongoing AML02 trial, 172 of 182 (94.5%) patients had AML cells with aberrant phenotypes at diagnosis (sensitivity was 10^{-3} in 90 cases and 10^{-4} in 82 cases). The success rate of MRD assays in this multicenter study was high, with 1296 of 1313 (98.7%) of samples received having adequate viability and cellularity for informative analysis. At day 22, 69 of 167 (41.3%) had ≥ 0.1% MRD. MRD positivity at day 22 was significantly associated with FAB type, cytogenetics, and FLT3 status. There was no significant difference in the rates of MRD positivity between samples obtained from patients treated at St Jude and those treated at other institutions, further supporting the adequacy of the MRD analysis performed on shipped material. The three-year survivals according to day 22 MRD level were as follows: MRD negative, 79% ± 9%, MRD 0.1% to 1%, 73% ± 22%, and MRD ≥ 1%, 43% ± 12% (p=0.003). In AML08, we will continue using MRD as determined by flow cytometry for risk assignment. In addition, we will explore whether day 8 MRD in peripheral blood is a practical approach that might allow early therapeutic intervention in future studies.

2.1.11 Rationale for the use of prophylactic antimicrobials

Patients with AML who receive intensive chemotherapy are at high risk for bacterial sepsis and are especially susceptible to viridans streptococcal infections (VSI).[159,160] We recently reviewed the charts of 66 patients who were treated at St. Jude on the AML02 protocol from October 2002 to June 2006. Overall, patients who received any prophylactic antibiotic regimen had an 86% (95% CI: 66-95%) reduction in VSI (P<0.001) and a 76% (95% CI: 62-85%) reduction in all bacterial infections (P<0.001), compared to those who did not receive prophylactic antibiotics. Oral cephalosporins were ineffective at preventing overall

bacterial infections (P=0.97) or VSI (P=0.70). Intravenous cefepime completely prevented VSI. Furthermore, there was an 84% (95% CI: 52-94%) reduction in all bacterial infections (P<0.001). However, because we observed the emergence of life-threatening resistant gram-negative infections in two patients receiving cefepime, we instituted the prophylactic use of intravenous vancomycin with oral ciprofloxacin. The use of a vancomycin regimen resulted in an 84% (95% CI: 66-92%) decrease in total bacterial infections (P<0.001) and a 97% (95 CI: 78-100%) decrease in VSI (P<0.001). Importantly, fungal infection rates were similar in patients who received prophylactic antibiotics compared to those who did not (0.9 vs. 1.0 infection per 1000 patient days). Patients who received prophylactic antibiotics had shorter lengths of hospital stay (LOS), with a median (range) of 7 days (0-39) vs. 15 days (0-56) for each course of chemotherapy. Patients who received a vancomycin regimen had a 46% (95% CI: 28-59%) reduction in LOS (P<0.001) and those who received intravenous cefepime a 31% (95% CI: 9-47%) reduction (P=0.008) compared to patients who did not receive prophylactic antibiotics. Overall, there was only one death from septicemia; this occurred in a patient who was not receiving prophylactic antibiotics. Based on these results, prophylactic antibiotics will be used for all patients on AML08.

2.1.12 CNS therapy

On AML02, treatment of clinical and subclinical CNS leukemia initially consisted of intrathecal cytarabine, which was used successfully on the POG 9421 and CCG 2961 protocols. However, among the first 26 patients enrolled on AML02, 3 suffered relapses. The protocol was then amended to include triple intrathecal therapy with methotrexate, hydrocortisone, and cytarabine (MHA). Among the subsequent 170 patients enrolled, only 1 suffered an isolated CNS relapse. Thus, we will continue to use IT MHA on AML08.

2.1.13 Treatment of patients less than 10 kg

To reduce the potential for cardiac toxicity, dosing of daunorubicin and mitoxantrone for patients less than 10 kg will be based on weight rather than body surface area. Similarly, for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation, dosing of cytarabine, clofarabine, asparaginase, and etoposide, will be based on weight.

2.2 Background and rationale for correlative and biologic studies

2.2.1 Minimal residual disease studies

MRD studies provide powerful prognostic information, but it is likely that their informative power could be increased by improving their sensitivity. Indeed, current methods can detect 1 leukemic cell in 10,000 normal cells in approximately half of patients with AML; in the remaining patients, the maximum sensitivity is 1 in 1000. We postulate that sensitivity could be improved by the availability of additional markers. To this end, we performed comparative studies of gene expression of AML cells and normal CD34+ CD13+ cells to identify new markers of AML to be used for MRD studies. These studies identified several promising candidates. A second approach to improve the sensitivity of MRD studies by flow cytometry is to analyze more parameters simultaneously. In AML02, we used 4-color flow cytometry but a 3-laser flow cytometer capable of visualizing 9 or

more fluorochromes is now available in the laboratory. In AML08 we plan to compare the MRD results obtained with these new markers and methodologies to the standard marker combinations used in AML02. Moreover, we intend to explore the notion that AML cells with immunophenotypic features of immaturity (e.g., CD34+ CD38- cells, cells expressing ALDH) have increased drug resistance as compared to more mature AML cells. Therefore, we will measure the prevalence of these AML subsets among AML cells before and during treatment, a possibility that has been opened by the availability of 9-plus color instruments. Finally, we will use the same approach and commercially available anti-phosphoprotein antibodies to determine the effect of sorafenib on cell signaling pathways of AML cells.

2.2.2 NK cell receptor studies

The normal physiological roles of NK cells are to control infection and prevent cancer. In fact, NK cells are the only immune cells that have been shown by prospective cohort study in healthy persons to have immunosurveillance capability against human cancer.[161] In a prospective study of more than 3000 healthy volunteers who were followed for 11 years, the risk of cancer was associated with decreased NK cell cytotoxicity against K562 leukemia cells, decreased expression of the NK receptor NKRP1, and decreased production of cytokines such as TNFα and IFNγ in NK cells.

Three recent studies of adult leukemia have demonstrated a direct link between leukemia and NK cell. In one study of patients with acute myeloid leukemia, the majority (16 of 18) of blood samples showed defective expression and function of NK cell – triggering receptors (NCRs) (dull).[162] The expression of NK cell surface receptors was low, and the cytolytic activity against autologous leukemia cells, autologous B lymphoblasts, and NK cell–sensitive cell lines was weak. The abnormal NCR (dull) phenotype was confirmed in another study of 71 patients with acute myeloid leukemia and was found to be present in various morphologic and genotypic subtypes of leukemia.[163] In the third study of 25 AML and 14 ALL cases, the expression of HLA class I was frequently downregulated and that of the NK cell receptor ligands PVR and Nectin-2 were upregulated.[164] Together, these results suggest that NK receptor – ligand interaction may be crucial for the development of leukemia.

The normalization of NK cell receptor expression may be of prognostic value as NK cells may be important for the control of leukemia relapse and infection. These roles of NK cells have been shown by killer cell inhibitory receptor (KIR)-mismatched allogeneic stem cell transplantation.[46] Longitudinal study of adult AML patients showed that the NCR (dull) phenotype acquired during leukemia development was reversible in patients achieving complete remission after induction chemotherapy.[163] Reversibility of the NCR (dull) phenotype after complete remission suggested that leukemia cells might be involved in NCR down-regulation. Alternatively, the recovery of normal NCRs may allow the recovery of normal NK cell function that contributes to the acquisition of remission status. Interestingly, a correlation was found between the NCR (dull) phenotype and poor survival in AML patients after chemotherapy,[163] suggesting that NK-deficient activation caused by NCR down-regulation could play a role in treatment outcome. Therefore, we will study NK cell receptor expression at diagnosis and before reinduction to elucidate the role of NK cell in the development and treatment response of childhood ALL.

2.2.3 Biological prognostic factors

To assess the prognostic importance of specific genetic features, all patients in this trial will be studied by conventional cytogenetics and screened at diagnosis for the presence of the *AML1-ETO*, *MLL-AF9* and *MYH11-CBFβ* fusion transcripts. In addition, cases with 11q23 rearrangements will be screened for *MLL-AF4*, *MLL-AF6*, *MLL-AF10*, *MLL-ELL*, and *MLL-ENL* (as indicated), and cases with M7 morphology will be screened for *OTT-MAL*. Cases without one of the known genetic abnormalities will be analyzed by fluorescence in situ hybridization (FISH) for presence of *MLL* rearrangements and by RT-PCR for presence of cryptic *NUP98-NSD1*. FLT3-ITD and FLT3 point mutations will be detected by PCR and direct sequencing. Wild-type to mutant ratios will be determined in order to assess whether it adds prognostic information as previously demonstrated in adult de novo AML.[121] Leukemic cells that express fusion transcripts will also undergo RT-PCR during and after therapy. In addition to FLT3-ITD, other genes known to be mutated and associated with outcome as well as novel candidate genes will/may be sequenced after whole genome amplification of genomic DNA in order to assess the frequency of mutation and the association of genotype with outcome.

2.2.4 High-resolution single nucleotide polymorphism (SNP) array and gene expression

Currently available risk stratification features, such as leukocyte count, morphology, karyotype, MRD, standard genetic features, and gene expression profiles alone probably do not correctly molecularly and prognostically classify all AML patients. Previous studies have shown that gene expression profiling using DNA microarrays is a powerful approach to molecularly classify acute leukemias and to distinguish subtypes of AML.[145,165] However, a predictive signature for outcome in pediatric AML still remains to be defined. We will continue our effort to identify signatures predicting outcome based on gene expression profiles which will be obtained for all cases with sufficient material.

At St. Jude, a high resolution study of DNA copy number abnormalities and loss-of-heterozygosity in acute lymphoblastic leukemia using SNP microarrays identified a high frequency of recurring deletions in ALL, most notably involving genes regulating B-lymphoid development in B-progenitor ALL.[166] Presence of some of the deletions was found to be associated with outcome. A pioneer study of 110 cases of pediatric AML from different St. Jude AML protocols showed that high-resolution SNP arrays identified numerous cryptic copy number abnormalities and copy-neutral loss of heterozygosity (LOH) or uniparental disomy in pediatric de novo AML. Because of the small number of samples in this pioneer cohort, recurring lesions in 5-10% of the cases may have been missed and association with outcome could not be studied. Similar frequencies of cryptic copy number abnormalities were observed in adult MDS and t-AML samples (Gondek et al. Blood 2008;111:epub) using SNP array as complementary approach to conventional cytogenetic analysis. We propose to obtain gene expression profiles and high-resolution SNP array data for patients enrolled in AML08 (Affymetrix 500K 6.0). Two vials with 2x 10^7 cells should be reposited for this purpose for each patient. We will explore the prognostic value of integrated analysis of somatic copy number abnormalities, copy-neutral LOH, gene expression profiles, sequence mutation and MRD. Profiles will be analyzed to determine whether they have a relationship with any morphologic, genotypic, immunophenotypic or clinical characteristic.

Most studies assess leukemic blasts in bulk by genome-wide expression or SNP array profiling. There is evidence for heterogeneity within each sample based on cytogenetic and FISH data. Some of the lesions and aberrant gene expression profiles might be only present in a subset of the blasts. For example, we identified cases in our pioneer AML cohort for which gains of one copy of a genetic locus was identified by SNP array analysis of bulk cells while FISH showed that a high level gain was only present in <30% of the cells. In a comparative study of copy number abnormalities at relapse and diagnosis in 17 pediatric AML cases, about 30% of cases had novel lesions at relapse which had not been detected at diagnosis (ASH abstract #984). These lesions might be present but not detectable at diagnosis because they might occur only in a subset of cells. Some of these subpopulations may represent leukemia-initiating or stem cells within each tumor bulk which maintain propagation of the disease. Studying cells with CD34+CD38-, a population shown to be enriched for leukemia-initiating cells, may provide information which cannot be obtained from the bulk of cells. A recent study revealed that within single tumor samples CD34+CD38- had a different gene expression profile than CD34+CD38+.[167] We propose to analyze gene expression, copy number and LOH within the subpopulations of single AML tumor samples to determine whether the genetic heterogeneity of the tumor contributes to their resistance to therapy and to the relapse of disease.

To investigate why patients fail to respond to Induction I, we plan to assess whether residual blasts late in or after Induction I have characteristic genetic features or expression profiles, different to those at time of diagnosis. Comparison between blasts at diagnosis and residual blasts after Induction I for each case individually would allow determine whether the treatment selected for a genetic feature or profile. To identify lesions with predictive value, we would also compare the constellation of genetic lesions at diagnosis in cases with failure to respond to Induction I to cases that undergo a complete response. To address this question, we propose to sort blasts from samples obtained from blood at day 8 or from marrow at Day 22 and extract DNA and RNA from the blast population to analyze gene expression, copy number and LOH.

2.2.5 Phospho-signaling network analysis

Recently, a quantitative method to profile aberrant phospho-signaling networks has been established in primary AML leukemia cells. Phospho-signaling has been shown to provide prognostic information as a summary read-out which might be a more accurate classifier than any single genetic or phenotypic feature identified so far.[168] Using this flow-based approach, the signaling response can be determined in different subpopulations by co-staining with surface markers and also after in vitro exposure to inhibitors. We would like to explore within AML08 whether characterization of the phospho-signaling networks can be used to classify within known genetic subtypes and to predict response to therapy.

Phospho-flow profiling will not be prospectively performed on all AML08 samples. Instead, all samples will be banked and selected samples will later be studied by Dr. Downing's laboratory at St. Jude and by Dr. Lacayo's laboratory at Stanford University. Dr. Lacayo's laboratory proposes to interrogate signaling mechanisms in training and validation set of banked AML patient samples from Pediatric Oncology Group (POG) studies and from the present study. Maps of signaling profiles for groups and individual AML patients will be created using 100 available banked samples from the POG 9421

study and additional samples from AML08. Dr. Lacayo will test the hypothesis that patients with similar signaling profiles will have similar responses to therapy and that gene expression differences will correlate with specific patterns of signaling in AML. If a profile of signaling is significantly associated with clinical outcome, they will then validate this panel in real-time with a second (banked samples) and third set (prospectively collected) of additional AML patient specimens. Using bioinformatic techniques the network signaling can be inferred by studying key nodal points. The analysis of POG 9421 samples will include a matrix of 196 data points. From this data matrix we will identify 5-10 nodal points that can be studied using banked AML08 samples. This approach will reduce the required number of cells to 5-10x10^6 viable cells in DMSO, from 50 additional banked samples. Dr. Lacayo will initially study a total of 25 AML08 banked samples for a pilot study, with release of 25 additional samples after review of data generated.

2.2.6 Pharmacokinetic studies

Sorafenib pharmacokinetics - Note: sorafenib pharmacokinetic studies were completed with LOA #4, May 29, 2015

After oral administration, maximum sorafenib plasma concentrations are reached between 6 and 12 hours, and the terminal half-life ranges from 20 to 40 hours.[169] Consequently, steady-state is reached after approximately 7 days with the achievement of concentrations 4- to 6-fold (approximately 1-log) higher than those on day 1 with twice daily dosing. Sorafenib is metabolized primarily by cytochrome P-450 3A4 (CYP3A4) with involvement of UGT-1A9 and shows extensive binding in human plasma (>99.5%). Like most drugs that are metabolized by CYP3A4, sorafenib exhibits wide pharmacokinetic variability with a coefficient of variation for AUC ranging from 50-90%. Associations between sorafenib exposure and side effects and outcome remain to be performed. The pharmacokinetics of sorafenib have not been evaluated in children. Therefore, it will be important to understand the disposition of this agent in the setting of pediatric AML and associations with toxicity and efficacy. Furthermore, availability of this information will allow evaluation of the possible associations of common germline variants in genes encoding transporters and enzymes and sorafenib pharmacokinetics.

L-carnitine analysis in urine

L-carnitine (vitamin Bt) is an essential nutrient, particularly in infants, and is involved in the mitochondrial oxidation of long-chain fatty acids, it stimulates mitochondrial metabolism, decreases oxidative stress, and inhibits apoptosis by preventing ceramide formation. Carnitine elimination takes place in the kidney mainly by glomerular filtration and secretion at the proximal tubules, and may return to the systemic circulation via a process of drug reabsorption. Our recent studies have indicated that the solute carrier OCTN2 is a key regulator of this reabsorption and that the function of OCTN2 can be substantially diminished by a number of commonly used anticancer drugs, including epipodophyllotoxins and anthracyclines. The subsequent loss of l-carnitine in the urine is potentially an important cause of secondary l-carnitine deficiency, which phenomenon will likely contribute to a variety of treatment related side effects.

Under amendment 4.0, this study will not be done.

Etoposide and daunorubicin pharmacokinetics

Pharmacokinetic studies of etoposide and daunorubicin will be performed in patients with urinary collection for l-carnitine concentrations. Availability of this information will allow evaluation of the possible associations of urinary l-carnitine loss with the pharmacokinetic profiles of etoposide and daunorubicin.

Under amendment 4.0, this study will not be done.

2.2.7 Pharmacogenetic studies

A growing body of research is highlighting the role that variations in genes encoding drug-metabolizing enzymes and drug transporters play in explaining the variability seen in treatment-related outcomes of several important anticancer agents. Drug metabolizing enzymes and transporters are localized to the intestines, liver, and kidneys and contribute to systemic pharmacokinetic variability, but they are also localized in cancer cells and can contribute to intracellular pharmacokinetic variability and drug action. For example, in childhood AML, studies have shown that overexpression of cellular drug efflux transporters from the ABC family such as BCRP (ABCG2) and MRP3 (ABCC3) is associated with a poor prognosis.[170] Cellular uptake carriers from the SLC family are also under expressed in AML. From microarray analysis of blasts cells from over 200 patients enrolled on six previous frontline AML trials at St. Jude, including AML02, we have some evidence that under expression of the uptake carriers ENT1 (SLC29A1), CNT3 (SLC28A3), and OCTN1 (SLC22A4) and overexpression of the efflux transporters p-glycoprotein (ABCB1) and MRP1 (ABCC1) are associated with worse long-term outcome. We also know that drugs used in AML therapy such as cytarabine, clofarabine, daunorubicin, and etoposide are substrates for several efflux transporters (e.g., p-glycoprotein, BCRP, MRP2, MRP4), uptake carriers (e.g., ENT1, CNT3, OCTN1, OCTN2) and/or drug metabolizing enzymes (e.g., CYP3A4). We propose to evaluate associations between genetic polymorphisms in drug-metabolizing enzymes and drug transporters and treatment-related outcomes, including both toxicity and efficacy.

Pharmacogenetics of nucleoside analogs

Cytarabine and clofarabine, nucleoside analogs that are used in the treatment of AML are prodrugs and must be converted to the triphosphate form by deoxycytidine kinase (DCK) for their anti-leukemic effect.

The genes of relevance to activation of cytarabine and clofarabine include equilibrative nucleoside transporter (hENT1), critical for intracellular uptake of ara-C. Once inside the cell, cytarabine and clofarabine are activated to their active tri-phosphate forms (ara-CTP or clofarabine-TP) by a series of intracellular enzymes, with deoxycytidine kinase (DCK) catalyzing the first rate limiting metabolic step. Cytidine deaminase (CDA) and 5'-Nucleotidase (NT5C2) are the primary inactivating enzymes. The primary mechanism of resistance appears to be the insufficient intracellular levels of the active triphosphate metabolite ara-CTP; this may be due to: a) inefficient cellular uptake due to low levels of the transporter, hENT1; b) reduced levels of the activating enzymes, primarily DCK; c) increased levels of inactivating enzymes, such as NT5C2 and CDA; and d) increased

cellular dCTP pools, that can compete with DNA incorporation of ara-CTP or clofarabine-TP and also inhibit DCK activity. The intracellular dCTP pools, in turn, are regulated by ribonucleotide reductase (RRM1 and RRM2).

We have shown that genetic variations in several of these genes integral to activation of these nucleoside analogs results in changes in expression or activity of proteins that affect drug uptake, activation and degradation. Previous unpublished analysis of data on pre-treatment gene expression profiles in 40 patients from AML97 have indicated that expression of the ENT1 and NT5C2 genes is associated with the clinical outcome. We have recently reported novel coding SNPs and other regulatory SNPs in DCK that affect its function and expression, respectively are were also associated with the leukemic blast ara-CTP levels in the patients enrolled in AML97 protocol undergoing treatment with ara-C.[171] Although the sample size was very less, interestingly the two AML patients heterozygous for coding changes in DCK also relapsed despite having favorable cytogenetic abnormalities [inv16 and t(8;21), respectively].[171] We have also identified coding and regulatory genetic polymorphism in NT5C2 that are associated with its mRNA expression, sensitivity to ara-C in lymphoblast cell lines and with blast ara-CTP levels in AML patients.

Thus, the observed inter-patient variation in the anticancer efficacy of cytarabine/clofarabine could be, in part, due to inter-patient variation in the expression and/or activity of proteins that affect drug uptake, activation and degradation. Further, since the activated triphosphate form of clofarabine inhibits DNA polymerases, inhibits ribonucleotide reductase, and disrupts mitochondria, genetic variation in RRM1 and RRM2 could have an influence on its therapeutic efficacy. Therefore, we propose to further explore whether genetic polymorphisms in the key candidate genes (DCK, hENT1, CDA, NT5C2, RRM1, RRM2 etc.) in the metabolic pathway of nucleoside analogs are associated with early blast clearance, treatment response or toxicity in AML patients.

2.2.8 Infectious disease aims

As described in section 2.1.11, a retrospective review of prophylactic antibiotics used in a subset of patients treated on AML02 demonstrated that certain regimens dramatically reduced the incidence of bacterial infections. In AML08, we will prospectively collect information and describe the impact of antibiotic/antifungal prophylaxis on the outcome measures summarized below. These include variables that are routinely collected as part of pediatric oncology protocols (CTCAE v 3.0) permitting cross-protocol comparisons as well as some data that will be collected specifically for this protocol. Monitoring for antimicrobial resistance using stool surveillance cultures will be done only on St. Jude participants since such surveillance is part of standard of care only at this institution. Where possible, comparisons will be made with similar immunosuppressed patients based on historical information or from other cooperative group protocols. Outcome measures for descriptive analysis in the study population include:

- Invasive bacterial infections
- Invasive fungal infections
- Fever with neutropenia episodes
- Rates of antimicrobial resistance (invasive infections)
- Hospitalization days

We will also measure the impact of antibiotic and antifungal prophylaxis in terms of rates of antimicrobial resistance (information for invasive infections from all participating sites and stool surveillance cultures from St. Jude data only).

A large number of episodes of fever with neutropenia remain with unclear etiology. We suspect that many such "fever of unknown origin" episodes are due to viral infections. The ability to diagnose viral respiratory infections significantly improves with use of a PCR based approach over conventional diagnostic techniques such as direct fluorescent assay or culture. Asymptomatic infection in immunocompromised patients due to respiratory viruses has been previously demonstrated.[172,173] However, the contribution of such asymptomatic viral infection to fever with neutropenia in patients being treated for an underlying malignancy remains unclear.[174,175] Therefore, we propose the routine use of respiratory PCRs[176] for influenza A, influenza B, respiratory syncytial virus, human parainfluenza virus 1-3, human adenovirus, human rhinovirus, coronavirus, enterovirus and human metapneumovirus (MultiCode-PLx Respiratory PCR panel; EraGen Biosciences, Madison, WI) on nasal wash specimens collected at admission for febrile neutropenia (all patients regardless of the presence of respiratory symptoms).

Under amendment 4.0, the respiratory PCR panel on nasal wash samples will not be done. Note that this research aim has been deleted in amendment 4.0.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

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

3.1 Diagnostic criteria

Patients must have one of the following three characteristics:

1. Acute myeloid leukemia fulfilling the criteria of the WHO Classification

2. <20% marrow myeloblasts and evidence of a clonal de novo AML genetic abnormality [e.g., t(8;21), inv(16), t(9;11)]

3. Myeloid sarcoma (also referred to as extramedullary myeloid tumor, granulocytic sarcoma, or chloroma), with or without evidence of a leukemic process in the bone marrow or peripheral blood, with confirmation of myeloid differentiation.

Patients with secondary AML following treatment of primary malignancy are eligible.

3.2 Inclusion criteria – all participants

3.2.1 Age ≤ 21 years at time of study entry.
3.2.2 No prior therapy for this malignancy except for one dose of intrathecal therapy and the use of hydroxyurea or low-dose cytarabine (100-200 mg/m^2 per day for one week or less) for hyperleukocytosis
3.2.3 Written informed consent according to institutional guidelines
3.2.4 Female patients of childbearing potential must have a negative pregnancy test within 2 weeks prior to enrollment
3.2.5 Male and female participants must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.

3.3 Exclusion criteria – all participants

3.3.1 Down syndrome
3.3.2 Acute Promyelocytic Leukemia (APL)
3.3.3 Juvenile Myelomonocytic Leukemia (JMML)
3.3.4 Fanconi anemia (FA)
3.3.5 Kostmann syndrome
3.3.6 Shwachman syndrome
3.3.7 Other bone marrow failure syndromes
3.3.8 Use of concomitant chemotherapy, radiation therapy, or immunotherapy other than as specified in the protocol.
3.3.9 Use of investigational agents within 30 days or any anticancer therapy for this malignancy within 2 weeks before study entry with the exception of IT therapy, hydroxyurea, or low-dose cytarabine as stated above. The patient must have recovered from all acute toxicities from any previous therapy.
3.3.10 Systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment).
3.3.11 Pregnant or lactating patients.
3.3.12 Any significant concurrent disease, illness, or psychiatric disorder that would compromise patient safety or compliance, interfere with consent, study participation, follow up, or interpretation of study results.

3.4 Criteria for randomization

Participants must meet the following criteria to qualify for HD-ADE versus Clo/AraC randomization. Participants who do not meet these criteria may still be enrolled, but will be treated on HD-ADE arm and will NOT be randomized.

3.4.1 Normal creatinine for age
3.4.2 Serum bilirubin ≤ 1.5 x upper limit of normal (ULN).
3.4.3 Aspartate transaminase (AST/SGOT) and alanine transaminase (ALT/SGPT) ≤ 2.5 x ULN
3.4.4 Alkaline phosphatase ≤ 2.5 x ULN

3.5 Enrollment on study

3.5.1 St. Jude Children's Research Hospital

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the 'Participant Eligibility Checklist'. The study team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent/assent form(s) must be faxed or emailed to the CPDMO at [redacted] to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 am to 5:00 pm. Enrollments may be requested during weekends or holidays by calling the CPDMO "On Call" cell phone [redacted] or referencing the "On Call Schedule" on the intranet).

3.5.2 Collaborating Sites

Collaborating Site research participants should be registered at St. Jude within 24 hours of enrollment at the site. The completed Eligibility Checklist and entire signed Informed Consent should be faxed to [redacted]. Please call [redacted] if confirmation of the enrollment information is needed. The Protocol Eligibility Coordinator will then register the research participant in the Patient Protocol Manager (PPM) system.

4.0 TREATMENT PLAN

4.1 General overview

Treatment will be based on cytogenetic and molecular characteristics, morphology, and response to therapy as assessed by flow cytometry. Risk groups are defined below. The general treatment plan will consist of chemotherapy for LR patients, chemotherapy ± NK cell therapy for SR patients, and chemotherapy + SCT for HR patients. HR patients who do not have a suitable stem cell donor or who decline SCT will be eligible for NK cell therapy, but will be analyzed separately. The definition of suitable stem cell donor and the conditioning regimens used for SCT will be determined by local institutional protocols or guidelines. In addition, although we do not recommend SCT for SR patients, the role of SCT for SR patients who have a matched sibling donor is controversial and is widely recommended by many pediatric oncologists and by the current COG protocol. Therefore, SR patients who have matched sibling donors may proceed to SCT after Induction II at the discretion of the treating physician. However, because of the favorable prognosis recently described for patients with wild-type *FLT3* and mutations of *NPM1* or *CEPBA*, these patients will not be candidates for SCT, even if a matched sibling donor is available.

Note about administration guidelines for all treatment phases: The timing and duration for administration for all commercially available agents are provided in the treatment phase sections as guidelines only. Variations in the timing and duration of chemotherapy infusions according to institutional practice or variations based on patient care needs are

acceptable, as long as the treating investigator and/or PI determines that there was no impact on patient safety.

These variations will not be considered protocol deviations, as long as the total dose is given within 10% of protocol specified dose

To reduce the potential for cardiac toxicity, dosing of daunorubicin and mitoxantrone for patients less than 10 kg will be based on weight rather than body surface area. Similarly, for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation, dosing of cytarabine, clofarabine, asparaginase, and etoposide, will be based on weight.

NK cell therapy for collaborating site patients, except Singapore, will be done at St. Jude. The patient, one parent, and the NK donor will need to travel to Memphis and stay for about 5 weeks. St. Jude will cover travel costs for the patient, donor and one parent, housing for up to 4 family members, and all medical costs during this time. St. Jude will also cover the travel costs for the NK cell donor.

Guidance during times of drug shortages and unavailability:

Treating investigators are urged to consult with the PI or co-PI and use their best clinical judgment in optimizing therapeutic intent and ensuring patient safety in managing the protocol-specified therapy. Although these decisions may constitute “Protocol Violations,” they are unavoidable and made in consideration of the best interest of an individual patient. These will not be considered monitoring/audit findings if appropriately documented. All protocol deviations must be noted in the research database and the alterations in therapy due to the agent shortage will be captured. This should be accomplished by entering “dose modified” and details noted in the comments field. These deviations will also be noted in the Deviation Log with the notation “Drug substitution/reduction due to unavoidable drug shortage/unavailability”.

4.1.1 Low-risk (LR) criteria (not eligible for SCT or NK cell therapy)

Core binding factor (CBF) leukemia [t(8;21)/*AML1-ETO* or inv(16)/t(16;16)/*CBFβ-MYH11*] and MRD < 0.1% at day 22, regardless of other genetic features. Patients with CBF leukemia who have MRD ≥ 0.1%, but less than 5%, at day 22 or who have increasing levels of fusion transcript will be considered SR and thus eligible for NK cell therapy.

4.1.2 Standard-risk (SR) criteria (eligible for NK cell therapy)

- CBF leukemia with MRD ≥ 0.1%, but less than 5%, at day 22 or increasing levels of fusion transcript
- *FLT3*-ITD and MRD < 0.1% at day 22
- Absence of low-risk or high-risk features

4.1.3 High-risk (HR) criteria (candidates for SCT; eligible for NK cell therapy)

- t(6;9), t(8;16), t(16;21), -7, -5, or 5q-
- FAB M0 or M6
- FAB M7 without t(1;22)
- Treatment-related (secondary) AML
- RAEB-2 or AML arising from prior MDS
- FLT3-ITD and MRD ≥ 0.1% at day 22
- All other patients with poor response to therapy (must have one of the following features)
  - MRD ≥ 5% at day 22
  - MRD ≥ 0.1% after Induction II

4.2 Induction therapy (2 courses)

All patients will receive two courses of induction therapy that will include one course of either high dose cytarabine, daunorubicin, and etoposide (HD-ADE) or one course of clofarabine and cytarabine (Clo/AraC), followed by one course of low dose cytarabine, daunorubicin, and etoposide (LD-ADE). Patients will be randomly assigned to receive one of the following induction regimens.

4.2.1 Induction I: HD-ADE

- Cytarabine: 3 g/m^2 (100 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 3 hours q12 hours x 6 doses (days 1, 3, 5)
- Daunorubicin: 50 mg/m^2 (1.67 mg/kg for patients less than 10 kg) IV over 6 hours on days 2, 4, 6 (3 doses)
- Etoposide: 100 mg/m^2 (3.3 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 4 hours on days 2-6 (5 doses)

4.2.2 Induction I: Clo/AraC

- Clofarabine: 52 mg/m^2 (1.7 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 2 hours on days 1-5 (5 doses).
- Cytarabine: 1 gram/m^2 (33 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 2 hours on days 1-5 (5 doses; each dose to start 4 hours after the start of clofarabine)

Clofarabine will be supplied by Genzyme.

<u>Special considerations for patients with hyperleukocytosis:</u>

In patients with leukocyte counts greater than 100 x 10^9/L or symptoms of hyperviscosity, leukapheresis or exchange transfusion should be performed according to local institutional

guidelines. In addition, patients with leukocyte counts greater than 50 x 10^9/L, especially those with M4, M4Eo, or M5 morphology, are at risk for severe cardiopulmonary and renal complications associated with rapid cell lysis during the initiation of chemotherapy with nucleoside analogues, including cytarabine and clofarabine.[177] Therefore, the administration of hydroxyurea (10 to 20 mg/kg/day PO; maximum 1000 mg PO BID) or cytarabine (100 mg/m^2/dose IV every 12 hours) should be considered in such cases. Hydroxyurea or low-dose cytarabine may be given prior to Induction I at the discretion of the treating physician.

Special considerations for administration of clofarabine

Clofarabine is excreted primarily by the kidneys. Therefore, drugs with known renal toxicity should be avoided during the 5 days of clofarabine treatment in each cycle. Additionally, the liver is a known target organ for clofarabine toxicity. Therefore, concomitant use of medications known to induce hepatic toxicity should be avoided. Hepatic and renal function should be assessed prior to and during treatment with clofarabine and it is recommended that the patient's fluid status and hepatic and renal function be carefully monitored during the drug administration period. All patients should receive hydration each day of clofarabine treatment, giving careful consideration to the cardiac and renal function of the patient. To the extent possible, use of nephrotoxic (e.g., vancomycin, amphotericin B, etc.) and hepatotoxic (e.g., voriconazole, cyclosporine, etc.) agents is to be avoided during clofarabine administration.

Prophylactic steroid administration has been administered by some investigators and has been reported to minimize the occurrence and/or severity of the following potential clofarabine-related toxicities: nausea, vomiting, skin rash/desquamation, and capillary leak syndrome. Therefore, methylprednisolone, 0.5 to 1 mg/kg/dose IV, should be given prior to each dose of clofarabine.

4.2.3 Evaluation of response and criteria for starting Induction II

All patients will undergo bone marrow aspirate and LPIT at day 22 (see Section 7.1) and will start Induction II at approximately day 29.

- Patients with MRD ≥ 0.1% or with definitive leukemic blasts by morphology may start Induction II prior to day 29 regardless of blood counts.
- In patients with MRD < 0.1% and hypocellular marrows, Induction II may be delayed until there are signs of hematopoietic recovery, but should not start later than day 36. A second bone marrow aspirate may be performed if clinically indicated.

4.2.4 Induction II: LD-ADE

- Cytarabine: 100 mg/m^2 (3.3 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 30 minutes q12 hours on days 1-8 (16 doses)
- Daunorubicin: 50 mg/m^2 (1.67 mg/kg for patients less than 10 kg) IV over 6 hours on days 2, 4, 6 (3 doses)

- Etoposide: 100 mg/m^2 (3.3 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 4 hours on days 1-5 (5 doses)

4.2.5 Induction II for patients with FLT3-ITD: LD-ADE + sorafenib

Sorafenib, a commercially available inhibitor of FLT3, will be incorporated into the treatment regimen of patients whose leukemic blasts carry FLT3-ITD. Patients must have total bilirubin, SGOT (AST), and SGPT (ALT) < two times the upper limit of normal (ULN) for age to be eligible to receive sorafenib.

Patients with FLT3-ITD will take sorafenib, 400 mg/m^2 per day, orally in two divided doses (200 mg/m^2/dose BID) starting one day after the completion of Induction II and continuing for 21 days. The maximum dose of sorafenib will be 400 mg PO BID. If needed, the sorafenib 200 mg tablet will be cut (halved or quartered) and the dose will be rounded up to the nearest 50 mg as shown in the table below. Sorafenib may be taken with or without food. If taken with food, sorafenib should be taken with a moderate to low fat meal. If a patient has vomiting within 30 minutes of taking sorafenib, the drug may be re-administered.

Sorafenib 400 mg/m^2/day rounded to the nearest 50 mg divided in two doses

BSA Range	Calculated Daily Dose	Actual Daily Dose	AM Dose	PM Dose
0.18 – 0.31 m^2	72 -124 mg	100 mg	50 mg	50 mg
0.32 – 0.43 m^2	125 -174 mg	150 mg	100 mg	50 mg
0.44 – 0.56 m^2	175 – 224 mg	200 mg	100 mg	100 mg
0.57 - 0.68 m^2	225 – 274 mg	250 mg	150 mg	100 mg
0.69 – 0.81 m^2	275 – 324 mg	300 mg	150 mg	150 mg
0.82 – 0.93 m^2	325 – 374 mg	350 mg	200 mg	150 mg
0.94 – 1.06 m^2	375 – 424 mg	400 mg	200 mg	200 mg
1.07 – 1.18 m^2	425 – 474 mg	450 mg	250 mg	200 mg
1.19 – 1.31 m^2	475 – 524 mg	500 mg	250 mg	250 mg
1.32 – 1.43 m^2	525 – 574 mg	550 mg	300 mg	250 mg
1.44 – 1.56 m^2	575 – 624 mg	600 mg	300 mg	300 mg
1.57 – 1.68 m^2	625 – 674 mg	650 mg	350 mg	300 mg
1.68 – 1.81 m^2	675 – 724 mg	700 mg	350 mg	350 mg
1.82 – 1.93 m^2	725 – 774 mg	750 mg	400 mg	350 mg
≥ 1.94 m^2	≥ 775 mg	800 mg*	400 mg	400 mg

**Maximum dose. (400 mg BID is the FDA approved adult dose)*

Patients with FLT3-ITD who do not experience toxicity related to Sorafenib after Induction II may also receive a 21-day course of sorafenib after subsequent courses of chemotherapy.

Voriconazole and other azoles should be avoided during sorafenib administration. Micafungin should be used for fungal prophylaxis in patients who are receiving sorafenib.

Sorafenib will be dose-reduced by 50% or discontinued in patients who experience the following adverse events if such events are felt to be probably or definitely related to

sorafenib. The treating physician, in consultation with the PI, will determine whether to reduce the dose or discontinue sorafenib.

Note that all dose reductions will be made by deleting the evening dose, thereby reducing the daily dose by approximately 50%.

- Any grade 4 non-hematological toxicity
- Any grade 3 non-hematological toxicity with the exception of
  - Grade 3 nausea and vomiting of less than < 5 days duration
  - Grade 3 transaminases that return to levels that meet initial eligibility criteria within 7 days of sorafenib interruption and that do not recur upon re-challenge with sorafenib
  - Grade 3 fever or infection < 5 days duration
  - Grade 3 hypokalemia, hypophosphatemia, hypomagnesemia, or hypocalcemia responsive to oral supplementation
- Any grade 2 non-hematological toxicity that persists for ≥ 7 days and is considered sufficiently medically significant or sufficiently intolerable by patients that it requires treatment interruption
- Hypertension defined as:
  - A diastolic blood pressure >25 mmHg above the 95th percentile for age and gender confirmed by repeated measurement
  - In patients on antihypertensive therapy, a diastolic blood pressure ≤ 25 mmHg above the 95th percentile for age and gender for > 14 days
  - Grade ≥ 4 hypertension

Suggested Dose Modifications for Sorafenib for Hand-Foot Skin Reaction and skin rash

Hand-foot skin reaction		
Grade	**Occurrence**	**Suggested Dose Modification**
Grade 1 Numbness, dysesthesia, paresthesia, tingling, painless swelling, erythema or discomfort of the hands or feet which does not disrupt the patient's normal activities	Any	Promptly institute supportive measures such as topical therapy for symptomatic relief and continue sorafenib treatment
Grade 2 Painful erythema and swelling of the hands or feet and/or discomfort affecting the patient's normal activities	First	Promptly institute supportive measures such as topical therapy for symptomatic relief and decrease sorafenib by 50% • If toxicity resolves to grade 0–1 after dose reduction, may increase sorafenib back to full dose. • If toxicity develops rapidly within several days or does not resolve to grade 0–1 despite dose reduction, interrupt sorafenib treatment for a minimum of 7 days and until toxicity has resolved to grade 0–1. • When resuming treatment after dose interruption, resume sorafenib at reduced dose • If toxicity is maintained at grade 0–1 at reduced dose for a minimum of 7 days, may increase back sorafenib to full dose.
	Second	Discontinue sorafenib

Suggested Dose Modifications for Sorafenib for Hand-Foot Skin Reaction and skin rash

Grade 3 Moist desquamation, ulceration, blistering or severe pain of the hands or feet, or severe discomfort that causes the patient to be unable to work or perform activities of daily living	First occurrence	Institute supportive measures such as topical therapy for symptomatic relief and interrupt sorafenib treatment for a minimum of 7 days and until toxicity has resolved to grade 0–1 • When resuming treatment after dose interruption, decrease sorafenib by 50%. • If toxicity is maintained at grade 0–1 at reduced dose for a minimum of 7 days, may increase back sorafenib to full dose.
	Second	Discontinue sorafenib
Skin rash		
Grade	**Occurrence**	**Suggested Dose Modification**
Grade 1 Macular or papular eruption or erythema without associated symptoms	Any	Promptly institute supportive measures such as topical therapy for symptomatic relief and continue sorafenib treatment
Hand-foot skin reaction		
Grade	**Occurrence**	**Suggested Dose Modification**
Grade 2 Macular or papular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area (BSA)	First	Promptly institute supportive measures such as topical therapy for symptomatic relief and decrease sorafenib by 50% • If toxicity resolves to grade 0–1 after dose reduction, may increase sorafenib back to full dose. • If toxicity develops rapidly within several days or does not resolve to grade 0–1 despite dose reduction, interrupt sorafenib treatment for a minimum of 7 days and until toxicity has resolved to grade 0–1. • When resuming treatment after dose interruption, resume sorafenib at reduced dose • If toxicity is maintained at grade 0–1 at reduced dose for a minimum of 7 days, may increase back sorafenib to full dose.
	Second	Discontinue sorafenib
Grade 3 Severe, generalized erythroderma or macular, papular or vesicular eruption; desquamation covering ≥50% BSA	First	Institute supportive measures such as topical therapy for symptomatic relief and interrupt sorafenib treatment for a minimum of 7 days and until toxicity has resolved to grade 0–1 • When resuming treatment after dose interruption, decrease sorafenib by 50% • If toxicity is maintained at grade 0–1 at reduced dose for a minimum of 7 days, may increase back sorafenib to full dose.
	Second	Discontinue sorafenib

See Section 5.9 for management of hypertension related to sorafenib

4.2.6 Induction II for other HR patients: LD-ADE + vorinostat*

Patients with M7 AML without t(1;22) and other HR patients without FLT3-ITD will be treated with a combination of vorinostat and LD-ADE. Vorinostat (100 mg/m^2/dose PO TID) will be given for 3 days (Days -2, -1, 0) prior to the initiation of Induction II chemotherapy.

Days -2, -1, 0: Vorinostat 100 mg/m^2/dose PO TID
Days 1-8: LD-ADE as described above

Histone acetylation will be assessed *in vivo* as a measure of the effects of vorinostat. Peripheral blood samples (collected in 3 mL of preservative-free heparin) will be drawn as follows:

1. Prior to the first dose of vorinostat on Day -2
2. On Day 1 prior to the initiation of chemotherapy

Suggested dosing table for vorinostat* capsule doses at 300 mg/m^2/**day**, given TID

BSA range (m^2)	Calculated dose range	Capsules per day (100 mg/capsule)	Dose divisions am/mid-day/pm (number of capsules)
0.9 to 1.15	270 mg to 345 mg	3	1/1/1
1.16 to 1.50	348 mg to 450 mg	4	2/1/1
1.51 to 1.83	453 mg to 549 mg	5	2/2/1
1.84 to 2.17	552 mg to 651 mg	6	2/2/2
2.18 to 2.49	654 mg to 747 mg	7	3/2/2
≥ 2.5		8	3/3/2

Patients with a BSA less than 0.9 m^2 and patients who cannot swallow whole capsules should take vorinostat liquid 100 mg/m^2/**dose**, rounded to the nearest 5 mg.

A suspension can be prepared locally by mixing 20 mL of OraPlus with the contents of twenty 100 mg vorinostat capsules in a 4 ounce glass bottle. After shaking for up to 3 minutes to disperse, add an additional 20 mL of OraSweet. Shake the container to disperse, resulting in a final concentration of 50 mg/mL. The suspension should be stored at room temperature for a maximum of 2 weeks.

*__Note__: Collaborating sites may elect to not participate in vorinostat exploratory objective of this study. If the decision is made that a site will opt out, then all applicable patients at that site will receive standard therapy for Induction II with LD-ADE.

Reference: Fouladi M, Park JR, Stewart CF, et al, "Pediatric Phase I Trial and Pharmacokinetic Study of Vorinostat: A Children's Oncology Group Phase I Consortium Report," J Clin Oncol, 2010, 28(22):3623-9. [PubMed 20606092]

4.2.7 Special subgroup

HR patients with MRD < 0.1% may proceed directly to SCT after Induction I if a suitable donor is available and the transplant can be performed without delay. Based on the results of AML02, this will apply to about 1% of patients.

4.3 Consolidation I for patients who will not receive SCT

All LR patients, those SR patients who will not receive SCT, and HR patients who do not have a suitable donor, will receive mitoxantrone, and cytarabine (MA) as consolidation I. Criteria for starting Consolidation I: ANC ≥ 300/µl and rising and platelet count ≥ 30,000/µl and rising.

4.3.1 MA (mitoxantrone/Ara-C)

- Mitoxantrone: 12 mg/m^2 (0.4 mg/kg for patients less than 10 kg) IV over 1 hour on days 3-5 (3 doses)
- Cytarabine: 1 g/m^2 (33 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 2 hours every 12 hours on days 1-4 (8 doses)

4.4 Consolidation therapy for HR patients who will undergo SCT

HR patients are eligible to undergo SCT after Induction II. HR patients who have < 0.1% MRD and for whom a donor has been identified should proceed directly to SCT after Induction II. HR patients who have ≥ 0.1% MRD or for whom a donor workup is still in progress are eligible to receive therapy prior to SCT as follows:

4.4.1 HR patients who have < 0.1% MRD and are awaiting SCT

4.4.1.1 HR patients who have <0.1% MRD, but for whom a SCT donor is not yet available (expected time from count recovery after Induction II to SCT greater than about 4 weeks), should receive a third course of chemotherapy consisting of mitoxantrone and cytarabine (MA) identical to consolidation I above

- Mitoxantrone: 12 mg/m^2 (0.4 mg/kg for patients less than 10 kg) IV over 1 hour on days 3-5 (3 doses)

- Cytarabine: 1 g/m^2 (33 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 2 hours every 12 hours on days 1-4 (8 doses)

4.4.1.2 HR patients who have <0.1% MRD who will undergo SCT less than 4 weeks after Induction II, may receive low-dose cytarabine and oral thioguanine, rather than MA, at the discretion of the treating physician.

- Cytarabine: 75 mg/m^2 (2.5 mg/kg for infants < 1 month of age or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 30 minutes on days 1-4 and 15-18

- Thioguanine: 60 mg/m^2 orally at bedtime on days 1-28

4.4.2 HR patients who have ≥ 0.1% MRD after Induction II

Because the optimal therapy for patients who will undergo SCT, but who have MRD ≥ 0.1% is unknown, this protocol will allow several therapeutic options. The therapy for each patient will be decided by the treating physician, with consultation from the local SCT team and the PI.

Option 1: SCT

Patients with low levels of MRD (e.g., < 1%) and for whom a SCT donor is ready, may proceed directly to SCT.

Option 2: NK cell therapy, followed by SCT

Patients who have a KIR-mismatched family member who is greater than 18 years old may undergo NK cell transplantation as described below. These patients will then undergo SCT after recovery from NK cell transplantation.

Option 3: Conventional therapy, followed by SCT

Patients who have ≥ 0.1% MRD are eligible to receive conventional chemotherapy with mitoxantrone and cytarabine as described above (section 4.4.1.1) prior to SCT.

4.5 Consolidation II (HD-AraC/Asp)

Criteria for starting Consolidation II: ANC ≥ 300/μl and rising and platelet count ≥ 30,000/μl and rising.

- Cytarabine 3 g/m^2 (100 mg/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV over 3 hours every 12 hours on days 1, 2, 8, 9 (8 doses)
- E. coli L-asparaginase 6000 (200 Units/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) Units/m^2 IM 3 hours after 4th and 8th doses of cytarabine.

*When E. coli L-asparaginase is no longer available, each dose during consolidation II should be replaced with:

- Erwinia asparaginase 25,000 Units/m^2 (833 Units/kg for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation) IV or IM (according to institutional standard) over 1 hour, 3 hours after the 4^{th} and 8^{th} doses of cytarabine.

Each institution should follow local administration guidelines for this commercially available agent. The following suggested guidelines may be used, but are not required.

Administration

For IV administration, *Erwinia* asparaginase will be diluted in 50 mL NS to infuse over 30 minutes to 1 hour via syringe. *Erwinia* asparaginase should be administered to run concurrently with IVF. (NS at TKO is sufficient.)

Erwinia asparaginase should be administered at least 2 hours after an LP/IT since some cases of over sedation were reported when given "simultaneously" with sedation and/or an LP/IT at other institutions. Further, it is desirable to avoid asparaginase directly before an IT because asparaginase could possibly interfere with the efficacy of methotrexate.

Erwinia asparaginase may also be administered IM.

Monitoring and concerns for anaphylaxis

Obtain vital signs pre infusion, and then remain at the patient's bedside for the first 5 minutes. Visually observe the patient at 10 minutes, and directly observe and obtain vital signs at 15 minutes and at the end of the infusion. Repeat vital signs at discharge. Patients must remain in the Medicine Room for 1 hour after administration to be observed for adverse effects. Keep NS at TKO during the 1 hour post watch time unless otherwise ordered. To prepare for anaphylaxis:

- Have oxygen, suction and pulse oximetry at bedside during and after infusion
- Have readily available* the following medications:
    - Diphenhydramine 1 mg/kg (max 50 mg) for IV administration
    - Epinephrine (1:1000) 0.01 mL/kg (max 0.3 mL) for **SQ** administration
    - Hydrocortisone 100 mg/m2 for IV administration
    - NS for IV administration

*Readily available means in the general area – such as the emergency medication box in the medicine room or inpatient areas.

4.6 NK cell therapy

Criteria for starting NK cell therapy: ANC ≥ 300/µl and rising and platelet count ≥ 30,000/µl and rising.

Standard risk patients who have a KIR-mismatched family member who is greater than 18 years old will undergo NK cell transplantation. In addition, HR patients who do not have a suitable stem cell donor or who decline SCT will be eligible for NK cell therapy if they have a KIR-mismatched family member, but will be analyzed separately. All other patients will receive no further therapy.

4.6.1 Donor selection and testing

Patients will receive NK cell transplantation from an adult family member who shares at least one HLA-haplotype with the recipient. The donor will be selected such that there is donor KIR – recipient KIR-ligand mismatch. Specific exclusion criteria include pregnancy and any other medical condition that, in the opinion of an independent physician, precludes performance of an apheresis procedure.

Donors will undergo screening, examination, and testing as described in 21 CFR 1271 and the Guidance "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" to determine eligibility.

Histocompatibility testing, including HLA and KIR genotyping, are performed in a CLIA-certified, ASHI- and CAP-inspected laboratory of the Department of Pathology, SJCRH. Molecular techniques will be utilized to characterize donor and recipient HLA Class I gene content to the level sufficient to determine the ligand repertoire for KIR recognition.

4.6.2 NK cell collection and selection

On day -1 the donor will undergo apheresis once. The cells obtained will be purified for CD56+ cells by the two-step procedure described previously.[58] The CliniMACS selection column will be operated using the Standard Operating Procedures of the Human Applications Laboratory. For this protocol, our goal is to infuse immediately after processing on Day 0 all the NK cells collected to give $>2x10^6$ $CD56^+$ cells/kg of recipient body weight, but allowing for a $CD3^+CD56^-$ cell dose of no greater than $0.05x10^6$ /kg. The maximum dose of cells that would be infused is 400 x 10^6 CD56+ cells/kg. The minimum dose that would be infused is 0.1 x 10^6 cells/kg**.** The lot release criteria of the NK cell product is detailed in the table below.

Parameter	Specification
CD56+ purity	≥ 70% CD56+ cells
CD3+ log depletion	≥ 2.5 logs
Viability	≥ 70%
Gram Stain	No organisms seen
*Calcofluor White stain	No fungal elements seen
*Sterility	Negative
*Endotoxin	≤ 2 EU/ml

**Product can be released for immediate infusion with results pending. Final review must include complete results*

4.6.3 Quality assurance for cellular products

The Department of Therapeutic Production and Quality has established an independent division of Quality Assurance (QA). This group is responsible for the management of Quality Control, Quality Assurance and Quality Improvement processes for the Human Applications Laboratory. Production and QA Systems that are in place include:

- Standard Operation Procedures for production and quality processes
- Documentation of Donor Eligibility
- Documentation of processes captured in Batch Records
- In-process quality control testing including sterility
- Release Specifications established for all products
- Out of Specification Reporting and Investigation Process
- Authorization by QA for the release of all products after review of records and release specification test results
- Product labeling procedures with multiple person review
- Variance Management Process
- Personnel Competence and Proficiency Program
- Inventory control and documentation of product history through patient infusion

Test results that are out of specification for products that are needed on a clinically urgent basis will be evaluated by the laboratory medical director. The patient's physician or attending transplant physician will be informed of the test result prior to infusion of the product. Notification regarding positive sterility results before or after infusion will be given to the primary attending physician and patient and/or parent/guardian. Notification to the FDA and St. Jude IRB will be given and will include testing results or adverse events and any required intervention. An investigation following TPQ/HAL SOPs will be completed, reviewed by TPQ Quality Assurance and outcomes of the investigation reported.

4.6.4 Treatment schema

- Day -7: Cyclophosphamide 60 mg/kg IV over 1 hour. Patients should receive IV hydration at 150 ml/m^2/hr for 2 hours prior to cyclophosphamide and 24 hours after cyclophosphamide. Mesna 15 mg/kg/dose IV should be given immediately before cyclophosphamide and at 3, 6, and 9 hours after cyclophosphamide.
- Days -6 through -2: Fludarabine 25 mg/m^2/day IV over 30 minutes (5 doses)
- Days -1, +1, +3, +5, +7, +9: IL-2 1 million units/m^2 given subcutaneously
- Day -1: Donor pheresis
- Day 0: NK cell infusion

NK cells may be infused in either the inpatient or outpatient setting by a physician, Physician Assistant, Nurse Practitioner, or qualified RN. Careful monitoring and supportive care during NK cell infusion will be guided in part by the Standard Operating Procedures for Lymphocytes Infusions in the St. Jude Nursing Policy & Procedure Manual (See Attached Nursing Policy and Procedure: Therapeutic Cell Infusion).

No steroids, including the use of hydrocortisone as pre-medication, may be given to patients during the 3 days prior to the NK cell infusion or during the first 7 days after the infusion.

MESNA dose and administration may vary based on physician recommendation and institutional standards. These variations will not be considered treatment deviations.

4.7 CNS therapy

All patients should undergo lumbar puncture and receive an age-appropriate dose of intrathecal (IT) chemotherapy at the time of diagnosis. Triple intrathecal therapy with methotrexate, hydrocortisone, and cytarabine (MHA) will be used for all CNS therapy at the doses shown below. However, patients who receive IT cytarabine prior to enrollment on AML08 will be eligible for treatment on AML08 and should not receive an additional dose of IT MHA at the time of enrollment.

To reduce the risk of neurotoxicity, we recommend separating IT therapy from high-dose cytarabine (≥ 1 g/m^2) by approximately 24 hours. If systemic therapy needs to begin urgently (e.g., in cases with hyperleukocytosis), the initial dose of IT therapy may be delayed as the discretion of the treating physician.

IT MHA Dosing

Patient Age	Methotrexate	Hydrocortisone	Cytarabine	Volume
< 1 year	6 mg	12 mg	18 mg	6 ml
1-2 years	8 mg	16 mg	24 mg	8 ml
2-3 years	10 mg	20 mg	30 mg	10 ml
> 3 years	12 mg	24 mg	36 mg	12 ml

Leucovorin rescue (5 mg/m^2 per dose; 5 mg maximum per dose) will be given orally or intravenously at 24 and 30 hours after each IT MHA treatment. Leucovorin dosing and intrathecal volume may be adjusted according to local institutional guidelines.

Patients with no evidence of CNS disease (CNS1: no leukemic blast cells on CSF cytospin) will receive 4 total doses of intrathecal therapy, given at approximately one month intervals or at the beginning of each of the first 4 courses of chemotherapy. IT therapy will not be given before NK cell therapy.

Patients with overt CNS leukemia (CNS3: ≥ 5 leukocytes per µl of CSF and the presence of leukemic blast cells on CSF cytospin) will receive weekly intrathecal therapy until the CSF is free of blast cells (minimum number of doses, 4). These patients will then receive3 additional doses of intrathecal therapy (minimum total number of doses, 7) at approximately 1-month intervals (generally given with each subsequent course of chemotherapy). IT therapy will not be given before NK cell therapy.

Patients with < 5 leukocytes per µl of CSF and the presence of leukemic blast cells on CSF cytospin (CNS2) will receive weekly intrathecal therapy until the CSF is free of blast cells. These patients will then receive 3 additional doses of intrathecal therapy at approximately

1-month intervals (generally given with each subsequent course of chemotherapy). IT therapy will not be given before NK cell therapy.

Patients who are unable to undergo lumbar puncture and receive intrathecal therapy prior to starting induction I should be treated as CNS2 unless they have overt CNS leukemia (CNS3).

5.0 SUPPORTIVE CARE GUIDELINES

5.1 Prophylaxis and treatment of metabolic derangement

Care should be taken to prevent hyperuricemia and hyperphosphatemia in patients with large tumor burdens. Such patients should receive IV hydration at 3000 ml/m^2/day before the initiation of therapy, oral phosphate binders (aluminum hydroxide, calcium carbonate, or sevelamer), and recombinant urate oxidase or allopurinol as needed.

5.2 Treatment of hyperleukocytosis

In patients with WBC > 100 x 10^9/L or symptoms of hyperviscosity, leukapheresis or exchange transfusion may be used according to local institutional guidelines. The administration of hydroxyurea (10 to 20 mg/kg/day PO; maximum 1000 mg PO BID) or cytarabine (100 mg/m^2/dose IV every 12 hours) is also allowed.

5.3 Prevention and treatment of complications related to tumor lysis

Patients with AML, especially those with high leukocyte counts and M4, M4Eo, or M5 morphology, are at risk for severe cardiopulmonary complications during the initiation of chemotherapy.[177] Although the mechanism of these complications is unknown, it is believed that rapid cell lysis may lead to a systemic inflammatory response syndrome (SIRS). We have also observed that this inflammatory response may respond to treatment with steroids and may be prevented by steroids. Therefore, for patients with elevated leukocyte counts, investigators should consider methylprednisolone, 0.5 to 1 mg/kg/dose IV every 12 hours, starting prior to the first dose of chemotherapy and continuing for 3 to 5 days. In addition, hydroxyurea, or low-dose cytarabine may also be given prior to induction therapy to reduce tumor burden and reduce the risk of SIRS. Hydroxyurea or low-dose cytarabine may be given prior to Induction I at the discretion of the treating physician.

5.4 Prophylaxis for *Pneumocystis jiroveci* pneumonia

All patients should receive TMP/SMZ (trimethoprim 150 mg/m^2/day and sulfamethoxazole 750 mg/m^2/day in 2 divided doses on Monday, Tuesday, and Wednesday of each week). For patients who cannot tolerate TMP/SMZ, daily oral atovaquone or monthly aerosolized pentamidine may be substituted.

5.5 Prophylaxis for fungal infections (REQUIRED)

Patients with AML are at especially increased risk for fungal infections, most commonly candidiasis and aspergillosis. Although there is no national standard for antifungal prophylaxis of these patients, effective regimens include voriconazole and posaconazole.

Micafungin and caspofungin are acceptable alternatives, but fluconazole and itraconazole are not recommended because of lack of activity against Aspergillus. Antifungal prophylaxis should be initiated at the completion of induction I and continue until count recovery after the final course of chemotherapy. Prophylaxis should be given daily except on days that patients are receiving daunorubicin, etoposide, or mitoxantrone.

Acceptable antifungal prophylaxis options are listed below, in order of preference. Currently, we recommend voriconazole, as we prefer to reserve posaconazole for breakthrough infections. **All patients on AML08 must receive prophylactic antifungal therapy**, although the agent used may be based on local institutional guidelines. Please call the PI if you wish to use an antifungal agent other than one listed below.

Voriconazole and other azoles should be avoided during sorafenib administration. Micafungin may be used for fungal prophylaxis in patients who are receiving sorafenib.

- Voriconazole PO

  ≥ 1 – 11 years: Loading dose: Not recommended
  Maintenance Dose: 7 mg/kg q12h (rounded up to 50 or 100 mg dose increments.)

  ≥ 12 years, < 40 kg: Loading dose: 200 mg PO q12h day 1
  Maintenance Dose: 100 mg PO q12h

  ≥12 years, ≥ 40 kg: Loading dose: 400 mg PO q12h day 1
  Maintenance Dose: 200 mg PO q12h

- Posaconazole

  Patients > 13 years old: 200 mg PO 3 times/day with meals
  Patients < 13 years old: dose not established

- Micafungin:

  ≤ 40 kg: 1 mg/kg/day IV (max 50 mg/day)
  > 40 kg: 50 mg/day IV

- Liposomal amphotericin B IV: 3-5 mg/kg/day

- Caspofungin: 1 mg/kg/day IV (max 50 mg/day)

5.6 Prophylaxis for viridans streptococcal infection (REQUIRED)

All patients should have a dental evaluation at the time of diagnosis. During and following each course of chemotherapy, we recommend oral rinsing with 10-15 ml undiluted chlorhexidine (Peridex) for 30 seconds 2-4 times per day. For infants and small children, a soft cloth saturated with rinse can be rubbed on gum and at tooth line.

Because patients with AML are at high risk for bacterial sepsis, especially sepsis due to viridans streptococci, **all patients on AML08 must receive prophylactic antibiotics** (see section 2.1.13 for rationale). Prophylactic antibiotics should be started when the ANC < 1000 and falling or predicted to fall, and continue until the ANC > 100 and rising. Antibiotics may be given by the parents or other caregivers at home, according to local institutional guidelines.

Acceptable prophylactic regimens, in order of preference, include the following:

- Vancomycin 400 mg/m^2/dose IV every 12 hours (maximum 1 gram per dose) plus ciprofloxacin 250-350 mg/m^2/dose PO every 12 hours (maximum 500 mg per dose)
- Cefepime 1500 mg/m^2/dose IV every 12 hours (maximum 2 gram per dose)
- Vancomycin 400 mg/m^2/dose IV every 12 hours (maximum 1 gram per dose) plus cefepime 1500 mg/m^2/dose IV every 12 hours (maximum 2 gram per dose). Note that this regimen is not recommended by the PI, but is acceptable if preferred by the local institution or treating physician.
- Please call the PI if you wish to use a prophylactic regimen other than one listed above. Because local infection rates, organisms, and susceptibilities vary, other prophylactic regimens will be allowed. However, regimens should be uniform at each collaborating site and data on each regimen will be captured to explore the efficacy of each regimen.

5.7 Management of febrile neutropenia

Episodes of fever and neutropenia should be managed according to institutional guidelines. Patients with fever (defined as any oral temperature ≥ 38.3° C or an oral temperature of ≥ 38.0° C that persists for one hour) and neutropenia (defined as ANC < 500 cells/μL) should be given IV antibiotics immediately. We recommend the following guidelines:

- Patients who develop fever while receiving prophylactic vancomycin and ciprofloxacin:
    - Begin cefepime (1500 mg/m^2/dose IV every 8 hours)
    - Increase vancomycin to 400 mg/m^2/dose IV every 8 hours
    - Discontinue ciprofloxacin
- Patients who develop fever while receiving prophylactic cefepime:
    - Begin vancomycin (400 mg/m^2/dose IV every 8 hours) and meropenem (20 mg/kg/dose IV every 8 hours)
    - Discontinue cefepime

Patients who have suspected catheter-related infection, have evidence of sepsis (including shock, hypotension, rigors, septic emboli, unexplained respiratory distress or hypoxemia, or poor peripheral perfusion), or are known to be colonized by *Pseudomonas aeruginosa*, should also receive tobramycin, 60 mg/m^2/dose IV every 6 hours.

Patients who have severe abdominal pain or radiographic findings suggesting typhlitis, severe abdominal pain with evidence of sepsis, focal findings suggestive of intra-abdominal infection on physical examination, or known or suspected infection with *Bacillus cereus* should receive meropenem, 20 mg/kg/dose IV every 8 hours, instead of cefepime.

5.8 Management of Capillary Leak Syndrome related to clofarabine

In pediatric studies, during or shortly after clofarabine administration a few patients developed signs and symptoms consistent with capillary leak syndrome. In these heavily pretreated patients, it has been difficult to separate potential drug-related cases of capillary leak syndrome from concurrent medical conditions such as infection/sepsis, progressive disease, or other underlying problems resulting from prior anti-leukemic therapies.

For these reasons, during and after each dose of clofarabine investigators are to assess patients for the onset of the following signs or symptoms ≥ grade 2:

- Tachypnea or other evidence of respiratory distress;
- Unexplained hypotension; and/or
- Unexplained tachycardia.

If one or more of these signs or symptoms occurs during study drug infusion, clofarabine administration is to be interrupted or held as clinically indicated. It is recognized that the total infusion time for this clofarabine dose in this circumstance may exceed 1 hour. Thus, if the patient's condition stabilizes or improves, clofarabine administration may resume. Pretreatment with steroids (methylprednisolone, 0.5 to 1 mg/kg/dose IV) is recommended for all subsequent doses during the remainder of that treatment cycle.

5.9 Management of hypertension related to sorafenib

A diastolic blood pressure (DBP) equal to the 95th percentile for age and gender will be defined as the upper limit of normal (ULN). Patients with elevated DBP at any time should have blood pressure measurements performed twice weekly until the DBP is less than the ULN. Patients with Grade 2/3 hypertension that is well controlled with oral medications may continue therapy. Sorafenib should be discontinued for persistent, symptomatic, or Grade 4 hypertension.

If the DBP remains above the ULN for age and gender on recheck, then start single agent antihypertensive therapy and monitor blood pressure at least every 3 days. If the DBP drops below the ULN for age within 14 days, continue single agent antihypertensive therapy. However, if the DBP remains above the ULN for age for more than 14 days after the institution of antihypertensive, sorafenib should be discontinued.

5.10 Drug interactions

Efforts should be made to avoid the use of enzyme-inducing anticonvulsants, such as phenytoin, phenobarbital, and carbamazepine. Because azole antifungals such as fluconazole, itraconazole, voriconazole, and posaconazole may have inhibitory effects on

drug-metabolizing enzymes, these agents should be held on days that patients are receiving daunorubicin, etoposide, or mitoxantrone.

5.11 Growth factors

Prophylactic use of hematopoietic growth factors (GM-CSF or G-CSF) is not recommended. However, GM-CSF (250 μg/m^2/day) or G-CSF (5-10 μg/kg/day) should be considered for patients who have documented or suspected fungal infections or bacterial sepsis.

5.12 Conjunctivitis prophylaxis

Dexamethasone ophthalmic solution (0.1%), 2 drops to both eyes four times per day, or artificial tears (e.g., hydroxymethylcellulose, hypromellose, polyvinyl alcohol), 2 drops to both eyes every 2-6 hours, should be used during HDAC administration and for 24 hours after completion to prevent conjunctival irritation.

5.13 Treatment modifications

5.13.1 Renal impairment

Patients who have received amphotericin B or nephrotoxic antibiotic regimens for at least 7 days, and patients in whom serum creatinine is greater than two times normal for age, should have their glomerular filtration rate (GFR) measured before they receive HDAC. In patients with a GFR ≤ 60 ml/min per 1.73m^2, we recommend decreasing the dosage of cytarabine from 3 g/m^2 to 2 g/m^2 every 12 hours. No decrease is necessary for standard dose cytarabine (≤ 1g/m^2/day) regimens.

5.13.2 CNS toxicity

Patients with grade 3 or greater cerebellar toxicity should not receive additional HDAC. These cases should be discussed with the PI to determine further management.

5.13.3 Cardiac toxicity

Patients with clinical evidence of congestive heart failure should receive no additional daunorubicin or mitoxantrone. In the event of a fractional shortening < 28%, consideration will be given to discontinuing administration of daunorubicin and mitoxantrone.

5.13.4 Hepatic toxicity

Patients who develop veno-occlusive (VOD) disease of the liver (manifested by greater than 5% increase in weight and a total bilirubin greater than 5 mg/dL) during induction I or induction II should not receive treatment with GO. The treatment of patients who are candidates for GO but who have developed VOD should be discussed with the Principal Investigator. These patients are eligible to remain on study.

Daunorubicin, mitoxantrone, and etoposide doses should be adjusted in patients who develop increased direct bilirubin levels during therapy.

Daunorubicin and mitoxantrone should be adjusted as follows:

- Direct bilirubin 2-4 mg/dl: 50% dose decrease
- Direct bilirubin 4-6 mg/dl: 75% dose decease
- Direct bilirubin > 6 mg/dl: hold dose

Similar dose reductions of etoposide should be considered in patients with elevated bilirubin, especially in the presence of serum albumin levels less than 2.5 g/dl.

At the time of diagnosis, elevated bilirubin levels may reflect leukemic infiltrate, rather than toxic liver damage. In such cases, full doses of chemotherapy may be given at the discretion of the treating physician.

5.13.5 Dosing of infants

To reduce the potential for cardiac toxicity, dosing of daunorubicin and mitoxantrone for patients less than 10 kg will be based on weight rather than body surface area. Similarly, for infants < 1 month of age, or for infants < 3 months of age who were born significantly prematurely defined as < 36 weeks gestation, dosing of cytarabine, clofarabine, asparaginase, and etoposide, will be based on weight.

6.0 DRUG INFORMATION

See Appendix I for Information on all agents to be used in this protocol.

7.0 EVALUATIONS, TESTS, AND OBSERVATIONS

7.1 Required evaluations for diagnosis and response (all patients)

	Baseline	Day 22 of Ind I	Prior to each course of therapy	End of therapy[g]	At time of relapse
CBCD	X	X	X	X	X
CMP	X		X	X	X
ECHO or MUGA	X		X[e]	X[e]	
EKG - **REQUIRED**	X		X[e]	X[e]	
BMA[a]	X[a]	X[f]	X[f]	X[f]	X[f]
Bone marrow biopsy[b]	**X**[b]				
Peripheral blood MRD		Days 8 and 22			
Cell count and cytospin of CSF[c]	X	X	X		X
Pregnancy test[d]	X				
HLA typing of patient, parents, and full siblings	X[h]				
NK typing of parents[i]			Prior to cons I		
NK cell receptor studies	X		Prior to cons I		
Pharmacogenetic studies	X		Prior to cons I		

CBCD, complete blood count with differential; CMP, complete metabolic panel, including electrolytes, BUN, creatinine, AST, ALT, bilirubin. Daily LFTs (ALT, AST, total and direct bilirubin) and serum ammonia levels during vorinostat administration for patients receiving vorinostat during Induction II.

[a] At the time of enrollment, a bone marrow aspirate should be obtained for morphologic, immunophenotypic (including the identification of a leukemia-associated phenotype for MRD studies), molecular, and cytogenetic analyses. For patients with elevated leukocyte counts and high blast percentages, and patients too ill to undergo bone marrow aspirate, all diagnostic studies may be performed on blood rather than bone marrow.

[b] Bone marrow biopsies may be omitted in cases in which a diagnostic bone marrow aspirate is not performed.

[c] Examination of the CSF should be performed with every dose of intrathecal therapy

[d] Applies only to female patients of childbearing potential

[e] ECHO or MUGA, and EKG should be performed prior to chemotherapy courses that include daunorubicin or mitoxantrone. Patients who have a normal baseline ECHO or MUGA and who receive clofarabine and cytarabine in Induction I do not need a cardiac evaluation prior to Induction II. The end of therapy cardiac evaluations may be performed anytime within the first year after completion of therapy.

[f] All subsequent bone marrow aspirates should be sent for morphologic and MRD analyses on all patients. In addition, for patients with leukemia-specific fusion transcripts, all subsequent bone marrow aspirates should be sent for RT-PCR analysis.

[g] The time of neutrophil and platelet recovery after consolidation II (or after NK cell therapy where applicable) will be considered the end of therapy.

[h] HLA typing of patients should include allele level resolution for HLA-A, B, Cw, and DR□1. HLA typing may be done at St. Jude at no cost to participant, family or collaborating institutions.

[i] For patients who are potential candidates for NK cell therapy (see section 4.1), NK and HLA typing must be obtained from both parents prior to consolidation I.

See section 10.3 for Pharmacokinetic Studies
See section 10.5 for Histone acetylation Studies

7.2 Additional required evaluations for patients receiving NK cells

7.2.1 Pre-study evaluation for recipients

- Pre-transplant chimerism
- NK cell phenotyping

7.2.2 Pre-study evaluation for donors

- HLA typing
- Pre-transplant chimerism
- NK cell phenotyping

7.2.3 Evaluations during treatment

- BMA for morphology, MRD, and RT-PCR (if applicable) 1 month after NK cell infusion
- NK cell chimerism studies should be performed on days 7, 14, 21, and 28

Note: because NK cell studies are performed only on weekdays (Monday through Friday), these tests may be sent within 48 hours of the time they are due (e.g., if day 2 falls on a Sunday, the tests may be drawn on day 3). Post-transplant chimerism studies should also be performed after day 28 in cases of persistent chimerism.

7.2.4 NK cell recipients will be monitored for a period of 45 days after the NK cell infusion for GVHD. GVHD will be evaluated and graded using the criteria found in Appendix II of this protocol.

7.3 Long-term follow-up evaluations

The time of neutrophil and platelet recovery after consolidation II or NK cell infusion will be considered the end of therapy.

7.3.1 CBC with differential and platelet count

As clinically indicated

7.3.2 Bone marrow examinations

Bone marrow aspirates for MRD (and RT-PCR if applicable) should be performed every 4 months for one year (i.e., 3 additional evaluations following the end of therapy evaluation). If there is evidence of an aberrant phenotype or leukemia-specific fusion transcript at one year, bone marrow examinations should continue to be performed every 4-6 months or as clinically indicated.

7.3.3 Echocardiograms and EKG should be performed yearly.

7.4 Routine tests

All studies listed in Table 7.1 and 7.3 are considered part of the routine care of AML patients, with exception of NK typing, NK cell receptor studies, pharmacokinetic, pharmacogenetic, and histone acetylation studies.

7.5 Research tests

NK typing studies, NK cell receptor (chimerism and phenotyping) studies, pharmacokinetic, pharmacogenetic, and histone acetylation studies, high-resolution single nucleotide polymorphism (SNP) array and gene expression analysis, phospho-flow profiling, *in vitro* drug sensitivity assays, and minimal residual disease studies are considered research tests in this study.

8.0 EVALUATION CRITERIA

8.1 Response criteria

The response after each course of chemotherapy will be determined by the examination of the bone marrow before the next course of chemotherapy is begun. If two bone marrow examinations are performed after a course of therapy, the second examination will be used to classify the response to that course. For the purposes of this protocol, MRD-negative is defined as < 0.1% blasts with a leukemia-associated phenotype detected by flow cytometry. Because morphologic examination of the bone marrow during periods of hematopoietic recovery after intensive chemotherapy may be unreliable, response will be based on blast percentage by flow cytometry (MRD). Blast percentages determined by morphology will be used in cases that are not evaluable by flow cytometry. If the blast percentage is less than 5% in such cases, they will be classified as Complete Response, MRD not evaluable.

8.1.1 Complete response, MRD-negative
- < 0.1% blasts by flow cytometry

8.1.2 Complete response, MRD-positive
- 0.1% to ≤ 5% blasts by flow cytometry

8.1.3 Partial response
- 5% to 25% blasts by flow cytometry

8.1.4 No response
- > 25% blasts in the bone marrow by flow cytometry

8.1.5 Relapse
- Subsequent appearance, after achievement of CR, of ≥ 5% blasts in the bone marrow by flow cytometry

8.2 Toxicity evaluation criteria

This study will utilize the CTCAE Version 3.0 for toxicity and performance reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov).

9.0 OFF THERAPY AND OFF-STUDY CRITERIA

9.1 Criteria for removal from protocol therapy (off therapy)

- Patients who have ≥ 5% MRD after two courses of therapy may be removed from protocol therapy and entered onto protocols for relapsed or refractory disease at the investigator's discretion. Alternatively, these patients may proceed directly to SCT
- Relapse at any site
- Second malignancy
- Refusal of therapy
- Development of unacceptable toxicity during therapy
- Completion of planned therapy
- Major deviation in protocol therapy not approved by the PI
- Enrollment on another therapeutic study or treatment plan

9.2 Criteria for removal from study (off study)

- Death
- Lost to follow-up
- Withdrawal of consent for further participation or data submission
- Found, after enrollment, to be ineligible

9.3 NK cell donors

NK cell donors will be followed for adverse events and serious adverse events on the day of apheresis and for seven days following apheresis. Donors will be removed from therapy (off therapy) and from study (off study) on the eighth day after apheresis.

9.4 NK cell recipients

Patients who receive NK cell infusions will receive no further therapy, other than supportive care, after the sixth dose of IL-2, which is given on day +9 after the infusion. Thus, they will essentially be off therapy at that time. We will, however, follow these patients until all toxicities have resolved to less than grade 2.

10.0 BIOLOGIC STUDIES

10.1 NK cell receptor study

Peripheral blood will be obtained prior to Induction I and prior to Consolidation I for immunophenotyping and genotyping for NK cell receptors as described previously.[46,164] We will assess NK cell receptors including KIRs, NCRs, NKG2D, DNAM-1, 2B4, and NTBA.

10.2 Minimal residual disease studies

This study will apply immunologic and molecular methods as performed in AML02. As in AML02, immunologic methods will be used for clinical purposes, but molecular results will be reported as well. All participants registered in AML08 whose leukemic cells are amenable to our assays will be eligible for testing. Peripheral blood samples (5-10 ml) will be taken only on days 8 and 22. Bone marrow samples will be analyzed at all time points.

10.3 Pharmacokinetic studies

Note: Pharmacokinetic studies were completed with LOA #4, 5/29/2015

In order to study the pharmacokinetics of sorafenib, 7 blood samples (3 mL each) will be collected in a green top tube prior to the first dose on day 1, then serial blood sampling on day 8 (at steady-state) at pre-treatment, then at 2, 4.5 and 7.5 hours. Weekly pre-treatment troughs before the morning dose will be obtained on days 15 ± 2 days and 21 ± 2 days for the first cycle. Samples for sorafenib pharmacokinetics should be obtained even if sorafenib is discontinued prior to the day of sampling. All pre-treatment trough samples should be obtained within a ± 3 hour window (9-15 hours after the previous dose but before the subsequent dose). Total sorafenib will be quantitated using a validated LC-MS-MS assay and unbound sorafenib will be measured using a validated micro-equilibrium dialysis method in the Pharmacokinetics Shared Resource with Dr. Sharyn Baker as the pharmacokinetic investigator for these studies. Pharmacokinetic data will be analyzed using model-independent and modeling techniques. See appendix II for sample processing and shipping instructions.

10.4 Pharmacogenetic studies

Note: Pharmacogenetic studies were completed with LOA #4, 5/29/2015

We will collect peripheral blood samples at diagnosis and at the start of consolidation (or at the time of count recovery after Induction II) for extraction of germline DNA. Assessment of germline polymorphisms in drug metabolizing enzymes and drug transporters will be performed by Dr. Sharyn Baker and genes involved in the activation pathway of nucleoside analogs will be performed by Dr. Jatinder Lamba using a multiplex assay such as a 48-plex format (DNAPrint genomics).

10.5 *In vitro* drug sensitivity assays

Drug sensitivity assays completed with LOA #4, 5/29/2015

As part of an ongoing project to develop new drugs for AML in Dr. Sharyn Baker's laboratory, we plan to evaluate the activity of novel agents in diagnostic blasts in a short-term co-culture system to mimic the bone marrow microenvironment. For example, new drugs to be tested include receptor tyrosine kinase inhibitors, STAT inhibitors, PI3K/mTOR inhibitors, IGF1-R inhibitors, HDAC inhibitors, and HSP90 inhibitors. 1 x 10^7 cells will be aliquoted and frozen until the time of *in vitro* testing. Blast cells will be thawed and plated on mesenchymal stromal cells, co-cultured overnight and treated with drug for 72 hours. Cell viability and apoptosis will be assessed by flow cytometry in the Pharmaceutical Sciences department. If enough cells are available a second aliquot of 1 x 10^7 cells will be used to assess changes in gene expression before and after drug treatment using the latest microarray technology in the Hartwell center. For example, we are interested in changes in genome wide changes in methylation status after *ex vivo* treatment of blasts cells with HDAC inhibitors.

10.6 Histone acetylation studies

Histone acetylation studies completed with Revision 10.1, 6/2/16

For patients who receive vorinostat (see section 4.6), histone acetylation will be assessed in peripheral blood samples (collected in 3 mL of preservative-free heparin) on days -2, 1, and 6 of Induction II as described in section 4.2.6. Histone H3 and H4 acetylation will be analyzed by Western blot using antibodies directed against acetylated histone H3 or H4.[100]

10.7 Other biology studies

High-resolution single nucleotide polymorphism (SNP) array and gene expression analysis will be performed as described in section 2.2.4. Phospho-flow profiling will be performed as described in section 2.2.5.

11.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Adverse event monitoring and reporting

This study will utilize the CTCAE Version 3.0 for toxicity and performance reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). Additionally, the toxicities are to be reported on the appropriate data collection forms or electronic data entry screens.

11.2 Reporting adverse events and deaths to St. Jude IRB

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

constitute unanticipated problems are reportable to the St. Jude IRB. As further described in the definition of unanticipated problem, this includes any event that in the PI's opinion was:

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

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

Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death. For the purposes of this study, **this includes all deaths of patients on active protocol therapy or within 30 days of receiving protocol therapy**.

The following definitions apply with respect to reporting adverse experiences:

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

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

Unexpected Adverse Event:

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

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

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

Unanticipated Problem Involving Risks to Subjects or Others: An unanticipated problem involving risks to subjects or others is an event which was not expected to occur and which increases the degree of risk posed to research participants. Such events, in general, meet all of the following criteria:

- unexpected;
- related or possibly related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

Consistent with FDA and OHRP guidance on reporting unanticipated problems and adverse events to IRBs, the St. Jude IRB does not require the submission of external events, for example IND safety reports, nor is a summary of such events/reports required; however, if an event giving rise to an IND safety or other external event report constitutes an "unanticipated problem involving risks to subjects or others" it must be reported in accordance with this policy. In general, to be reportable external events need to have implications for the conduct of the study (for example, requiring a significant and usually safety-related change in the protocol and/or informed consent form).

Although some adverse events will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected adverse events.

Examples of unanticipated problems involving risks to subjects or others include:

- Improperly staging a participant's tumor resulting in the participant being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug. Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others.

11.3 Reporting requirements from St. Jude to FDA

Any unexpected fatal or unexpected life-threatening event (e.g. progressive multifocal leukoencephalopathy or pulmonary toxicity as described on page 47) judged by the PI to possibly be due to the investigational agent, will be reported to the FDA by telephone or fax as soon as possible but no later than seven calendar days after notification of the event and followed by a written safety report as complete as possible within eight additional calendar days (i.e. full report 15 calendar days total after notification of event).

Unexpected, non-fatal and non-life-threatening SAEs, which occur in on-study participants during the time periods specified in Section 9.1 that are considered due to or possibly due to the investigational agent, will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs, even unexpected fatal SAEs, considered by the PI to be not related to the study, will be reported to the FDA in the Annual Review Report along with non-serious AEs. All FDA correspondence and reporting will be conducted through the St. Jude Office of Regulatory Affairs.

11.4 Reporting to St. Jude Regulatory Affairs Office (RAO)

Copies of all correspondence to the St. Jude IRB, including SAE reports, are provided to the St. Jude Regulatory Affairs office by the St. Jude study team. FDA-related correspondence and reporting will be conducted through the Regulatory Affairs office.

11.5 Reporting from St. Jude to Sanofi Group Pharmacovigilance

The following reporting requirements apply only to **participants treated with clofarabine during Induction I**, and are applicable only during the time period from first dose of clofarabine until recovery from Induction I and start of Induction II.

Serious adverse event (SAE): any untoward medical occurrence that at any dose:

- Results in death,
- Is life threatening, (Note: the term "life-threatening" refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe),

- Requires inpatient hospitalization or results in prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Is a medically important event or reaction. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above.

Related Adverse Event, i.e. Adverse Drug Reaction (ADR): There is a reasonable possibility according to the IST/ISS sponsor that the product may have caused the event.

Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product or package insert/summary of product characteristics for an approved product). An expected ADR with a fatal outcome should be considered unexpected unless the local/regional product labeling specifically states that the ADR might be associated with a fatal outcome.

AESI: An adverse event of special interest (AESI) is an adverse event (serious or non-serious) of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them. AESIs may be added or removed during a study by protocol amendment.

St. Jude PI and study team (Investigator-sponsored trial or IST) responsibilities include:

- The IST/ISS sponsor warrants that the study will be performed in compliance with all applicable local and international laws and regulations, including without limitation ICH E6 guidelines for Good Clinical Practices.
- The IST/ISS sponsor shall be responsible for the respect of all obligations required by applicable local and international laws and regulations.
- The sponsor shall be responsible for ensuring submission of required expedited and periodic reports to the appropriate Health Authority (HA), the Ethics Committee and investigators of each country participating in the IST/ISS (based on applicable regulations).
- The IST/ISS sponsor is responsible for providing any "Dear Investigator Letter" (DIL) for new safety finding received from Sanofi group entity to the investigators and Ethics Committee in each country participating in the study.
- The sponsor must report the following information in English to the Sanofi group entity Pharmacovigilance contact:

1. Routine transmission of: Only related SAEs must be transmitted within 1 working day of the Investigator's awareness or identification of the event.

2. Other events or periodic reports (e.g. Development Safety Update Report (DSUR)), submitted to Regulatory Authority must be transmitted at the time of submission.
3. Other significant safety issues or findings in a study pertaining to safety of product must be transmitted within 1 working day. (e.g., Data Safety Monitoring Board recommendations).
4. The study report of any IST/ISS must contain a section describing safety review and conclusion.
5. The reference safety information to be used by the IST/ISS sponsor for evaluation of expectedness of adverse events shall be the current approved product label available in the country.

Sanofi Group Entity Pharmacovigilance Contact:

IST/ISS Investigators will notify Sanofi via fax or email, attention: Sanofi Pharmacovigilance (PV):

Fax: [redacted]
E-mail: [redacted]

11.6 Other reporting mechanisms

Copies of all correspondence to the St. Jude Institutional Review Board (IRB), including SAE reports, are provided to the St. Jude Office of Research Compliance and Regulatory Affairs (ORCRA). All FDA related correspondence and reporting will be conducted through the ORCRA. Continuing review reports of protocol progress and summaries of adverse events will be filed with the St. Jude IRB, Institutional Biosafety Committee (IBC), and FDA at least annually.

11.7 Process for reporting AEs to/from St. Jude and collaborating sites

Adverse events from collaborating sites will also be reviewed by the PI and discussed in study team meetings as described above. SAE reports from collaborating sites for AEs that are serious, unexpected, and at least possibly related to protocol treatment or interventions will be reported to site IRB and the St. Jude IRB within the reporting requirements described above. The PI will determine if this is an event that will need to be reported expeditiously to all participating sites, considering the following criteria:

- Is the AE serious, unexpected, and related or possibly related to participation in the research?
- Is the AE expected, but occurring at a significantly higher frequency or severity than expected?
- Is this an AE that is unexpected (regardless of severity that may alter the IRB's analysis of the risk versus potential benefit of the research *and*, as a result, warrant consideration of substantive changes in the research protocol or informed consent process/document?

With the submission of the "Reportable Event" in St. Jude TRACKS application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or

consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects).

Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI.

A cumulative summary of Grade 3-5 AEs and expected/unrelated deaths that occur more than 30 days after protocol treatment will be reported to all sites with study progress report at the time of continuing review.

For collaborating sites: Serious AND unexpected events are to be reported to the St. Jude PI (Dr. Jeffrey Rubnitz) within 72 hours via phone call or email. Unexpected deaths must be reported to the St. Jude PI via phone call or email within 24 hours of the event. A written report must follow. In addition, the study coordinator [redacted] should also be contacted regarding the event.

Jeffery Rubnitz
Leukemia/Lymphoma Division
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Phone: [redacted]
Email: [redacted]

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For the purposes of this protocol, the following events will NOT be considered serious nor unexpected adverse events:

- Hospitalization for treatment related febrile neutropenia
- Hospitalization for expected complications of treatment or expected toxicities of the commercially available agents used in this study (except for grade 4 non-hematologic toxicities)
- Hospitalization for treatment of expected signs or symptoms of disease complications or progression of disease
- Death not related to therapy (e.g. disease progression) > 30 days after last dose of protocol therapy

12.0 DATA COLLECTION, STUDY MONITORING AND CONFIDENTIALITY

12.1 Data collection and management

Data Management will be supervised by the Director of Clinical Trials Management, Oncology Programs, working with Dr. Rubnitz or his designee. All protocol-specific data and all grade 3-5 non-hematological adverse events will be recorded by the clinical research associates into the CRIS database, *ideally* within 2-4 weeks of completion of study phase. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart.

Regular (at least monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review. Biostatistics will work with the PI in generating the progress reports for the IRB and the DSMB.

12.2 Study monitoring

The Eligibility Coordinators will verify 100 % of the informed consent documentation on all participants and verify 100% of St. Jude participants' eligibility status within 10 working days of the completion of enrollment.
The study team will meet at appropriate intervals to review case histories or quality summaries on participants.

The Clinical Research Monitor will assess protocol and regulatory compliance as well as the accuracy and completeness of all data points for the first two participants then 15% of study enrollees every six months. Accrual will be tracked continuously for studies that have strata. All SAE reports will be monitored for type, grade, attribution, duration, timeliness and appropriateness on all study participants *semi-annually.*

The monitor will also verify 100% of all data points on the first two participants and on 15% of cases thereafter. Protocol compliance monitoring will include participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, participant protocol status, off-study, and off-therapy criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC). Monitoring may be conducted more frequently if deemed necessary by the CPDMO or the IMC.

Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in TRACKS (Total Research and Knowledge System) are reviewed in a timely manner by the IRB/ OHSP.

Monitoring of this protocol is considered to be in the High-Risk 3 (HR-3) category, according to the St. Jude Data Safety and Monitoring Plan (DSMP). The Monitoring Plan is outlined in a separate document from this protocol, but has been submitted for review and approval by the Clinical Trials Scientific Review Committee and the Institutional Review Board.

St. Jude affiliates and domestic collaborating study sites will be monitored on-site by a representative of St. Jude at intervals specified in the Data and Safety Monitoring Plan. International collaborators will be monitored by a Contract Research Organization (CRO) under contractual agreement with St. Jude.

12.3 Confidentiality

Study numbers will be used in place of an identifier such as a medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and St. Jude clinical research monitors.

13.0 STATISTICAL CONSIDERATIONS

13.1 Design and analysis for primary objective

The primary objective of this study is to compare two initial courses of therapy in terms of the proportion of patients who have MRD levels of less than 0.1% (MRD-) as measured by flow cytometry. The two courses to be compared are HD-ADE and Clo/AraC. HD-ADE was studied as an initial course in the AML02 protocol. As of February 2008, 57 of 91 (62.6%) MRD-evaluable patients on AML02 had no detectable MRD after treatment with one course of HD-ADE.

In AML02, we observed a strong correlation between MRD levels at day 22 and event-free survival. In AML02, the 3-year EFS of MRD- and MRD+ AML02 patients are 74.3% (+/- 7.0% SE) and 47.2% (+/- 9.2% SE), respectively ($p = 0.001$). Clearly, MRD after one course is strongly correlated with long-term prognosis. As such, MRD serves as a useful measure of treatment efficacy.

The MRD- rate among AML02 patients varies substantially by risk-group. When classified according to the risk definitions of AML08, 21 of 23 (91.3 %) low-risk patients, 27 of 41 (65.9%) standard-risk patients, and 9 of 27 (33.3%) high-risk patients were MRD- after one course. Therefore, the statistical analysis for the primary objective of this protocol will be stratified by risk group.

In this study, patients will be randomized to receive HD-ADE or Clo/AraC as their initial course of therapy and MRD will be evaluated at day 22 of this course. The design calls for enrollment of up to 240 MRD-evaluable patients (Table 13.1). The design allows for 4 interim analyses and one final analysis. In each analysis, an exact risk-stratified Mantel-Haenzel test will be used to test the null hypothesis that the ratio of the odds of MRD- on Clo/AraC to the odds of MRD- on HD-ADE is equal to one. The risk-stratification categories for the analysis are defined in section 4.1 of the protocol. MRD-status will not be considered in assigning subjects to risk-strata for purposes of this statistical analysis. In the four interim analyses, the result will be considered statistically significant if the p-value is less than 0.005. If an interim analysis gives a significant p-value at the 0.005 level, we will temporarily cease accrual and consult the St. Jude DSMB on how to best proceed. In

the final analysis, the result will be considered statistically significant if the p-value is less than 0.0429. The level of the final analysis was determined using the Haybittle-Peto method as implemented in East software, windows version 5.2.

We performed simulations to further evaluate the level and power of the selected design. The simulation is based on one million replications. With risk-specific MRD-rates equal to those observed on the HD-ADE arm of AML02, the approximate level is 0.0396 (99.9% CI = 0.0389 – 0.0402). The design provides a power of approximately 0.8013 (99.9% CI = 0.7999 – 0.8026) to declare statistical significance if the odds of MRD- of the Clo/araC are 0.4 times that of the HD-ADE arm. Assuming an equal odds ratio for all risk groups, an odds ratio of 0.4 corresponds to MRD- rate of 62.6% in the HD-ADE arm and an MRD- rate of 45.0% in the Clo/AraC arm. For the Clo/AraC arm, this corresponds to assumed MRD- rates of 80.7%, 43.5%, and 16.7% among low, standard, and high risk patients, respectively.

Also, the design provides power of approximately 0.8297 (99.9% CI = 0.8284 – 0.8309) to detect the setting in which the odds of MRD- on HD-ADE are 2.5 times the odds of MRD- on HD-ADE. An odds ratio of 2.5 corresponds to an MRD- rate of 62.6% in the HD-ADE arm and an MRD- rate of 78.1% in the Clo/AraC arm. For the Clo/AraC arm, this corresponds to assumed MRD- rates of 96.3%, 82.8%, and 55.5% among low, standard, and high risk patients, respectively.

Table 13.1. Statistical Characteristics of the Design. The table gives the number of subjects in each arm at each interim analysis (n/arm), the p-value threshold used in each interim analysis (α), the cumulative level, i.e., the cumulative probability of a Type I error over the interim analyses (Cum. Level), the cumulative power (Cum. Power) over the interim analyses for an odds ratio of 2.5, and the cumulative power over the interim analyses for an odds ratio of 0.4. Estimates of level and power are based on simulations with one million replications.

n/arm	a	Cum. Level	Cum. Power (OR = 2.5)	Cum. Power (OR = 0.4)
48	0.005	0.002861	0.044709	0.038920
96	0.005	0.005752	0.162844	0.145386
144	0.005	0.008301	0.316510	0.287142
192	0.005	0.010505	0.472528	0.435433
240	0.0429	0.039559	0.829673	0.801282

Accrual targets are based on the number of MRD-evaluable patients. As of February 2008, 23 of 209 (11%) patients treated on AML02 were not evaluable for MRD after their first course of therapy. If the same proportion of patients is not evaluable for MRD in AML08, a total of 270 patients will need to be randomized to obtain 240 MRD-evaluable patients. At the accrual rate of 40 patients per year observed on AML02, roughly 7 years are required to accrue 270 eligible patients.

The randomization will be stratified according to risk features of the disease. For patients for whom karyotype is available, the randomization will be stratified by inv(16) vs. t(8,21) vs. 11q23 vs. M7 vs. others. If the karyotype is not available, the randomization will be

stratified primarily by FAB type: M2 with Auer rods vs. M4Eo vs. M5 vs. M7 vs. others. The randomization is stratified to maintain a balance between the two arms with respect to factors that are known to affect the secondary outcome measures such as CR rate and EFS.

The randomization will be performed using a program that implements the block-randomization scheme proposed by Zelen.[179] The program resides on our network and has been routinely used for randomization since 1992. The Pharmacy will be provided access to the program and will be responsible for randomizing patients. The system stores all required data for randomization in a secure Access database. Once a patient is randomized, all related data are frozen in the database and cannot be changed. All eligible patients who are registered and randomized will be included in the analyses consistent with the intent-to-treat principle.

13.2 Monitoring rules

13.2.1 Monitoring for unequal survival in the two arms

We will compare the event-free and overall survival of the two arms concurrently with each interim analysis. At each interim analysis, we will perform the exact log-rank test at the 0.005 level to compare event-free and overall survival. If the results are significant, we will temporarily cease accrual and seek the guidance of the St. Jude DSMB in determining whether to continue the study. Additionally, a level of 0.0429 will be used in the final analysis of the secondary objective of comparing survival distributions. This will result in a Type I error rate of less than or equal to 0.05 according to the Haybittle-Peto method as implemented in East software, windows version 5.2. For these stopping rules, EFS is defined as the time elapsed from the date of study enrollment to death, relapse, or diagnosis of a second malignancy, with event-free patients censored at last follow-up. OS is defined as the time elapsed from enrollment date to date of death, with living patients censored at last follow-up.

Table 13.2 gives simulation-based estimates of the probability of rejecting the null hypothesis and the expected enrollment of the study of the EFS monitoring rule for specific values of the 3-year EFS. Each simulation transformed the Kaplan-Meier EFS of the HD-ADE arm of AML02 by raising it to the power that gives the value of the 3-year EFS for each arm to be simulated. Data was generated from the transformed survival curve for the simulation. The simulation used one million independent realizations for each set of EFS-values for HD-ADE and Clo/AraC. The simulation indicates that the monitoring rule has a Type I error probability of less than 0.05 (see probability of rejection in highlighted cells).

Table 13.3 shows simulation-based estimates for the OS monitoring rule. The simulation was performed using the historical OS experience in the HD-ADE arm of AML02 in an analogous manner as described above. One-million independent realizations were generated and the simulation indicates that the Type I error probability is less than or equal 0.05.

Table 13.2. Simulation Estimates of the Statistical Characteristics of the EFS monitoring rule. For each scenario, the table provides simulation estimates of the probability of rejecting the null hypothesis of equal EFS at some point in the study [Pr(Rej)] and the expected number of subjects enrolled [E(n)] on the study prior to rejection of the null hypothesis as part of the monitoring.

	HD-ADE									
3-yr EFS	45%		50%		55%		60%		65%	
Clo/araC	Pr(Rej)	E(n)	Pr(Rej)	E(n)	Pr(Rej)	E(n)	Pr(Rej)	E(n)	Pr(Rej)	E(n)
30%	0.431	237	0.716	209	0.907	178	0.982	150	0.998	129
35%	0.165	257	0.401	240	0.687	213	0.893	182	0.978	153
40%	0.048	265	0.155	258	0.382	241	0.671	215	0.886	184
45%	0.022	267	0.046	265	0.150	258	0.375	242	0.666	216
50%	0.046	265	0.021	267	0.045	265	0.148	259	0.376	243
55%	0.150	258	0.046	265	0.021	267	0.045	266	0.150	259
60%	0.374	242	0.149	258	0.045	266	0.020	267	0.044	266
65%	0.667	216	0.376	243	0.151	259	0.044	266	0.019	268
70%	0.889	184	0.678	216	0.388	242	0.156	258	0.045	266
75%	0.980	154	0.901	182	0.701	214	0.412	241	0.167	258
80%	0.998	130	0.986	151	0.921	178	0.743	210	0.453	238

Table 13.3. Simulation Estimates of the Statistical Characteristics of OS monitoring rule. The table provides simulation estimates of the probability of rejecting the null hypothesis of equal EFS [Pr(Rej)] and the expected number of subjects enrolled on the protocol prior to rejection of the null hypothesis [E(n)] under the monitoring rule.

	HD-ADE									
3-yr EFS	45%		50%		55%		60%		65%	
Clo/araC	Pr(Rej)	E(n)	Pr(Rej)	E(n)	Pr(Rej)	E(n)	Pr(Rej)	E(n)	Pr(Rej)	E(n)
30%	0.404	241	0.685	217	0.887	189	0.975	163	0.997	142
35%	0.154	259	0.374	244	0.654	221	0.870	193	0.970	166
40%	0.045	266	0.144	259	0.354	246	0.637	223	0.863	195
45%	0.020	267	0.043	266	0.138	260	0.348	247	0.633	224
50%	0.043	266	0.020	268	0.042	266	0.137	260	0.350	247
55%	0.138	260	0.042	266	0.019	268	0.041	266	0.138	260
60%	0.347	247	0.137	260	0.041	266	0.018	268	0.041	266
65%	0.633	224	0.350	247	0.138	260	0.041	266	0.017	268
70%	0.865	195	0.643	224	0.360	246	0.143	260	0.041	267
75%	0.972	167	0.879	194	0.667	222	0.382	245	0.154	260
80%	0.997	144	0.979	165	0.901	190	0.707	219	0.421	243

13.2.2 Monitoring for deaths due to causes other than disease progression

It is important to monitor the study for an unacceptable rate of death due to causes other than disease progression (i.e., infection, toxicity, transplant-related complications, etc.). On the AML02 study, as of February 2008, patients randomized to HD-ADE had a cumulative incidence of deaths due to causes other than disease progression of 5.7% (± 2.5% SE) at 1 year, 8.6% (±3.2% SE) at 2 years, and 10.4% (± 3.6% SE) at 3 years post-enrollment. Patients randomized to LD-ADE had a similar outcome with a cumulative incidence of 2.1% (± 1.5% SE) at 1 year, 10.2% (± 3.5% SE) at 2 years, and 11.9% (± 3.9% SE) at 3

years post-enrollment. We wish to monitor the AML08 study to ensure that the rate of deaths due to causes other than disease progression is similar to or less than that of the AML02 study.

Therefore, concurrently with each interim analysis, we will compute the cumulative incidence of death due to causes other than disease progression for each arm of the AML08 study. If the cumulative incidence estimate for either arm is greater than 10% at 1 year or 15% at 2 years, then we will temporarily halt accrual while determining whether the study should continue. Gray's method will be used to compute cumulative incidence estimates.[180]

13.2.3 Monitoring for futility

In the spring of 2013, the St. Jude DSMB recommended that stopping rules for futility be incorporated into the protocol. In response to their request, Dr. Cheng Cheng developed the futility stopping rules shown in Table 13.4. The stopping rule will be evaluated concurrently with the fourth planned interim analyses of the primary MRD endpoint (i.e., when 192 evaluable patients have been enrolled). At that time, two one-sided tests of the odds ratio will be performed and futility will be declared if the p-value of each one-sided test crosses the boundary indicated in Table 13.4. The St. Jude DSMB will be notified of the result of this analysis. If futility is declared, we will consult the St. Jude DSMB to determine subsequent actions to be taken. The futility analysis design is powered for each H1 at the effect size OR=2.5 and 0.4 respectively. Tables 13.5 and 13.6 show the statistical properties of the rule determined by EAST 5.

Table 13.4. Futility Analysis Stopping Rules

Sample size = 192	Declare futility if
H1: OR>1	P>=0.177
H1: OR<1	AND P>=0.184

Table 13.5. Statistical properties of the futility analysis with OR=2.5.

Information Fraction	Cumulative Accrual	Beta Spent	Boundary to Reject H1	Boundary Crossing Probabilities		
				Under H0	Under H1	Under H1/2
0.600	144.000	0.067	0.473	0.527	0.067	0.317
0.800	192.000	0.132	0.177	0.309	0.064	0.232
1.000	240.000	0.200	0.059	0.113	0.068	0.158

Table 13.6. Statistical properties of the futility analysis with OR=0.4.

Information Fraction	Cumulative Accrual	Beta Spent	Boundary to Reject H1	Boundary Crossing Probabilities		
				Under H0	Under H1	Under H1/2
0.600	144.000	0.043	0.518	0.482	0.043	0.264
0.800	192.000	0.093	0.184	0.344	0.050	0.238
1.000	240.000	0.149	0.057	0.122	0.054	0.169

13.3 Comparison and stratification for amendment 6

Amendment 6 eliminates the 5th course of therapy from AML08 on the basis of a randomized MRC study showing no statistically or clinically meaningful difference in EFS or OS according to completion of 4 or 5 courses of chemotherapy. Similar results were obtained in a retrospective comparison between those completing 4 or 5 courses of therapy among AML02 patients in remission following 4 courses of therapy.

At the conclusion of the AML08 study, we will use the log-rank test to compare EFS and OS of patients enrolled before amendment 6 to that of patients enrolled after amendment 6. If a significant difference is observed, then we will report the finding, stratify comparisons of EFS and OS between the Clo/AraC and HD-ADE arms according to this amendment, and include the amendment as a predictor in Cox regression analyses.

Amendment 6 only impacts therapy given after the primary endpoint (Day 22 MRD) is evaluated, so there is no need to modify statistical analysis plans for the primary objective.

13.4 Analyses for secondary objective

Brief analysis strategies for each secondary objective are provided below. Several secondary objectives involve extensive multiple-testing. Multiple-testing issues should be addressed via control or estimation of the false discovery rate (FDR). Published recommendations developed at St. Jude will be used to select the most appropriate FDR method for each analysis.[181,182]

Objective 1.2.1 To describe the event-free survival (EFS) of standard-risk patients who receive chemotherapy relative to that of standard-risk patients who chemotherapy followed by an infusion of natural killer (NK) cells from a KIR-mismatched donor.

For this objective, we will compute Kaplan-Meier estimates for each group and compare those estimates with the exact log-rank test. For this analysis, event-free survival will be defined as the time elapsed from the completion of the final course of chemotherapy to death or diagnosis of relapse or a second malignancy, with event-free patients censored at last follow-up.

To date, ten AML patients have received NK cell therapy during first remission under the NKAML protocol. Most of these patients had KIR-mismatched donors and to date none have experienced relapse. The therapy is promising but the outcome is not yet well characterized. Additionally, biologic studies suggest that NK cell therapy should be most effective in the KIR-mismatch setting. Therefore, there is interest to gain more experience with NK-cell therapy and better characterize prognosis of patients receiving this therapy.

Based on experience of AML02 patients, we expect that 28% of enrolled patients will be standard-risk and complete 5 courses and be available to contribute to this objective. Thus, we anticipate a total of 76 patients to contribute to this objective and expect one-half (38) patients to have a KIR-mismatched donor and receive NK-cell therapy. In the HD-ADE arm of AML02, 24 patients enrolled prior to July 1, 2007 met the requirements to

contribute to this component of AML08. As of February 2008, the estimated 3-year event-free survival of this group was 74.7% (± 15.3% SE).

13.5 Analyses for exploratory objectives

Objective 1.3.1 To genotype natural killer (NK) cell receptors and measure their expressions at diagnosis and after induction therapy, and to explore the associations these features with treatment outcome.

We will use arm-stratified exact chi-square tests to explore the association of the NK cell receptor genotypes with response to induction therapy (CR vs. no CR or MRD+ vs. MRD-). Additionally, we will explore the possibility of genotype-treatment interactions with logistic regression models.

We will explore the association of NK cell receptor genotype and expression with event-free and overall survival using Cox models, a rank-based test that accounts for censoring, or the log-rank test.[183,184]

Objective 1.3.2 To explore the prognostic value of levels of minimal residual disease in peripheral blood at day 8 of induction I.

We will use logistic regression models to explore the association of day 8 MRD in peripheral blood with response and post-induction I MRD. We will use Cox regression models and Jung's procedure to explore the association of day 8 MRD in peripheral blood with event-free and overall survival. The analysis will account for initial arm. We will also account for subsequent therapy if sample sizes allow.

Objective 1.3.3 To validate new markers and methods for minimal residual disease (MRD) detection.

We will compute the correlation of MRD measurements using new markers with the MRD measurements determined in the same way as in AML02. Additionally, we will use Cox regression, a rank-based procedure, or the log-rank test to explore the prognostic value of MRD measured with the new markers.[183,184]

Objective 1.3.4 To identify new prognostic factors by applying new technologies to study patient material

We will use SNP microarrays to identify genomic lesions. Summary signals will be computed for each probe set and then reference-normalized using the method described by Mullighan et al.[166] The summary signal profile for each tumor will be compared to a control signal profile obtained from one or multiple control samples by subtraction or taking the ratio. The resulting signal difference will be segmented using circular binary segmentation or Bayesian change-point analysis. Segments will be then characterized as showing evidence of amplification, evidence of deletion, or no evidence of alteration. The inferred copy number states will be compared across different cell subpopulations from the same individual's tumor and across samples from different subjects. Additionally, we will

use log-rank tests, a rank-based procedure, or Cox models to explore the association of identified lesions with event-free and overall survival.[183,184]

We will use mRNA microarrays to compare the expression of leukemic blasts prior to therapy to that of residual blasts following induction I. We will measure expression at diagnosis and post-therapy in each subject with sufficient material for the assays. For each pair of arrays from the same subject, we will compute the log-ratio of expression after treatment to that prior to treatment. Within each arm, we will apply the signed-rank test to the expression change values to test for significant expression changes. Additionally, we will compare the expression changes across arms with the rank-sum test. Also, we will use linear models to explore the possibilities of that the differences in expression response to therapy between the two arms vary according to disease subtype. Finally, we will use a rank-based procedure or Cox regression to explore the association of expression with event-free survival or overall survival.[183,184]

Objective 1.3.5 To identify pharmacogenetic, pharmacokinetic and pharmacodynamic factors that associate with clinical outcomes in the context of the systemic therapy used in the protocol.

We will use logistic regression models to explore the association of PK variables (AUC, clearance, etc.), PD variables (IC50, etc.) and pharmacogenetic (PG) variables with toxicity and response to therapy (MRD+/- or CR). We will use Cox models or a rank-based procedure to explore the association of PK, PD, and PG variables with event-free and overall survival.[183,184] We will use correlation coefficients and linear models to explore the association of PK variables with PD variables. We will use ANOVA models or the Kruskall-Wallis test to explore the association of PG variables with PD and PK variables.

Objective 1.3.6 To describe the impact of antibiotic and antifungal prophylaxis on invasive bacterial and fungal infections, febrile neutropenia, hospitalization, and antibiotic resistance.

We will use Gray's method estimate the cumulative incidence of each type of infection in the protocol.[180] Additionally, we will use Gray's test to compare the cumulative incidence of infection of patients assigned to HD-ADE to that of patients assigned to Clo/AraC.

14.0 OBTAINING INFORMED CONSENT

14.1 Consent/Assent at Induction and Post-Induction

The process of informed consent for AML08 will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research study), blood transfusion and other procedures as necessary. After the diagnosis of AML is established, we will invite the patient to participate in the AML08 protocol. After completion of induction, we will seek informed consent for their participation in risk-directed post-induction therapy.

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at SJCRH and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care.

We will also obtain verbal assent from children 7 to 14 years old and written assent for all patients ≥14 years of age. Patients who reach the age of majority while on study will be re-consented for continued participation on AML08, according to Cancer Center and institutional policy.

14.2 Consent at the age of majority

The age of majority in the state of Tennessee is 18 years old. Research participants on active therapy must be consented at the next clinic visit after their 18th birthday. Participants, who have reached age of majority and have completed all protocol-directed therapy, will be re-consented with a separate consent specifically for this purpose (AOM consent). Participants, who reach age of majority after the 5 year protocol required follow-up, will be followed for survival and late effects as per the SJLTFU. A waiver for AOM consent will be requested for these participants at St. Jude.

If an affiliate site is located in a state where a different age of majority applies, that location must consent the participants according to their local laws. Collaborating sites may follow this guidance for AOM, or may follow institutional policy (and will provide institutional policy to St. Jude).

14.3 Consent when English is not the primary language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information will be documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites. Collaborating Sites will follow institutional policy for consenting non-English speaking participants (and will provide institutional policy to St. Jude).

15.0 DATA SAFETY MONITORING BOARD

The protocol progress will be reviewed and monitored by the St. Jude Children's Research Hospital Data Safety Monitoring Board (DSMB). Data summaries will be provided to the DSMB by the Department of Biostatistics after review by the Principal Investigator. The data will include patient accrual, demographic summaries, grade 3/4 toxicities, major adverse events (i.e. deaths, relapses, second malignancies) and results of interim and final analyses as specified in the protocol. The data are retrieved from the database and are reviewed at every AML08 meeting, which is generally held twice a month, and are reflected in the minutes, which are provided to the biostatistician. When the appropriate number of patients have failed or accrued, triggering interim or final analysis, the

biostatistician will be informed by the PI. Should a safety stopping rule be exceeded, the protocol will be temporarily closed until the DSMB can review the situation. Reporting will comply with Cancer Center and St. Jude institutional guidelines for monitoring.

16.0 REFERENCES

1. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med 2004;350:1535-1548.
2. Crews KR, Gandhi V, Srivastava DK, et al. Interim comparison of a continuous infusion versus a short daily infusion of cytarabine given in combination with cladribine for pediatric acute myeloid leukemia. J Clin Oncol 2002;20:4217-4224.
3. Woods WG, Neudorf S, Gold S, et al. A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission. Blood 2001;97:56-62.
4. Stevens RF, Hann IM, Wheatley K, Gray RG. Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukemia: results of the United Kingdom Medical Research Council's 10th AML trial. MRC Childhood Leukaemia Working Party. Br J Haematol 1998;101:130-140.
5. Creutzig U, Ritter J, Zimmermann M, et al. Improved treatment results in high-risk pediatric acute myeloid leukemia patients after intensification with high-dose cytarabine and mitoxantrone: results of Study Acute Myeloid Leukemia-Berlin-Frankfurt- Munster 93. J Clin Oncol 2001;19:2705-2713.
6. Rubnitz JE, Lensing S, Zhou Y, et al. Death during induction therapy and first remission of acute leukemia in childhood: the St. Jude experience. Cancer 2004;101:1677-1684.
7. Creutzig U, Zimmermann M, Reinhardt D, et al. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98. J Clin Oncol 2004;22:4384-4393.
8. Reichelova V, Liliemark J, Albertioni F. Liquid chromatographic study of acid stability of 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues. J Pharm Biomed Anal 1995;13:711-714.
9. Carson DA, Wasson DB, Esparza LM, et al. Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine. Proc Natl Acad Sci U S A 1992;89:2970-2974.
10. Lotfi K, Mansson E, Spasokoukotskaja T, et al. Biochemical pharmacology and resistance to 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine, a novel analogue of cladribine in human leukemic cells. Clin Cancer Res 1999;5:2438-2444.
11. Parker WB, Shaddix SC, Chang CH, et al. Effects of 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5'-triphosphate. Cancer Res 1991;51:2386-2394.
12. Xie KC, Plunkett W. Deoxynucleotide pool depletion and sustained inhibition of ribonucleotide reductase and DNA synthesis after treatment of human lymphoblastoid cells with 2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) adenine. Cancer Res 1996;56:3030-3037.
13. Genini D, Adachi S, Chao Q, et al. Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. Blood 2000;96:3537-3543.
14. Kantarjian HM, Gandhi V, Kozuch P, et al. Phase I clinical and pharmacology study of clofarabine in patients with solid and hematologic cancers. J Clin Oncol 2003;21:1167-1173.
15. Kantarjian H, Gandhi V, Cortes J, et al. Phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia. Blood 2003;102:2379-2386.

16. Foran JM, Faderl S, Wetzler M. A phase II open-label study of clofarabine in older adult patients with refractory or relapsed acute myelogenous leukemia. [abstract]. Proc Am Soc Clin Oncol 2003;22:587.
17. Foran J, Wetzler M, Kantarjian H. A phase II, open label study of Clorarex in adult patients with refractory or relapsed acute myelogenous leukemia. [abstract]. Blood 2002;100:271B.
18. Faderl S, Gandhi V, O'Brien S, et al. Results of a phase 1-2 study of clofarabine in combination with cytarabine (ara-C) in relapsed and refractory acute leukemias. Blood 2005;105:940-947.
19. Agura E, Berryman B, Brougher L. Phase II study of clofarabine and cytosine arabinoside in adult patients with relapsed AML and in elderly patients with untreated AML who are at high risk of anthracycline toxicity. [abstract]. Blood 2006;108:552a.
20. Burnett A, Kell W, Hills R. The feasibility of combining daunorubicin, clofarabine and Gemtuzumab Ozogamicin is feasible and effective. A pilot study. [abstract]. Blood 2006;108:511a-552a.
21. Faderl S, Ferrajoli A, Rivandi-Kashani F. Clofarabine plus anthracycline combinations in acute myelogenous leukemia. [abstract]. Blood 2006;108:51a.
22. Faderl S, Verstovsek S, Cortes J, et al. Clofarabine and cytarabine combination as induction therapy for acute myeloid leukemia (AML) in patients 50 years of age or older. Blood 2006;108:45-51.
23. Faderl S, Ravandi F, Ferrajoli A. Clofarabine and clofarabine plus low-dose cytarabine (ara-C) as inductin therapy for patients (pts) ≥60 years with newly diagnosed acute myeloid lekemia (AML). [abstract]. Blood 2005;106:
24. Foran JM, The AS, Devetten M. Phase I/II study of clofarabine combined with standard dose cytarabine as remission induction therapy of de novo AML in elderly patients ≥60 years. [abstract]. Blood 2005;106:
25. Burnett A, Russell N, Kell J. Evaluation of single agent clofarabine as first line treatment for older patients with AML who are not considered fit for intensive chemotherapy. [abstract]. Blood 2004;103:
26. Burnett A, Baccarani M, Johnson P. A phase II study (Biov-121) of clofarabine monotherapy first line in patients aged 65 years or older with acute myeloid leukemia for whom standard intensive chemotherapy is not considered suitable. [abstract]. Blood 2006;108:
27. Jeha S, Gandhi V, Chan KW, et al. Clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia. Blood 2004;103:784-789.
28. Jeha S, Gaynon PS, Razzouk BI, et al. Phase II study of clofarabine in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. J Clin Oncol 2006;24:1917-1923.
29. Jeha S, Razzouk B, Gaynon P et al. Clofarabine in Children with Refractory/Relapsed Acute Leukemia: Results of 2 Phase II Open Label Studies [abstract]. Blood 2006;108:
30. Coustan-Smith E, Ribeiro RC, Rubnitz JE, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukaemia. Br J Haematol 2003;123:243-252.
31. Woods WG, Kobrinsky N, Buckley JD, et al. Time-sequential induction therapy improves postremission outcome in acute myeloid leukemia: a report from the Children's Cancer Group. Blood 1996;87:4979-4989.
32. Bishop JF, Matthews JP, Young GA, et al. A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. Blood 1996;87:1710-1717.
33. Weick JK, Kopecky KJ, Appelbaum FR, et al. A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study. Blood 1996;88:2841-2851.

34. Kern W, Estey EH. High-dose cytosine arabinoside in the treatment of acute myeloid leukemia: Review of three randomized trials. Cancer 2006;107:116-124.
35. Bernstein ID. Monoclonal antibodies to the myeloid stem cells: therapeutic implications of CMA-676, a humanized anti-CD33 antibody calicheamicin conjugate. Leukemia 2000;14:474-475.
36. Bross PF, Beitz J, Chen G, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clin Cancer Res 2001;7:1490-1496.
37. Sievers EL, Appelbaum FR, Spielberger RT, et al. Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 calicheamicin immunoconjugate. Blood 1999;93:3678-3684.
38. Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33- positive acute myeloid leukemia in first relapse. J Clin Oncol 2001;19:3244-3254.
39. Tack DK, Letendre L, Kamath PS, Tefferi A. Development of hepatic veno-occlusive disease after Mylotarg infusion for relapsed acute myeloid leukemia. Bone Marrow Transplant 2001;28:895-897.
40. Gordon LI. Gemtuzumab Ozogamicin (Mylotarg) and hepatic veno-occlusive disease: take two acetaminophen, and. Bone Marrow Transplant 2001;28:811-812.
41. Giles FJ, Kantarjian HM, Kornblau SM, et al. Mylotarg (gemtuzumab ozogamicin) therapy is associated with hepatic venoocclusive disease in patients who have not received stem cell transplantation. Cancer 2001;92:406-413.
42. Kell WJ, Burnett AK, Chopra R, et al. A feasibility study of simultaneous administration of gemtuzumab ozogamicin with intensive chemotherapy in induction and consolidation in younger patients with acute myeloid leukemia. Blood 2003;102:4277-4283.
43. Moretta A, Bottino C, Mingari MC, Biassoni R, Moretta L. What is a natural killer cell? Nat Immunol 2002;3:6-8.
44. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature 1986;319:675-678.
45. Farag SS, Fehniger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 2002;100:1935-1947.
46. Leung W, Iyengar R, Turner V, et al. Determinants of antileukemia effects of allogeneic NK cells. J Immunol 2004;172:644-650.
47. Moretta A, Bottino C, Vitale M, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 2001;19:197-223.
48. Long EO. Regulation of immune responses through inhibitory receptors. Annu Rev Immunol 1999;17:875-904.
49. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood 1999;94:333-339.
50. Giebel S, Locatelli F, Lamparelli T, et al. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood 2003;102:814-819.
51. Hsu KC, Keever-Taylor CA, Wilton A, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood 2005;105:4878-4884.
52. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002;295:2097-2100.
53. Leung W, Iyengar R, Triplett B, et al. Comparison of killer Ig-like receptor genotyping and phenotyping for selection of allogeneic blood stem cell donors. J Immunol 2005;174:6540-6545.

54. Bishara A, De Santis D, Witt CC, et al. The beneficial role of inhibitory KIR genes of HLA class I NK epitopes in haploidentically mismatched stem cell allografts may be masked by residual donor-alloreactive T cells causing GVHD. Tissue Antigens 2004;63:204-211.
55. Davies SM, Ruggieri L, DeFor T, et al. Evaluation of KIR ligand incompatibility in mismatched unrelated donor hematopoietic transplants. Killer immunoglobulin-like receptor. Blood 2002;100:3825-3827.
56. Bornhauser M, Schwerdtfeger R, Martin H, et al. Role of KIR ligand incompatibility in hematopoietic stem cell transplantation using unrelated donors. Blood 2004;103:2860-2861.
57. Lowe EJ, Turner V, Handgretinger R, et al. T-cell alloreactivity dominates natural killer cell alloreactivity in minimally T-cell-depleted HLA-non-identical paediatric bone marrow transplantation. Br J Haematol 2003;123:323-326.
58. Iyengar R, Handgretinger R, Babarin-Dorner A, et al. Purification of human natural killer cells using a clinical-scale immunomagnetic method. Cytotherapy 2003;5:479-484.
59. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med 1998;339:1186-1193.
60. Muller S, Schulz A, Reiss U, et al. Definition of a critical T cell threshold for prevention of GVHD after HLA non-identical PBPC transplantation in children. Bone Marrow Transplant 1999;24:575-581.
61. Leung W, Iyengar R, Leimig T, et al. Phenotype and function of human natural killer cells purified by using a clinical-scale immunomagnetic method. Cancer Immunol Immunother 2005;54:389-394.
62. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood 2005;105:3051-3057.
63. Santos GW, Tutschka PJ, Brookmeyer R, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. N Engl J Med 1983;309:1347-1353.
64. Forman SJ, Spruce WE, Farbstein MJ, et al. Bone marrow ablation followed by allogeneic marrow grafting during first complete remission of acute nonlymphocytic leukemia. Blood 1983;61:439-442.
65. Dahl GV, Kalwinsky DK, Mirro J, Jr., et al. Allogeneic bone marrow transplantation in a program of intensive sequential chemotherapy for children and young adults with acute nonlymphocytic leukemia in first remission. J Clin Oncol 1990;8:295-303.
66. Cassileth PA, Harrington DP, Appelbaum FR, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N Engl J Med 1998;339:1649-1656.
67. Appelbaum FR. Allogeneic hematopoietic stem cell transplantation for acute leukemia. Semin Oncol 1997;24:114-123.
68. Reiffers J, Stoppa AM, Attal M, et al. Allogeneic vs autologous stem cell transplantation vs chemotherapy in patients with acute myeloid leukemia in first remission: the BGMT 87 study. Leukemia 1996;10:1874-1882.
69. Blaise D, Maraninchi D, Archimbaud E, et al. Allogeneic bone marrow transplantation for acute myeloid leukemia in first remission: a randomized trial of a busulfan-Cytoxan versus Cytoxan-total body irradiation as preparative regimen: a report from the Group d'Etudes de la Greffe de Moelle Osseuse. Blood 1992;79:2578-2582.

70. Jourdan E, Maraninchi D, Reiffers J, et al. Early allogeneic transplantation favorably influences the outcome of adult patients suffering from acute myeloid leukemia. Societe Francaise de Greffe de Moelle (SFGM). Bone Marrow Transplant 1997;19:875-881.
71. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med 1994;331:896-903.
72. Mayer RJ. Intensive chemotherapy versus allogeneic bone marrow transplantation in first-remission acute myeloid leukemia. Bone Marrow Transplant 1990;6 Suppl 1:48-51.
73. Feig SA, Lampkin B, Nesbit ME, et al. Outcome of BMT during first complete remission of AML: a comparison of two sequential studies by the Children's Cancer Group. Bone Marrow Transplant 1993;12:65-71.
74. Nesbit ME, Jr., Buckley JD, Feig SA, et al. Chemotherapy for induction of remission of childhood acute myeloid leukemia followed by marrow transplantation or multiagent chemotherapy: a report from the Childrens Cancer Group. J Clin Oncol 1994;12:127-135.
75. Chen AR, Alonzo TA, Woods WG, Arceci RJ. Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation?--an American view. Br J Haematol 2002;118:378-384.
76. Creutzig U, Reinhardt D. Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation?--a European view. Br J Haematol 2002;118:365-377.
77. Kornblau SM, Womble M, Qiu YH, et al. Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. Blood 2006;108:2358-2365.
78. Liang DC, Shih LY, Fu JF, et al. K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. Cancer 2006;106:950-956.
79. Christiansen DH, Andersen MK, Desta F, Pedersen-Bjergaard J. Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. Leukemia 2005;19:2232-2240.
80. Meshinchi S, Alonzo TA, Stirewalt DL, et al. Clinical implications of FLT3 mutations in pediatric AML. Blood 2006;108:3654-3661.
81. Zwaan CM, Meshinchi S, Radich JP, et al. FLT3 internal tandem duplication in 234 children with acute myeloid leukemia: prognostic significance and relation to cellular drug resistance. Blood 2003;102:2387-2394.
82. Shimada A, Taki T, Tabuchi K, et al. KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21); a study of the Japanese Childhood AML Cooperative Study Group. Blood 2006;107:1806-1809.
83. Smith BD, Levis M, Beran M, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. Blood 2004;103:3669-3676.
84. Wilhelm S, Carter C, Lynch M, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov 2006;5:835-844.
85. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099-7109.
86. Carlomagno F, Anaganti S, Guida T, et al. BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst 2006;98:326-334.

87. Kolch W, Kotwaliwale A, Vass K, Janosch P. The role of Raf kinases in malignant transformation. Expert Rev Mol Med 2002;4:1-18.
88. Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356:125-134.
89. Steelman LS, Pohnert SC, Shelton JG, et al. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia 2004;18:189-218.
90. Ricciardi MR, McQueen T, Chism D, et al. Quantitative single cell determination of ERK phosphorylation and regulation in relapsed and refractory primary acute myeloid leukemia. Leukemia 2005;19:1543-1549.
91. Pui CH, Carroll AJ, Head D, et al. Near-triploid and near-tetraploid acute lymphoblastic leukemia of childhood. Blood 1990;76:590-596.
92. Auclair D, Miller D, Yatsula V, et al. Antitumor activity of sorafenib in FLT3-driven leukemic cells. Leukemia 2007;21:439-445.
93. Zhang W, Konopleva M, Shi YX, et al. Mutant FLT3: a direct target of sorafenib in acute myelogenous leukemia. J Natl Cancer Inst 2008;100:184-198.
94. Athale UH, Razzouk BI, Raimondi SC, et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. Blood 2001;97:3727-3732.
95. Barnard D, Alonzo TA, Gerbing R, Lange B, Woods W. Comparison of Childhood Myelodysplastic Syndrome, AML FAB M6 or M7, CCG 2891: Report From the Children's Oncology Group. Pediatr Blood Cancer 2007;49:17-22.
96. Dastugue N, Lafage-Pochitaloff M, Pages MP, et al. Cytogenetic profile of childhood and adult megakaryoblastic leukemia (M7): a study of the Groupe Francais de Cytogenetique Hematologique (GFCH). Blood 2002;100:618-626.
97. Taub JW, Ge Y. Down syndrome, drug metabolism and chromosome 21. Pediatr Blood Cancer 2005;44:33-39.
98. Kuendgen A, Gattermann N. Valproic acid for the treatment of myeloid malignancies. Cancer 2007;110:943-954.
99. Soriano AO, Yang H, Faderl S, et al. Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood 2007;110:2302-2308.
100. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. Blood 2006;108:3271-3279.
101. Bug G, Ritter M, Wassmann B, et al. Clinical trial of valproic acid and all-trans retinoic acid in patients with poor-risk acute myeloid leukemia. Cancer 2005;104:2717-2725.
102. Kuendgen A, Knipp S, Fox F, et al. Results of a phase 2 study of valproic acid alone or in combination with all-trans retinoic acid in 75 patients with myelodysplastic syndrome and relapsed or refractory acute myeloid leukemia. Ann Hematol 2005;84 Suppl 1:61-66.
103. Gerstner T, Bell N, Longin E, Konig SA. Oral rapid loading of valproic acid--an alternative to the usual saturation scheme? Seizure 2006;15:630-632.
104. Garderet L, Labopin M, Gorin NC, et al. Hematopoietic stem cell transplantation for de novo acute megakaryocytic leukemia in first complete remission: a retrospective study of the European Group for Blood and Marrow Transplantation (EBMT). Blood 2005;105:405-409.
105. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 1998;92:2322-2333.

106. Raimondi SC, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative Pediatric Oncology Group study-POG 8821. Blood 1999;94:3707-3716.
107. Hasle H, Alonzo TA, Auvrignon A, et al. Monosomy 7 and deletion 7q in children and adolescents with acute myeloid leukemia: an international retrospective study. Blood 2007;109:4641-4647.
108. Dimartino JF, Cleary ML. Mll rearrangements in haematological malignancies: lessons from clinical and biological studies. Br J Haematol 1999;106:614-626.
109. Rubnitz JE, Raimondi SC, Tong X, et al. Favorable Impact of the t(9;11) in Childhood Acute Myeloid Leukemia. J Clin Oncol 2002;20:2302-2309.
110. Schoch C, Schnittger S, Klaus M, et al. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood 2003;102:2395-2402.
111. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. Blood 2007;107:3463-3468.
112. Schnittger S, Kohl T, Haferlach T, et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overll survival. Blood 2006;107:1791-1799.
113. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. J Clin Oncol 2006;24:3904-3911.
114. Goemans BF, Zwaan CM, Miller M, et al. Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. Leukemia 2005;19:1536-1542.
115. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia 2006;20:965-970.
116. Meshinchi S, Stirewalt DL, Alonzo TA, et al. Activating mutations of RTK/ras signal transduction pathway in pediatric acute myeloid leukemia. Blood 2003;102:1474-1479.
117. Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood 1999;93:3074-3080.
118. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001;98:1752-1759.
119. Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res 2001;61:7233-7239.
120. Schnittger S, Schoch C, Dugas M, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood 2002;100:59-66.
121. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 2002;99:4326-4335.
122. Meshinchi S, Woods WG, Stirewalt DL, et al. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood 2001;97:89-94.

123. Iwai T, Yokota S, Nakao M, et al. Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group, Japan. Leukemia 1999;13:38-43.
124. Pollard JA, Alonzo TA, Gerbing RB, et al. FLT3 internal tandem duplication in CD34+/. Blood 2006;108:2764-2769.
125. Leith CP, Kopecky KJ, Chen IM, et al. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. Blood 1999;94:1086-1099.
126. Legrand O, Simonin G, Beauchamp-Nicoud A, Zittoun R, Marie JP. Simultaneous activity of MRP1 and Pgp is correlated with in vitro resistance to daunorubicin and with in vivo resistance in adult acute myeloid leukemia. Blood 1999;94:1046-1056.
127. den Boer ML, Pieters R, Kazemier KM, et al. Relationship between major vault protein/lung resistance protein, multidrug resistance-associated protein, P-glycoprotein expression, and drug resistance in childhood leukemia. Blood 1998;91:2092-2098.
128. Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood 2002;100:2717-2723.
129. Frohling S, Schlenk RF, Stolze I, et al. CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol 2004;22:624-633.
130. Inokuchi K, Yamaguchi H, Hanawa H, et al. Loss of DCC Gene Expression Is of Prognostic Importance in Acute Myelogenous Leukemia. Clin Cancer Res 2002;8:1882-1888.
131. De Bont ES, Fidler V, Meeuwsen T, et al. Vascular Endothelial Growth Factor Secretion Is an Independent Prognostic Factor for Relapse-free Survival in Pediatric Acute Myeloid Leukemia Patients. Clin Cancer Res 2002;8:2856-2861.
132. Kohler T, Schill C, Deininger MW, et al. High Bad and Bax mRNA expression correlate with negative outcome in acute myeloid leukemia (AML). Leukemia 2002;16:22-29.
133. Karakas T, Miething CC, Maurer U, et al. The coexpression of the apoptosis-related genes bcl-2 and wt1 in predicting survival in adult acute myeloid leukemia. Leukemia 2002;16:846-854.
134. Del Poeta G, Venditti A, Del Principe MI, et al. Amount of spontaneous apoptosis detected by Bax/Bcl-2 ratio predicts outcome in acute myeloid leukemia (AML). Blood 2003;101:2125-2131.
135. Baldus CD, Thiede C, Soucek S, et al. BAALC expression and FLT3 internal tandem duplication mutations in acute myeloid leukemia patients with normal cytogenetics: prognostic implications. J Clin Oncol 2006;24:790-797.
136. Marcucci G, Maharry K, Whitman SP, et al. High Expression Levels of the ETS-Related Gene, ERG, Predict Adverse Outcome and Improve Molecular Risk-Based Classification of Cytogenetically Normal Acute Myeloid Leukemia: A Cancer and Leukemia Group B Study. J Clin Oncol 2007;25:3337-3343.
137. Marcucci G, Baldus CD, Ruppert AS, et al. Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. J Clin Oncol 2005;23:9234-9242.
138. Boissel N, Renneville A, Biggio V, et al. Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. Blood 2005;106:3618-3620.
139. Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 2006;107:4011-4020.

140. Brown P, McIntyre E, Rau R, et al. The incidence and clinical significance of nucleophosmin mutations in childhood AML. Blood 2007;110:979-985.
141. Schnittger S, Kinkelin U, Schoch C, et al. Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia 2000;14:796-804.
142. Dohner K, Tobis K, Ulrich R, et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. J Clin Oncol 2002;20:3254-3261.
143. Yagi T, Morimoto A, Eguchi M, et al. Identification of a gene expression signature associated with pediatric AML prognosis. Blood 2003;102:1849-1856.
144. Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 2004;350:1617-1628.
145. Bullinger L, Dohner K, Bair E, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. N Engl J Med 2004;350:1605-1616.
146. Radmacher MD, Marcucci G, Ruppert AS, et al. Independent confirmation of a prognostic gene-expression signature in adult acute myeloid leukemia with a normal karyotype: a Cancer and Leukemia Group B study. Blood 2006;108:1677-1683.
147. Wilson CS, Davidson GS, Martin SB, et al. Gene expression profiling of adult acute myeloid leukemia identifies novel biologic clusters for risk classification and outcome prediction. Blood 2006;108:685-696.
148. Renneville A, Boissel N, Gachard N, et al. The favorable impact of CEBPA mutations in patients with acute myeloid leukemia is only observed in the absence of associated cytogenetic abnormalities and FLT3 internal duplication. Blood 2009;113:5090-5093.
149. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358:1909-1918.
150. Ho PA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic implications of CEBPA mutations in pediatric AML: a report from the Children's Oncology Group. Blood 2009;113:6558-6566.
151. Verhaak RG, Goudswaard CS, van PW, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood 2005;106:3747-3754.
152. Schnittger S, Schoch C, Kern W, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood 2005;106:3733-3739.
153. Hollink IH, Zwaan CM, Zimmermann M, et al. Favorable prognostic impact of NPM1 gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. Leukemia 2009;23:262-270.
154. Creutzig U, Zimmermann M, Ritter J, et al. Definition of a standard-risk group in children with AML. Br J Haematol 1999;104:630-639.
155. 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 2003;101:3398-3406.
156. Buccisano F, Maurillo L, Gattei V, et al. The kinetics of reduction of minimal residual disease impacts on duration of response and survival of patients with acute myeloid leukemia. Leukemia 2006;20:1783-1789.

157. Langebrake C, Creutzig U, Dworzak M, et al. Residual disease monitoring in childhood acute myeloid leukemia by multiparameter flow cytometry: the MRD-AML-BFM Study Group. J Clin Oncol 2006;24:3686-3692.
158. Kern W, Voskova D, Schoch C, et al. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. Blood 2004;104:3078-3085.
159. Okamoto Y, Ribeiro RC, Srivastava DK, et al. Viridans streptococcal sepsis: clinical features and complications in childhood acute myeloid leukemia. J Pediatr Hematol Oncol 2003;25:696-703.
160. Gamis AS, Howells WB, DeSwarte-Wallace J, et al. Alpha Hemolytic Streptococcal Infection During Intensive Treatment for Acute Myeloid Leukemia: A Report From the Children's Cancer Group Study CCG-2891. J Clin Oncol 2000;18:1845-1855.
161. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 2000;356:1795-1799.
162. Costello RT, Sivori S, Marcenaro E, et al. Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia. Blood 2002;99:3661-3667.
163. Fauriat C, Just-Landi S, Mallet F, et al. Deficient expression of NCR in NK cells from acute myeloid leukemia: Evolution during leukemia treatment and impact of leukemia cells in NCRdull phenotype induction. Blood 2007;109:323-330.
164. Pende D, Spaggiari GM, Marcenaro S, et al. Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the Poliovirus receptor (CD155) and Nectin-2 (CD112). Blood 2005;105:2066-2073.
165. Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. Blood 2004;104:3679-3687.
166. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007;446:758-764.
167. Gal H, Amariglio N, Trakhtenbrot L, et al. Gene expression profiles of AML derived stem cells; similarity to hematopoietic stem cells. Leukemia 2006;20:2147-2154.
168. Irish JM, Hovland R, Krutzik PO, et al. Single cell profiling of potentiated phospho-protein networks in cancer cells. Cell 2004;118:217-228.
169. Strumberg D, Clark JW, Awada A, et al. Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. Oncologist 2007;12:426-437.
170. Steinbach D, Legrand O. ABC transporters and drug resistance in leukemia: was P-gp nothing but the first head of the Hydra? Leukemia 2007;21:1172-1176.
171. Lamba JK, Crews K, Pounds S, et al. Pharmacogenetics of deoxycytidine kinase: identification and characterization of novel genetic variants. J Pharmacol Exp Ther 2007;323:935-945.
172. Peck AJ, Englund JA, Kuypers J, et al. Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. Blood 2007;110:1681-1688.
173. Debiaggi M, Canducci F, Sampaolo M, et al. Persistent symptomless human metapneumovirus infection in hematopoietic stem cell transplant recipients. J Infect Dis 2006;194:474-478.
174. Antoniadou A, Giamarellou H. Fever of unknown origin in febrile leukopenia. Infect Dis Clin North Am 2007;21:1055-90, x.

175. Engelhart S, Glasmacher A, Exner M, Kramer MH. Surveillance for nosocomial infections and fever of unknown origin among adult hematology-oncology patients. Infect Control Hosp Epidemiol 2002;23:244-248.
176. Freymuth F, Vabret A, Cuvillon-Nimal D, et al. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. J Med Virol 2006;78:1498-1504.
177. Hijiya N, Metzger ML, Pounds S, et al. Severe cardiopulmonary complications consistent with systemic inflammatory response syndrome caused by leukemia cell lysis in childhood acute myelomonocytic or monocytic leukemia. Pediatr Blood Cancer 2005;44:63-69.
178. Panetta JC, Wilkinson M, Pui CH, Relling MV. Limited and optimal sampling strategies for etoposide and etoposide catechol in children with leukemia. J Pharmacokinet Pharmacodyn 2002;29:171-188.
179. Zelen M. The randomization and stratification of patients to clinical trials. J Chronic Dis 1974;27:365-375.
180. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 1988;16:1141-1154.
181. Cheng C, Pounds S. False discovery rate paradigms for statistical analyses of microarray gene expression data. Bioinformation 2007;1:436-446.
182. Pounds SB. Estimation and control of multiple testing error rates for microarray studies. Brief Bioinform 2006;7:25-36.
183. Cox DR. Regression models and life-tables. J R Stat Soc B 1972;34:187-220.
184. Jung SH, Owzar K, George SL. A multiple testing procedure to associate gene expression levels with survival. Stat Med 2005;24:3077-3088.

APPENDIX I – DRUG INFORMATION

1. CYTARABINE (Ara-C) (Cytosar-U®)

Source and pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. ra-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml respectively resulting in a final concentration of 50mg/ml. The 1000 and 2000mg vials are reconstituted with 20ml and 40 ml respectively resulting in a final concentration of 50mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: Commercially available.

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

Dosage and route of administration: See Treatment sections 4.2 – 4.6 and 4.9.

2. CYTARABINE (High Dose Ara-C)

Source and pharmacology: Cytarabine is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ARA-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is cell cycle, S phase specific. Cytarabine does penetrate the blood brain barrier. It is converted to its inactive form, uracil arabinoside, by pyrimidine nucleoside deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation and stability: Cytarabine is available in multi-dose vials containing 100, 500, 1000 and 2000mg of lyophilized drug. Intact vials can be stored at room temperature. For IV use, either sterile water for injection or bacteriostatic water for injection can be used to reconstitute the lyophilized drug. For intrathecal use, only sterile water for injection should be used for reconstitution. The 100 and 500 mg vials are reconstituted with 2 and 10 ml respectively resulting in a final concentration of 50mg/ml. The 1000 and 2000mg vials are reconstituted with 20ml and 40 ml respectively resulting in a final concentration of 50mg/ml. After reconstitution, the drug is stable for 8 days at room temperature.

Supplier: Commercially available.

Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other adverse effects reported commonly include nausea and vomiting (may be severe at high doses), diarrhea, mucositis, anorexia, alopecia, skin rash and liver dysfunction. A flu-like syndrome characterized by fever, muscle and bone aches is common. Less common side effects include allergic reactions and cellulitis at the injection site. High doses of cytarabine can cause conjunctivitis, hepatitis, and a group of CNS symptoms including somnolence, peripheral neuropathy, ataxia and personality changes. CNS symptoms are usually reversible and are more common in patients who have received previous cranial irradiation. In addition, a syndrome of sudden respiratory distress progressing to pulmonary edema has occurred.

Dosage and route of administration: See Treatment Sections 4.2 – 4.6.

3. DAUNORUBICIN (Daunomycin, Cerubidine®)

Source and pharmacology: Daunorubicin is an anthracycline antibiotic derived from *Streptomyces coeruleorubidus*. Daunorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, daunorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Even though daunorubicin exerts its major effects in the S phase, it is considered to be cell cycle phase non-specific. Daunorubicin is widely distributed in tissues but does not cross the blood brain barrier. It is metabolized to daunorubicinol which is the major active metabolite and aglycones (inactive). The major route of elimination is through the bile (40%) with additional elimination through the urine. Dosages should be reduced in patients with liver dysfunction (bilirubin > 1/2 mg/dL) or renal dysfunction (creatinine > 3 mg/dL).

Formulation and stability: Daunorubicin is supplied in vials containing 20 mg of reddish colored lyophilized powder and 100 mg of mannitol. The intact vials should be stored at room temperature. Each vial can be reconstituted with 4 ml of sterile water for injection to give a final concentration of 5 mg/ml. Reconstituted solutions are stable for 24 hours at room temperature and 48 hours if refrigerated.

Supplier: Commercially available.

Toxicity: Dose-limiting toxicities of daunorubicin include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550mg/m^2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred. Typhlitis can occur when combined with cytarabine.

Dosage and route of administration: See Treatment Section 4.2.

4. ETOPOSIDE (VP-16) (Vepesid®)

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum.* It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P450 3A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide is available in multi-dose vials containing 100mg, 150mg, 500mg and 1000mg of etoposide as a 20mg/ml solution and 30% alcohol. Etoposide is also available as a 50 mg capsule. The intact vials of etoposide solution should be stored at room temperature. The capsules should be stored under refrigeration. Etoposide solution should be diluted in D5W or 0.9% NaCl prior to administration. Solutions with a final concentration of 0.2 and 0.4 mg/ml are stable at room temperature for 96 hours and 24 hours respectively.

Supplier: Commercially available.

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis and peripheral neuropathy. Secondary leukemia has been reported.

Dosage and route of administration: See Treatment Sections 4.2 and 4.6.

5. CLOFARABINE (Clolar™, Clofarex)

Source and pharmacology: Clofarabine is a purine nucleoside analog. It is intracellulary metabolized to the active metabolite clofarabine 5'-triphosphate which competes with deoxyadenosine triphosphate for binding to ribonucleotide reductase and DNA polymerase. It inhibits DNA synthesis, terminates DNA chain elongation and inhibits DNA repair. Clofarabine also disrupts the mitochondrial membrane which results in the release of proteins, cytochrome C and apoptosis-inducing factor leading to cell death. It is mainly excreted in the urine as unchanged drug.

Formulation and stability: Clofarabine is available in the parenteral form as a preservative-free solution that is 1 mg/mL. It is available in 20 mL vials. The undiluted drug should be stored at room temperature. The diluted solution is stable for 24 hours at room temperature. Clofarabine injection should be filtered through sterile 0.2 micrometer syringe filter and then further diluted with 5% dextrose or 0.9% NaCl containing solutions.

Supplier: The injection is commercially available, however for this study clofarabine will be supplied by Genzyme.

Toxicity: The most common side effects are nausea, vomiting, diarrhea, headache, fever and pruritus. Also reported are pericardial effusion, tachycardia, hypotension, left ventricular systolic dysfunction, edema, flushing, hypertension, fatigue, anxiety, pain, dizziness, depression, irritability. Patients who receive clofarabine are at risk for tumor lysis syndrome and need to be monitored closely. Patients may also experience a systemic inflammatory response syndrome (SIRS) or capillary leak syndrome. Patients should be monitored for this during the infusion.

Dosage and route of administration: See Treatment Sections 4.2.

6. MITOXANTRONE (Novantrone®)

Source and pharmacology: Mitoxantrone is an anthracenedione that is structurally similar to the anthracyclines. It is thought to act by intercalating into DNA, causing template disorder, steric obstruction and inhibition of DNA and RNA synthesis. In addition, mitoxantrone inhibits the action of topoisomerase II. Mitoxantrone is active throughout the cell cycle. Mitoxantrone is about 78% protein bound and does cross the blood brain barrier. Mitoxantrone is metabolized in the liver to inactive metabolites. The parent drug and metabolites are excreted primarily via hepatobiliary excretion with small amounts excreted in the urine. Dosage adjustment is recommended for patients with severe hepatic dysfunction (total bilirubin > 3.4 mg/dl).

Formulation and stability: Mitoxantrone is available in multi-dose vials containing 20 mg, 25 mg or 30 mg of mitoxantrone as a dark blue, aqueous solution at a concentration of 2 mg/ml. The intact vials should be stored at room temperature. Refrigeration may result in precipitation of mitoxantrone, which will re-dissolve upon warming to room temperature. The drug should be further diluted to at least 50 ml in 5% dextrose or 0.9% NaCl prior to administration. These solutions are chemically stable for at least 7 days when stored at room temperature.

Supplier: Commercially available.

Toxicity: The major dose-limiting toxicity of mitoxantrone is leukopenia with thrombocytopenia and anemia occurring much less frequently. Nausea and vomiting are usually moderate in severity. Other side effects reported commonly include alopecia, diarrhea, headache, fever and stomatitis. Blue to green discoloration of urine and other body fluids occurs. Other side effects reported less commonly include elevated liver function tests, allergic reactions, seizures, jaundice and renal failure. Congestive heart failure has been reported, but is much less common than with doxorubicin. CHF has been reported primarily in patients receiving prior therapy with anthracyclines. Patients with an increased risk of cardiotoxicity include those having received prior therapy with anthracyclines, those with previous mediastinal radiotherapy and those with pre-existing cardiac conditions.

Dosage and route of administration: See Treatment Sections 4.3 and 4.4.

7. THIOGUANINE

Source and pharmacology: Thioguanine is a purine antimetabolite. It is intracellularly converted to ribonucleotides which are incorporated into DNA and RNA. Absorption of thioguanine is variable and poor and is decreased by food. Thioguanine undergoes extensive metabolism in the liver and other tissues to the inactive, methylated derivative and to 6-thiouracil by xanthine oxidase. Thioguanine is excreted in the urine almost completely as metabolites.

Formulation and stability: Thioguanine is available as a 40 mg scored tablet. It may be stored at room temperature.

Supplier: Tablets are commercially available.

Toxicity: The major dose-limiting toxicity is myelosuppression. Nausea and vomiting are usually mild. Other toxicities reported include diarrhea, rash, anorexia, stomatitis, and hyperuricemia. Jaundice and elevated liver function tests have been reported rarely

Guidelines for administration: See Treatment Section 4.4.

8. L-ASPARAGINASE (Elspar®)

Source and pharmacology: Asparaginase is an enzyme. It is derived from *Escherichia coli*. Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract and must be given parenterally. Asparaginase does not cross into the CSF. The plasma half-life of L-asparaginase when given IV is ≈ 8-30 hours. When given IM the half-life is ≈30 hours. Only minimal urinary and biliary excretion occurs. Clearance is unaffected by age, renal function or hepatic function.

Formulation and stability: E. Coli asparaginase is available in vials containing 10,000 units of lyophilized drug and 80 mg mannitol. Unused vials should be refrigerated. The contents of each vial should be diluted with 1 cc of preservative-free normal saline or sterile water, giving a resultant solution of 10,000 units/ml. Once in solution, it is recommended that it be used within 8 hours as no preservative is added. Occasionally a small number of gelatinous-like fibers may develop upon standing. If this occurs, the solution can be filtered through a 5 micron filter to remove the particles with no change in potency.

Supplier: Commercially available

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFT's, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in hemorrhage. Thrombosis and/or pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

Dosage and route of administration: See Treatment Section 4.5.

9. CYCLOPHOSPHAMIDE (Cytoxan®)

Source and pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Cyclophosphamide is cell-cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of > 75%. Cyclophosphamide is a prodrug that requires activation. It is metabolized by mixed-function oxidases in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with aldofosfamide. Aldofosfamide spontaneously splits into cyclophosphamide mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldofosfamide may be enzymatically metabolized to carboxyphosphamide, which are generally considered to be inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dosage adjustments should be made in patients with a creatinine clearance of < 50 ml/min.

Formulation and stability: Cyclophosphamide is available in 25 and 50 mg tablets. Cyclophosphamide is also available in vials containing 100, 200, 500, 1000 and 2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection respectively to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.

Supplier: Commercially available.

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of MESNA decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.

Dosage and route of administration: See Treatment Section 4.7.

10. FLUDARABINE (Fludara®)

Source and pharmacology: Fludarabine phosphate is a synthetic purine nucleoside analog. It acts by inhibiting DNA polymerase, ribonucleotide reductase and DNA primase by competing with the physiologic substrate, deoxyadenosine triphosphate, resulting in inhibition of DNA synthesis. In addition, fludarabine can be incorporated into growing DNA chains as a false base, thus interfering with chain elongation and halting DNA synthesis. Fludarabine is rapidly dephosphorylated in the blood and transported intracellularly via a carrier mediated process. It is then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate form. Approximately 23% of the dose is excreted as the active metabolite in the urine (with dosages of 18-25 mg/m^2/day for 5 days). Renal clearance appears to become more important at higher doses, with approximately 41-60% of the dose being excreted as the active metabolite in the urine with dosages of 80-260 mg/m^2.

Formulation and stability: Fludarabine is supplied in single-dose vials containing 50 mg fludarabine as a white lyophilized powder and 50 mg of mannitol. The intact vials should be stored under refrigeration. Each vial can be reconstituted by adding 2 ml of sterile water for injection resulting in a final concentration of 25mg/ml. Because the reconstituted solution contains no antimicrobial preservative, the manufacturer recommends that it should be used within 8 hours of preparation. The solution should be further diluted in 5% dextrose or 0.9% NaCl prior to administration.

Supplier: Commercially available.

Toxicity: The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly include, anorexia, fever and chills, alopecia and rash. Neurotoxicity can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness and coma and is more common at high doses. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia and

pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.

Dosage and route of administration: See Treatment Section 4.7.

11. METHOTREXATE

Source and pharmacology: Methotrexate is a folate analogue that acts by inhibiting dihydrofolate reductase. Dihydrofolate reductase is an enzyme important in the conversion of folic acid to tetrahydrofolic acid, which is necessary in the synthesis of purine nucleotides and thymidylate. By inhibiting the production of tetrahydrofolic acid, methotrexate interferes with DNA, RNA and protein synthesis. Methotrexate is poorly and variably absorbed orally, with an average of ≈ 40% for doses of $\leq$ 30 mg/m^2. At higher dosages, the extent of absorption decreases. Methotrexate is approximately 50% protein bound. It distributes widely into body tissues and fluids with sustained concentrations in the kidney and the liver. Methotrexate undergoes metabolism by cytosolic aldehyde oxidase to hydroxy methotrexate. It is excreted mainly in the urine as unchanged drug with small amounts being excreted in the bile and feces. The percent recovered as unchanged drug in the urine is higher with short infusions than with prolonged infusions. Methotrexate has a biphasic elimination with an initial half-life of ≈ 2-3 hours and a terminal half-life of 10-12 hours. Methotrexate may be "sequestered" in body fluid collections and eliminated slowly from these areas. Patients with effusions or GI obstruction should have plasma levels monitored closely for delayed excretion following high-dose methotrexate.

Formulation and stability: Methotrexate is supplied in single-dose vials containing 50mg, 100mg, 200mg, and 250 mg of methotrexate as a 25 mg/ml preservative-free solution and in vials containing 20mg, 50 mg, 100mg, 250 mg and 1000mg of lyophilized drug. It is also available in 2.5 mg tablets. Methotrexate preservative-free solution and lyophilized drug should be stored at room temperature and protected from light. Methotrexate tablets can also be stored at room temperature. The vials containing 20, 50, 100 and 250 mg of lyophilized product can be reconstituted by adding sterile water, 0.9% NaCl or D5W to a final concentration not exceeding 25 mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50 mg/ml.

Supplier: Commercially available.

Toxicity: The dose limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea or acute nephrotoxicity. Toxicities reported frequently include nausea and vomiting, diarrhea, anorexia, alopecia, hepatic toxicity and alopecia. Less common side effects include blurred vision, photosensitivity, anaphylaxis, headache, pneumonitis, skin depigmentation or hyperpigmentation, rash, vasculitis and encephalopathy. During high-dose methotrexate therapy, most patients experience a transient decrease in GFR, but renal failure can occur, particularly if the patient does not receive urinary alkalinization and aggressive hydration before, during and after receiving high dose methotrexate. Leucovorin rescue should be initiated within 48 hours of starting high-dose methotrexate and adjusted based on MTX levels to prevent bone marrow toxicity and mucositis. Leucovorin may also be

necessary after IT administration, especially if IT methotrexate therapy is given to patients with renal dysfunction. Patients with Down syndrome have a tendency to have delayed methotrexate clearance and a greater risk of toxicity, despite increased leucovorin rescue.

Dosage and route of administration: Intrathecal, See Treatment Section 4.9.

12. HYDROCORTISONE, Intrathecal (Cortef, Solu-Cortef)

Source and pharmacology: Hydrocortisone is a synthetic steroid akin to the natural adrenal hormone cortisol. Hydrocortisone has phase-specific cytotoxicity, killing lymphoblasts primarily during S phase. It has catabolic effect on proteins and alters the kinetics of peripheral blood leukocytes. It is excreted in the urine and catabolized in the liver.

Formulation and stability: Solu-Cortef sterile powder is supplied in the following package: 100 mg plain, and 100 mg, 250 mg, 500 mg, and 1000 mg ACT-O-VIAL (MIX-O-VIAL). Store unreconstituted product at controlled room temperature 15-30°C (59-86°F). Store reconstituted solution in the refrigerator and protect from light. Unused solution should be discarded after 3 days. Use Solu-Cortef (plain vial) for intrathecal use, and reconstitute with 0.9% sodium chloride, USP for injection.

Supplier: Commercially available.

Toxicity: If given intrathecally, sterile arachnoiditis may occur. Headache, seizures, unusual feelings or sensations, loss of feeling or ability to move arms or legs, and difficulty with urination or bowel movements may also occur.

Dosage and route of administration: Intrathecal, See Treatment Section 4.9

13. MESNA (Mesnex®)

Source and pharmacology: Mesna is a synthetic sulfhydryl (thiol) compound. Mesna contains free sulfhydryl groups that interact chemically with urotoxic metabolites of oxazaphosphorine derivatives such as cyclophosphamide and ifosfamide. Oral bioavailability is ≈50%. Upon injection into the blood, mesna is oxidized to mesna disulfide, a totally inert compound. Following glomerular filtration, mesna disulfide is rapidly reduced in the renal tubules back to Mesna, the active form of the drug. Mesna and mesna disulfide are excreted primarily via the urine.

Formulation and stability: Mesna is available in 2 ml, 4 ml and 10 ml amps containing 100 mg/ml of mesna solution. The intact vials can be stored at room temperature. Mesna may be further diluted in 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.

Supplier: Commercially available.

Toxicity: Mesna is generally well tolerated. Nausea and vomiting, headache, diarrhea, rash, transient hypotension and allergic reactions have been reported. Patients may complain of a bitter taste in their mouth during administration. Mesna may cause false positive urine dipstick readings for ketones.

Dosage and administration: See Treatment Section 4.7.

14. SORAFENIB (Nexavar®) – Participants with FLT3-ITD

Source and pharmacology: Sorafenib (BAY 54-9085) is a kinase inhibitor (Raf, VEGF-R, and PDGF-R). The ras/raf signaling pathway is an important mediator of responses to growth signals and angiogenic factors. This pathway is often aberrantly activated in human tumors due to presence of activated ras, mutant b-raf, or over expression of growth factor receptors. Sorafenib is a potent inhibitor of c-raf, and wild-type and mutant b-raf in vitro. Additionally, further characterization of BAY 43-9006 tosylate revealed that this agent inhibits several receptor tyrosine kinases (RTKs) that are involved in tumor progression (VEGF-R, PDGF-R, Flt3, and c-KIT) and p38α, a member of the MAPK family

Formulation and stability: Sorafenib is supplied as a salmon-colored 200 mg, round, immediate-release film-coated tablet

Sorafenib 200 mg tablets are supplied in bottles of 120 tablets. Sorafenib should be stored at 59 - 86°F(15°C – 30°C) in a dry place. Tablets are stable until the date of expiration listed on the manufacturer's container.

Supplier: Commercially available.

Dosage and route of administration: Sorafenib is administered orally as tablets BID approximately every 12 hours for 21 days after a course of chemotherapy. See Treatment Section 4.2.5.

Tablets can be cut into fourths and the dose rounded to the nearest 50 mg. Tablets should be taken with clear liquids (approximately 2 to 4 ounces for children < 12 and 4 to 8 ounces for patients ≥12 years). It is recommended that sorafenib be taken on an empty stomach. If taken with food, sorafenib should be taken with a moderate to low fat meal.

Toxicity: The most frequently occurring side effects (> 20% of patients) include: fatigue (asthenia, lethargy, malaise), rash/desquamation, hand-foot syndrome, diarrhea. Hypertension, is frequently seen during the first 6 weeks of therapy and should be monitored and treated. Elevated lipase and amylase were commonly reported during adult clinical trials, as was hypophosphatemia. Patients on sorafenib have also experienced cardiac ischemia or infarction, increased risk of hemorrhage, wound healing complications and gastrointestinal perforation. Based on animal studies sorafenib is expected to be teratogenic.

CYP3A4 inhibitors have not been demonstrated to alter the metabolism of sorafenib.

CYP 450 inducers: Intake of the following cytochrome P450 enzyme-inducing agents is expected to increase the metabolism of sorafenib: Antiepileptic drugs (phenytoin, carbamazepine or phenobarbital), rifampin, dexamethasone or St. Johns Wort. These agents should be used with caution as they are likely to decrease the concentration of sorafenib.

Drug interactions: Sorafenib is metabolized by the P450 CYP3A4 and UGT1A9 enzymes and has been shown in preclinical studies to inhibit multiple CYP isoforms. Therefore, it is possible that sorafenib may interact with drugs that are metabolized by the P450 CYP isoenzymes or with drugs that inhibit CYP 3A. Close monitoring is recommended for patients taking agents with narrow therapeutic indices and metabolized by the liver, such as warfarin, quinidine, cyclosporine, and digoxin. Drug interactions (increase in AUC) have been noted with doxorubicin, docetaxel, and irinotecan.

Sorafenib is 97% to 99% protein bound.

15. VORINOSTAT (ZOLINZA®, SUBEROYLANILIDE HYDROXAMIC ACID, SAHA)

Source and pharmacology: vorinostat is a histone deacetylase (HDAC) inhibitor. Its chemical name is N-hydroxy-N'-phenyl-octane-1, 8-diotic acid diamide, N-hydroxy-N'-phenyl (9CI) octanediamide. The HDAC enzymes catalyze the removal of acetyl groups from the lysine residues of proteins, such as histones and transcription factors. In some cancer cells, there is an overexpression of HDACs or an abnormal recruitment of HDACs to oncogenic transcription factors causing hypoacetylation of core nucleosomal histones. Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription.

Vorinostat inhibits HDAC by binding directly to the catalytic pocket of HDAC1, HDAC2, and HDAC3 (Class I) and HDAC6 (Class II) enzymes. Inhibition of HDAC activity allows for the accumulation of acetylated histones. This accumulation influences the regulation of gene expression. In vitro, exposure of cultured transformed cell to vorinostat led to G1 or G2 phase cell-cycle arrest, apoptosis, or differentiation and demonstrated synergistic and additive activity in combination with other cancer therapies (including radiation, kinase inhibitors, cytotoxic agents, and differentiating agents). The mechanism of the antineoplastic effect of vorinostat has not been fully characterized.

After oral administration, vorinostat is rapidly absorbed, however, administration with a high-fat meal resulted in a 33% increase in the extent of absorption and a 2.5-hour delay in the rate of absorption compared to the fasted state. Vorinostat is approximately 71% bound to human plasma protein. It is extensively metabolized to inactive metabolites, primarily by glucuronidation and hydrolysis followed by beta-oxidation. The two metabolites, O-glucuronide of vorinostat and 4-anilino-4-oxobutanoic acid are pharmacologically inactive. In vitro studies indicate that vorinostat is not metabolized by and does not inhibit the activity of cytochrome P-450 enzymes. Less than 1% of an administered dose is excreted unchanged in the urine. Approximately 35-52% of an oral dose of vorinostat is excreted in the urine as the two major metabolites. The mean terminal half-life of vorinostat and the O-glucuronide metabolite is approximately 2 hours, while that of the 4-anilino-4-oxobutanoic acid metabolite it is 11 hours.

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) were observed in patients receiving vorinostat with coumarin-derivative anticoagulants (e.g., warfarin). Therefore PT and INR should be monitored when coumarin-dervative anticoagulants are started or discontinued. When vorinostat was administered with other HDAC inhibitors (e.g., valproic acid), severe thrombocytopenia and gastrointestinal bleeding have been reported.

Formulation and stability: Vorinostat is supplied as a white, opaque gelatin, size 3 capsule, containing 100 mg of vorinostat. The inactive ingredients in each capsule include icrocrystalline cellulose, sodium croscarmellose, and magnesium stearate. Vorinostat 100 mg capsules are supplied in bottles containing 120 capsules.

Store vorinostat capsules at room temperature, 15 to 30ºC (59 to 86ºF). Do not store above 30ºC and avoid exposure to excessive moisture.

Guidelines for administration: See Treatment sections of the protocol. Vorinostat should be taken with food. The capsules should not be opened or crushed. A suspension can be prepared by the pharmacy for patients that cannot swallow pills. If a patient needs less than 120 capsules for a treatment cycle, the exact number of capsules needed for treatment can be counted into a prescription bottle.

Direct contact of the powder in vorinostat capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly. Clean powder spills from broken or damaged vorinostat capsules carefully minimizing inhalation. Wash spill area at least 3 times with ethyl alcohol, followed by water.

Toxicity: Likely (>20%) adverse events include anemia, diarrhea, nausea, vomiting, fatigue, thrombocytopenia, anorexia. See package insert for full information.

Supplier: commercially available.

16. INTERLEUKIN-2 (IL-2, ALDESLEUKIN, PROLEUKIN®)

Source and pharmacology: Aldesleukin is a biosynthetic cytokine (a lymphokine) of recombinant DNA origin. It differs from human interleukin-2 by the absence of an N-terminal alanine, the replacement of cysteine with serine at position 125 of the sequence, and the absence of glycosylation. It is a biologic response modifier with complex antineoplastic and immunomodulating activities.

Formulation and stability: Aldesleukin vials contain 22 million units of lyophilized recombinant interleukin. Each single use vial is reconstituted with 1.2 ml of sterile water for injection to give 18 million units/ml. Aldesleukin should be admixed with D5W for infusion, with albumin 0.1% added to decrease adsorption. Do not use an in-line filter. Do not mix in saline.

Supplier: Commercially available.

Toxicities: Aldesleukin is a highly toxic drug. Most adverse effects are dose related and schedule dependent, with fewer toxicities associated with low dose, subcutaneous or continuous IV infusions as compared to high dose, rapid IV infusions. Most adverse effects are self-limiting and reversible within 2 to 3 days of drug discontinuance. Many of the adverse effects of aldesleukin are related to capillary leak syndrome, which has been associated with this drug. The most frequently reported serious adverse effects include hypotension, renal dysfunction with oliguria/anuria, dyspnea or pulmonary congestion, and mental status changes (lethargy, somnolence, confusion, agitation). Additional serious adverse effects reported include myocardial ischemia, myocarditis, gangrene, respiratory failure leading to intubation, GI bleeding, intestinal perforation, ileus, coma, seizures, sepsis, and renal impairment requiring dialysis. Most patients receiving aldesleukin develop some degree of a flu-like syndrome that may include fever, chills, rigors, fatigue, weakness, malaise, arthralgia and myalgia.

Dosage and administration: NK Cell transplant participants, see Section 4.7.

17. LEUCOVORIN (Folinic Acid) -

Source and pharmacology: Leucovorin is a racemic mixture of tetrahydrofolic acid, which is involved as a cofactor for 1-carbon transfer reactions in the synthesis of purine and pyrimidines. Leucovorin is a potent antidote for both the hematopoietic and reticuloendothelial toxic effects of folic acid antagonists by replenishing reduced folate pools. It is postulated that in some cancers, leucovorin enters and "rescues" normal cells from the toxic effects of folic acid antagonists, in preference to tumor cells, because of differences in membrane transport and affinity for polyglutamylation. Leucovorin is converted in the intestinal mucosa and the liver to 5-methyl-tetrahydrofolate, which is also active as a reduced folate. It is excreted primarily in the urine with minor excretion occurring in the feces.

Formulation and stability: Leucovorin is supplied in 5, 15 and 25 mg tablets and vials containing 50, 100 or 350 mg of leucovorin as a lyophilized powder. The tablets and the lyophilized powder can be stored at room temperature. The 50 mg and 100 vials can be reconstituted by adding 5 or 10 ml of sterile water or bacteriostatic water for injection respectively to yield a final concentration of 10 mg/ml. The 350 mg vials can be reconstituted with 17 ml of sterile water or bacteriostatic water for injection to yield a final concentration of 20 mg/ml. The reconstituted solution is stable for at least 7 days at room temperature. Leucovorin may be further diluted in 5% dextrose or 0.9% NaCl containing solutions.

Supplier: Commercially available.

18. HYDROXYUREA – ORAL (Hydrea, HU)

Source and pharmacology: Hydroxyurea is well absorbed after oral administration with the peak serum concentration achieved in two hours. The drug is excreted primarily in the urine, either as urea or as the unchanged compound.

Formulation and stability: capsules, white crystalline powder. Store at room temperature, avoid excessive heat and keep bottle tightly closed.

Administration: PO.

Supplier: Commercially available.

19. ERWINIA L-ASPARAGINASE (Erwinaze®)

Source and pharmacology: Erwinia asparaginase is an enzyme. It is derived from *Erwinia chrysanthemi* and may be useful in patients with an allergy to the *E. coli* derived product. Asparaginase hydrolyzes serum asparagine (an amino acid required to synthesize proteins) to aspartic acid and ammonia, and is therefore lethal to cells that cannot synthesize asparagine. Asparaginase is active during all phases of the cell cycle. Asparaginase is not absorbed from the GI tract and must be given parenterally. Asparaginase does not cross into the CSF. The plasma half-life of Erwinia asparaginase when given IM is approximately 16 hours. Only minimal urinary and biliary excretion occurs. Clearance is unaffected by age, renal function or hepatic function.

Formulation and stability: Erwinia asparaginase is available in vials containing 10,000 units of lyophilized drug. Unused vials should be refrigerated. The contents of each vial should be diluted with 1 ml of preservative-free normal saline, giving a resultant solution of 10,000 units/ml. Once in solution, it is recommended that it be used within 8 hours as no preservative is added. Occasionally a small number of gelatinous-like fibers may develop upon standing. If this occurs, the solution can be filtered through a 5 micron filter to remove the particles with no change in potency.

Supplier: Commercially available.

Toxicity: Acute toxicity includes anaphylactic reactions that occur most commonly when the drug is given IV. These can be characterized by laryngeal constriction, hypotension, diaphoresis, fever, chills, edema and loss of consciousness. Allergic reactions at the site of IM injection include pain, swelling and erythema. Other adverse effects include neutropenia and associated immunosuppression, mild nausea and vomiting, malaise, anorexia, elevated LFTs, pancreatitis and hyperglycemia. A decrease in protein synthesis including albumin, fibrinogen and other coagulation factors may occur which can result in hemorrhage. Thrombosis and/or pulmonary embolism can also occur. Less common side effects include renal dysfunction and CNS complications including somnolence, weakness, lethargy, coma and seizures.

For additional information about this drug, please see package insert.

APPENDIX II

Criteria for Acute Graft-vs.-Host Disease

Organ staging:

Organ	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin	No rash	Rash on < 25% body surface area[a]	Rash ≥ 25% to ≤ 50%	Rash >50% generalized erythroderma	Plus bullae and desquamation
Gastro-intestinal	Diarrhea < 500 mL/day	Diarrhea 501 to 1000 mL/day[c] or persistent nausea[c]	Diarrhea 1001 to 1500 mL/day	Diarrhea >1500 mL/day	Severe abdominal pain with or without ileus
Liver	Bilirubin < 2.0 mg/dl	Bilirubin 2.1 to 3.0 mg/dl[b]	Bilirubin 3.1 to 6.0 mg/dl	Bilirubin 6.1 to 15 mg/dl	Bilirubin 15 mg/dl

Overall Grading for acute GVHD[e]

Grade	Skin	Liver	Gut
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage2-4
IV[f]	Stage 4 or	Stage 4	-

a) Use rule of Nines" or burn chart to determine extent of rash.
b) Range given as total bilirubin. Downgrade one stage if additional cause of elevated bilirubin is documented.
c) Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if additional cause of diarrhea has been documented.
d) Persistent nausea with histological evidence of GVHD in stomach or duodenum.
e) Criteria for grading given as minimum degree of organ involvement required to confer that grade.
f) Grade IV may also include lesser organ involvement but with extreme decrease in performance status.

APPENDIX II (continued)

CRITERIA FOR GRADING CHRONIC GVHD GRADE

Staging of Chronic GVHD:

Limited - Localized skin and/or hepatic dysfunction.

Extensive - One or more of the following (as clinically judged by a physician and deemed as chronic GVHD by the PI):

- Generalized skin involvement
- Liver histology showing chronic aggressive hepatitis, bridging necrosis and/or cirrhosis.
- Eye dryness with Schirmer's test <5 mm wetting
- Oral: involvement of salivary glands or oral mucosa.
- Other: another target organ involvement.

APPENDIX III – SPECIMEN SUBMISSION GUIDELINES

Diagnostic studies

Morphology review

As of Amendment 9.0, central review of morphology is no longer required. However, Dr. John Choi [redacted] is available for review or assistance if requested by any collaborating site.

Cytogenetic review

Conventional karyotyping and *MLL* FISH analysis will be performed at local institutions and the results will be reviewed by Dr. Susana Raimondi at St. Jude. Please submit two karyotypes of each abnormal line (this can be done electronically) and a final report to the following:

Dr. Susana Raimondi
Department of Pathology
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Phone: [redacted]
FAX: [redacted]
Email: [redacted]
*Please include FedEx tracking number

Immunophenotyping, molecular studies, MRD, and cell banking

Immunophenotyping for diagnostic purposes may be performed at local institutions or centrally, as desired by the treating physician. If the local institution prefers immunophenotyping to be done at St. Jude, please indicate this at the time the diagnostic bone marrow is shipped. Immunophenotyping to determine a leukemic-specific phenotype for future MRD studies will be performed at St. Jude.

The Molecular Pathology laboratory (Dr. Sheila Shurtleff, technical director) will carry out molecular studies, including testing for the presence of the *AML1-ETO*, *MLL-AF9*, and *MYH11-CBFβ* fusion transcripts, *FLT3*-ITD, and mutations in the *FLT3*, *NPM1*, and *CEPBA* genes. Selected cases will also be screened for the presence of *MLL* gene rearrangements by FISH and for the *MLL-ENL*, *MLL-ELL*, *MLL-AF10*, or *RBM15/MKL1* fusions as needed.

At diagnosis, please collect a minimum of 10 mL of anti-coagulated bone marrow (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 (bovine serum albumin). Label the tube(s) with patient name, date of birth, and date sample obtained. Seal centrifuge tube with parafilm or equivalent. Complete the AML08 Specimen Submission Form and

include with specimen(s) for shipping. Clinical specimens with a low probability of containing an infectious agent must be "triple packaged" in accordance with Dangerous Goods Regulations (DRG), International Air Transport Association (IATA) and OSHA guidelines (see http://www.iata.org, http://osha.gov, and AML08 Submission Form for additional information). Specimens should be shipped at ambient temperature.

Note: If bone marrow is unattainable (dry tap) or is less than 5 mL, peripheral blood containing leukemic blasts should submitted along with any bone marrow obtained. 5 to 10 mL of peripheral blood collected in preservative free heparin should be added to RPMI 1640 with 20% FCS or BSA, processed and packaged for shipment as described for bone marrow specimens.

Ship by Federal Express for next day delivery to:

Tissue Resources
Department of Pathology, Room C5013B
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Phone: [redacted]
On call mobile: [redacted]
Email: [redacted]
*Please include FedEx tracking number

Please notify the Tissue Resources lab (Matthew Lear, technical director, [redacted]) and Dr. John Choi [redacted] via email when shipping MRD samples.

For Saturday delivery (new patients only), please notify the Tissue Resources Lab by email and by phone on Friday before 4:30 PM Central Standard time at [redacted].

Ship by Federal Express to:

Tissue Resources
Delivery to Guards desk (PCC)
Department of Pathology
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Tel: [redacted]
Fax: [redacted]
On call mobile: [redacted]

For unantipicated Saturday deliveries (e.g., diagnostic samples from new patients who arrive on a Friday evening), please inform the Tissue Resources director (Matthew Lear, [redacted]) to inform him of the Saturday delivery.

Minimal Residual Disease studies

At days 8 and 22, please send 5 ml of blood for MRD studies. Samples for MRD should be sent to the Tissue Resources Lab in preservative-free heparin stored at ambient temperature.

At day 22 and all subsequent time points, please send 5 ml of bone marrow for MRD studies. Samples for MRD should be sent to the Tissue Resources Lab in preservative-free heparin stored at ambient temperature. In addition, for patients with leukemia-specific fusion transcripts, all bone marrow aspirates should be sent for RT-PCR analysis.

Note that MRD studies are not performed on weekends. Hence, samples for MRD should be drawn Monday through Thursday and sent by overnight delivery. MRD samples that arrive on weekends are processed on Monday, but such delay may affect the quality of the assay. Only diagnostic specimens will be accepted on weekends and holidays with prior notice.

Please notify the Tissue Resources lab and Dr. John Choi [redacted]) via email when shipping MRD samples.

*Please include FedEx tracking number

NK cell studies

All samples related to NK cell research and therapy should be sent at room temperature by FEDEX priority overnight delivery to:

Barbara Rooney
St. Jude Children's Research Hospital
262 Danny Thomas Place
Rm D5032
Memphis, TN 38105
Phone [redacted]
Email: [redacted]
*Please include FedEx tracking number

Please have the blood drawn Mon-Thurs, for delivery Tuesday-Friday.

NK cell receptor studies (all patients)

At the time of diagnosis and prior to consolidation I, all patients should have blood sent for NK cell receptor genotyping and phenotyping. Please send 3 ml at diagnosis and 8.5 ml at consol I in yellow top (ACD) tubes.

In addition please send 8.5 ml yellow top (ACD) tube for HLA typing or include current HLA typing with shipment.

NK typing of parents (all patients who are standard risk or high risk who will recieve NK cell transplantation)

For NK typing, 8cc blood should be drawn in yellow top (ACD) tubes. If HLA typing needs to be performed, include one extra yellow top tube for each donor. Tubes must to be clearly labeled with the donor's full name and date of birth. Please include the completed NK HLA shipping form

NK cell studies for patients who have recieved NK infusions

Following the NK cell infusions, NK chimerism and phenotyping will be performed on days 7, 14, 21, and 28. Please send 2 yellow top tubes to Barbara Rooney at each of those time points. *Note: because NK cell studies are performed only on weekdays (Monday through Friday), these tests may be sent within 48 hours of the time they are due (e.g., if day 2 falls on a Sunday, the tests may be drawn on day 3). Post-transplant chimerism studies should also be performed after day 28 in cases of persistent chimerism.*

Pharmacokinetic studies – *(completed with LOA #4, 5/29/15)*

Sample processing for sorafenib plasma pharmacokinetic studies: after obtaining whole blood, the sample should be placed on ice. The sample is centrifuged at 3000 rpm for 10 minutes, plasma transferred to a pre-labeled cryovial, and then frozen at -20 °C until the time of shipment.

Sample shipment: Samples, and copies of completed pharmacokinetic sample collection forms and sorafenib dosing diaries for cycle 1 should be shipped on dry ice in batch every 4-6 months to Dr. Sharyn Baker. A FedEx account number will be provided to individual sites. Please notify Dr. Baker's laboratory by email before shipment and provide a FedEx tracking number. Ship to:

Dr. Sharyn Baker c/o Shelley Orwick
Pharmaceutical Sciences Department
St. Jude Children's Research Hospital
262 Danny Thomas Place
CCC Room I5504
Memphis, TN 38105
Phone (1): [redacted]
Phone (2): [redacted]
[redacted]
*Please include FedEx tracking number

Pharmacogenetic studies – *(completed with LOA #4, 5/29/15)*

Peripheral blood (10 ml) will be collected at the time of diagnosis and at the time of count recovery prior to consolidation I and sent to the Tissue Resources laboratory. Plasma will be banked for future proteomic studies and DNA will be extracted from lymphocytes for genotyping studies. These samples should be sent in purple top tubes and may be shipped with the bone marrow specimens.

Histone acetylation studies – *(completed with Revision 10.1, 6/2/16)*

For patients who receive vorinostat (see section 4.2.6), histone acetylation will be assessed in peripheral blood samples (collected in 3 mL of preservative-free heparin) on days -2 and 1 of Induction II (section 4.2.6). Samples may be ficolled, frozen and batched for shipping to:

Drs. Yubin Ge and Larry Matherly
Karmanos Cancer Institute
110 East Warren Avenue
Detroit, Michigan 48201
[redacted]

APPENDIX IV: MOLECULAR DIAGNOSTICS FOR FEVER AND NEUTROPENIA (St. Jude patients only)

Note: As of *Amendment 9.0, this research study will no longer be done.*

Background – A significant challenge among patients with neutropenia and fever is identification of causative infectious agents. Present technology is limited in this regard, with an etiology typically identified in only 30-50% of cases. Detection of bacterial agents continues to largely rely upon culture-based methods. In recent years, we have improved our blood culture methodologies and have markedly improved the positive culture rate at St. Jude; however, even with these enhancements, bacterial or fungal pathogens are isolated from less than 25% of cases. While viral diagnostics have made much greater use of molecular diagnostic techniques; most assays in routine use target only a single agent or class of agents, again potentially limiting their sensitivity.

We propose the application of broad-range molecular diagnostic assays, targeting bacterial, fungal, and viral pathogens, in an effort to identify infectious agents in patients with febrile neutropenia. Several small studies in the literature provide evidence that such techniques may result in a substantial increase in sensitivity over culture; however, these methods have not been broadly applied to pediatric oncology patients.

Hypothesis – The use of broad-range molecular diagnostic methods will enable the direct detection of infectious agents associated with febrile neutropenia, resulting in an increased speed and sensitivity of detection of pathogens currently recovered by culture, and allowing the identification of non-cultivable organisms.

Objectives – Determine the performance characteristics of broad-range, molecular diagnostic methods for the detection of bacterial, fungal, and viral agents, in comparison to methods currently in routine clinical use.

Methods – Concomitant with initial bacterial blood culture for each episode of febrile neutropenia, two (2) 4 ml EDTA vacutainer tubes of whole blood will be collected in an aseptic manner (patients less than 5 years of age will have only one (1) 4 ml EDTA tube of blood collected). Samples will transported to the Clinical Molecular Microbiology Laboratory at room temperature. Samples will be held at 4°C and processed within 7 days or frozen at -80° C until processing. Samples will be coded and de-identified prior to all processing and analysis. Processing will be performed in large batches. Following rapid thawing, nucleic acid extraction will be performed on each sample. Broad-range molecular detection methods will be performed for the detection of bacterial, fungal, and viral agents. Results of analysis will remain coded and unavailable to clinical care providers, as this methodology remains experimental. Results of testing will be correlated to the underlying clinical condition and history of patients, clinical course, and results of routine laboratory testing, including, but not limited to, culture, antigen detection, and viral PCR. Discrepant results will be reconciled based on chart review, together with results of tissue pathology and other laboratory results. Comparisons of current methods will be made relative to test sensitivity, specificity, clinical predictive value, time required to

detect a positive result, and ability to detect novel or non-cultivable organisms. The ability to risk-stratify pathogens based on subspecies-level variation will also be examined.

Agreement and discrepancies between the molecular and standard methods will be assessed by the study pathologist. Probability (rates) of agreement and discrepancies will be estimated with a 95% confidence interval. Of particular interest will be the probability (rate) of infections immediately following the febrile neutropenia episode among the patients tested positive by the molecular method but negative by the standard method; this will be estimated with a 95% confidence interval. Rates of infection immediately following the febrile neutropenia episode among the patients tested positive vs. negative by the two methods will be estimated and compared as well using models of paired categorical (binary) responses.

References

1. Ammann RA, Zucol F, Aebi C, Niggli FK, Kuhne T, Nadal D. Real-time broad-range PCR versus blood culture. A prospective pilot study in pediatric cancer patients with fever and neutropenia. Support Care Cancer 2007 June;15(6):637-641.
2. Hakim H, Flynn PM, Knapp KM, Srivastava DK, Gaur AH. Etiology and Clinical Course of Febrile Neutropenia in Children With Cancer. J Pediatr Hematol Oncol 2009 July 28.
3. Seng P, Drancourt M, Gouriet F et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009 August 15;49(4):543-551.
4. Sjoholm MI, Dillner J, Carlson J. Multiplex detection of human herpesviruses from archival specimens by using matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2008 February;46(2):540-545.
5. Varani S, Stanzani M, Paolucci M et al. Diagnosis of bloodstream infections in immunocompromised patients by real-time PCR. J Infect 2009 May;58(5):346-351.
6. Xu J, Moore JE, Millar BC et al. Improved laboratory diagnosis of bacterial and fungal infections in patients with hematological malignancies using PCR and ribosomal RNA sequence analysis. Leuk Lymphoma 2004 August;45(8):1637-1641

APPENDIX V: RANDOMIZATION FORM

AML08 RANDOMIZATION FORM

Please call the St. Jude Pharmacy at [redacted] to let them know you have enrolled a new patient and for randomization.

Check only one box below and **FAX** this information to the St. Jude pharmacy at [redacted]. If you are unable to send this form by **FAX**, the information may be given by phone and sent by **FAX** later.

MRN: ☐☐☐☐☐☐☐☐☐ Initials: ☐☐☐ Date: ☐☐ ☐☐ ☐☐
MM DD YY

Institution:
- ☐ St. Jude Children's Research Hospital
- ☐ Children's Hospital of Michigan
- ☐ Dana Farber Cancer Institute
- ☐ University of Chicago
- ☐ Stanford University Medical Center
- ☐ Cook Children's Medical Center
- ☐ Singapore
- ☐ Rady Children's Hospital

If Karyotype is available, select one category below. If the patient has a high-risk feature listed below and one of the favorable translocations, please check the box for high-risk only.

- ☐ inv(16)
- ☐ t(8,21)
- ☐ 11q23
- ☐ M7
- ☐ Others

If Karyotype is not available, select one category below.

- ☐ M2 with Auer rods
- ☐ M4Eo
- ☐ M5
- ☐ M7
- ☐ Other

Criteria for Randomization:

- ☐ Normal creatinine for age
- ☐ Serum bilirubin ≤ 1.5 x ULN (bili _____ mg/dl)
- ☐ AST and ALT ≤ 2.5 x ULN (AST/SGOT ____ mg/dl AND ALT/SGPT _____ mg/dl)
- ☐ Alkaline phosphatase ≤ 2.5 x ULN (alkaline phosphatase ____ mg/dl)

Patients with hepatic dysfunction who do not meet all of the above criteria **WILL NOT BE RANDOMIZED**, but can be enrolled on AML08 and treated with Induction I (HD-ADE).

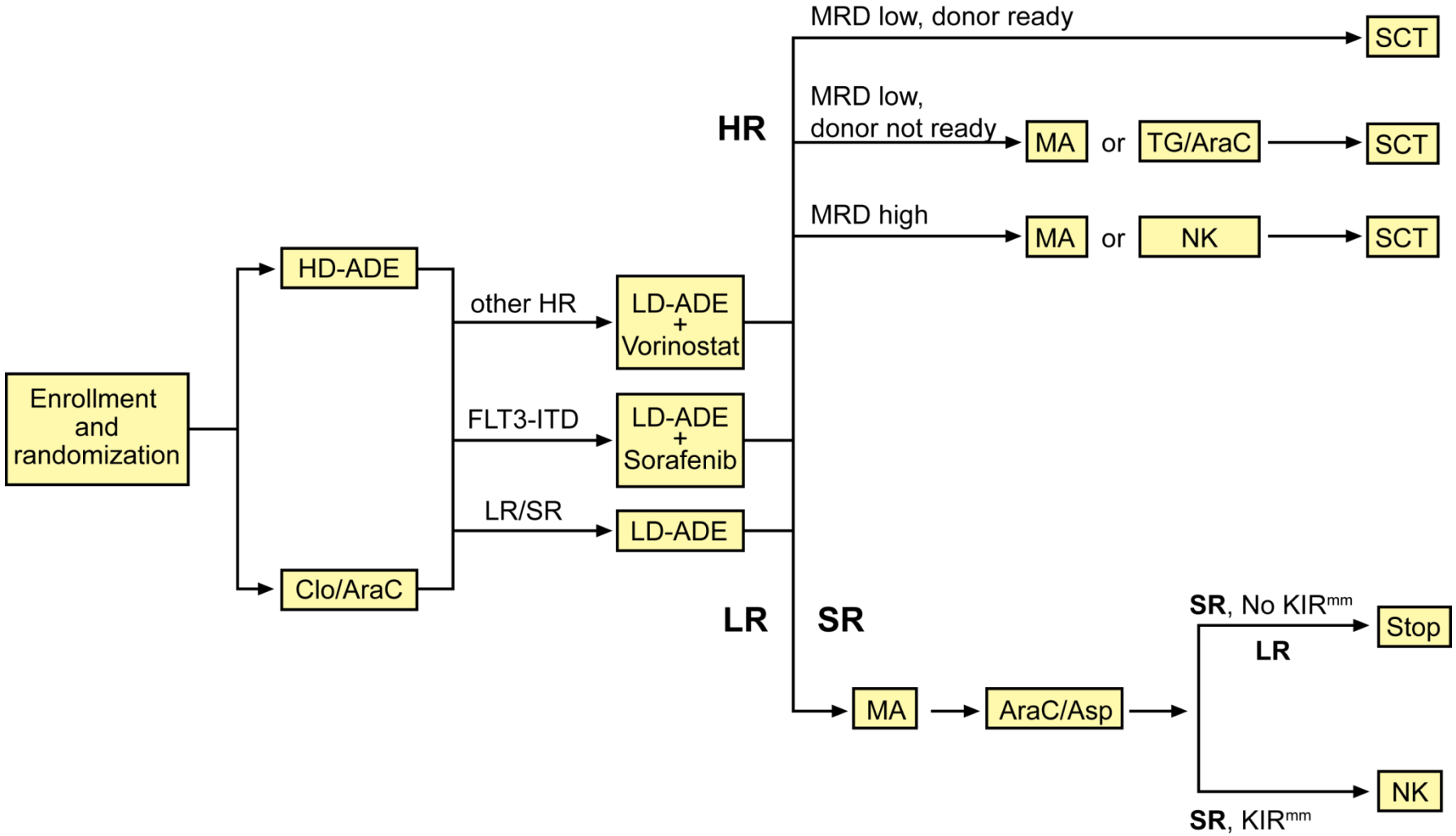
☐ Do NOT randomize this patient.

Physician: _____
Please print.

Contact number for randomization results:
☐☐☐ ☐☐☐ ☐☐☐☐

(Be sure to provide your contact number (phone or pager) so that the pharmacist can give you the randomization results.)

APPENDIX VI: TREATMENT SCHEMA



Note: collaborating sites may elect to opt out of vorinostat administration during Induction II. Participants at these sites will receive Induction II with LD-ADE alone.