ECOG-ACRIN cancer research group
Reshaping the future of patient care

E2906

Phase III Randomized Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)

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Version Date: November 27, 2018
NCI Update Date: July 25, 2013

Rev. 7/14	***STUDY PARTICIPANTS***	***ACTIVATION DATE***
Rev. 4/11	**ALLIANCE** / Alliance for Clinical Trials in Oncology – US Sites Only	December 28, 2010
	NRG / NRG Oncology Foundation, Inc – US Sites Only	Addendum #1 – Prior to Activation
Rev. 4/11	**SWOG** / SWOG – US Sites Only	Addendum #2 – Prior to Activation
Rev. 2/13	Shaare Zedek Medical Center	Update #1 – Prior to Activation
Rev. 4/11	Rambam Medical Center	Update #2 – 4/11
Rev. 4/11	**NOTE:** This study is supported by the NCI Cancer Trials Support Unit (CTSU). Institutions not aligned with ECOG-ACRIN will participate through the CTSU mechanism.	Update #3 – 7/11 Addendum #3 – 2/12 Update #4 – 6/12 Addendum #4 – 2/13 Addendum #5 – 2/13 Update #5 – 2/13 Addendum #6 – 8/13 Update #6 – 7/13 Addendum #7 – 12/13 Addendum #8 – 7/14 Addendum #9 – 5/17

Clofarabine will be provided by Genzyme
Decitabine will be provided by Eisai Inc.

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Rev. 7/14, Add10

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	**For patient enrollments:**	**Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:**
CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone – 1-866-651-CTSU Fax – 215-569-0206 Email: CTSURegulatory@ctsu.coccg.org (for submitting regulatory documents only)	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Netwok (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com	ECOG-ACRIN Operations Office - Boston 28 State St, Suite 1100 Boston, MA 02109 (ATTN: DATA). Phone # 857-504-2900 Fax # 617-589-0914 Data should be sent via postal mail. Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.
The most current version of the **study protocol and all supportingdocuments** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.		
For clinical questions (i.e. patient eligibility or treatment-related) contact the Study PI of the Coordinating Group.		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
For detailed information on the regulatory and monitoring procedures for CTSU sites please review the CTSU Regulatory and Monitoring Procedures policy located on the CTSU members' website https://www.ctsu.org > education and resources tab > CTSU Operations Information >CTSU Regulatory and Monitoring Policy		
The CTSU Web site is located at https://www.ctsu.org		

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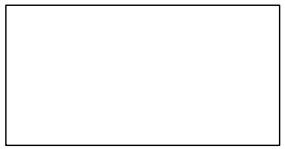
Schema



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NON-ABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION TREATMENT SCHEMA

ELIGIBLE PATIENTS AFTER INDUCTION WITH MATCHED DONOR

Preferred Patient Preparative Regimen & Stem Cell Infusion

Prior to initiating therapy, placement of a multi-lumen, indwelling Silastic catheter is required.

	F →																			
				B →																
				ATG →																
					A →	for patients with a history of herpes simplex (see below) to Day +100												→		
						T →	begin taper between Day +90 - +120 to stop between Day +150 - +180												→		
								PBSCT												
									M		**M**			**M**					**M**	
						Fluconazole →							*through Day +100* →							
																				G-CSF
Cotrimoxazole BID two days weekly beginning on Day +28 through Day +100 (see below).																				
Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

F Fludarabine 30 mg/m^2/day IV over 30 minutes x 5 days on Days -7 through -3.

B Busulfan 0.8 mg/kg IV over 2 hours q 6 hours x 8 doses on Days -4 and -3.

ATG Rabbit antithymocyte globulin (thymoglobulin) 2.5 mg/kg/day IV over 6 hours x 3 doses on Days -4 through -2. After the first dose, thymoglobulin may be administered over 4 hours.

A Acyclovir 200-400 P0 TID on Days -3 through Day +100 for patients with a history of herpes simplex infection or seropositivity. Valacyclovir 500 mg P0 QD may be used instead of acyclovir. Prophylaxis may be extended beyond Day +100 at the discretion of the treating physician.

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TMP/SMX DS TMP/SMX DS (Bactrim DS) BID on 2 days weekly beginning on Day +28 through Day +100. If, at this time, CD4 lymphocytes are < 200/µL, then prophylaxis should continue until CD4 lymphocytes ≥200/µL. In patients who develop chronic GVHD, PCP prophylaxis should be extended at the discretion of the physician.

T Tacrolimus target serum levels are 5-10 ng/mL. Serum levels are not to exceed 15 ng/mL. The suggested starting dose is 0.03 mg/kg P0 BID beginning on Day -2. Begin tapering between Day +90 to +120 with a goal of stopping by Day +150 to +180.

PBSCT Peripheral Blood Stem Cell Transplant. On Day 0 a minimum **total** CD34+ cell dose of 2 x 10^6/kg (actual weight - recipient) and a maximum of 8 x 10^6/kg (actual weight - recipient) will be infused.

M Methotrexate 5 mg/m^2/day IV on Days +1, +3, +6 and +11. Hydrate intravenously and induce diuresis .

Fluconazole Fluconazole or itraconazole 200-400 mg P0 daily or voriconazole 200-300 mg P0 twice daily (or 3-6 mg/kg IV q 12 hours) on Days -2 through +100 based on institutional standard. Low-dose amphotericin B (10-20 mg/day IV) also may be used.

G-CSF Recipients will receive 5 mcg/kg G-CSF SQ daily beginning on Day +12 and continuing until ANC >1500/µL for two consecutive days or> 5000/µL for one

day. If ANC decreases to < 1000/µL then resume G-CSF at 5 mcg/kg/day.

HLA-Identical Sibling Donor Stem Cell Collection

			G-CSF---see below -----													
							Donor Pheresis									
Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

G-CSF Donors will receive 10 mcg/kg SQ on Days-5 through-2 (and, if necessary -1).

Donor Pheresis On Days -1 (and 0) donors will undergo leukapheresis for 1-2 days to achieve a CD34+ cell dose of 2-8 x 10^6/kg (actual weight - recipient). If the yield of CD34+ cells is less than 2 x 10^6/kg on Day -1, an additional pheresis will be performed on Day 0 to achieve a total or set dose of 2-8 x $l0^6$/kg CD34+ cells.

1. Introduction

There have been significant advances in the management of acute myeloid leukemia (AML) in younger adults (age <60 years). The strategy of intensive remission induction therapy (sometimes comprising high dose cytarabine) to achieve a complete remission (CR), followed by post-remission therapy with high-dose cytosine arabinoside (cytarabine; Ara-C) or autologous or allogeneic blood or marrow transplantation has been developed progressively over the past 20 years, and has achieved measurable improvements in survival, particularly for favorable risk groups. A recent review of the ECOG-ACRIN Cancer Research Group (ECOG-ACRIN) experience demonstrated a significant improvement in 5-year survival for younger AML patients from 11% in 1973-79, to 37% from 1989-97 [1,2](see Figure 1).

However, similar progress has not been achieved in older patients (≥ 60 yrs), where the median survival in those receiving therapy is approximately 7 months, and the 5 year survival over the past three decades remains <15% [2-6](Figure 1). The poor survival in this population is predominantly on the basis of de novo chemo-resistance, manifest in a substantially lower rate of CR following induction chemotherapy (approximately 40-50% in most series, compared with 70-80% CR rate in younger adults), and early relapse [2,6,7]. Older patients with AML also have a significantly higher incidence of adverse prognostic and karyotypic features, which are associated with a worse outcome[8-12]. Other factors that contribute to the poor response to induction chemotherapy include the expression and function of multi-drug resistance proteins, such as p-glycoprotein, which is expressed in the majority (approx. 70%) of older patients presenting with AML[12], and which confers chemo-resistance.

It must be noted that some older patients presenting with a 'favorable' karyotypic abnormality (albeit rare – incidence ≤5%) may be cured with standard therapy (long-term disease-free survival approximately 20-30%)[13]. It is not known whether intensified Ara-C-based consolidation regimens are of benefit in this small subgroup of patients[11].

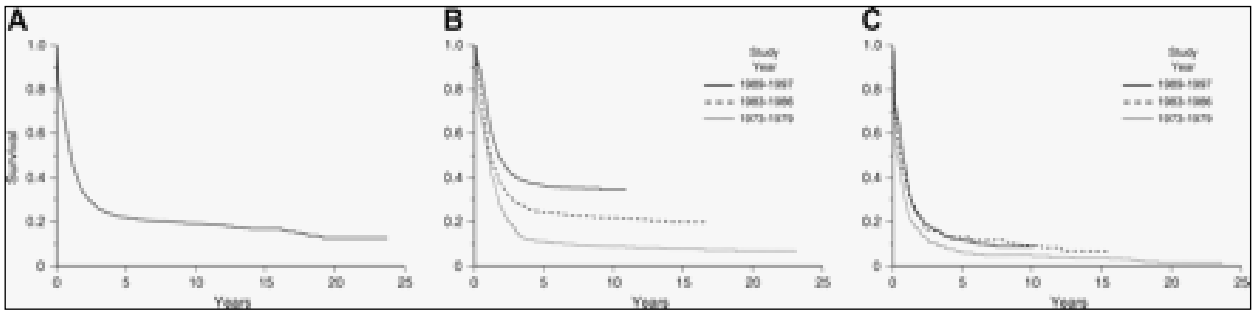

Figure 1. Overall survival of newly-diagnosed AML patients treated on ECOG protocols 1973-1997.

(A: all patients; B: patients age ≤55 yrs; C: patients >55 yrs)[14]

In addition, older patients with AML tolerate intensive treatment less well, with a reported treatment-related mortality (TRM) ranging from 15-25%[5,6]. In a recent ECOG study, the TRM was 13% for patients age 55-70 yrs, and 22% for patients age >70 years[15]. Similar retrospective data in older adults (> 55-65 years) receiving intensive therapy have recently been published from the MD Anderson Cancer Center (n=998), CALGB (n=635) and SWOG (n=968), demonstrating the limitations of intensive chemotherapy[10,11,16]. The

SWOG analysis in particular highlights the importance of performance status in predicting induction mortality, with 30 day induction mortality rates of approximately 50% in patients age 65-75 with ECOG PS > 2, or in patients age > 75 years with PS > 1[10], suggesting that intensive therapy regimens in that population are inappropriate.

In addition to the issue of increased induction mortality and *de novo* chemotherapy resistance (characterized by low CR rates), early relapse from CR also remains a major problem in those patients who do achieve CR. The median duration of response following CR is short (approximately 7-8 months), and there are very few long-term survivors (<15% at 5 years)[10,11,15,16].

This experience is particularly relevant as AML is a disease of older adults, with a median age of over 65 years. It is clear therefore that older adults with AML do not benefit from the advances in therapy demonstrated for younger adults, and are considered by many to have a disease similar to secondary or relapsed AML in younger patients[2,5,6].

Recent studies have attempted to address the problems of poor CR rate, excess treatment-related mortality, and high relapse rate in older patients, with little success[4,15,17-26]. As in younger adults, Ara-C remains the most important chemotherapeutic agent in the treatment of AML in older adults[27]. Recent studies replacing Ara-C with etoposide have been disappointing. Despite encouraging phase II results, a randomized study conducted by the Southwest Oncology Group comparing mitoxantrone & etoposide to standard daunorubicin and Ara-C demonstrated no advantage to the etoposide combination, and in fact suggested an inferior overall survival[17]. Similarly, older patients do not appear to benefit from the substitution of idarubicin for daunorubicin in remission induction therapy[15,28], or from pre-chemotherapy priming with GM-CSF[15], and derive no survival advantage from replacing daunorubicin with mitoxantrone[18]**.** The addition of 6-thioguanine improved the rate of CR in one study to 62%, but did not influence survival[20]. Similarly, efforts to reverse the effects of p-glycoprotein with agents such as PSC-833 have been largely unsuccessful, and in some cases have been associated with excess toxicity[29]. ECOG has recently completed a randomized study (E3999) evaluating the MDR inhibitor *zosuquidar trihydrochloride* (LY335979) in older patients with AML receiving standard remission induction therapy, and unfortunately there was no improvement in overall survival[30]. The incorporation of newer anti-leukemic agents, such as gemtuzumab-ozogamicin (humanized anti-CD33–calicheamicin immunotoxin), with or without interleukin-11 support, has also failed to improve the CR rate in older patients[21]. Despite a significant decrease in the duration of neutropenia, the use of the hematopoietic growth factors G-CSF and GM-CSF after chemotherapy has little overall impact on treatment-related mortality, and with the exception of the ECOG experience[22] has demonstrated no effect on survival [4,23-25].

Unlike younger patients, intensified Ara-C in consolidation ± mitoxantrone does not improve survival, and very high doses of Ara-C in induction are in general poorly tolerated in this population[3,26,31]. In contrast, previous ECOG studies have shown that intermediate dose (ID) Ara-C given as consolidation therapy is remarkably well tolerated in older patients in remission, and while there is no clear survival advantage ID Ara-C continues to be a standard ECOG consolidation therapy even for older patients [15,22,30]. Prolonged therapy with standard chemotherapy has also been evaluated, but similarly does not appear to improve survival[20,32].

It is nevertheless important to note that in general older patients do still benefit from therapy: while there is probably an advantage favoring more intensive therapy when

appropriate[19,33], it was recently demonstrated in a randomized study from the UK that even in older patients considered '*unfit for chemotherapy*' there was a survival benefit associated with low intensity treatment with low dose Ara-C (LDAC) over supportive care alone[34]. In an analysis from the Swedish Leukemia Registry, there was a superior 2-year survival for patients age 70-79 who were treated with the *intent to induce remission*, suggesting that more patients may indeed benefit from therapy than who actually receive it at present[35]. Current estimates in the United States are that despite evidence of a survival advantage with treatment, only approximately 35% of AML patients over the age of 65 years receive chemotherapy[131].

In summary, AML in older patients (≥60 years) is characterized by lower complete remission rates, shorter remission duration, and a very poor prognosis despite therapy. This represents a significant barrier to treatment, and there is therefore a clear need for AML therapy that is both less toxic and of superior efficacy, particularly in high risk subgroups (such as poor-risk cytogenetics). In view of the lack of progress in this group, older patients, who constitute more than 50% of adults with AML, represent an ideal group in whom novel treatment strategies should be evaluated.

1.1 Clofarabine

Clofarabine ([2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine]; Cl-F-ara-A) is a rationally designed, second-generation purine nucleoside analog. Designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of fludarabine phosphate and cladribine, clofarabine has a chloro group at the 2-position of adenine, and its chemical structure therefore more closely resembles CdA than F-ara-A. Halogenation at the 2-position of adenine renders this class of compounds resistant to cellular degradation by the enzyme adenine deaminase[36].

Clofarabine is cytotoxic to CCRF-CEM (human T-lymphoblast leukemia) cells *in vitro* in a time and concentration dependent manner[37]. It is a more efficient substrate for deoxycytidine kinase (dCK; rate-limiting step in intracellular phosphorylation of purine nucleosides) than the natural substrate deoxycytidine, and phosphorylated clofarabine is incorporated in the DNA of cells, the degree of incorporation correlating with cytotoxicity. There is no apparent cell-cycle specificity for the accumulation of clofarabine nucleotide metabolites. Compared to *cladribine*, there is prolonged intracellular retention of clofarabine-triphosphate, which exerts its cytotoxic activity[38]. Furthermore, clofarabine-triphosphate inhibits both DNA polymerase α and ribonucleotide reductase (RNR), and is superior to cladribine in inhibition of DNA synthesis at similar concentration[36,38,39].

Widely distributed in murine tissue, clofarabine is bound to plasma proteins (approx. 47% in humans). There is limited human pharmacokinetic (PK) data available, although in recent phase I studies the concentrations at the end of infusion appeared to be dose-proportional across all doses[40-42]. Clofarabine exhibits biphasic kinetics, with an effective t½ of about 1.5hrs, and a terminal t½ of about 7hrs[41]. Based upon phase II data, clofarabine has been approved by the FDA for the treatment of relapsed and refractory acute lymphoblastic leukemia in pediatric patients[40,43].

1.1.1 Clofarabine in Relapse/Refractory AML

Clofarabine has significant activity in AML. In a phase I study performed at the MD Anderson Cancer Center (MDACC) in patients

with relapsed and refractory acute leukemia, the dose-limiting toxicity was hepatotoxicity, and the maximum tolerated dose (MTD) was established as 40mg/m^2/day x 5 days as a short intravenous infusion. Importantly, objective responses were seen, including complete remission in 2 patients with AML[41].

To confirm its activity a phase II study was subsequently performed in patients with high grade myeloid malignancy, including 31 patients with relapsed and refractory AML[44]. Thirteen of the 22 patients achieved a CR (42%), and 4 further patients achieved CR with incomplete platelet recovery ('CRp'), for an overall response rate (RR) of 55%. The median duration of response was almost 6 months. Significant (≥ grade III) toxicity included rash (n=6), reversible elevation of hepatic transaminases (n=5), and nausea/vomiting (n=1).

A concurrent multi-institutional phase II study was performed in 40 patients with early relapse (within 12 months of achieving CR) or refractory AML, of whom 29 were evaluable and had complete clinical data[45]. This included patients with primary refractory disease (n=9), and those with an initial remission of either <6 months duration (n=14) or 6-12 months duration (n=8), thus representing a group with high risk disease and a very poor prognosis. The median age was 63 years (range 19-78).

At interim analysis, the response rate was substantially lower in this refractory AML population than that reported in *relapsed* disease, particularly those with longer duration of 1st CR who had been shown to have a higher chance of responding[44]. Only 1/21 evaluable patients achieved a complete remission (4 months duration), and another achieved a partial remission (duration 2 months). Most patients received only a single treatment course with clofarabine, although 8 patients received a 2nd course, and 1 patient each received a 3rd and 4th course. Grade III/IV hematologic toxicity was common, including neutropenia (29/29), anemia (24/29) and thrombocytopenia (29/29). Grade III/IV non-hematologic toxicity in the latter study included elevated AST (n=8) or bilirubin (n=4). Other toxicity included paresthesiae (20), nausea and vomiting (19), confusion (10), diarrhea (8), fatigue (5), hand-foot syndrome (3), and pancreatitis in 1 patient. There were 2 treatment-related deaths due to sepsis (n=1) and multi-organ failure (n=1). These results demonstrate that clofarabine alone is not active in *refractory* AML, but rather suggest that it is more likely to exert clinical activity earlier in the course of the disease.

1.1.2 Clofarabine in Newly-Diagnosed AML

Much more encouraging are the results of a recent phase II study performed by the UK Medical Research Council (MRC) employing clofarabine as a single agent as remission induction therapy and post-remission therapy for older adults who were considered 'unfit' for intensive induction therapy[46]. In this trial, thirty patients with newly-diagnosed AML were treated (median age 72 yrs, range 61-82), including 17 with 'intermediate risk' and 7 with 'high risk' cytogenetics. Clofarabine was administered as a single agent at a dose of

30mg/m2/day x 5, every 4-5 wks for 2-4 cycles. Remarkably, 13 patients achieved CR (43%), and 4 others achieved CRp (13%). There were 4 treatment-related deaths (13%). Toxicity (≥ grade III) was predictable, and consisted predominantly of reversible hepatotoxicity (↑ALT 24%, ↑ bilirubin 15%), handfoot syndrome (11%), rash (10%), and nausea and vomiting (3%).

Based upon the encouraging MRC experience in newly-diagnosed patients, a follow-up multicenter phase II trial was recently completed in Europe to confirm the activity of single agent clofarabine as induction and consolidation therapy in patents age ≥65 years (n=66) who were considered *unsuitable* for chemotherapy on the basis of age, comorbidity or performance status[47,48]. Clofarabine was given for 2-4 courses at 30mg/m^2/day on days 1-5, however most patients were only able to receive 2 courses due to toxicity with consolidation; the last 16 patients were treated at a lower clofarabine dose of 20mg/m^2/day beginning with the 2nd course, and the treatment was much better tolerated (A. Burnett, personal communication). The activity of single agent clofarabine was confirmed in this study, with an overall CR rate of 48%, and similar TRM to that reported in the initial MRC trial referenced above (9% clofarabine-related early death rate). Importantly, significant activity was noted in high-risk patient subsets, including those with 'poor risk' cytogenetics (CR 46%), and in those >70 years (CR 44%). The median disease-free survival was 6 months, and the median overall survival was 5 months (A. Burnett, personal communication).

In Summary, the experience in older AML patients considered 'unsuitable' for standard intensive therapy suggest that single agent clofarabine may be better tolerated than standard therapy, and that it has significant single agent activity particularly in high risk populations where standard therapy has been demonstrated to be inadequate. Clofarabine therefore represents an excellent candidate drug for study in newly-diagnosed patients who are candidates for intensive therapy, in whom the activity would likely be equivalent or better, to address the clinical problems of low CR rates and high induction mortality encountered with standard daunorubicin & cytarabine.

1.1.3 Clofarabine & Ara-C in AML

Clofarabine inhibits RNR, and is a superior substrate for dCK, with increased cytotoxic effects *in vitro* compared to fludarabine and cladribine, and therefore may be an appropriate combination agent with Ara-C[36,38,39]. *In vitro* studies suggest that clofarabine is more effective at increasing Ara-CTP in leukemic blasts than fludarabine[49]. Therefore, the combination of clofarabine and Ara-C is theoretically attractive, and several such combinations have been studied in AML in both the relapse/refractory and newly-diagnosed populations.

An MDACC phase I/II pilot study of clofarabine & 'intermediate dose' Ara-C (1g/m^2/day x 5) in relapsed/refractory AML patients demonstrated that the combination was reasonably well tolerated, although grade III/IV toxicity was observed, including diarrhea (n=4,

20%), rash (n=2, 10%), impaired liver function (n=2, 10%), hand-foot syndrome (n=1, 5%) and palpitations/arrhythmia (n=1, 5%). A febrile episode associated with myelosuppression was experienced by 20 patients (63%) including 1 treatment-related death from bacterial sepsis. The overall response rate was 38%[50].

The combination of clofarabine & intermediate-dose Ara-C was subsequently evaluated in a phase II study in 60 *newly-diagnosed* patients with AML and high grade MDS at the MTD of clofarabine (40mg/m^2 d1-5)[51]. The median age was 61 years (range 50-74). Myelosuppression following treatment was 'ubiquitous', and there were 9 deaths on study (15%), virtually all related to infectious complications. The most frequent side effects were diarrhea, emesis, palmoplantar erythrodysesthesia, and reversible liver function test abnormalities (most commonly grade III/IV hyperbilirubunemia and elevation in transaminases). The CR rate was 52%, and another 5 achieved CRp (8%). At a median follow-up of 18 months, the median duration of CR and CRp were 8 months and <2 months, respectively. Median overall survival was 10 months, while the median survival of patients achieving CR was almost 2 years.

A phase I study of clofarabine (starting dose 30mg/m^2 d1-5) with '*standard dose*' cytarabine (100mg/m^2 by 24hr continuous infusion d1-7) is ongoing at the University of Alabama at Birmingham Comprehensive Cancer Center in newly-diagnosed AML patients, although dose-limiting toxicity has been encountered at the starting dose necessitating 2 consecutive clofarabine dose reductions to 15mg/m^2/day x 5 days[52]. That study is currently continuing in phase I, and the maximum tolerated dose has not yet been established.

Therefore, while feasible in selected patients, the clinical results and toxicity of clofarabine combined with infusional Ara-C (at either standard or intermediate dose) do not presently support the incorporation of this intensive treatment strategy into phase III trials in elderly patients.

Investigators at MDACC have recently completed a small randomized phase II study comparing clofarabine to clofarabine & subcutaneous low-dose Ara-C (LDAC) in patients age ≥60 years with newly-diagnosed AML[53]. Using a Bayesian (adaptive) randomization, which progressively favors the treatment arm with higher CR rate, 76 patients (median age 76 years, range 60-83) were randomized to either clofarabine alone (30 mg/m^2 d1-5 induction; d1-3 consolidation) (n=16); or to clofarabine combined with LDAC (20mg/m^2 SQ d1-14 induction; d1-7 consolidation) (n=60). The overall CR rate was 57%, with 20% induction deaths. Interestingly, there was a difference in the CR rate observed between the clofarabine (CR 31%) and clofarabine & LDAC (CR 63%, p<0.05), although strangely there was an increase in induction deaths noted with the single agent clofarabine (TRM 31%) compared with the more intensive combination (TRM 17%). Unfortunately the unbalanced randomization (only n=16 in the clofarabine arm) and the unexplained high induction death rate in the

single agent clofarabine arm (which would have been predicted to be less toxic) confound the interpretation of this single center study. In addition, the low CR rate with single agent clofarabine in this trial is at odds with 2 multicenter European studies with n=95 patients[46,48], probably reflecting patient selection and the small number of patients randomized to the single agent arm. There was not a statistically significant difference in overall survival between the 2 arms.

In summary, the available experience of combination clofarabine and cytarabine, despite representing a more intensive therapy (particularly for infusional Ara-C), does not demonstrate a clear benefit in survival over single agent clofarabine and appears substantially more toxic. These results therefore do not justify incorporating Ara-C (either infusional or subcutaneous LDAC) into comparative trials of clofarabine versus standard therapy at this time.

In Summary, these findings indicate that single agent clofarabine has significant activity in newly-diagnosed AML in the elderly population, particularly in high risk populations, and may be better tolerated than 'standard' therapy. These data strongly support the rationale for a randomized trial comparing clofarabine as both remission induction and post-remission therapy versus 'standard' daunorubicin & cytarabine consolidation to determine its effect on overall survival.

1.2 Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation for AML

Allogeneic hematopoietic stem cell transplantation (AlloSCT) is curative for many younger patients with relapsed or poor prognosis acute myeloid leukemia. This treatment modality has until recently had only a small impact on overall AML survival given that the median age of diagnosis with AML is beyond that at which ablative transplants are generally offered. A number of publications have documented the activity of reduced intensity conditioning regimens in advanced AML[54-58]. Non-ablative conditioning has reportedly been successful for treatment of patients in remission[59,60]. The composite experience suggests that GVHD risks are lower following AlloSCT with reduced intensity conditioning [130].Using a low-dose total body irradiation (TBI) ± fludarabine non-ablative conditioning regimen, investigators in Seattle have recently reported a 2-year disease-free & overall survival of 44% and 48%, respectively, for a cohort of n=122 patients (median age 57.5 yrs, range 17-74) with AML undergoing allogeneic transplantation from matched related or unrelated donors[61]. The 100 day and 1-year non-relapse mortality (NRM) was 3% and 14%, respectively (higher in those with an unrelated donor). Using a graft-versus-host disease (GVHD) prophylaxis regimen of mycophenolate mofetil and cyclosporine, with the former being tapered beginning day 40 and the latter beginning day 100[62], the incidence of acute GVHD was 40%, and the incidence of chronic GVHD (cGVHD) was 36% at 2 years. Importantly, for the 51 patients transplanted in 1st complete remission the 2-year survival was 51%. In a similar experience, in a cohort of n=112 patients (median age 55 yrs, range 22-74) with AML or MDS undergoing allogeneic transplantation following reduced intensity conditioning with melphalan & fludarabine at the MDACC, the 2-year survival was 66% for those patients in remission at the time of transplant[63]. The 100 day and 2-year NRM was 0% and 20%, respectively. Finally, a recent multicenter study employing fludarabine-cyclophosphamide

reduced intensity conditioning in 34 patients with AML in 1st CR demonstrated a 2-year disease-free and overall survival of 56% (95% confidence intervals (CI) 39-71%) and 68% (95% CI 50-81%), respectively[64]. There were 3 deaths from acute GVHD (9%), and the 12 month chronic GVHD rate was low at 24%. Most survivors were reported to have an excellent performance status.

Recently, Claxton et al[66] reported the use of allogeneic hematopoietic transplant following non-ablative conditioning with fludarabine & cyclophosphamide (Flu-Cy) combined with sirolimus and tacrolimus as GVHD prophylaxis. In this strategy, to facilitate GVL, tacrolimus was tapered early in patients without graft versus host disease at day 30, the intent being to leave sirolimus, an agent with know anti-leukemic activity, as the only immunosuppressive agent. In an update of that experience, n=91 patients have undergone AlloSCT using Flu-Cy conditioning, including patients with advanced AML. The median age was 60 years, and all patients achieved successful engraftment (full donor chimerism in peripheral blood in majority). Only n=2 of 39 patients of all diagnoses receiving matched sibling transplants have suffered NRM (non-relapse related mortality), at day 295 & day 567 respectively. Thus 100 day TRM has been 0%, and 2 year TRM has been ~5%. For 52 unrelated donor recipients, many with advanced and multiply pretreated malignancy and most with <10/10 matched donors, there were 5 transplant-related deaths by day 100 (10%) and 13/52 (23%) by 2 years. (D. Claxton, personal communication). Therefore, using an immunosuppressive conditioning regimen that does not require pharmacokinetic targeting or the use of TBI, effective donor engraftment with low NRM can be achieved in an older patient population.

These experiences demonstrate that non-ablative AlloSCT with reduced-intensity conditioning (RIC) is both feasible in a multicenter fashion, and associated with improved outcomes in selected patients, predominantly through a graft-versus-leukemia (GVL) effect. In contrast more intensive preparative regimens have often been substantially more toxic[58,67]. However a recent single center feasibility analysis from MDACC suggests that this strategy may have only limited applicability in this population, and that only a small number of older AML patients may ultimately benefit from AlloSCT[65]. In that prospective single center study, only 26 of 259 (10%) newly-diagnosed patients age ≥50yrs with AML or high-grade myelodysplastic syndrome achieved CR, had a BMT consultation, and had a matched donor identified. Furthermore, only 14/26 (~5% of cohort) ultimately proceeded to AlloSCT. Therefore, a critical question remains as to the true feasibility and impact of an AlloSCT strategy in older AML patients, and whether the benefits of AlloSCT can be effectively extended to an older population in a multicenter study. The present study (E2906) affords a unique opportunity to evaluate the role and impact of AlloSCT in the management of older patients with AML by formally incorporating a 'biological randomization' to AlloSCT into the primary treatment strategy. Specifically, employing a 'donor vs. no donor' analysis, this strategy allows a true estimate of the 'denominator' of newly-diagnosed older AML patients who may be considered for AlloSCT, thereby accounting for the apparent selection bias of patients who actually proceed to AlloSCT inherent in such reports in this population.

Based upon the favorable experience and tolerability of the Reduced Intensity Conditioning (RIC) regimen employed in the recently completed prospective multicenter BMT CTN 0502 (CALGB 100 103) study in older AML patients in

CR1 (personal communication, Drs. S. Devine & H. Lazarus), we plan to employ an identical busulfan-fludarabine conditioning regimen with similar GVHD prophylaxis. This will allow comparability of our AlloSCT results with those of the BMT CTN 0502 study, but in a more uniformly-treated population. However, the systematic early consideration of AlloSCT from the time of diagnosis in E2906, and inclusion of HLA-typing at presentation, has an important advantage over previous pilot RIC studies, and will allow a more definitive evaluation of the role of AlloSCT in this population.

The non-ablative Flu-Cy conditioning regimen has limited anti-leukemic activity, and although the same may be said for other regimens the proposed reduced-intensity busulfan-fludarabine regimen has the advantage of having been used successfully in a recent multi-center study. Both regimens share low morbidity and non-relapse-related mortality (NRM), and both share the intent of inducing early engraftment and a GVL effect.

As applied to better matched, less heavily-treated AML patients in CR after only 1 regimen as proposed in E2906, we may expect superior results. In contrast more intensive preparative regimens have often been substantially more toxic[58,67].

1.2.1 In Summary, these observations strongly suggest that allogeneic hematopoietic transplantation may yield durable remissions in older AML patients who would without transplant have died of their leukemia. Thus the formal testing of this therapy in a large series of patients 'biologically' randomized to receive or not to receive transplantation based on donor availability is logical and timely. Disease free and overall survival will be examined in these treatment groups in comparison with patients who are treated with chemotherapy in a 'donor vs. no donor' analysis.

1.3 Decitabine in AML

Early relapse from complete remission remains a significant problem in AML in the elderly, with fewer than 15% long-term disease-free survivors among those who actually achieve CR. Previous maintenance strategies with low-dose Ara-C alone or LDAC-based regimens have unfortunately failed to improve the survival of older patients with AML[18,32].Therefore, an effective and well tolerated maintenance strategy is urgently needed.

Decitabine (5-aza-2′-deoxycytidine; DAC) is a deoxycytidine analog which requires DNA synthesis for activity. Cytotoxic at high doses, low-dose DAC inhibits DNA methyltransferase, and is associated with gene hypomethylation and reactivation of target gene expression, favoring differentiation, reduced proliferation, and/or increased apoptosis[68,69]. Phase I studies have demonstrated that low-dose daily DAC was well tolerated and had significant activity in patients with AML[70] and myelodysplastic syndrome (MDS) (overall response rate 32%)[69]. The degree of DNA hypomethylation *in vivo* correlated with response in AML patients treated with low dose DAC; interestingly, there was a dose-response relationship, with a linear decrease in methylation with increasing DAC dose from 5 to 20mg/m^2/day, although no further decrease in methylation was noted at doses higher than 20mg/m^2 [71].

In a randomized study in intermediate and high-risk MDS, DAC (15mg/m^2 IV over 4 hours, q8 hours for 3 days - total dose 135mg/m^2) at 6 week intervals significantly improved patient outcomes, with 17% overall response rate, and a longer median time to AML progression or death in those with higher risk disease compared to supportive care (p=0.03)[72]. Alternative dosing schedules have also been evaluated, and a randomized study from MDACC in patients with high grade MDS has demonstrated improved CR rate of 39% with a once daily DAC dosing schedule (20mg/m^2/day IV d1-5)[73]. DAC is therefore well tolerated and active in advanced MDS and AML, and was recently approved by the FDA for the treatment of intermediate and high risk MDS.

Re-treatment or prolonged treatment with DAC has recently been evaluated in 2 myeloid malignancy studies, including an analysis of re-treatment in high-grade MDS (including some patients with AML)[74], and more recently as an induction and 'maintenance' strategy in *de novo* AML in older adults considered ineligible for intensive chemotherapy[75]. In the earlier trial of 108 patients[74], 22 of 65 responding patients were re-treated with low-dose DAC at the time of progression (a median of 11 months (range 3-27) from completion of initial DAC therapy). A 2nd response of short duration (median 4 months) was achieved in 10/22 (45%). In the opinion of the investigators, this experience highlighted the importance of extended or 'maintenance' therapy with DAC in responding patients to prolong response and prevent secondary resistance.

The more recent study more directly addressed the question of both induction and maintenance therapy with DAC in older adults (age >60 years) with newly-diagnosed AML who were not eligible for intensive induction chemotherapy[75]. In this trial, 51 newly-diagnosed AML patients (median age 72, range 63-85 years) including those with complex karyotype (65%) and antecedent MDS (51%), were treated with standard low-dose DAC (15mg/m^2 IV q8 hours d1-3, repeated q6weeks) up to 4 courses as induction, followed by maintenance DAC. The response rate to induction DAC was 62%, including CR 14%, partial response 17%, and hematologic benefit 31%. The toxicity was reported to be very similar to that described for MDS, and there was only 1 induction death. Maintenance DAC 20mg/m^2/day d1-3 was then administered as an outpatient every 8 weeks; it was reported to be very well tolerated, and there were no hospitalizations during maintenance. Remarkably, the median survival was 7.5 months, and the 1 year survival was 24%. The investigators concluded that maintenance with DAC was associated with prolonged anti-leukemia effect, the occurrence of some late responses, and limited hospitalizations, and overall had good feasibility in the outpatient setting.

In a recent update of that experience, the investigators have amended the maintenance DAC dosing schedule to address the emergence of secondary resistance, decreasing the treatment interval from q8 weeks to q4 weeks. This is supported by correlative observations in chronic myeloid leukemia and MDS patients receiving decitabine, where re-methylation of LINE1 repeats in peripheral blood mononuclear cells occurred about 4 weeks after initial dosing73,76. The study has continued to accrue (n=>100), and the 4 week interval has been well tolerated (M. Lübbert personal communication).

The present proposed study therefore represents a unique opportunity to address the important clinical problem of early relapse in patients who achieve

CR following both standard and experimental therapy. In view of the promising phase II German experience with prolonged DAC, we therefore propose to prospectively evaluate this novel maintenance strategy by adding a second randomization to observation (standard) or low-dose decitabine (experimental) in patients who remain in CR following completion of assigned consolidation therapy.

1.3.1 In Summary, AML in older adults is characterized by early relapse, with < 15% long-term disease-free survival. Prolonged low dose decitabine therapy has demonstrated apparent anti-leukemia activity is both feasible and well tolerated in the outpatient setting, and there is a suggestion that it may prolong remission duration. We therefore propose to incorporate a 2nd randomization following completion of consolidation to decitabine versus observation to evaluate the effect of prolonged decitabine maintenance on disease-free survival following completion of therapy.

1.3.2 Gender and Ethnicity

Entry to this study is open to both men and women, and to persons of any racial or ethnic group. Previous ECOG studies in this patient population enrolled 56% men, 44% women, 95% white, 4% black and 1% other from institutions. We anticipate that entry of women and minorities to this study will show similar proportions.

We are aware of no data that would lead us to expect differential treatment effects by gender and ethnicity and therefore have not incorporated separate accrual goals for these subgroups.

1.4 Non-Inferiority Trial Design

As reviewed extensively above, standard chemotherapy treatment is both toxic and ineffective for most older AML patients, and in particular for those who have poor performance status, comorbidities, or poor-risk cytogenetics. These poor results represent *de facto* a significant barrier to treatment for older AML patients, as evidenced by current estimates that only approximately 35% of those over age 65 years currently receive chemotherapy (131). The preliminary data suggest that clofarabine may have a similar complete remission rate with lower induction mortality compared with standard chemotherapy, and that it may have greater activity in those with poor-risk cytogenetics. This suggests that if clofarabine has a similar overall survival to standard therapy but with less toxicity or with lower induction mortality, it may allow the possibility of extending therapy to a larger population of older AML patients than who presently receive treatment. Considering the impact on both induction mortality and overall survival, E2906 is therefore designed to test both non-inferiority of clofarabine compared with standard induction chemotherapy, and if found to be non-inferior than to test its superiority.

1.5 Quality of Life Assessment

1.5.1 Background and Significance

Quality of life (QOL) is an important concept in the oncology patient population and is frequently studied, including in the context of clinical

trials. However, with the exception of some studies of patients who received bone marrow transplant, there is a striking lack of published data that captures QOL in the AML patient population. Moreover, those published are fraught with small sample sizes, high attrition rates, and thus, inconclusive findings.

QOL has been compared in elderly AML patients receiving intensive versus low intensity therapy. In a small non randomized trial, no differences were noted between baseline assessment and 6 weeks later or between treatment arms [88]. However, these patients scored lower overall in QOL measurement than the general population and scored higher in depression. Another study of 60 patients also noted stability in QOL assessments over a six month time period in patients receiving either induction therapy or low intensity therapy [89] Yet in a study of 28 younger patients who successfully completed a full course of AML treatment (induction, consolidation, and two cycles of maintenance therapy), patients reported an improvement in QOL and a decrease in symptoms [90].

Fatigue is an important component of QOL [91] and can be significant in patients with AML. In two studies of AML patients, fatigue was common [92], persistent over time [93], and inversely correlated with QOL [92,93], and with self-reported activities of daily living [93].

The elderly AML patient may be more susceptible to complications from disease as well as from treatment. The complications that arise may be influenced by physiologic changes associated with aging [94]. Nutritional deficiencies are common in both the elderly as well as in the patient with AML [94,95]. The etiology for these deficiencies varies and the resultant physiologic and emotional consequences can be profound. Cognitive changes also occur with normal aging and have the potential to affect an elderly AML patient's ability to make informed treatment choices, understand prognosis, and treatment-related side effects [94]. Such cognitive changes were found in a one study of AML and MDS patients [96]. In contrast, level of available social support has been shown to be a significant predictor of improved QOL [97] and even survival [98] in AML survivors.

The ability to fully assess an elderly patient's functional status is crucial to better anticipate potential problems and identify those who are at greater risk for complications. To date, there are is only one published study describing a fully comprehensive geriatric assessment in individuals with AML [89].

For the elderly patient, increasing or maintaining QOL can be viewed as more important than prolonged survival [99]. This viewpoint was also demonstrated in a group of elderly patients with AML [88]. Yet how to best improve or maintain QOL is not yet known. Clearly more research is needed in this area.

1.5.2 Rationale for QOL

The proposed study provides the unique opportunity to assess QOL and level of functioning in a large cohort of elderly AML patients who

are randomized to two different treatment arms. The toxicity profile of each treatment varies considerably; hence the impact of each treatment on patients' symptoms and quality of life is important, particularly if efficacy in treating the underlying disease is similar. Baseline assessments will serve to further describe QOL and functioning in this patient population and the findings will also be used to compare with those from subsequent measurements. Thus analysis will be performed both within and between treatment groups.

Questionnaires to be administered have been carefully selected to evaluate the most clinically relevant patient-reported health outcomes. The assessment battery includes questionnaires that have been previously used with AML patients and/or elderly cancer patients (Functional Assessment of Cancer Therapy – Leukemia and FACT Fatigue as well as the abbreviated Comprehensive Geriatric Assessment (aCGA). Additional measurements of nutritional status, social support, co-morbidity, and sexual function will further enhance the aCGA as these areas are known to be problematic in the elderly leukemia patient population [94].

Assessment time-points were selected to capture data on patients' QOL at critical time points in the clinical trial so that the impact of each treatment phase can be assessed vis-à-vis QOL. The first assessment will be administered at the time of randomization. The second assessment will occur at the time hematopoietic nadir (~ two weeks after beginning induction therapy). The third assessment will occur 28 days after the patient begins induction therapy. The fourth assessment will occur at the time consolidation therapy is to begin. The fifth assessment will occur at the time patients are randomized to maintenance treatment or observation. Efforts will be made to obtain QOL assessments at the time a patient is removed from the study. For the subset of patients who are is eligible for transplant, QOL assessments will be made at the time of conditioning therapy, and 100 days post transplant.

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1.6 Additional Correlative Studies

1.6.1 Mechanisms of Resistance to Therapy

MDR1/P-glycoprotein expression is an established adverse prognostic factor in AML, particularly in older adults [12,118]. ECOG-ACRIN has extensive experience in the evaluation of MDR status, unfortunately recent efforts to target MDR have failed to impact on clinical outcomes [86,119]. Nevertheless, it remains an important prognostic factor in AML, and important preliminary studies suggest that clofarabine is not an MDR substrate and that the mechanisms of resistance to clofarabine appear to be different than cytarabine *in vitro* [120]. We therefore plan to prospectively evaluate P-glycoprotein expression by flow cytometry [86,119] to determine its effect on the CR rate and on overall survival.

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1.6.2 CXCR4 Expression

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The intensity of CXCR4 expression by AML blasts has recently been associated with inferior survival in retrospective studies in younger adults [87], but its relevance has not been evaluated in older adults nor in a large prospective study. We therefore plan to evaluate

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CXCR4 expression prospectively by flow cytometry in order to better characterize its prognostic impact (in terms of CR, disease-free survival & overall survival) in relation to other established prognostic factors, in particular cytogenetics.

1.6.3 Epigenetics

E2906 incorporates a randomization to maintenance therapy with decitabine, a methyltransferase inhibitor, and affords a unique opportunity to study the epigenetic phenotype in AML at presentation and in remission. The following clinical & basic hypotheses will be studied:

1.6.3.1 Clinical

Our preliminary data show that AML comes in different epigenetic subtypes, and that a phenotype of epigenetic instability seems to correlate with responsiveness with DNA methylransferase inhibitors. In addition to the epigenetic unstable phenotype, many of the more defined AML epigenetic subtypes had different medical outcomes in retrospective studies and so may be highly clinically relevant. Interestingly, gene expression profiling failed to identify several of these subtypes alone, demonstrating the power of epigenetic analysis to capture important biological differences in AML. Finally, the integration of gene expression and epigenetic signatures were shown to synergistically capture biological differences between patients with leukemia.

Based on these data we predict that patients who present with AML and epigenetic instability will be the better candidates to respond to decitabine maintenance. Therefore we propose to perform expression and methylation profiling on all patients enrolled in 2906 and correlate their integrated epigenetic signatures with response to decitabine. Of equal importance we also predict that specific integrated epigenetic subtypes associated with differential clinical outcomes will be confirmed in this prospective study and could lead to an important advance in leukemia classification. The integrated signatures captured upon enrollment of these patients will be used to prospectively validate, confirm and

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possibly expand our ability to accurately segregate patients into biologically distinct cohorts. We plan to use these data to direct the design of future ECOG-ACRIN clinical trials.

1.6.3.2 Basic

Data from the published literature and our laboratory indicate that progressive alteration of epigenetic signatures in bone marrow cells occurs during normal aging. We hypothesize that certain epigenetic signatures associated with aging might constitute an epigenetic field defect that could result in leukemogenesis. We will examine the epigenetic profiles of remission marrow in patients randomized to observation vs decitabine in E2906 to determine whether epigenetic signature of apparently morphologically normal bone marrow is predictive of relapse or response to decitabine maintenance. Our data performed in fractionated vs unfractionated marrow from the same individuals indicate that epigenetic signatures indicative of aberrant epigenetic programming are equally readily evident in either case, and are readily distinguishable between normal and abnormal samples. Therefore, we expect our experimental approach to successfully capture indications of epigenetic field defects in this patient cohort.

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1.6.4 Somatic Mutation Analysis

1.6.4.1 Background

Somatic mutation analyses have been extensively studied in younger AML cohorts; the biologic and therapeutic complexity in the elderly, where the disease is most common, has been less extensively investigated. Any previous studies have not been sufficiently broad in scope or of sufficiently high resolution to elucidate in any detail the spectrum of somatic alterations in elderly AML patients and to determine which altered pathways are the major determinants of chemoresistance and of adverse outcome in this disease. The constellation of genetic lesions that collectively mediate the chemoresistant phenotype are likely heterogenous in nature and require integrated, high-resolution exploration in a sufficiently large number of cases in order to identify recurrently perturbed biologic pathways with clinical, prognostic, and therapeutic relevance. While it is possible that thousands of coding and non-coding genes are affected by mutations, these genes often have in common the perturbation of common pathways, such as DNA repair, RNA splicing, etc. Discovering which pathways contribute to chemoresistance and how they are perturbed is fundamentally important to address this important clinical phenomenon.

1.6.4.2 Genetic Characterization of E2906 Leukemias

This study will perform a complete genetic characterization of patient leukemia cells using state of the art next

generation sequencing methods. The entire spectrum of somatic coding mutations will be identified, using Agilent Sure-Select exome capture followed by Illumina HiSeq2000 sequencing, the most powerful and economical approach for sequencing the coding sequence of genomes on a large scale. DNA from the patients' own T-lymphocytes or remission cells will be used as the matched germline control, using whole genome amplification to amplify small amounts of DNA that will be generated from these control cells, in order to distinguish true somatic variants. In addition, we will perform base-pair resolution DNA methylation sequencing across gene regulatory regions encompassing > four million individual cytosine residues. This will be achieved using an improved version of the reduced representation bisulfate sequencing protocol followed by Illumina HiSeq2000 sequencing. All mutations detected will be validated by repeat PCR and sequencing on the unamplified diagnostic sample. Wherever possible, matched control DNA (remission or T-lymphocyte DNA) will be resequenced to determine if candidate disease alleles are somatic or present in germline.

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1.6.5 Epidemiology of AML & Clinical Outcomes in E2906

The etiology of AML is poorly understood and in the large majority of cases is unknown. Similarly, with the exception of therapy-related AML, the impact of prior exposures or lifestyle factors which may have contributed to its development on subsequent AML survival is also not known. In addition to rare cases of "familial" leukemia (associated with familial monosomy 7, GATA-1 mutations, germline CEBPA mutation, and others) and well-characterized genetic syndromes more closely associated with childhood disease (e.g. Down's syndrome, Bloom's syndrome, Schwachman-Diamond syndrome, Kostmann syndrome, etc.)[132,1332], observational and case-control studies have identified several factors strongly associated with the development of AML. Among the best characterized of these is prior exposure to cytotoxic chemotherapy (in particular alkylating agents & topoisomerase II inhibitors) and/or ionizing or therapeutic radiation – so-called therapy-related AML (t-AML) - which is associated with inferior survival[134].

However more recent studies have identified important lifestyle and occupational exposures that are significantly associated with increased relative risk (RR) of developing AML. These include obesity[135] (approximately 2-fold RR of developing AML), regular acetaminophen use[136] (RR 1.5), and smoking[137] (RR 1.2) which has been particularly linked with the t(8;21) cytogenetic abnormality. In addition, benzene exposure[132] and living in a rural/farm environment (personal communication, J. Ross) have also been strongly linked with an increased risk of developing AML in some studies. A recently completed NIH-funded population-based case-control study (the "*Predictors of Acute Leukemia Minnesota*" - PALM - study) designed

and run by Dr. Julie Ross at the University of Minnesota identified > 600 new cases of myeloid leukemia (including n=393 AML) and 682 controls. The PALM study employed a straightforward self-administered questionnaire, and preliminary analyses confirm the significant association between prior regular aspirin (RR 0.6 in women) and acetaminophen (RR 1.5) use and development of AML (*in press*, Ross JA, et al Cancer Epi Biom Prev, July 2011), and has also identified prior obesity at ages 35 & 50 years with an increased risk of adult AML[138] (RR ~ 2). Importantly, a significant association between both previous medical history[139] and family history and the development of AML has been identified from the PALM study, although that data is still being analyzed and has not yet been presented. The prevalence of common exposures among n=682 controls from the PALM study is included in the Table (below).

Table: Prevalence of common exposure from PALM study control population (*personal communication, Dr. J. Ross, Univ. of Minnes*ota)

Exposure	***PALM*** Question #	Prevalence in Controls (n=682)
Benzene	85c	7%
Ever Smoked in lifetime	10	48%
Aspirin*	31a	27%
Rural/Farm – ever lived	67	**58%

* *more than occasionally*

** *Rural/Farm is expected to be significantly lower in ECOG trial, as PALM study included only Minnesota residents*

While some of these factors are potentially modifiable, a critical question remains as to the impact of these common exposures on subsequent AML survival. Patients with t-AML have been shown to have an inferior outcome, although this is highly dependent on the specific cytogenetic abnormality[134]. However, the impact of other potential etiologic factors (in particular prior exposures, lifestyle factors, and medical/family history) on survival after AML is not known. The PALM study was itself not designed to study subsequent leukemia clinical outcomes.

A large multicenter cooperative group study affords a unique and highly attractive opportunity to prospectively study the impact of these established prior exposures/potential etiologic factors on AML survival in a controlled clinical setting. Indeed, E2906 is uniquely positioned to prospectively study their impact on AML survival for several reasons: it is a large prospective trial; it is focused on the largest group of patients with AML (age >60 years) and is broadly inclusive; the clinical endpoints are well-defined; genetic and molecular subtypes of AML will be extremely well-characterized; and it controls strictly for therapy. The questionnaire is designed to be self-administered in < 20 minutes

(unless significant farm exposures - 20-30 minutes), and will add minimal additional burden to site research personnel and patients during their initial 1-month hospitalization.

We therefore propose to formally study the impact of underlying exposures and lifestyle factors associated with AML development on subsequent clinical outcomes, and specifically on overall survival. Adapting the self-administered PALM tool for use in E2906 in collaboration with Dr. Julie Ross, Univ. of Minnesota (the "*Acute Leukemia Epidemiology & Survival in ECOG"* - "**ALESE**" questionnaire), we will offer patients the opportunity to participate in a prospective study which will evaluate the presence of these previously-identified exposures/potential etiologic factors among AML patients and study their impact on subsequent survival after therapy with curative intent. The use of the ALESE tool will allow an opportunity to normalize our case data from E2906 with the results of the PALM Minnesota population-based case-control study to determine important similarities and differences, in order to better understand the prevalence of individual exposures & lifestyle factors in the broader AML population receiving therapy with curative intent. In addition, the availability of this prospective clinical data on lifestyle, family/medical history & exposure will be an invaluable resource in the future for the correlation of specific exposures with well-characterized genetic & molecular subgroups of AML (as will be performed in E2906), allowing a planned future study of the molecular epidemiology of AML & clinical outcomes.

METHODS

Data coordination (including QA & secure data entry) will be performed in the Cancer Epidemiology program at Mayo Clinic, Florida. Patients will be given the self-administered ALESE questionnaire to complete within the first week of hospitalization on E2906, and the site research coordinator will return it to the investigator. A contact e-mail & telephone number will be provided in the questionnaire for questions and assistance to patients or site research personnel. While the ALESE questionnaire does not need to be completed immediately at baseline prior to initiating therapy (unlike the QOL correlative studies) - as it pertains to previous exposures and lifestyle/medical/family history factors which are stable - we will ask them to complete it in the first week of hospitalization to ensure that we can QA the questionnaire during their initial 1-month hospitalization. Also the ALESE questionnaire should optimally be completed within the first week of initiating therapy to minimize any potential bias of early mortality on the analysis.

High patient participation rates are anticipated, as in the earlier PALM study the case *Cooperation Rate* with the questionnaire was 83% (personal communication, Dr. J. Ross). Similarly, in E1Z03 (an unrelated QOL study) the compliance (with the help of close coordination and follow-up by a clinical coordinator) was >90% (personal communication, L. Wagner). This is not expected to be a

significant additional burden for patients, and based on the experience from the PALM study it is anticipated that most patients will be able to complete the self-directed questionnaire in <20 minutes with minimal or no help (personal communication, Dr. J. Ross) unless they have extensive farm exposures (20-30 minutes). Indeed in the PALM study there was significant enthusiasm among patients to participate in the study (personal communication, Dr. J. Ross), and a similar response is anticipated in E2906. No additional laboratory or genetic studies are required, as appropriate leukemia & germline samples are already being collected.

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ALESE Questionnaires will be returned directly to the Investigator in stamped sealed envelope where QA & secure data entry (using scanning or double-key entry) will be performed by a trained clinical coordinator in the Cancer Epidemiology program at Mayo Clinic Florida under the supervision of Investigator. A detailed analysis plan will include clinical (treatment, induction mortality, remission, survival) & cytogenetic data as part of a dedicated grant application including collaboration with the ECOG-ACRIN Patient Outcomes Committee, Cytogenetic, and Leukemia Lab Committees, and Dr. Ross.

2. Objectives

2.1 Primary Objective

2.1.1 To evaluate the effect of clofarabine induction and consolidation therapy on overall survival in comparison with standard therapy (daunorubicin & cytarabine) in newly-diagnosed AML patients age ≥ 60 years.

2.2 Secondary Objectives

2.2.1 To evaluate complete remission (CR) rates, duration of remission, and toxicity/treatment-related mortality of clofarabine in comparison with standard therapy (daunorubicin & cytarabine) in newly-diagnosed AML patients age ≥60 years.

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2.2.2 To evaluate the feasibility of consolidation with reduced-intensity conditioning and allogeneic hematopoietic stem cell transplantation from HLA-identical donors in patients who achieve a response to induction therapy, including the incidence of successful engraftment, acute and chronic graft-versus-host disease, transplant-related mortality, and its impact on overall survival in comparison to patients receiving chemotherapy.

2.2.3 To evaluate the duration of remission and disease-free survival of patients in complete remission following completion of consolidation therapy who are subsequently randomized to receive scheduled low-dose decitabine maintenance in comparison with observation.

2.2.4 To perform expression and methylation profiling on all patients receiving decitabine and to correlate their integrated epigenetic signatures with response to decitabine.

2.2.5 To examine the epigenetic profiles of remission marrow in patients randomized to observation vs. decitabine to determine whether epigenetic signature of apparently morphologically normal bone marrow is predictive of relapse or response to decitabine maintenance.

2.2.6 To explore the possible association of response to clofarabine with ABC-transporter P-glycoprotein (Pgp).

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2.2.7 To assess the intensity of expression of CXCR4 on diagnostic leukemia cells and to correlate this parameter with other established prognostic factors

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2.2.8 To assess the entire spectrum of somatic mutations and affected pathways at diagnosis of AML and elucidate the association between gene mutation and outcome.

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2.2.9 To examine the impact of smoking, obesity, regular acetaminophen use, regular aspirin use, benzene exposure, living in a rural/farm environment and some other underlying exposures and lifestyle factors associated with AML development on OS.

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2.2.10 To investigate potential correlative results between array CGH findings and acute myeloid leukemia patient characteristics.

2.3 Quality of Life Objectives

2.3.1 To compare health-related QOL (physical, functional, leukemia-specific well-being) and fatigue in elderly AML patients receiving standard induction therapy with those receiving clofarabine.

2.3.2 To measure the change in health-related QOL that occurs over time (within treatment groups).

2.3.3 To comprehensively assess patient function at the time of study enrollment.

2.3.4 To determine if components of a comprehensive geriatric assessment or QOL scales predict ability to complete AML treatment.

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2.3.5 To describe the impact of transplant on QOL in AML patients above age 60.

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3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

ECOG-ACRIN Patient No. _____

Patient's Initials (L, F, M) _____

Physician Signature and Date _____

NOTE: All questions regarding eligibility should be directed to the study chair or study chair liaison.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration/randomization by the treating physician.

NOTE: This study involves pre-registration (see Section 4). Bone marrow and peripheral blood specimens must be submitted for centralized immunophenotyping and results will determine the patient's eligibility.

3.1 Pre-Registration

Diagnostic bone marrow and peripheral blood specimens must be submitted for eligibility testing by multiparameter flow cytometry. Testing will be performed by the ECOG-ACRIN Leukemia Translational Studies Laboratory and reported to the institution.

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3.2 Eligibility Criteria

_____ 3.2.1 Age ≥ 60 years.

_____ 3.2.2 Sexually active males must be strongly advised to use an accepted and effective method of contraception.

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_____ 3.2.3 AST, ALT, total bilirubin ≤ Grade 1.

AST: _____ Date of test: _____ ULN: _____

ALT: _____ Date of test: _____ ULN: _____

Total bilirubin: _____ Date of test: _____ ULN: _____

NOTE: If total bilirubin is 2 to 3 mg/dL, but direct bilirubin is normal, then the patient will be considered eligible.

_____ 3.2.4 Patient must not have a concurrent active malignancy for which they are receiving treatment (other than MDS).

_____ 3.2.5 Patient must not have an active, uncontrolled infection.

_____ 3.2.6 Additional Induction Eligibility Criteria

_____ 3.2.6.1 Newly-diagnosed AML patients according to WHO classification who are considered candidates for intensive chemotherapy based upon examination of peripheral blood or bone marrow aspirate specimens or touch preparations of the bone marrow biopsy obtained within two weeks prior to randomization. A bone marrow aspirate is required for enrollment. However, on occasion there is discordance between percentage of myeloblasts on the differential of the peripheral blood or aspirate. The peripheral blood criteria are sufficient for diagnosis. Confirmatory immunophenotyping will be performed centrally.

_____ 3.2.6.2 ECOG performance status 0-3 (restricted to ECOG PS 0-2 if age ≥70 years)

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_____ 3.2.6.3 Patients with APL confirmed either by the presence of t(15;17)(q22;q21) or PML/RAR∀ transcripts will be excluded

_____ 3.2.6.4 Patients must not have blastic transformation of chronic myelogenous leukemia.

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_____ 3.2.6.5 Patients with secondary AML are eligible for enrollment onto the trial. Secondary AML is defined as AML that has developed in a person with a history of antecedent blood count abnormalities, or myelodysplastic syndrome (MDS), or a myeloproliferative disorder (excluding Chronic Myeloid Leukemia); or a history of prior chemotherapy or radiation therapy for a disease other than AML.

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NOTE: Prior therapy of MDS with decitabine, low-dose cytarabine, or azacitidine is excluded.

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_____ 3.2.6.6 Patients may not have received prior chemotherapy for AML with the exception of hydroxyurea for increased blast count or leukapheresis for leukocytosis. Patients who have received a limited and short-term exposure of ATRA (all trans retinoic acid) while AML-M3 (Acute Promyelocytic Leukemia) was being ruled out, and which has been discontinued, will be eligible.

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_____ 3.2.6.7 Patients must have a total serum bilirubin ≤ 1.5 X ULN (grade ≤ 1) and a serum creatinine ≤ 1 mg/dL. If total bilirubin is 2 to 3 mg/dL, but direct bilirubin is normal, then the patient will be considered eligible. Patients with a serum creatinine >1 are eligible if they have a calculated GFR of ≥ 60 mL/min (i.e. class I or class II chronic kidney disease) using the MDRD formula [77,78]. The values must be obtained within 48 hours prior to randomization (for MDRD extended version with defaults to SI units please refer to http://mdrd.com).

NOTE: Daily creatinine and MDRD formula are only for the 1st induction cycle.

_____ 3.2.6.8 Patients must have a cardiac ejection fraction of ≥ 45% or within institutional normal limits. A nuclear medicine gated blood pool examination is preferred. A 2-D ECHO scan is acceptable if a calculated ejection fraction is obtained and follow-up measurement of the cardiac ejection fraction will also be performed by echocardiography. Measurement of cardiac ejection fraction should be within two weeks prior to receiving treatment.

NOTE: When a MUGA or echocardiogram cannot be obtained due to weekend or holiday, then patients may be enrolled provided there is no history of significant cardiovascular disease and a measurement of cardiac ejection fraction will be performed within 5 days of study enrollment.

_____ 3.2.6.9 Patients with suspected CNS involvement should undergo lumbar puncture. Those with documented CNS involvement will be excluded.

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_____ 3.2.6.10 Cytogenetic analysis must be performed from diagnostic bone marrow (preferred) or if adequate number of circulating blasts (>10^9/l) from peripheral blood.

_____ 3.2.6.11 Patients who have received previous treatment for AHD with 5-azacitidine, decitabine, or low dose cytarabine will be excluded.

_____ 3.2.6.12 Patients with known HIV infection are excluded due to the potential immunosuppressive effects of clofarabine and allogeneic transplantation.

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_____ 3.2.6.13 HLA typing should be performed at registration, if possible.

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_____ 3.2.6.14 Diagnostic bone marrow and peripheral blood specimens must be submitted for immunophenotyping and selected molecular testing.

3.2.7 Consolidation Criteria, Step 2 for Arms C and D

NOTE: All patients achieving CR or CRi will receive consolidation when fit.

NOTE: Patients proceeding to transplant are allowed up to one cycle of consolidation treatment.

_____ 3.2.7.1 Consolidation Cycle 1 must commence within sixty days of the bone marrow aspirate and biopsy that confirmed the presence of a CR or CRi.

_____ 3.2.7.2 Patients must have achieved a CR or Cri (or morphologic leukemia-free state for those patients proceeding to Arm G transplant) as defined in Section 6.

_____ 3.2.7.3 Patients who have achieved a CR or CRi must have maintained peripheral blood evidence of a CR or CRi as defined in Section 6.

_____ 3.2.7.4 Patients must have an ECOG performance status of 0-2.

_____ 3.2.7.5 Patients must have resolved any serious infectious complications related to induction.

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NOTE: Patients with an HLA-matched donor and proceeding to transplant will be allowed up to one cycle of consolidation treatment.

_____ 3.2.7.6 Any significant medical complications related to induction must have resolved.

_____ 3.2.7.7 Patients must have a creatinine and AST ≤ grade 1 (refer to Section 5.4 for dose modifications). The values must be obtained within 48 hours prior to registration.

3.2.8 Maintenance Criteria, Step 3 for Arms E and F

_____ 3.2.8.1 Maintenance should commence within 60 days of recovery of peripheral blood counts after Consolidation Cycle 2. Patients must begin Consolidation Cycle 2 within 60 days of recovery to be eligible for further therapy.

_____ 3.2.8.2 Patients must have maintained peripheral blood evidence of a remission as defined in Section 6 and must have a CR or CRi, confirmed on restaging BM aspirate and biopsy and cytogenetic analysis.

_____ 3.2.8.3 Patients must have an ECOG performance status of 0 -2.

_____ 3.2.8.4 Patients must have resolved any serious infectious complications related to Consolidation Cycle 2.

_____ 3.2.8.5 Any significant medical complications related to Consolidation Cycle 2 must have resolved.

_____ 3.2.8.6 Patients must have a total serum bilirubin ≤ 1.5 x ULN and a serum creatinine ≤ grade 1. The values must be obtained within 48 hours prior to randomization.

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NOTE: If total bilirubin is 2-3 mg/dL, but direct bilirubin is normal, then the patient will be considered eligible.

_____ 3.2.8.7 The ANC must be > 1000 mm^3 and the platelet count > 75,000 mm^3 prior to starting every cycle of treatment with decitabine. Decitabine may be delayed for up to 4 weeks between cycles (i.e. may be administered as infrequently as q8 weeks) while waiting for counts to recover.

3.2.9 Allogeneic Transplantation, Step 3 for Arm G

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_____ 3.2.9.1 Patients must be > 28 days from the start of induction or re-induction chemotherapy, or from the start Consolidation Cycle 1 (if received) and < 90 days following recovery from most recent treatment; and they must have achieved and maintained a response to induction therapy (CR, CRi, or

"morphologic disease-free state") in accordance with Section 6 of the protocol.

_____ 3.2.9.2 Patients must have recovered from the effects of induction, re-induction, or consolidation chemotherapy (all toxicities ≤ grade I with the exception of reversible electrolyte abnormalities), and have no ongoing active infection requiring treatment.

_____ 3.2.9.3 Patients must have a total serum bilirubin ≤ 1.5 x ULN (grade ≤ 1) and a serum creatinine ≤ grade1. The values must be obtained within 48 hours prior to registration.

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_____ 3.2.9.4 An eligible HLA-identical donor (either related or unrelated) should be available. In sibling donors, low resolution HLA typing (A,B,DR) will be considered sufficient. In the case of unrelated donors, high-resolution class I and II typing (A,B,C,DRB1 and DQ) should be matched at all 10 loci. Donors must be willing and able to undergo peripheral blood progenitor mobilization.

- HLA-Identical Sibling (6/6): The donor must be determined to be an HLA-identical sibling (6/6) by serologic typing for class (A, B) and low resolution molecular typing for class II (DRB1).
- Matched Unrelated Donor (10/10): High resolution molecular typing at the following loci is required: HLA-A, -B, -C, -DRBL, and –DQB1.

  NOTE: For matched donors – will allow select 1 antigen mismatched sibling donors and unrelated donors in accordance with site institutional standard, as long as matched at HLA-A, HLA-B, HLA-C, and DRB1, and with advanced discussion/approval by the Study Chair and the BMT co-chair.

_____3.2.9.5 Patients must be considered reliable enough to comply with the medication regimen and follow-up, and have social support necessary to allow this compliance.

_____3.2.9.6 Patients must have a cardiac ejection fraction of ≥ 40%, or within institutional normal limits. A nuclear medicine gated blood pool examination is preferred. A 2-D ECHO scan is acceptable if a calculated ejection fraction is obtained and follow-up measurement of the cardiac ejection fraction will also be performed by echocardiography. Measurement of cardiac ejection fraction should be within two weeks prior to allogeneic transplantation.

_____ 3.2.9.7 DLCO > 40% with no symptomatic pulmonary disease.

_____ 3.2.9.8 No known hypersensitivity to E.coli-derived products

_____ 3.2.9.9 No HIV infection. Patients with immune dysfunction are at a significantly higher risk of toxicities from intensive immunosuppressive therapies.

_____ 3.2.9.10 Initial Required Laboratory Data

Creatinine	≤ Grade 1
Bilirubin*	≤ Grade 1
AST	≤ Grade 1

*If bilirubin is 2-3 mg/dL, but direct bilirubin is normal then patient will be considered eligible.

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4. Registration Procedures

CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at <http://ctep.cancer.gov/investigatorResources/investigator_registration.htm>. For questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at <pmbregpend@ctep.nci.nih.gov>.

CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

Additional information can be found on the CTEP website at <http://ctep.cancer.gov/branches/pmb/associate_registration.htm>. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at <ctepreghelp@ctep.nci.nih.gov>.

CTSU Registration Procedures

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

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site by entering credentials at https://www.ctsu.org. For sites under the CIRB initiative, IRB data will automatically load to RSS.

Downloading Site Registration Documents

Site registration forms may be downloaded from the **E2906** protocol page located on the CTSU members' website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Click on the **ECOG-ACRIN** link to expand, then select trial protocol **E2906**
- Click on the Site Registration Documents link

Requirements for E2906 site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

Submitting Regulatory Documents

Submit completed forms along with a copy of your IRB Approval and Model Informed Consent to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office
Coalition of National Cancer Cooperative Groups
1818 Market Street, Suite 1100
Philadelphia, PA 19103
PHONE: 1-866-651-2878
FAX: (215) 569-0206
E-MAIL: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

Required Protocol Specific Regulatory Documents

1. **CTSU Regulatory Transmittal Form.**
2. **Copy of IRB Informed Consent Document.**

   NOTE: Any deletion or substantive modification of information concerning risks or alternative procedures contained in the sample informed consent document must be justified in writing by the investigator and approved by the IRB.

3. **A. CTSU IRB Certification Form.**
   Or
   B. Signed HHS OMB No. 0990-0263 (replaces Form 310).
   Or
   C. IRB Approval Letter

NOTE: The above submissions must include the following details:

- **Indicate all sites approved for the protocol under an assurance number.**
- **OHRP assurance number of reviewing IRB**
- **Full protocol title and number**
- **Version Date**

- **Type of review (full board vs. expedited)**
- **Date of review.**
- **Signature of IRB official**

Checking Your Site's Registration Status

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Patient Enrollment

Patients must not start protocol treatment prior to registration.

Treatment should start within five working days after registration.

Patient registration can occur only after pre-treatment evaluation is complete, eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS. Patients must have signed and dated all applicable consents and authorization forms.

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Please refer to Section 4.1 for the registration information that will be requested.

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

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

Prior to accessing OPEN site staff should verify the following:

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

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

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

The following information will be requested

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4.1 Pre-registration

NOTE: Patients who are only pre-registered must not begin treatment.

To proceed to randomization, patients must have centralized immunophenotyping performed by the ECOG-ACRIN Leukemia Translational Studies Laboratory.

4.1.1 Protocol Number

4.1.2 Investigator Identification

- 4.1.2.1 Institution and affiliate name (Institution CTEP ID)
- 4.1.2.2 Investigator's name (NCI number)
- 4.1.2.3 Cooperative Group Credit
- 4.1.2.4 Credit Investigator
- 4.1.2.5 Protocol specific contact information

4.1.3 Patient Identification

- 4.1.3.1 Patient's initials (first and last)
- 4.1.3.2 Patient's Hospital ID and/or Social Security number
- 4.1.3.3 Patient demographics
  - 4.1.3.3.1 Gender
  - 4.1.3.3.2 Birth date
  - 4.1.3.3.3 Race
  - 4.1.3.3.4 Ethnicity
  - 4.1.3.3.5 Nine-digit ZIP code
  - 4.1.3.3.6 Method of payment
  - 4.1.3.3.7 Country of residence

4.1.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3.1.

4.1.5 Additional Requirements

- 4.1.5.1 Patients must provide a signed and dated, written informed consent form.
- 4.1.5.2 Bone marrow and peripheral blood specimens **must be submitted** at pre-registration for centralized immunophenotyping.

NOTE: Copies of the consent are not collected by the ECOG-ACRIN Operations Office. However the ECOG-ACRIN Leukemia Translational Studies Laboratory (LTSL) requires institutions to submit a copy of the E2906 consent and a copy of the HIPAA Authorization form.

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4.2 Step 1: Randomization to Induction (ARMS A or B)

4.2.1 Protocol Number

4.2.2 Investigator Identification

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4.2.2.1 [Deleted in Addendum #4]

4.2.2.2 Investigator's name (NCI number)

4.2.2.3 Cooperative Group Credit

4.2.2.4 Credit Investigator

4.2.2.5 Protocol specific contact information

4.2.3 Patient Identification

4.2.3.1 Patient's initials (first and last)

4.2.3.2 Patient's Hospital ID and/or Social Security number

4.2.3.3 Patient demographics

4.2.3.3.1 Gender

4.2.3.3.2 Birth date

4.2.3.3.3 Race

4.2.3.3.4 Ethnicity

4.2.3.3.5 Nine-digit ZIP code

4.2.3.3.6 Method of payment

4.2.3.3.7 Country of residence

4.2.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3. An eligibility checklist has been appended to the protocol. A confirmation of registration will be forwarded by the ECOG-ACRIN Operations Office - Boston.

4.2.5 Stratification

- Age 60-69 vs. ≥ 70 years

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- [Deleted in Addendum #3]
- Therapy-related AML (any prior treatment with chemotherapy or radiation therapy for another malignancy)
- Antecedent Hematological Disorders (AHD) (prior diagnosis of MDS, myeloproliferative disorder or other hematologic disorder)

4.2.6 Additional Requirements (1st Randomization)

4.2.6.1 Patients must provide a signed and dated, written informed consent form.

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4.2.6.2 Baseline and follow-up peripheral blood and buccal swabs are to be submitted as outlined in Section 10 for banking, per patient consent.

4.2.6.3 Baseline and follow-up karyotypes must be submitted for central review as outlined in Section 10.

Rev. 12/13

NOTE: Specimens submitted for mandatory central reviews will also be used for the optional correlative studies indicated in Section 10 per patient consent.

NOTE: ECOG-ACRIN requires that biological samples submitted from patients be entered and tracked via the online ECOG-ACRIN Sample Tracking System (STS).

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NOTE: Institutions outside of the United States and Canada must confer with the receiving laboratory and the ECOG-ACRIN Operations Office - Boston regarding logistics for submission of fresh or frozen samples.

4.3 Step 2: Registration to Consolidation (ARMS C or D)

All patients must register to step 2. Patients who are proceeding to transplant and are not receiving consolidation therapy may proceed immediately to Registration to Transplant (Step 3).

4.3.1 Protocol Number

4.3.2 Investigator Identification

4.3.2.1 Institution and affiliate name (Institution CTEP ID)

4.3.2.2 Investigator's name (NCI number)

4.3.2.3 Cooperative Group Credit

4.3.2.4 Credit Investigator

4.3.2.5 Protocol specific contact information

4.3.3 Patient Identification

4.3.3.1 Patient's initials (first and last)

4.3.3.2 Patient's Hospital ID and/or Social Security number

4.3.3.3 Patient demographics

4.3.3.3.1 Gender

4.3.3.3.2 Birth date

4.3.3.3.3 Race

4.3.3.3.4 Ethnicity

4.3.3.3.5 Nine-digit ZIP code

4.3.3.3.6 Method of payment

4.3.3.3.7 Country of residence

4.3.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3. An eligibility checklist has been appended to the protocol. A

confirmation of registration will be forwarded by the ECOG-ACRIN Operations Office - Boston.

4.3.5 Classification

- Induction Treatment (Arm A vs Arm B)

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4.3.6 Additional Requirements (Randomization)

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4.3.6.1 Follow-up karyotypes, smears, bone marrow and peripheral blood must be submitted for central review as outlined in Section 10.

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4.3.6.2 Follow-up peripheral blood is to be submitted as outlined in Section 10 for banking, per patient consent.

NOTE: Specimens submitted for central review will also be used for the optional correlative studies outlined in Section 10 per patient consent.

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NOTE: ECOG-ACRIN requires that all biological samples submitted be entered and tracked via the online ECOG-ACRIN Sample Tracking System (STS).

4.4 Step 3: 2nd Randomization-Maintenance (Arms E or F), or Registration to Transplant (Arm G)

4.4.1 Protocol Number

4.4.2 Investigator Identification

4.4.2.1 Institution and affiliate name (Institution CTEP ID)

4.4.2.2 Investigator's name (NCI number)

4.4.2.3 Cooperative Group Credit

4.4.2.4 Credit Investigator

4.4.2.5 Protocol specific contact information

4.4.3 Patient Identification

4.4.3.1 Patient's initials (first and last)

4.4.3.2 Patient's Hospital ID and/or Social Security number

4.4.3.3 Patient demographics

4.4.3.3.1 Gender

4.4.3.3.2 Birth date

4.4.3.3.3 Race

4.4.3.3.4 Ethnicity

4.4.3.3.5 Nine-digit ZIP code

4.4.3.3.6 Method of payment

4.4.3.3.7 Country of residence

4.4.4 Eligibility Verification

Patients must meet all of the eligibility requirements listed in Section 3. An eligibility checklist has been appended to the protocol. A confirmation of registration will be forwarded by the ECOG-ACRIN Operations Office - Boston.

4.4.5 Stratification and Classification Factors

4.4.5.1 Classification for Registration to Transplant

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- [Deleted in Addendum #4]
- Transplant donor Identified: HLA-Identical Sibling (6/6) or Matched Unrelated Donor (10/10)

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NOTE: For matched donors – will allow select 1 antigen mismatched sibling donors and unrelated donors in accordance with site institutional standard, as long as matched at HLA-A, HLB, HLA-C, and DRB1, and with advanced discussion/approval by the Study Chair and the BMT co-chair.

4.4.5.2 Stratification for Randomization to Maintenance

- Age 60-69 vs. ≥ 70 years
- Cytogenetics unfavorable vs. “other”
- Induction Treatment (Arm A vs. B)

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4.4.6 Additional Requirements (2nd Randomization)

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4.4.6.1 Follow-up karyotypes, smears, bone marrow and peripheral blood must be submitted for central review as outlined in Section 10.

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4.4.6.2 Follow-up peripheral blood is to be submitted as outlined in Section 10 for banking, per patient consent.

NOTE: Specimens submitted for central review will also be used for the optional correlative studies outlined in Section 10 per patient consent.

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NOTE: ECOG-ACRIN requires that all biological samples submitted from patients be entered and tracked via the online ECOG-ACRIN Sample Tracking System (STS).

NOTE: FACT (Foundation for the Accreditation of Cellular Therapy) credentialing certificate must be submitted to the CTSU Patient Registrar at 1-888-691-8039 at time of patient registration to Arm G.

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4.5 Instructions for Patients who Do Not Start Assigned Protocol Treatment

If a patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted according to the instructions in the E2906 Forms Packet. Document the reason for not starting

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protocol treatment on the Off Treatment form. Also report the date and type of the first non-protocol treatment that the patient receives.

4.6 Investigator's Drug Brochure and Safety Alerts

A copy of the Investigator's Drug Brochure (IDB) for Clofarabine and the drug package insert for Decitabine are available for download from the ECOG webpage. The IDB and drug package insert provide relevant and current scientific information about the drug products. The IDB and drug package insert should be submitted to your IRB/EC according to GCP regulations. The Clofarabine IDB, the Decitabine drug package insert and any correspondence to the Institutional Review Board (IRB)/Ethics Committee (EC) should be kept in the E2906 regulatory files.

Should any SAE report on this study qualify as a safety alert report requiring expedited reporting, the SAE report will be sent by the sponsors to regulatory authorities globally (including the FDA) and ECOG-ACRIN. If applicable, ECOG-ACRIN will disseminate these safety alert reports to all participating investigators in the bimonthly group mailings. These reports should be forwarded to your IRB/EC within 90 days of receipt for review. Reporting instructions are provided with each safety alert. These safety alerts and any correspondence to your IRB/EC should be maintained in your E2906 study file.

4.7 IND Status

The use of Clofarabine and Decitabine in this trial is classified as "off-label" or unapproved use of FDA-approved drugs. When a drug or combination of drugs is used in an off-label manner as part of a clinical trial, it is, by rule, considered as an investigational treatment regimen. However, while these treatment regimens are not approved by the FDA, their use is exempt from the requirements for an IND to conduct this study as defined under Title 21 CFR 312.2(b) of the codified FDA regulations

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5. Treatment Plan

5.1 Induction Therapy and Premedications

NOTE: Doses to be calculated using actual body weight.

5.1.1 Initial Considerations

Patients should begin treatment with Allopurinol 300 mg/day orally. Reversible abnormalities of renal or metabolic function should be treated aggressively and corrected prior to institution of therapy. Immediate measures to diagnose and begin treatment of infections should be instituted prior to induction therapy.

Patients should be adequately hydrated with NS or equivalent^{-} and achieve a target urine output ≥ 100ml/hour average prior to starting induction therapy. This should be maintained throughout the first 5-7 days of induction therapy, with adjustments as clinically indicated to maintain volume status and prevent volume overload. Appropriate monitoring for evidence of tumor lysis should also be performed for the first 3-5 days of induction therapy (e.g. measurement of K^+, PO_4^{3-}, serum creatinine, calcium and uric acid q 8-12 hours)

Patients with high circulating blast cell counts > 50,000/µl, should be given hydroxyurea 750 mg/m^2 (or more) orally every 6-12 hours until the absolute blast count falls < 50,000/µl.

NOTE: Patients who present with a clinically significant increase in the peripheral blast count may receive hydroxyurea prior to the start of induction. A suggested dose is 1 to 2 gm po every six hours, at the discretion of the physician. The hydroxyurea must be discontinued prior to administration of induction. Hydroxurea suggested minimum 500–1000 mg PO twice a day. Suggested maximum dose is not to exceed 4,000 mg twice a day.

NOTE: Patients with M4 or M5 disease and who have CNS signs or symptoms should have a lumbar puncture once blasts have cleared from the peripheral blood smear.

NOTE: Up to two courses of induction therapy are permitted to achieve marrow aplasia. Patients who have not achieved CR, CRi, or a morphologic leukemia-free state after the second cycle of induction are taken off study and further treatment is at the discretion of the investigator.

NOTE: Allopurinol 300 mg daily or the dose adjusted for renal insufficiency should be administered prior to starting induction through the completion of the cytarabine infusion during induction.

Patients will be randomized to one of the following induction therapy arms:

5.1.2 **ARM A** Standard Therapy with “7&3 schedule”

Daunorubicin 60 mg/m²/day by 10 - 15 minute intravenous infusion for 3 days (days 1, 2, and 3).

Cytarabine 100 mg/m²/day by 24 hour continuous intravenous infusion for 7 days (days 1-7).

NOTE: There are no dose modifications during initial induction.

Dexamethasone 10 mg IV QD (or equivalent) and Ondansetron 16 mg IV, or PO, or equivalent, is suggested as an anti-emetic.

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Patients with residual AML by morphologic review or who do not achieve an aplastic BM on day 12-14 (i.e. < 5% blasts and < 20% cellularity or markedly/moderately hypocellular) are eligible to receive a 2nd cycle of induction therapy with the same dose & schedule of daunorubicin & cytarabine beginning no sooner than day 14.

5.1.3 **ARM B** Clofarabine

Clofarabine 30mg/m²/day IV by 1 hour infusion QD x 5 days

Dexamethasone 10 mg IV QD (or equivalent) and Ondansetron 16 mg IV, or PO, or equivalent, is suggested as an anti-emetic.

Patients with residual AML or who do not achieve an aplastic BM on day 12-14 (i.e. < 5% blasts and < 20% cellularity or markedly/moderately hypocellular) are eligible to receive a 2nd cycle of induction therapy with clofarabine at the **lower 'consolidation' dose of 20mg/m²/day IV QD x 5 days**.

The creatinine must be measured daily during the first cycle of clofarabine treatment. Dose adjustments for an increase in serum creatinine > grade I during clofarabine treatment will be according to Dose Modification, Section 5.4.4.

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NOTE: Dose adjustments for the first induction cycle of clofarabine will follow Section 5.4.4.

The 2nd cycle of induction with clofarabine must not commence sooner than day 21 and no later than day 56, and must be withheld until creatinine, AST & total bilirubin are ≤ grade I (See Section 5.4.4 for dose modifications).

5.1.4 Restaging

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A restaging BM will be performed on day 12 -14 (+/- 1 day) to determine whether patients have achieved an aplastic BM (i.e. < 5% blasts and < 20% cellularity, or markedly/moderately hypocellular). Patients who achieve an aplastic BM should start GM-CSF or G-CSF, or pegfilgrastim (unless contraindicated). Those with residual leukemia or who do not achieve an aplastic or markedly/moderately hypocellular BM are eligible for re-induction with a 2nd cycle of chemotherapy as noted above.

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A repeat BM for outcome will be performed between day 28 – day 56 from the start of initial therapy after 1st or 2nd induction either when the ANC is > 1000 and platelets > 100,000, or if there is evidence of delayed hematologic recovery and it is felt that the ANC will not reach 1000 or the platelet count > 100,000, to document response to induction therapy. Those with an HLA-matched donor identified who achieve a "morphologic leukemia-free state" on or before day 56 may

proceed to allogeneic transplantation if medically appropriate and per physician discretion. Patients proceeding to transplant are allowed up to 1 cycle of consolidation before the transplant. See Sections 5.1.6 and 5.1.7.

5.1.5 Criteria for Second Induction Cycle

A bone marrow aspirate and biopsy is performed on day 12-14 (± 1 day), i.e. 5-7 days after the completion of the cytarabine infusion or 7-9 days after completion of clofarabine treatment. The decision to administer a second induction cycle is based on the following criteria:

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NOTE: Patients undergoing 2nd induction who do not achieve complete response by day 56 of the start of re-induction will be taken off protocol treatment.

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5.1.5.1 If the marrow cellularity is judged to be greater than 20%, or not markedly/moderately hypocellular on the marrow biopsy, or with > 5% blasts, or the bone marrow is indeterminate, then repeat induction is indicated.

If the marrow cellularity is less than markedly hypocellular, or moderately hypocellular, or there are persistent circulating blasts, then the patient's status should be followed with a repeat marrow assessment within 5 to 7 days.

5.1.5.2 Second Induction Treatment Dose Arm A & Arm B

For Arm A, the 2nd induction cycle is a repeat of the initial induction and at the same schedule of chemotherapy as the 1st induction, however, for Arm B, clofarabine is administered at the reduced dose of **20** mg/m^2/day IV by 1 hour infusion days 1-5, and should not start before day 21. Prior to administering a 2nd cycle of clofarabine induction, abnormalities in hepatic function must have returned ≤ grade I.

5.1.6 Consolidation

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All patients achieving CR or CRi will receive consolidation therapy when fit. Patients with an HLA-matched donor who achieve at least a 'morphologic leukemia-free state' [79] (< 5% blasts without hematologic recovery, and negative flow cytometry if performed) will be eligible for AlloSCT (see Section 5.1.7). Patients will be assigned to consolidation based upon the 1st randomization: those randomized to 'standard' daunorubicin & cytarabine induction will receive 2 cycles at 4-6 week intervals of 'standard' intermediate-dose Cytarabine 1500 mg/m^2 q 12 hours for either 12 doses per cycle (age 60-69 years) or 6 doses per cycle (age ≥ 70 years); those randomized to 'clofarabine' will receive 2 cycles of clofarabine 20 mg/m^2/day x 5 days at 4-6 week intervals.

Arm C 'Intermediate-Dose Cytarabine' Consolidation

- All patients randomized to 'standard' daunorubicin & cytarabine induction

- 2 cycles of consolidation therapy
- Cytarabine 1500 mg/m^2 IV over 1 hour stratified by age for either twice a day (q 12 hours) 12 doses per cycle (age 60-69 years) or once daily days 1-6, 6 doses per cycle (age ≥ 70 years).

Cycles will be repeated at 4-6 week intervals.

Arm D 'Clofarabine' Consolidation

- All patients randomized to clofarabine induction
- 2 cycles of consolidation therapy

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- Clofarabine 20 mg/m^2/day infused over 1 hour x 5 days per cycle

Cycles will be repeated at 4-6 week intervals.

NOTE: Consolidation for Patients Proceeding to Transplant

- Up to 1 cycle of consolidation treatment is allowed
- Treatment determined by induction

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5.1.6.1 Consolidation Cycle 1

Patients who achieve a CR or CRi and have no residual significant toxicities from the induction course are eligible for further protocol therapy. In general, consolidation therapy should start within 1 month of documentation of CR/CRi when possible, after the resolution of any non-hematologic toxicity of previous chemotherapy (to </= grade I), and with recovery of performance status to >/= baseline in the judgment of the Investigator.The first cycle of post-remission therapy must begin within sixty days of documentation of a CR or CRi. A repeat measurement of ejection fraction should be performed for patients on Arm C and Arm D. Patients on Arm C will receive dose-escalated cytarabine.

The schedule of cytarabine administration is modified based upon age < or ≥ 70 years.

5.1.6.2 Consolidation Cycle 2

Patients must have maintained peripheral blood evidence of a remission as defined in Section 6.1. Consolidation cycle 2 should commence within thirty days of recovery of peripheral blood counts after consolidation cycle 1. Patients who do not begin consolidation cycle 2 within 60 days of recovery are ineligible for further protocol therapy.

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5.1.7 Allogeneic Stem Cell Transplantation with Reduced Intensity Conditioning – Arm G

All patients with an HLA-matched donor identified (either sibling or unrelated) are eligible for AlloSCT. HLA typing should be performed on all patients and eligible siblings pre-induction treatment.

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5.1.7.1 Allogeneic Transplant

An eligible HLA-identical donor (either related or unrelated) should be available. In sibling donors, low resolution HLA typing (A,B,DR) will be considered sufficient. In the case of unrelated donors, high-resolution class I and II typing (A,B,C,DRB1 and DQ) should be matched at all 10 loci. Donors must be willing and able to undergo peripheral blood progenitor mobilization.

- HLA-Identical Sibling (6/6): The donor must be determined to be an HLA-identical sibling (6/6) by serologic typing for class (A, B) and low resolution molecular typing for class II (DRB1).
- Matched Unrelated Donor (10/10): High resolution molecular typing at the following loci is required: HLA-A, -B, -C, -DRBL, and –DQB1.

  NOTE: For matched donors – will allow select 1 antigen mismatched sibling donors and unrelated donors in accordance with site institutional standard, as long as matched at HLA-A, HLA-B, HLA-C, and DRB1, and with advanced discussion/approval by the Study Chair and the BMT co-chair.

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Patients allocated to allogeneic transplant will proceed directly to transplant after induction therapy on Arms A or B, or after 1 cycle of consolidation (post-remission) therapy on Arms C or D. They will undergo the therapy no sooner than 28 days after initiation of induction therapy; after documentation of CR, CRi, or morphologic disease-free state; and not later than three months after recovery of peripheral blood counts following induction and post remission therapy, providing all eligibility guidelines in Section 3.1.9 are met.

Those patients not felt to be fit for transplant or who do not have a suitable matched donor as above or who decline transplant may be allocated to consolidation.

5.1.7.2 Selection of Allogeneic Transplant Center

Eligible patients will be referred to one of the approved allogeneic blood and marrow transplant institutions. Arrangements will be made by contacting the marrow transplant patient coordinator at the chosen institution.

NOTE: ECOG-ACRIN institutions may refer to the ECOG website for information about BMT centers.

5.1.7.3 Transplant Regimen

Preferred Preparative Conditioning regimen will consist of:

Fludarabine	30 mg/m^2 I.V. days -7, -6, -5, -4, -3, (5 doses)
Busulfan**	0.8 mg/kg I.V. q6 hours days -4 thru -3, (8 doses)
Thymoglobulin	2.5 mg/kg/day I.V. days -4, -3, -2, (3 doses)

****Busulfan Pharmacokinetic:** While not being formally tested in E2906, participating BMT centers may perform Busulfan (Bu) Pharmacokinetic (PK) testing per institutional standards. Busulfan may be dose reduced in individual patients in accordance with results of Bu PK results only after consultation with the Study Chair or BMT Chairs, but may not be dose escalated. Please inform Study Chair prior to AlloSCT if Bu PK will be performed.

NOTE: Bu-Flu-ATG is the preferred preparative conditioning regimen. Alternative equivalent reduced-intensity or non-myeloblative conditioning regimens may be used at the discretion of the investigator only after discussion with the Study Chair (or Co-Chair) and BMT Co-Chair.

Allogeneic Stem Cells - Collection from Donors

HLA-Identical Sibling Donors

HLA-identical sibling donors should be treated with G-CSF 10 mcg/kg subcutaneously daily on Days -5, -4, -3, -2 (and -1, if the initial collection is inadequate). G-CSF should continue throughout mobilization, but will be reduced by 50% if WBC > 50,000/µL.

On Day -1 HLA-identical sibling donors will have vein to vein apheresis. CD3 and CD34 cells as fractions of the peripheral mononuclear (PMN) cells will be determined from the established PMN cell collection according to institutional flow cytometry. If the yield of CD34+ cells is less than 2 x 10^6/kg (actual weight -recipient) on Day -1, an additional apheresis will be performed on Day 0 to achieve a total or set dose of 2-8 x 10^6/kg (actual weight -recipient) CD34+ cells.

The PMN cells will be separated and collected, while all the other separated blood components will be returned to the donor.

Cells from the stem cell collection either may be stored frozen or collected fresh.

Matched Unrelated Donors

Collection of peripheral blood stem cells will be performed on Days -1 (and 0) according to existing NMDP collection center protocols (or equivalent for other registries). Donors that are unwilling or unable to have PBSC collected will not be eligible. Matched unrelated donors are not required to complete Donor Consent Form.

Allogeneic Stem Cells - Infusion to Recipients

On Days 0 (and +1) a minimum total cell dose of CD34+ cells of 2 x 10^6/kg (**actual** weight - recipient) and a maximum of 8 x 10^6/kg (actual weight -recipient) will be infused.

G-CSF 5 mcg/kg/day subcutaneously beginning on Day +12 and continuing until ANC > 1500/µL for two consecutive days or > 5000/µL for one day. If the ANC subsequently falls to < l000/µL then resume G-CSF at 5 mcg/kg/day.

Patients will be monitored weekly for engraftment and toxicity. Chimerism studies should be performed on Days +30, +60, +90, + 120, + 180, +270, +365, and at time of relapse.

5.1.7.4 Infusion of Peripheral Blood Stem Cells

Infusion of stem cells is to proceed as described below or per institutional standards.

See Section 5.5 for treatment supportive care.

5.1.7.4.1 Stem Cells

2-8 X 10^6/kg CD34+ cells cryopreserved peripheral blood stem cells are rapidly thawed at 37^0 C and immediately infused on day 0.

5.1.7.4.2 Stem Cells

Cryopreserved stem cells are rapidly thawed at 37□C and immediately infused 36-48 hours after the last dose of Cyclophosphamide.

5.1.7.4.3 PBSC Infusion: The cells are not washed before reinfusion, and the osmolality of the cell suspension requires the use of a flowing central venous catheter. Before reinfusion of PBSC, hydration must be maintained to have an output of at least 3 cc/kg/hour. Emergency drugs (Benadryl, epinephrine, Solu-Medrol) in appropriate doses must be at the bedside. Baseline vital signs, forced vital capacity and EKG are recorded.

Fifteen minutes prior to administration of the thawed PBSC, the patient is to receive:

- 50 mL of 25% mannitol solution IV
- 50 mg of diphenhydramine IV
- 250 mg of hydrocortisone IV
- An antiemetic drug of choice (optional)

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NOTE: Institutional standard allowed for PBSC pre-meds.

5.1.8 Maintenance

5.1.8.1 Observation vs. Decitabine Maintenance (2nd Randomization) – Arm E and Arm F

Patients who complete consolidation therapy will undergo restaging with a BM aspiration & biopsy and repeat BM cytogenetics to confirm CR or CRi. Patients will then be randomized to maintenance therapy with decitabine vs. monthly observation for 12 months.

- Decitabine 20 mg/m^2/day IV over 1 hour QD x 3 days, repeated Q4 weeks x 12 months total[75], up to 13 cycles, but not to continue beyond 12 months. Regardless of treatment delays, the total duration of maintenance therapy will be 12 months.
- There are no planned dose reductions in decitabine. Patients unable to receive treatment on schedule (i.e. planned q4 weeks, but delays up to 4 weeks allowed) due to cytopenias or persistent side effects will discontinue decitabine and subsequently be observed per the 'observation ' group (Arm E), although they will be analyzed on an 'intention to treat' basis as having been assigned to Arm F.
- The ANC must be > 1000 mm^3 and the platelet count > 75,000 mm^3 prior to starting every cycle of treatment with decitabine. Decitabine may be delayed for up to 4 weeks between cycles (i.e. may be administered as infrequently as q8 weeks) while waiting for counts to recover.
- Monthly CBC and examination will be performed on all patients assigned to decitabine or observation for 24 months

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5.1.8.2 Patients who do not meet eligibility for Randomization to Observation vs. Decitabine Maintenance after recovery from Consolidation should be followed according to Arm E.

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5.2 Adverse Event Reporting Requirements

5.2.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (please refer to the E2906 Forms Packet for the list of forms with directions for routine adverse event reporting). Additionally, certain adverse events must be reported in an expedited manner for more timely monitoring of patient safety and care. The following sections provide information about expedited reporting.

5.2.2 Determination of Reporting Requirements

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study therapy (attribution), and the prior experience (expectedness) of the adverse event; 3) the phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

Steps to determine if an adverse event is to be reported in an expedited manner:

Step 1: *Identify the type of event:* The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov).

Step 2: *Grade the event using Version 4.0 of the NCI CTCAE*.

Step 3: *Determine whether the adverse event is related to the protocol therapy (investigational or commercial).* Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: *Determine the prior experience of the adverse event*. *Expected* events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered *unexpected*, for expedited reporting purposes only, when either the type of event or the severity of the event is **NOT** listed in:

- **Arm A, B, C, D and F** – the drug package insert or protocol

Step 5: *Review Section 5.2.6 for E2906 and/or ECOG-ACRIN specific requirements for expedited reporting of specific adverse events that require special monitoring.*

NOTE: For general questions regarding expedited reporting requirements, please contact the the AEMD Help Desk at aemd@tech-res.com or 301-897-7497.

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5.2.3 Reporting Procedure

This study requires that expedited adverse event reporting use CTEP's Adverse Event Reporting System (CTEP-AERS). CTEP's guidelines for CTEP-AERS can be found at http://ctep.cancer.gov. A CTEP-AERS report must be submitted electronically to ECOG-ACRIN and the appropriate regulatory agencies via the CTEP-AERS Web-based application located at http://ctep.cancer.gov.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made by telephone to

- the AE Team at ECOG-ACRIN (617-632-3610) and
- the FDA (1-800-FDA-1088)

An electronic report MUST be submitted immediately upon re-establishment of internet connection.

Supporting and follow up data: Any supporting or follow up documentation must be faxed to ECOG-ACRIN (617-632-2990), Attention: AE within 48-72 hours. In addition, supporting or follow up documentation must be faxed to the FDA (800-332-0178) in the same timeframe.

NCI Technical Help Desk: For any technical questions or system problems regarding the use of the CTEP-AERS application, please contact the NCI Technical Help Desk at ncictephelp@ctep.nci.nih.gov or by phone at 1-888-283-7457.

5.2.4 When to Report an Event in an Expedited Manner

When an adverse event requires expedited reporting, submit a full CTEP-AERS report within the timeframes outlined in Section 5.2.6.

NOTE: Adverse events that meet the reporting requirements in Section 5.2.6 and occur within 30 days of the last dose of protocol treatment must be reported on an expedited adverse event report form (using CTEP-AERS). For any adverse events that occur more than 30 days after the last dose of treatment, only those that have an attribution of possibly, probably, or definitely AND meet the reporting requirements in Section 5.2.6 must be reported on an expedited adverse event report form (using CTEP-AERS)

5.2.5 Other Recipients of Adverse Event Reports

Adverse events determined to be reportable must also be reported by the institution, according to the local policy and procedures, to the Institutional Review Board responsible for oversight of the patient.

A drug supporter representative may call a site for additional information regarding a serious adverse event. Any additional written AE information requested by the drug supporter MUST be submitted to BOTH ECOG-ACRIN and the drug supporter.

5.2.6 Expedited Reporting for Commercial Agents

NOTE: Although Arms B, D, and F use agents considered to be investigational, their use is exempt from the requirements for an IND to conduct this study as defined under Title 21 CFR 312.2(b) of the codified FDA regulations. Please see Section 4.6 for IND status information.

Commercial reporting requirements are provided below. The agents used in arms A, B, C, D, and F of this study are Daunorubicin, Cytarabine, Decitabine and Clofarabine.

Expedited reporting requirements for adverse events experienced by patients on arm(s) with commercial agents only – Arms A, B, C, D and F.

Attribution	**Grade 4**		**Grade 5[a]**		**ECOG-ACRIN and Protocol-Specific Requirements**
	Unexpected	Expected	Unexpected	Expected	See footnote (b) for special requirements.
Unrelated or Unlikely			7 calendar days	7 calendar days	
Possible, Probable, Definite	7 calendar days		7 calendar days	7 calendar days	

7 Calendar Days: Indicates a full CTEP-AERS report is to be submitted within 7 calendar days of learning of the event.

a This includes all deaths within 30 days of the last dose of treatment regardless of attribution. **NOTE: Any death that occurs > 30 days after the last dose of treatment and is attributed possibly, probably, or definitely to the treatment must be reported within 7 calendar days of learning of the event**.

b Protocol-specific expedited reporting requirements: The adverse events listed below also require expedited reporting for this trial:

Serious Events: Any event following treatment that results in *persistent or significant disabilities/incapacities, congenital anomalies, or birth defects* must be reported via CTEP-AERS within 7 calendar days of learning of the event. For instructions on how to specifically report these events via CTEP-AERS, please contact the the AEMD Help Desk at aemd@tech-res.com or 301-897-7497. These will need to be discussed on a case by case basis'

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5.2.7 Reporting Second Primary Cancers

All cases of second primary cancers, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), that occur following treatment on NCI-sponsored trials must be reported to ECOG-ACRIN:

- **A second malignancy is a cancer that is UNRELATED to any prior anti-cancer treatment (including the treatment on this protocol). Second malignancies require ONLY routine reporting as follows:**
  1. Submit a completed Second Primary Form within 30 days to ECOG-ACRIN at

     ECOG-ACRIN Operations Office - Boston
     FSTRF
     900 Commonwealth Avenue
     Boston, MA 02215
  2. Submit a copy of the pathology report to ECOG-ACRIN confirming the diagnosis.
  3. If the patient has been diagnosed with AML/MDS, submit a copy of the cytogenetics report (if available) to ECOG-ACRIN
- **A secondary malignancy is a cancer CAUSED BY any prior anti-cancer treatment (including the treatment on this protocol). Secondary malignancies require both routine and expedited reporting as follows:**
  1. Submit a completed Second Primary Form within 30 days to ECOG-ACRIN at

     ECOG-ACRIN Operations Office - Boston
     FSTRF
     900 Commonwealth Avenue
     Boston, MA 02215
  2. Report the diagnosis via CTEP-AERS at http://ctep.cancer.gov

     Report under a.) leukemia secondary to oncology chemotherapy, b.) myelodysplastic syndrome, or c.) treatment related secondary malignancy.
  3. Submit a copy of the pathology report to ECOG-ACRIN and NCI/CTEP confirming the diagnosis.
  4. If the patient has been diagnosed with AML/MDS, submit a copy of the cytogenetics report (if available) to ECOG-ACRIN and NCI/CTEP.

NOTE: The Second Primary Form and the CTEP-AERS report should not be used to report recurrence or development of metastatic disease.

NOTE: If a patient has been enrolled in more than one NCI-sponsored study, the Second Primary Form must be submitted for the most recent trial. ECOG-ACRIN must be provided with a copy of the form and the associated pathology report and cytogenetics report (if available) even if ECOG-ACRIN was not the patient's most recent trial.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted via CTEP-AERS or by the Second Primary Form.

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5.3 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Decitabine (5-aza-2'-deoxycytidine) (NSC 127716)

The Comprehensive Adverse Events and Potential Risks List (CAEPR) provides a thorough and detailed list of reported and/or potential adverse events associated with the Decitabine. The CAEPR is developed and continuously monitored by the CTEP Investigational Drug Branch (IDB). The information listed in the CAEPR below, as well as the other resources described in the 'Determination of reporting requirements' part of the Adverse Event Reporting section in this protocol, can be used to determine expectedness of an event when evaluating if the event is reportable via CTEP-AERS. Frequency is provided based on 1658 patients. Below is the CAEPR for Decitabine (5-aza-2'-deoxycytidine).

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Version 2.4, January 4, 2017[1]

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Adverse Events with Possible Relationship to Decitabine (5-aza-2'-deoxycytidine) (CTCAE 4.0 Term) [n= 1832]		
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Anemia		
	Febrile neutropenia	
GASTROINTESTINAL DISORDERS		
	Abdominal pain	
	Anal mucositis	
	Constipation	
	Diarrhea	
	Mucositis oral	
Nausea		
	Rectal mucositis	
	Small intestinal mucositis	
	Vomiting	
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	Chills	
	Edema limbs	
Fatigue		
Fever		
	Non-cardiac chest pain	
	Pain	
IMMUNE SYSTEM DISORDERS		
	Autoimmune disorder	
INFECTIONS AND INFESTATIONS		
Infection[2]		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS		
	Bruising	
INVESTIGATIONS		
	Alanine aminotransferase increased	
	Aspartate aminotransferase increased	
	Blood bilirubin increased	
	Creatinine increased	

	Lymphocyte count decreased	
Neutrophil count decreased		
Platelet count decreased		
White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS		
	Anorexia	
	Hyperglycemia	
	Hyperuricemia	
	Hypoalbuminemia	
	Hypocalcemia	
	Hypokalemia	
	Hypomagnesemia	
	Hyponatremia	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Arthralgia	
	Back pain	
	Bone pain	
	Pain in extremity	
NERVOUS SYSTEM DISORDERS		
	Dizziness	
	Headache	
		Intracranial hemorrhage
	Somnolence	
PSYCHIATRIC DISORDERS		
	Anxiety	
	Insomnia	
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	Cough	
	Dyspnea	
	Laryngeal mucositis	
	Pharyngeal mucositis	
	Pharyngolaryngeal pain	
	Respiratory hemorrhage[3]	
	Tracheal mucositis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Alopecia	
	Pruritus	
	Purpura	
	Rash maculo-papular	
VASCULAR DISORDERS		
	Hematoma	
	Phlebitis	
	Vascular disorders - Other (hemorrhage with decreased platelets)	

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

[2]Infection may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

[3]Respiratory hemorrhage includes Bronchopulmonary hemorrhage, Epistaxis, Laryngeal hemorrhage, Mediastinal hemorrhage, Pharyngeal hemorrhage, and Pleural hemorrhage under the RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS SOC.

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

[5]Gastrointestinal obstruction includes Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Obstruction gastric, Rectal obstruction, and Small intestinal obstruction under the GASTROINTESTINAL DISORDERS SOC.

Adverse events reported on decitabine (5-aza-2'-deoxycytidine) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that decitabine (5-aza-2'-deoxycytidine) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (coagulaopathy); Blood and lymphatic system disorders - Other (lymphadenopathy); Blood and lymphatic system disorders - Other (pancytopenia); Bone marrow hypocellular; Hemolysis; Leukocytosis; Spleen disorder

CARDIAC DISORDERS - Acute coronary syndrome; Atrial fibrillation; Atrial flutter; Atrioventricular block complete; Cardiac arrest; Cardiac disorders - Other (cardiac murmur); Cardiac disorders - Other (dilation atrial); Chest pain - cardiac; Heart failure; Myocardial infarction; Restrictive cardiomyopathy; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia; Ventricular arrhythmia

EAR AND LABYRINTH DISORDERS - Ear pain; Vertigo

EYE DISORDERS - Blurred vision; Eye disorders - Other (eye hemorrhage); Eye disorders - Other (eye swelling); Eye pain

GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Dyspepsia; Dysphagia; Enterocolitis; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (anal fissure); Gastrointestinal disorders - Other (diverticulitis); Gastrointestinal disorders - Other (mouth ulceration); Gastrointestinal disorders - Other (oral mucosal blistering); Gastrointestinal hemorrhage[4]; Gastrointestinal obstruction[5]; Gastrointestinal pain; Hemorrhoids; Ileus; Oral pain; Periodontal disease; Proctitis; Rectal pain; Toothache; Typhlitis

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Gait disturbance; Infusion related reaction; Injection site reaction; Localized edema; Malaise; Multi-organ failure

HEPATOBILIARY DISORDERS - Cholecystitis; Hepatic failure; Hepatobiliary disorders - Other (cholethiasis); Hepatobiliary disorders - Other (hepatomegaly)

IMMUNE SYSTEM DISORDERS - Allergic reaction

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Fracture; Injury, poisoning and procedural complications - Other (catheter site pain); Injury, poisoning and procedural complications - Other (hernia); Injury, poisoning and procedural complications - Other (procedural pain); Injury, poisoning and procedural complications - Other (stent occlusion)

INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alkaline phosphatase increased; CPK increased; Cardiac troponin I increased; Ejection fraction decreased; Electrocardiogram QT corrected interval prolonged; Fibrinogen decreased; GGT increased; INR increased; Investigations - Other (blood bicarbonate decreased); Investigations - Other (blood bicarbonate increased); Investigations - Other (blood bilrubin decreased); Investigations - Other (blood chloride decreased); Investigations - Other (blood chloride increased); Investigations -

Other (blood lactate dehydrogenase increased); Investigations - Other (blood urea increased); Investigations - Other (elevated ammonia); Investigations - Other (eosinophilia); Investigations - Other (platelet count increase); Investigations - Other (protein total decreased); Lipase increased; Serum amylase increased; Weight loss

METABOLISM AND NUTRITION DISORDERS - Acidosis; Dehydration; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypoglycemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (hyperphosphatemia); Metabolism and nutrition disorders - Other (malnutrition)

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Chest wall pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (muscle spasms); Myalgia

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Leukemia secondary to oncology chemotherapy; Tumor pain

NERVOUS SYSTEM DISORDERS - Amnesia; Aphonia; Ataxia; Cognitive disturbance; Dysesthesia; Dysgeusia; Ischemia cerebrovascular; Lethargy; Paresthesia; Peripheral sensory neuropathy; Seizure; Stroke; Syncope; Transient ischemic attacks

PSYCHIATRIC DISORDERS - Confusion; Delirium; Depression; Personality change

RENAL AND URINARY DISORDERS - Acute kidney injury; Cystitis noninfective; Hematuria; Urinary fistula; Urinary frequency; Urinary retention; Urinary tract pain; Urinary urgency

REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Uterine hemorrhage; Vaginal hemorrhage

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Allergic rhinitis; Atelectasis; Bronchospasm; Hypoxia; Nasal congestion; Pleural effusion; Pneumonitis; Postnasal drip; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (breath sounds abnormal/decreased); Respiratory, thoracic and mediastinal disorders - Other (crepitations); Respiratory, thoracic and mediastinal disorders - Other (pulmonary congestion); Sinus disorder; Wheezing

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Bullous dermatitis; Dry skin; Erythema multiforme; Hyperhidrosis; Skin and subcutaneous tissue disorders - Other (hyperkeratosis); Skin and subcutaneous tissue disorders - Other (Sweet's Syndrome); Skin hyperpigmentation; Skin hypopigmentation; Skin ulceration; Stevens-Johnson syndrome; Urticaria

VASCULAR DISORDERS - Flushing; Hypertension; Hypotension ;Thromboembolic event; Vascular disorders - Other (aortic anyeurism); Vascular disorders - Other (catheter site hemorrhage); Vascular disorders - Other (circulatory collapse); Vascular disorders - Other (hemorrhage); Vascular disorders - Other (splenic infarct vs hemorrhage/rupture); Vascular disorders - Other (veno-occlusive disease); Vasculitis

NOTE: Decitabine (5-aza-2'-deoxycytidine) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent

5.4 Dose Modifications

All toxicities should be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov). .

5.4.1 Dose Modification for Cytarabine – Arm C Consolidation

For patients ≥ 70 years of age the cytarabine dose is identical but the patient will receive one dose daily for a total of 6 doses (days 1-6).

Patients should have a daily creatinine and total bilirubin. The dose of cytarabine will be adjusted. The dose of cytarabine will be adjusted if there is a change in the serum creatinine or bilirubin during that cycle after the start of therapy.

Serum Creatinine (mg/dL)	Serum Total Bilirubin	Dose (mg/m^2)
< 2	< 3	1500
> 2 (or an increase of > 0.5 mg/dL from baseline)	< 3	750
> 3	< 3	500
Any	> 3	500

Modified from Smith, et al. JCO 15:833

5.4.2 Dose Modification for Daunorubicin During Induction Cycle II- Arm A

Patients who do not achieve aplasia with Cycle I are eligible for a second cycle of induction. The total bilirubin should be determined on the days of the administration of daunorubicin. The dose of daunorubicin is adjusted according to the following table.

If Total Bilirubin	Administer % of Daunorubicin Dose
≤ 1.5 mg/dL	100%
1.5 to 3 mg/dL	75%
3.1 to 5 mg/dL	50%
> 5 mg/dL	No Daunorubicin
Reference: The Chemotherapy Source Book, second edition. Perry, M. Publisher: Williams and Wilkins	

5.4.3 Decitabine Arm F Maintenance

There are no planned dose reductions in decitabine. Patients unable to receive treatment on schedule (i.e. planned q4 weeks, but delays up to 4 weeks allowed) due to cytopenias or persistent side effects will discontinue decitabine and subsequently be observed per the 'observation ' group (Arm E), although they will be analyzed on an 'intention to treat' basis as having been assigned to Arm F.

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5.4.4 Clofarabine Dose Modifications – Arm B Induction and Re-induction, and Arm D

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5.4.4.1 Hematologic (Blood/Bone Marrow) Toxicity

Patients who achieve a CR or CRi should receive the consolidation cycle no sooner than 28 days from Day 1 of the previous induction or re-induction cycle, provided their peripheral blood counts have recovered (ANC $\geq 1.0 \times 10^9$/l and platelet count $\geq 100 \times 10^9$/l (or $\geq 50 \times 10^9$/l if previously achieved CRi). If the peripheral count recovery is delayed beyond Day 42 from Day 1 of the prior induction or re-induction cycle and the delay is presumed to be secondary to the induction or re-induction therapy, the clofarabine dose for the consolidation cycle, if administered, must be reduced by 25% for all subsequent doses.

5.4.4.2 Non-Hematologic Toxicity

<u>Dose Modifications During the Treatment Period (Intracycle Dose Modifications)</u>

Patients who experience a grade 3 or greater drug-related non-hematologic toxicity or asymptomatic grade 2 serum creatinine or serum total bilirubin during clofarabine administration should have clofarabine withheld until recovery to baseline or grade 1 before resuming treatment. Dose omissions due to the above noted toxicities that occur during the drug administration period will only be made up if the toxicity resolves such that a dose delay does not exceed 48 hours. If study drug administration resumes, proceed at 25% dose reduction for all subsequent doses and cycles of clofarabine. Only one interruption during study drug(s) administration is allowed per treatment cycle. Excluded from this rule are:

- Grade 3 or greater anorexia, rash/desquamation, infection, transient isolated elevations in hepatic transaminases (AST and ALT) or alkaline phosphatase do not require withholding or reducing clofarabine.
- Grade 3 or greater nausea, vomiting, diarrhea, or mucositis may require that the clofarabine be withheld. This decision should be guided by the investigator based on best clinical judgment. If clofarabine is being held it can be restarted if, for these toxicities, recovery to grade 2 or less occurs within 48 hours (with or without supportive care) These toxicities do not require dose reduction of clofarabine.

5.4.5 Dose Modifications for Subsequent Cycles of Study Treatment

Clofarabine doses for re-induction and consolidation cycles will be modified (delayed/reduced) or discontinued for non-hematologic toxicity according to the criteria listed in the table:

Criteria for Treatment Clofarabine Delays, Dose Reductions, and Discontinuations in Patients Experiencing Non-Hematologic Toxicities for Re-Induction and Consolidation Cycles

Non-Hematologic Toxicities	
Description of Event	**Treatment Delays, Dose Reductions, or Discontinuations**
Infection any grade	Re-induction Cycle: No delay is required. Consolidation Cycle: If a patient develops a clinically significant infection of any grade, consideration should be given to delaying the consolidation cycle until the infection is clinically controlled (defined as improvement of signs/symptoms related to the infection with appropriate antibiotics or other treatment)—however, the judgment ultimately rests with the investigator. Treatment (ie, subsequent cycle) may then resume without dose reduction. At the discretion of the investigator, prophylactic therapy to prevent recurrence of infection may be instituted as clinically indicated.
Drug-related non-infectious event(s): Grade 2 toxicity	Re-induction and Consolidation Cycles: Initiation of a treatment cycle will be delayed until returned to baseline or ≤ Grade 1. If this has not occurred by Day 56 from Day 1 of the treatment cycle, further study drug treatment cannot be administered. No dose reduction is required for subsequent cycles after recovery of Grade 2 toxicities to ≤ Grade 1.
Drug-related non-infectious event(s): ≥ Grade 2 neurologic toxicity	Re-induction and Consolidation Cycles: The patient's study drug doses are to be re-evaluated in consultation with the PI, and may be reduced or discontinued according to the parameters below defined for a drug-related Grade 2 or higher toxicity.
Drug-related non-infectious, non-neurologic event(s): Grade 3 toxicity	Re-induction and Consolidation Cycles: If toxicity recovers to baseline or Grade 1 by Day 56 from Day 1 of the treatment cycle, study treatment is to be restarted at a 25% dose reduction with no alteration in schedule for clofarabine for all subsequent cycles. If treatment has not resumed by Day 56 from Day 1 of the treatment cycle, further clofarabine cannot be administered.
Drug-related non-infectious event: Grade 4 toxicity	Re-induction and Consolidation Cycles: The patient must not receive additional clofarabine except under extraordinary circumstances and only after consultation with the PI. If study treatment is restarted, toxicity must recover to baseline or Grade 1 by Day 56 from Day 1 of the treatment cycle, and either a 25% or 50% dose reduction with no alteration in schedule for clofarabine should be instituted for all subsequent cycles.

5.5 Supportive Care

All supportive measures consistent with optimal patient care will be given throughout the study.

5.5.1 Cytarabine Consolidation- Arm C- Cycles 1 and 2

Patients must receive corticosteroid eye drops, 2 drops in each eye at least 4 times a day starting on day 1 before the first dose of cytarabine and continuing until at least 48 hours after completion of cytarabine, i.e. for a minimum of 8 days.

Cytarabine Age 60-69 1500 mg/m² as a one hour intravenous infusion every 12 hours for a total of 12 doses (days 1-6) times 2 cycles.

Age ≥ 70 1500 mg/m^2 as a one hour intravenous infusion every 24 hours for a total of 6 doses (days 1-6) times 2 cycles.

Corticosteroid 2 drops in each eye, 4 times a day for 10 days. The eye drops should be applied within one hour of the administration of high dose cytarabine.

GM-CSF 250 mcg/m²/day by intravenous or subcutaneous injection starting one day after the last dose of cytarabine through recovery of absolute neutrophil count (ANC) ≥ 500 cells/mcL is sustained for 3 consecutive days. The dose may be rounded to the nearest vial size.

OR
G-CSF 5 mcg/kg/day by either intravenous or subcutaneous injection starting one day after the last dose of cytarabine, through recovery of absolute neutrophil count (ANC) ≥ 500 cells/mcL is sustained for 3 consecutive days. The dose may be rounded to the nearest vial size.

OR
Pegfilgrastim 6mg SC on day 7 (1 day after completing the last dose of cytarabine).

5.5.2 Supportive Care with GM-CSF, G-CSF & Prophylactic Antibiotics for Restaging Bone Marrow

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Patients who achieve an aplastic BM following the 1st or 2nd course of induction therapy should receive cytokine support with GM-CSF (Leukine™) at a dose of
250 mcg/m^2/day subcutaneously every day until calculated ANC ≥ 5000/mcL or discharge from hospital. Alternatively, patients may receive G-CSF at a dose of 5 mcg/kg (rounded to the nearest vial size) at the discretion of the treating physician.

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Patients should receive an oral Quinolone, Fluconazole, and Acyclovir to prevent infection once the ANC drops below 1000 beginning sooner than day 7 of induction.

5.5.3 **Arm D** Clofarabine Consolidation

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Patients should receive an oral Quinolone, Fluconazole or equivalent based on institutional standard, and Acyclovir to prevent infection once the ANC drops below 1000 mm^3.

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GM-CSF 250 mcg/m²/day by intravenous or subcutaneous injection starting one day after the last dose of clofarabine through recovery of absolute neutrophil count (ANC) ≥ 500 cells/mcL is sustained for 3 consecutive days. The dose may be rounded to the nearest vial size.

OR

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G-CSF 5 mcg/kg/day by either intravenous or subcutaneous injection starting one day after the last dose of clofarabine, through recovery of absolute neutrophil count (ANC) ≥ 500 cells/mcL is sustained for 3 consecutive days. The dose may be rounded to the nearest vial size.

OR

Pegfilgrastim 6 mg SC on day 6 (1 day after completing the last dose of clofarabine).

5.5.4 **Arm G** Supportive Care for Allogeneic Transplant

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NOTE: Patients must be premedicated with acetaminophen 650 mg P0, diphenhydramine 25-50 mg P0/IV prior to thymoglobulin, and methylprednisone 1 mg/kg at initiation and midway through thymoglobulin administration each day.

Patients will be evaluated on an outpatient basis for evidence of toxicity, in particular graft vs. host disease (GVHD) and cytopenias.

GVHD Prophylaxis

Tacrolimus dosing is to be based on target serum levels of 5-10 ng/mL. **Serum levels are not to exceed 15 ng/mL.** Tacrolimus is to be initiated on Day -2 with a suggested starting dose of 0.03 mg/kg P0 BID (Institutional standard allowed if equivalent). Begin tapering between Day +90 to +120 in the absence of GVHD as tolerated with a goal of stopping by Day +150 to + 180. The rate of taper will be adjusted for the presence of signs and symptoms of GVHD. In patients who are unable to take oral tacrolimus, the intravenous dose is generally 1/4 to 1/3 of the oral dose.

Please note that concurrent use of agents such as itraconazole, voriconazole or fluconazole (at doses > 200 mg) may inhibit the metabolism of tacrolimus. and thus increase tacrolimus levels. Hence, it is recommended to check tacrolimus levels twice weekly when these agents are initiated concurrently. In addition, the initial dose of tacrolimus may be decreased according to institutional policies.

Methotrexate 5 mg/m^2/day IV on Days +1, +3, +6 and Day +11. Hydrate intravenously and induce diuresis. Methotrexate will be held if the serum creatinine > 3.0 mg/dL. If the creatinine level is > 1.5 mg/dL, then administer leucovorin 10 mg IV or P0 q6 hours for four doses beginning 24 hours after methotrexate.

Rabbit antithymocyte globulin (Thymoglobulin) at 2.5 mg/kg/day IV over 6 hours x 3 doses on Days -4 through -2. Patients must be premedicated with acetaminophen 650 mg P0, diphenhydramine 25-50 mg P0/IV, and methylprednisone 1 mg/kg at initiation and midway through thymoglobulin administration each day. After the first dose, subsequent administration of antithymocyte globulin may be infused over 4 hours.

Patients with progressive disease will have met the primary study endpoint and will be removed from protocol therapy. Such patients may receive additional treatment at the discretion of the investigator, although patients will be followed for response to further therapy, survival and new primary malignancies.

Mucosal Evaluation and Care: Mucositis is expected to be very mild with this chemotherapy regimen. Stomatitis and esophagitis due to herpes virus may be confused with drug-induced mucositis and viral cultures should be obtained frequently. Patients with a history of herpes simplex infection or seropositivity will receive acyclovir 200-400 mg PO TID Days -3 through Day + 100. Valacyclovir 500 mg P0 QD may be used instead of acyclovir. Prophylaxis may be extended beyond Day +100 at the discretion of the treating physician.

Candida Prophylaxis: Fluconazole or itraconazole 200-400 mg P0 daily or voriconazole 200-300 mg P0 twice daily (or 3-6 mg/kg IV q12 hours) on Days -2 through +100 per institutional standards. Low dose amphotericin B (10-20 mg/day) IV also may be used.

Pneumocystis Pneumonia (PCP) Prophylaxis: In an attempt to prevent PCP, cotrimoxazole (Bactrim®) will be administered to all patients as one double strength tablet BID on 2 days weekly, beginning on Day +28 through Day + 100. If, at this time. CD4 lymphocytes are < 200/μL, then prophylaxis should be continued until CD4 lymphocytes are ≥ 200/μL. Patients allergic to cotrimoxazole should receive dapsone or inhaled pentamidine instead. In patients who develop chronic GVHD, PCP prophylaxis should be extended at the discretion of the physician.

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CMV Infections: No routine prophylaxis for CMV will be initiated. Surveillance for CMV using CMV Ag (e.g.. immunofluorescence) or CMV PCR (or Digene Hybrid Capture® assay or equivalent) is required weekly beginning on Day +7 through Day +100, and then every other week through Day + 180. Patients with positive CMV PCR or positive CMV Ag'emia should receive treatment with ganciclovir 5 mg/kg IV BID x 14 days (or appropriate doses of valganciclovir or foscarnet).

DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

Tacrolimus

Tacrolimus may cause hypertension, renal insufficiency (usually reversible), seizures, liver function abnormalities, hemolytic uremic syndrome (rare), hyperglycemia, and hypomagnesemia.

Tacrolimus dose adjustments will be made to achieve target trough levels of 5-10 ng/mL. Serum levels are not to exceed 15 ng/mL.

Methotrexate

Methotrexate may cause mucositis and cytopenias. In the presence of worsening renal insufficiency or the development of effusions or ascites, the addition of leucovorin is permitted at the discretion of the

physician. **If the creatinine is > 1.5 mg/dL, leucovorin 10 mg IV or P0 may be given every 6 hours for four doses beginning 24 hours after methotrexate. Methotrexate will not be given if the creatinine is > 3.0 mg/dL. Individual doses of methotrexate will not be altered.**

Graft Versus Host Disease

It is expected that some patients will develop mild-moderate graft versus host disease. It is precisely this effect that may be associated with an antineoplastic outcome.

The diagnosis of acute graft versus host disease rests on clinical presentation (rash, diarrhea, liver function abnormalities) and histopathologic evidence via skin, gastrointestinal, or liver biopsy. Chronic GVHD is associated with rash, sicca syndrome, hepatitis. In addition grade 2-4 GVHD or extensive chronic GVHD will be treated with methylprednisone 2 mg/kg/day and subsequently tapered according to institutional standard. The ultimate treatment approach, i.e., the addition of cyclosporine, tapering of immunosuppressive medications, etc., will be left to the discretion of the transplant or hematology/oncology physician according to institutional standard allogeneic guidelines for the management of these conditions. The following tables should be used for reporting these toxicities.

Clinical Grading of Acute GVHD

Organ Grade	Skin Changes	Bilirubin (mg/dL)	Gut Changes (diarrhea [ml/day])
0	No rash	< 2.0	None
1	Erythematous macular rash over < 25% body surface	2 - <3.0	> 500 - ≤ 1000
2	Over 25-50% of Body surface	≥ 3 - <6	> 1000 - ≤ 1500
3	≥ 50% body surface	≥ 6 - < 15	> 1500
4	Bullae, exfoliation Ulcerative dermatitis	≥ 15	Severe abdominal pain with or without ileus

Organ Grade (see table above)			
Skin Changes	**Hepatic**	**Gut Changes**	**Overall Grade**
0	0	0	0
1 or 2	0	0	1
1, 2, 3	1	1	2
2 or 3	2 or 3	2 or 3	3
Patients with Grade 4 toxicity in any organ system are considered overall Grade 4.			

Clinical Grading of Chronic GVHD

Limited Chronic GVHD:

1. Localized skin involvement,

and/or

2. Hepatic dysfunction due to chronic GVHD.

Extensive Chronic GVHD:

1. Generalized skin involvement,

or

2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD

Plus

3a. Liver histology showing chronic aggressive hepatitis. bridging necrosis, or cirrhosis, **or**

3b. Involvement of eye (Schirmer's test with less than 5 mm wetting), **or**

3c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, **or**

3d. Involvement of any other target organ.

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Instructions for Dosing by Corrected Body Weight (Arm G)

High-dose chemotherapy can adversely impact the outcomes of obese patients when dosing is performed according to actual body weight. **Therefore, all therapy (including methotrexate and tacrolimus) and growth factor drug doses will be determined using a corrected body weight formula. The corrected body weight is calculated based on the following formula with all weights in kg:**

Corrected Weight = (0.25)(actual weight - ideal weight)+(ideal weight)

Thus, for patients whose actual weight is > 150% of ideal, their "actual" weight will be capped at 150% of ideal (i.e., their corrected weight will be 112.5% of ideal). For patients whose actual weight is less than ideal, use their actual weight as the corrected weight.

5.5.4.1 Colony Stimulating Factors

All patients should receive G-CSF 5 mcg/kg SC daily from day 12 until achievement of an ANC of > 1.5 x 10^9/lt.

5.5.4.2 Antimicrobial Supportive Care

Antibacterial, antifungal and antiviral prophylaxis and treatment should follow conventional guidelines or institutional protocols. Patients should receive an oral Quinolone, Fluconozole, and Acyclovir to prevent infection once the ANC drops below 1000 mm^3.

Management of immunosuppression and donor lymphocyte infusions: Therapy will be directed to promote engraftment, and minimize GVHD without undue immunosuppression.

5.5.4.3 Treatment of Acute GVHD

Patients who develop Grade 2 or greater acute GVHD will receive methylprednisolone or prednisone at least 2 mg/kg daily and subsequently tapered according to institutional standard. Other immunosuppression may be administered as needed.

5.5.4.4 Immunosuppression

Patients with < 5% engraftment by day 35 may have immunosuppression tapered early in accordance with institutional standards.

5.5.4.5 Treatment of Residual Disease

Patients with evidence of residual disease as defined by clinical, radiological, cytogenetic or molecular techniques may have immunosuppression tapered rapidly and may subsequently receive treatment with 10^6 to 10^8 CD3+ donor lymphocyte cells/kg ("donor lymphocyte infusion") as often as every 6-12 weeks for up to 3 courses at the discretion of the treating physician and in accordance with institutional standards.

If a patient experiences a creatinine > 3, Tacrolimus will be decreased whenever possible. If in the judgment of the primary investigator Tacrolimus cannot be maintained at an adequate level then other immunosuppression should be added.

5.5.4.5.1 GVHD Prophylaxis

Standardizing GVHD prophylaxis regimen will be utilized comprising ATG, Tacrolimus, and methotrexate. See Section 5.5.

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5.5.4.5.2 Tacrolimus Administration

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Tacrolimus will be started 2 days before the marrow infusion at a dose of 0.03 mg/kg/day intravenously infused continuously over a period of 24 hours (Institutional standard allowed if equivalent). IV tacrolimus can be discontinued when patient starts to eat and PO tacrolimus substituted at a dose four times the IV dose (0.12 mg/kg) in two divided doses. Monitor blood concentrations to keep in therapeutic range per institutional guidelines. Unless toxicity is encountered, tacrolimus can be

continued at the same dose until day 100, after which the drug can be tapered and discontinued on day 180 after grafting.

5.5.4.5.3 Methotrexate Administration

Methotrexate is to be administered at doses of 5 mg/m² I.V. on days 1, 3, 6 and 11.

5.6 Duration of Therapy

Patients will receive protocol therapy unless:

- Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued. In this event submit forms according to the instructions in the E2906 Forms Packet.
- Patient withdraws consent.

5.6.1 Induction

The induction phase is considered to include the period of time from when a patient is enrolled on trial until one of the following endpoints:

- Death.
- Failure to achieve marrow aplasia after two courses of induction therapy or after one course of induction and judged to be unable to receive any further therapy.
- Achievement of CR and discharge from the hospital. Any repeat hospitalization within two weeks of induction needs to be reported.
- Patients who fail to experience recovery of peripheral blood counts to remission status by day 56 will undergo a repeat marrow aspirate and biopsy. Patients should be followed, at a minimum, with weekly CBCs until it is determined that there is recovery of peripheral blood counts, refractory AML, or death.
- Patient withdraws consent.
- A toxicity which in the opinion of the investigator precludes further protocol therapy. The patient should be followed until resolution of the toxicity.

5.6.2 Consolidation Cycle 1

Consolidation I is considered to include the period of time from when a patient receives therapy until one of the following endpoints:

- Death
- Recovery of peripheral blood counts and discharge from the hospital. Any repeat hospitalization within two weeks of discharge after Consolidation I needs to be reported

Patients who do not begin Consolidation II within 60 days of recovery are ineligible for further protocol therapy.

5.6.3 Consolidation Cycle 2

The Second Consolidation Cycle is considered to include the period of time from when a patient receives therapy until one of the following endpoints:

- Death.
- Recovery of peripheral blood counts and discharge from the hospital. Any repeat hospitalization within two weeks of discharge after Consolidation II needs to be reported.

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5.7 Duration of Follow-up

Surviving patients will be followed for at least 5 years. Following completion of consolidation, all patients will have a CBC performed monthly ***as well as a BM biopsy/aspirate performed every 3 months, for the first 24 months*** (including 12 months of observation or maintenance decitabine in 2nd randomization), then q3 months for ***the next*** 2 years, then q6 months. Following 5 years, patients will have a CBC performed annually and followed for survival.

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed for response until progression, even if non-protocol therapy is initiated, and for survival for 5 years from the date of registration. All patients must also be followed through completion of all protocol therapy.

6. Measurement of Effect

6.1 Complete Remission (CR)

Requires that all of the following be present:

6.1.1 Peripheral Blood Counts

6.1.1.1 Neutrophil count $\geq$ 1.0 x 10^9 /L.

6.1.1.2 Platelet count ≥ 100 x 10^9 /L.

6.1.1.3 Reduced hemoglobin concentration or hematocrit has no bearing on remission status.

6.1.1.4 Leukemic blasts must not be present in the peripheral blood.

6.1.2 Bone Marrow Aspirate and Biopsy

6.1.2.1 Cellularity of bone marrow biopsy must be > 20% with maturation of all cell lines.

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6.1.2.2 < 5% blasts by morphologic review.

6.1.2.3 Auer rods must not be detectable.

6.1.3 Extramedullary leukemia, such as CNS or soft tissue involvement, must not be present.

6.2 Complete Remission incomplete (CRi)

All the same response criteria in peripheral blood and bone marrow as CR with the exception that there is incomplete platelet recovery (platelets > 50 x 10^9/L independent of platelet transfusions) over a period of observation without evidence of ongoing platelet recovery and no sooner than day 35 after the preceding cycle of treatment.

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6.2.1 Patients who achieve criteria for CR or CRi and who have recovered from the effects of therapy but who have **< 20% cellularity in BM** will be deemed a "Hypocellular CR/CRi" and will be allowed to continue protocol therapy at the discretion of the Investigator and after discussion with the Study Chair as long as they meet other eligibility criteria for Step 2

6.3 Partial Remission (PR)

6.3.1 Requires that all of the criteria for complete remission be satisfied except that the bone marrow may contain > 5% blasts but < 25% blasts.

6.3.2 If all other criteria for CR are met, then a value of ≤5% blasts with Auer rods or abnormal morphology is considered a partial remission.

6.4 Relapse

Relapse following complete remission is defined as:

6.4.1 Peripheral Blood Counts

6.4.1.1 Reappearance of blasts in the blood.

6.4.2 Bone Marrow Aspirate and Biopsy

6.4.2.1 Presence of > 5% blasts, not attributable to another cause (e.g., bone marrow regeneration).

6.4.2.2 If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, then a repeat bone marrow performed ≥ 1 week later documenting more than 5% blasts is necessary to meet the criteria for relapse.

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6.5 Leukemia Free State

6.5.1 Peripheral Blood Counts

6.5.1.1 No circulating blasts in peripheral blood by morphologic review.

6.5.2 Bone Marrow Aspirate & Biopsy

6.5.2.1 < 5% blasts, without clustering of blasts, and without Auer rods by morphologic review.

6.5.3 Flow Cytometry of BM Aspirate

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6.5.3.1 If one or more unique leukemia-associated immunophenotypes (LAIP) were identified at diagnosis, minimal residual disease (MRD) levels will be analyzed by multiparameter flow cytometry.

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6.5.3.2 Immunologic MRD must be below the level of detection (< 0.001) to qualify for “Leukemia-Free State”.

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6.5.3.3 Any detectable immunologic MRD levels will be quantified and retrospectively correlated with relapse-free and overall survival.

6.5.4 Extramedullary leukemia must not be present.

6.5.5 If indeterminant, the BM Aspirate & biopsy should be repeated 1 week later.

6.6 Quality of Life Measurement

6.6.1 Assessment Battery

Patient reported outcomes measuring health related quality of life, physical and functional well-being, and fatigue are important components of a clinical trial. As the population of interest is elderly, it is also appropriate to perform a comprehensive geriatric assessment. Moreover, as the leukemia experience results in unique issues and concerns for patients, it is useful to choose an instrument that captures these unique issues.

6.6.2 Health-related Quality of Life

QOL will be assessed using the Functional Assessment of Cancer Therapy – Leukemia. This instrument combines the General version of the Functional Assessment of Cancer Therapy (FACT-G) with a

leukemia-specific subscale [100]. The FACT-G is intended to be a self-administered questionnaire that assesses four QOL domains: physical, social/family, emotional, and functional well being [101]. The FACT has been tested for reliability and validity. Test-retest correlation coefficients ranged from .82 (emotional well-being) to .88 (functional well-being) and .92 (total score) [101]. Convergent and discriminant validity were evaluated as well as construct validity. The FACT has good reliability and validity data, and has been shown to discriminate patients based on their stage of disease and performance rating as well as demonstrated sensitivity in detecting changes in QOL over time.

For this study, the Leukemia subscale will also be added to the FACT-G [100]. Comprised of 17 items, this subscale examines issues and concerns typically experienced by the leukemia patient population, such as “I am bothered by fevers; I worry about getting infections; and I feel isolated from others because of my illness or treatment.” The Leukemia subscale is scored in the same manner as the FACT-G, so that higher scores reflect greater well-being; scores range from 0 – 68. Internal consistency is good (Cronbach’s alpha 0.86-0.88). The Leukemia subscale was also successful in discriminating patients based on ECOG Performance status ratings, including the ability to detect changes in performance status over time.

The FACT Fatigue Scale has been developed by Cella and colleagues to assess the impact of fatigue on the multiple dimensions of QOL and will provide additional important QOL data in these patients [102]. The FACT Fatigue scale has been tested for reliability and validity with good results. Internal consistency was strong (alpha coefficient = .93-.95); convergent and discriminant validity were demonstrated by a strong positive relationship with other measures of fatigue and a strong negative relationship with vigor.

6.6.3 Comprehensive Geriatric Assessment

The abbreviated Comprehensive Geriatric Assessment (aCGA) is a brief, 15-item instrument developed from instruments used in the Comprehensive Geriatric Assessment: [103]. Specific items taken from known valid and widely used instruments: the Geriatric Depression Scale, the Activities of Daily Living, Instrumental Activities of Daily Living, and Mini-mental Status Examination; thus functional, emotional, and cognitive domains are represented. Specific item selection was based on highest item-to-total correlations in a retrospective review of 500 elderly cancer patients who had a Comprehensive Geriatric Assessment performed. Cronbach’s α was 0.70-0.93. The aCGA is designed to function as a screening tool for use in the outpatient setting; patients who test positive should have more comprehensive testing [104]. Feasibility within the context of a clinical trial is high and can better capture the functionality of this patient population beyond the inclusion and exclusion criteria for this clinical trial. Moreover, it takes only 5 minutes to complete. For the proposed study, the aCGA will be augmented with the following

components: a brief nutritional assessment, social support assessment, co-morbidity, and sexual function.

6.6.4 Nutritional Status

Body Mass Index, a well-known and clinically used tool will be used to assess nutritional status for this trial. In a study of 214 elderly adults living in a community setting, a low BMI (< 22 kg/m^2) was associated with dependency in ADLs and decreased survival at one year [105].

6.6.5 Unintentional Weight Loss

The percentage of unintentional weight loss is well documented as a prognostic factor in the context of chemotherapy treatment. Such weight loss occurring during the six months proceeding chemotherapy treatment was associated with a lower response rate to treatment, decreased performance status, and poorer survival [106,107].

6.6.6 Social Support

Medical Outcomes Study Social Support Survey: Emotional/Information and Tangible subscales

These subscales from the MOS Social Support Survey [108] assess the expression of empathetic understanding and positive affect; the offering of information, advice, feedback, or guidance (emotional/information) and behavioral assistance or access to material aid (tangible). Twelve items, they are two of four subscales from the larger instrument. They have excellent internal consistency (Cronbach alpha ? 0.90); convergent validity was also demonstrated. All but one item uses a 5-point Likert scale. These scales were chosen as they are brief, relevant, and moreover, are part of the comprehensive cancer-specific geriatric assessment battery developed by Hurria and colleagues [109].

6.6.7 Co-Morbidity

The Comorbitiy Questionnaire [110]. This instrument is a modification of the well-established Charlson Comorbidity Index (CCI) [111]. Designed to be a self-administered questionnaire; it identifies several medical conditions commonly encountered in the elderly patient population. It is scored like the CCI, which reflects not only the number of conditions, but the seriousness of each. Test-retest reliability was 0.91 (and 0.92 for the CCI [110]. The scale correlated significantly with health professionals' ratings via the CCI (Spearman correlation = 0.70) with item-specific agreement 90 – 100%, excluding the presence of tumor [110]. This instrument has not been extensively used in the elderly, nor in the oncology patient population, but the original Charlson Comorbidity Index has [112-114]. The CCI has demonstrated predictive value for evaluating tolerance for cancer treatment [112,113] an, in some studies, cancer survival [114]. For this study, the instrument will be modified to include some additional commonly occurring conditions (e.g. back pain, osteoporosis, depression, anemia) as well as some that commonly cause complications with AML management (chronic sinus infection, poor

dentition). The questionnaire will be further modified by asking the respondent to identify the number of medications taken, both prescribed and over-the-counter.

6.6.8 Sexual Function

Clinically, it has been observed that elderly AML patients who remain sexually active may better tolerate the rigor associated with induction therapy [115]. A literature review did not provide any data to support or refute this observation. Therefore, two additional questions related to sexual activity will be added to the Geriatric Assessment Battery but scored separately to address this important question.

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6.7 Timing of QOL Assessment

The Geriatric Assessment Battery will be administered at the time of randomization for a baseline assessment. This battery includes the aCGA, Body Mass Index, Unintentional Weight Loss, MOS Social Support Survey (Emotional/Information and Tangible Subscales only), Comorbidity Questionnaire and Sexual Function.

The FACT-Leukemia and FACT –Fatigue instruments will be administered at the following time points:

T1: Baseline before induction. (Beginning Step 1)
T2: At day 14 (+/- 2 days) following 1st induction. (Nadir of Step 1)
T3: Following the outcome restaging BM (day 35-56) prior to consolidation therapy. (End of Step 1, beginning Step 2)
T4: At the beginning of randomization to Arm E or F. (Beginning Step 3)
T5: At the end of the observation/maintenance decitabine (i.e. after 12 months) after randomization to Arm E or F). (End of Step 3)

NOTE: QOL is to be assessed at any time the patient is removed from or elects to withdraw from the study

For those patients who are eligible and elect to go on to transplant, the FACT-Leukemia and FACT Fatigue instruments will be administered at the following time points:

$T1_{tx}$: At the beginning of the conditioning regimen
$T2_{tx}$: 100 days (± 14 days) post transplant

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6.8 Timing of "ALESE" Questionnaire (see Appendix V)

The ALESE questionnaire will be administered at patient registration, or during the first week of induction therapy, whenever possible.

Administration of the ALESE questionnaire at later dates is permissible in surviving patients.

NOTE: Return instructions for sites are in Appendix V on page 34.

7. Study Parameters

7.1 Therapeutic Parameters

1. Prestudy scans and x-rays used to assess all measurable or non-measurable sites of disease must be done within **4 weeks** prior to randomization/registration.
2. Prestudy CBC (with differential and platelet count) should be done ≤ **4 weeks** before randomization/ registration.
3. All required prestudy chemistries, as outlined in Section 1.6, should be done ≤ **4 weeks** before randomization/registration – unless specifically required on Day 1 as per protocol.

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4. The diagnostic bone marrow aspirate must be obtained within two weeks prior to randomization.
5. The required prestudy chemistries, as enumerated in Section 3, should be obtained within forty-eight hours prior to randomization. Please note that the dose of daunorubicin is adjusted, for cycle 2 induction only, based upon the total bilirubin as indicated in Section 5.4.2.

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6. Prestudy CBC should be repeated ≤ 48 hours prior to randomization.
7. The cardiac ejection fraction will be determined prior to induction chemotherapy, and prior to re-induction (if required). The cardiac ejection fractions should be determined by either MUGA or echocardiography throughout the study.

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	Induction & Re-Induction			Consolidation I and II		Maintenance Observation vs. Decitabine	Follow-Up		
	Prior	Daily	Weekly	Prior	Daily	Monthly	Monthly	Quarterly	Annually
History and Physical	X	X[1]		X[1]	X[1]	X[2]	X[12]		
Weight, Height (baseline)	X	X[1]		X[1]	X[1]				
CBC	X[1]	X[1]		X[1]	X[1]	X[2]	X[16]		
HLA Typing	X[11]								
Serum Potassium, Calcium, Creatinine, Serum Phosphorus, Serum Uric Acid		X[10]							
Serum Creatinine, Uric Acid, Bilirubin, SGPT, (ALT) LDH, Alk Phosphatase, SGOT (AST) Mg	X	X[3]	X[3]	X[3]	X[3]				
EKG	X			X					
Bone Marrow Aspirate, Biopsy and Cytogenetics [9,4]	X		X[4]	X[19]		X[8]		X[17]	X[13]
Cardiac Ejection Fraction (MUGA/Echocardiogram)	X[5,7]			X[7]		X[7]			
Cerebellar Neuro Checks					X[6]				
QOL[14]	X	X[15]				X			
Geriatric Assessment[18]	X								
ALESE Questionnaire	X[20]								

1 The daily evaluations should be performed until discharge from the hospital. If the patient is discharged prior to recovery of ANC or platelet count the values should be obtained no less than twice weekly until the outcome BM is performed

2 It is required that complete blood counts be obtained every 4 weeks for the first twenty-four months after the conclusion of consolidation therapy. Complete blood counts should be obtained every three months in the third year. The frequency in subsequent years is at the discretion of the investigator.

3 During the administration of daunorubicin or high-dose cytarabine the serum creatinine and total bilirubin are required daily to adjust dose of medications. After the administration of chemotherapy the values must be determined and reported at least weekly or more frequently as clinically indicated.

4 A bone marrow is required on day 12-14 (+/- 1 day) to assess whether the patient has achieved an aplastic BM and will receive GM-CSF or G-CSF or a repeat course of induction. Subsequent marrows until recovery of peripheral blood counts are at the discretion of the treating physician. An outcome BM must be performed no sooner than day 28 and no later than day 56 to determine response to induction therapy at the time of recovery of ANC to >1000 & platelets (>100,000) or if the patients is suspected to have residual AML, or if no further recovery is expected (e.g. plateau in platelet recovery).

5 If the cardiac ejection fraction cannot be obtained due to weekend or holiday then patients may be enrolled provided there are no clinical signs or there is no history of significant cardiovascular disease and a measurement of cardiac ejection fraction will be performed within 5 days of study enrollment.

6 Prior to each dose of cytarabine during consolidation for patients randomized to Arms A and C.

Rev. 2/13 7 The Cardiac Ejection Fraction must be measured prior to Induction. For patients on Arm A, a repeat measurement is recommended before receiving re-induction (if indicated). Repeat measurement prior to Consolidation Cycle 1 (Arms C & D) is recommended per institutional guidelines and as clinically indicated. For **patients who proceed to Arm G (Allogeneic stem cell transplantation) a repeat measurement of Cardiac Ejection Fraction must be performed after recovery from** last cycle of chemotherapy and prior to start of transplant conditioning regimen

Rev. 2/12
Rev. 2/13 8 Routine bone marrow aspirate and biopsy is required every 3 months during maintenance and observation (Arms E and F) for first 24 months.

Rev. 12/13 9 A repeat bone marrow to confirm the maintenance of a CR prior to Consolidation II is not required provided a complete blood count with differential and platelet count remains within the range anticipated during a CR. Bone marrow (or peripheral blood if bone marrow is unavailable) should be sent to the institution's local cytogenetics laboratory for analysis. Please refer to the table in Section 7.3 for the time points for the submission of karyotypes for central review.

10 Monitoring for tumor lysis syndrome at the initiation of induction therapy is strongly suggested at least every 8-12 hours for at least the first 3-5 days of treatment, and at further intervals at the discretion of the investigator.

Rev. 2/13 11. HLA -typing (Class I & Class II) should be performed prior to therapy on all patients and on siblings who are eligible donors. The number of full siblings with ages and reason for ineligibility (including refusal) must be noted.

12. Physical only

13. If clinical or hematological evidence of relapse.

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12/13 14. QOL must be presented to all patients that consented to the QOL componentat the following time points:

- Baseline before induction. (Beginning Step 1)
- At day 14 (+/- 2 days) following 1st induction. (Nadir of Step 1)
- Following the outcome restaging BM (day 35-56) prior to consolidation therapy. (End of Step 1, beginning Step 2)
- At the beginning of randomization to Arm E or F. (Beginning of Step 3)
- At the end of the observation/maintenance decitabine (i.e. after 12 months after randomization to Arm E or F). (End of Step 3)

NOTE: QOL is also to be assessed at the time a patient is removed from or elects to withdraw from the study.

Rev. 12/13,
7/14 15. QOL should be done at day 14 nadir and following the outcome restaging BM (day 35-56). These must presented to all patients that consented to the QOL component of the protocol.

16. Monthly after 2nd randomization for 2 years, every 3 months for 2 years, every 6 months for a year and annually afterwards up to five years.

17. A surveillance BM aspirate and biopsy every 3 months for 2 years beginning at the time of the second randomization, and after that, when clinically indicated (e.g., symptoms of relapse or abnormal CBC).

18. Geriatric Assessment will be measured at Baseline before Induction.

19. BM required prior to consolidation cycle 1 only.

Rev. 2/13 20. ALESE: "Acute Leukemia Epidemiology and Survival in ECOG-ACRIN" Questionnaire should be administered early during induction therapy, preferably in the first week whenever possible. (See Section 1.6.6, 6.8, and Appendix V). For instructions on returning the questionnaire, please refer to Appendix V, page 34.

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7.2 Arm G Only (Allogeneic Stem Cell Transplantation)

	Prior to AlloSCT	Twice a week to Day +28 Weekly to Day +100 Monthly to Day +365	Restaging*	Post-treatment Follow-Up**
Tests & Observation				
History & Progress Notes	X	X	X	X
Physical Examination	X	X	X	X
Vital Signs & Weight	X	X	X	X
BSA	X			
ECOG PS	X	X	X	X
Toxicity Assessment		X	X	X
QOL[1]				
Lab Studies				
CBC, Diff, Plts	X	X	X	X
Serum creatinine, BUN	X	X		X
Creat. Clearance & Urinalysis	X			
Serum Electrolytes	X	X		
AST, Alk Phos, Bilirubin	X	X		X
Total Protein, Albumin	X			
EKG	X			
MUGA or Echo	X			
PFT (Lung Function)	X			
Hepatitis screen, CMV, HIV, EBV, HSV	X			
CMV Ag or PCR		A		
Tacrolimus		D		
Serologies & HLA Typing (donor & recipient)	X			
Staging				
CXR (PA/Lat)	X			
BM Asp. & Biopsy	X		C	X[2]
Chimerism Samples (+/- 7 days)	B	B	B	B

* Restaging will occur between days +90-100, and +150-180, then q3 months for the 1st year.

** After restaging, at least q6 months for a maximum of 5 years from AlloSCT.

1. QOL will be performed at the following time points: At the beginning of the conditioning regimen and 100 days (+/- 14 days) post transplant- TOTAL 2 time points.

2. Within 1 month after Allo SCT

A Weekly beginning day +7 through day +100, then every other week until day +180.

B Samples to be collected prior to study (from both donor & recipient), and form recipient on days+30, +60, +90, +120, +180, +270, +365, and at relapse.

C BM aspirate & biopsy (& cytogenetics & flow cytometry) will be required at days +90-100, and day +150-180, then q3 months for 2 years, then as clinically indicated.

D Twice weekly beginning days -2 until day+28, then weekly until day +150 or until drug stopped.

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7.3 Biological Sample Submissions

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1. Peripheral blood, bone marrow, and smears must be submitted for centralized immunophenotyping during pre-registration for eligibility testing
2. Karyotypes must be submitted at baseline for central review as outlined in Section 10.

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3. Follow-up karyotypes, smears, bone marrow, and peripheral blood must be submitted for review.

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4. Peripheral blood and buccal swabs/rinse are to be submitted for banking per patient consent.

NOTE: It is required that biological sample submissions be logged into the ECOG-ACRIN Sample Tracking System (STS) (see Section 10.6) for purposes of monitoring compliance.

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	Pre-registraion	Step 1 - Randomization (Baseline)[1]	Time of Outcome Following Induction (Prior to Consolidation)[8]	End of Consolidation (Prior to 2nd Randomization - Step 3)[4]	Relapse	Send To
MANDATORY – *Submissions required for central review*						
Karyotypes		X	X	X	X	Cyto lab[2]
Bone Marrow/ Peripheral Blood Smears (Wright-Giemsa stained)	X		X	X	X	LTSL[3]
Bone Marrow Aspirate (heparin, FIRST PULL)[6,9]	X		X	X	X	
Peripheral Blood (heparin, green or EDTA purple top tubes, (4) 10 mL tubes, 30-40 mL)[6]	X		X	X	X	
From patients who answer "YES" to "*I agree to provide additional specimens for research.*"						
Peripheral Blood (red top tubes, (2) 10 mL tubes, 15-20 mL)		X	X	X	X	LTSL[3]
Buccal Rinse (preferred) or Swab[7]		X				LTSL[3]

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1. Pre-study bone marrow, peripheral blood, and smears must be submitted for centralized immunophenotyping to determine patient eligibility. This testing is mandatory for participation in this study.
2. Submit to the ECOG-ACRIN Cytogenetic laboratory at Mayo Clinic.
3. Submit to the ECOG-ACRIN Leukemia Translational Studies Laboratory (LTSL). Signed E2906 patient consents and HIPAA authorizations must be submitted to the LTSL prior to or at time of submission of pre-registration specimens to the LTSL.
4. At time of evaluation response.

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5. [Deleted in Addendum #8]

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6. Bone marrow and peripheral blood will also be used for the optional correlative studies per patient consent.

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7. Buccal rinse (preferred) or swabs are strongly encouraged to be collected at baseline, but can be collected at any other time during the study, if necessary.

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8. At time of response assessment or off-study, whichever occurs first.

9. The laboratory will accept any amount as long as it represents a first pull. It is imperative that aspirate from a separate first pull be submitted. DO NOT SUBMIT THE SECOND OR THIRD PULL OF ASPIRATE FROM THE SAME ASPIRATION SITE.

8. Drug Formulation and Procurement

8.1 Cytarabine

8.1.1 Other Names

Cytosar-U™, Ara-C, Arabinosyl, cytosine arabinoside.

8.1.2 Classification

Antimetabolite.

8.1.3 Mode of Action

Converted to cytarabine triphosphate (Ara-CTP), a competitive inhibitor of DNA polymerase. The drug is also incorporated into cellular DNA and RNA. It is active against cells in S-phase and is considered to be phase specific.

8.1.4 Storage and Stability

The dry powder is stored at room temperature. Solutions reconstituted with sterile water without preservative should be used immediately; solutions reconstituted with Bacteriostatic of Water are stable up to 48 hours at controlled room temperature (15° to 30°C). Solutions with a slight haze should be discarded.

8.1.5 Dose Specifics

Induction Arm A

100 mg/m²/day by 24 hour continuous infusion for 7 days (days 1-7).

Consolidation I

1500 mg/m² as a one hour intravenous infusion every 12 hours for a total of 12 doses (days 1-6) for patients < 70 years of age, or a total of 6 doses for patients ≥ 70 years of age. See Section 5.4.1 for dose modifications for Consolidation I. See Section 5.6.3 for duration of Consolidation II therapy.

8.1.6 Preparation

For IV use, reconstitute the 100 mg vial with 5 mL bacteriostatic water for injection to achieve a concentration of 20 mg/mL. Add 10 mL of bacteriostatic water to the 500 mg vial to achieve a final concentration of 50 mg/mL. Add 10 and 20 mL of bacteriostatic water to the 1 and 2 gm vials respectively to achieve a final concentration of 100 mg/mL. For subcutaneous use, reconstitute the powder with sterile water or saline to a concentration of 50-100 mg/mL. For IT use, mix with lactated Ringer's solution or normal saline without preservatives.

8.1.7 Administration

IV push, IV continuous infusion, subcutaneous, or IT. Cytarabine is not absorbed when given orally.

8.1.8 Incompatibilities

Possible interaction with fluorouracil.

8.1.9 Compatibilities

Cytarabine (0.25 mg/mL), daunorubicin (0.03 mg/mL) and etoposide (0.4 mg/mL) are stable in D5/0.45% NaCl for 72 hours at room temperature. Cytarabine is also compatible with sodium chloride, potassium chloride, calcium, and magnesium sulfate.

8.1.10 Availability

Commercially available in 100 mg, 500 mg, 1 gm, and 2 gm vials.

8.1.11 Side Effects

1. Hematologic: Leukopenia, thrombocytopenia, anemia, and phlebitis. Nadir occurs in 5-7 days with recovery in 2-3 weeks.
2. Dermatologic: Rash, alopecia.
3. Gastrointestinal: Nausea, vomiting, diarrhea, dysphagia, mucositis, anorexia.
4. Hepatic: Transient increase in liver enzymes.
5. Renal: Urinary retention.
6. Other: Flu-like syndrome, fever. Profound hyperuricemia may occur in leukemia patients with high white blood counts. Conjunctivitis, dizziness, shortness of breath, headache, urticaria, pruritis, abdominal pain, pericarditis.
7. After intrathecal administration, the most common side effects are nausea, vomiting, fever, and headache, usually mild and self-limiting. Meningism, paresthesia, paraplegia, seizures, blindness, necrotizing encephalopathy have occurred.

8.2 Daunorubicin

8.2.1 Other Names

Daunomycin, Rubidomycin, Cerubidine.

8.2.2 Classification

Anthracycline antibiotic.

8.2.3 Mode of Action

Anthracycline mechanism of action results in a very tight binding of the drug to the DNA molecule. The ultimate effect is interference with nucleic acid synthesis, both RNA and DNA.

8.2.4 Storage and Stability

Intact vials are stored at room temperature and protected from direct sunlight. Reconstituted solutions are stable for 48 hours when refrigerated and 24 hours at room temperature, when protected from sunlight.

8.2.5 Dose Specifics Induction Arm A

60 mg/m²/day by 10-15 minute intravenous infusion for 3 days (days 1, 2 and 3).

NOTE: No dose modification during initial induction. See Section 5.4.2 for dose modification during induction cycle II.

Doses should be reduced in presence of impaired hepatic or renal function. Patients with serum bilirubin of 1.5 to 3 mg/dl should receive 75% of the usual dose; patients with serum bilirubin or serum creatinine 3.1 to 5 mg/dl should receive 50% of the usual dose.

8.2.6 Preparation

Each 20 mg vial is reconstituted with 4 mL of sterile water to give a final concentration of 5 mg/mL. The desired dose is drawn into a syringe containing
10-15 mL of normal saline. Protect from sunlight.

8.2.7 Administration

10-15 minute intravenous infusion.

8.2.8 Incompatibilities

Sodium heparin.

8.2.9 Availability

Commercially available in 20 mg glass vials of red colored lyophilized drug. Also available in 50 mg vials.

8.2.10 Side Effects

1. Hematologic: Myelosuppression (leukopenia with a nadir between 1-2 weeks).
2. Dermatologic: Rash; alopecia; chemical thrombophlebitis or local necrosis if extravasation occurs.
3. Gastrointestinal: Nausea, vomiting, commonly occurring one hour after a dose and lasting for several hours; diarrhea, stomatitis.
4. Cardiovascular: Arrhythmias, usually transient; congestive cardiomyopathy; maximum total (lifetime) dose of 500-600 mg/m² is recommended because of cumulative cardiotoxicity.
5. Renal: Red urine; not hematuria.
6. Other: Fever; transient elevations in serum bilirubin, AST, alkaline phosphatase.

8.2.11 Nursing Implications

1. Vesicant - avoid extravasation. Refer to extravasation protocol if inadvertent infiltration occurs.
2. Monitor CBC, platelet counts.
3. Advise patient of red coloration of urine.
4. Administer antiemetics as needed.

8.2.12 References

Von Hoff DD, Rozencweig M, Layard M, *et al.* Daunomycin-induced cardiotoxicity in children and adults: A review of 110 cases. Am J Med 1977; 62:200-208.

Yates J, Glidewell O, Wiernik P, *et al.* Cytosine arabinoside with daunorubicin or Adriamycin for therapy of acute myelocytic leukemia: A CALGB study. Blood 1982; 60:454-462.

8.3 Decitabine

8.3.1 Other Names

Dacogen®, 5-aza-2'-deoxycytidine, 5-aza-CdR

8.3.2 Classification

Antineoplastic, DNA hypomethylating agent

8.3.3 Mode of Action

A cytosine nucleoside analog.The drug inhibits DNA methyltransferase at low doses promoting DNA hypomethylation and reactivation of previously silenced genes. At higher doses, the drug has a direct cytotoxic effect. It is an S-phase specific agent.

8.3.4 Storage and Stability

Intact vials are stored at room temperature. Unless used within 15 minutes of reconstitution, diluted decitabine solution must be prepared using cold (2° to 8°C) infusion fluids and stored at 2° to 8°C for up to a maximum of 7 hours until administration.

8.3.5 Dose Specifics

Decitabine 20 mg/m²/day IV over 1 hour QD x 3 days, repeated Q 4 weeks x 12 months total. See Section 5.4.3 for duration.

In the absence of a hypoplastic marrow (5% or less cellularity), clearly progressive increase in bone marrow blast count, febrile neutropenia, or unresolved systemic neutropenic infection, dosing should be continued at the recommended dose without delay. In the event of delayed recovery to pre-dose ANC and platelet counts for more than two cycles, the dose should be reduced by approximately 25% upon restarting therapy.

8.3.6 Preparation

Each vial should be reconstituted with 10 mL of sterile water giving a final concentration of 5 mg/mL. Immediately after reconstitution, the solution should be further diluted with 0.9% sodium chloride injection, 5% dextrose injection or lactated ringer's injection to a final drug concentration of 0.1-1 mg/mL. Unless used within 15 minutes of reconstitution, the diluted decitabine solution must be prepared using cold (2°C to 8°C) infusion fluids and stored at 2°C to 8°C (36°F-46°F) for up to a maximum of 7 hours until administration.

8.3.7 Route of Administration

Intravenous infusion over 1 hour.

8.3.8 Compatibilities

Compatible with 0.9% sodium chloride injection, 5% dextrose injection or lactated ringer's injection.

8.3.9 Availability

Decitabine is an investigational agent available free of charge from Eisai Inc. and distributed by Fisher Clinical Services. Decitabine will be available in 50 mg vials. **Decitabine should only be requested for patients who are treated on Arm F of the Maintenance step.**

Initial Drug Orders for Each Patient

Following randomization to Arm F a supply of Decitabine may be ordered. Investigators must email a completed E2906 Decitabine Study Drug Request Form (See Appendix IV) to the ECOG-ACRIN Drug Team at 900.drugorder@jimmy.harvard.edu who will then forward the drug request to Fisher Clinical Services. If email is not available, the completed form may be faxed to ATTN: ECOG-ACRIN Drug Team at 617-632-2063. **No starter supplies are available for this protocol.**

Decitabine will be shipped to a responsible person (e.g., a pharmacist) at the investigator's institution. Drug is ambient and will use a normal ambient shipper. Quantities must be ordered per cycle (keeping in mind that you will need 3 vials per cycle.

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NOTE: Decitabine is provided in kits that contain 5 vials per kit. Each order should cover, at minimum, 2 cycles - 10 vials.

Institutions should allow 3-4 business days for shipment of drug from Fisher Clinical Services from receipt of the E2906 Decitabine Drug Request Form by the ECOG-ACRIN Drug Team. Shipments will be made fromFisher Clinical Services. on Monday through Thursday for delivery onsite Tuesday through Friday. There will be no weekend or holiday delivery of drugs. The E2906 Decitabine Drug Request Form can be downloaded from the ECOG website in WORD format.

IMPORTANT REORDER INSTRUCTIONS

Once it is determined that the patient will continue treatment, please reorder **2 cycles** of study drug immediately. Institutions should keep in mind the number of vials used per cycle, and that decitabine kits contain 5 vials per kit.

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Institutions should allow 3-4 business days for shipment of drug from Fisher Clinical Services from receipt of the E2906 Decitabine Drug Request Form by the ECOG-ACRIN Drug Team. Shipments will be made fromFisher Clinical Services. on Monday through Thursday for delivery onsite Tuesday through Friday. There will be no weekend or

holiday delivery of drugs. The E2906 Decitabine Drug Request Form can be downloaded from the ECOG website in WORD format.

Drug Destruction and Return

At the completion of all patients' treatment randomized to Arm F at your institution, all unused drugs, partially used, or empty containers must be destroyed at the site according to the institution's policy for drug destruction. Please maintain appropriate records of the disposal, including dates and quantities.

Drug Inventory Records

Investigational Product Records at Investigational Site(s): It is the responsibility of the Investigator to ensure that a current record of investigational product disposition is maintained at each study site where investigational product is inventoried.

8.3.10 Side Effects

See CAEPR (Section 5.3)

8.3.11 Nursing/Patient Implications

1. Monitor CBC, platelet counts.
2. Obtain liver chemistries and serum creatinine prior to initiation of treatment.
3. Premedicate with antiemetics as needed (emetogenic potential: minimal risk, < 10%).
4. Not an irritant or vesicant.

8.3.12 References

Kantarjian H, Oki Y, Garcia-Manero G, et al. Results of a randomized study of three schedules of low-dose decitabine in higher risk myelodysplastic syndrome and chronic myelomonocytic leukemia. *Blood*. 2007;109:52-57.

Kantarjian HM, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer*. 2006;106:1794-1803.

Data on file, MGI PHARMA, INC.

Dacogen [package insert]. Bloomington, MN: MGI PHARMA, INC; 2006.

8.4 Clofarabine

8.4.1 Other Names

CLOLAR™, Cl-F-Ara-A, 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine

8.4.2 Classification

Purine nucleoside analog, anti-metabolite\

8.4.3 Mode of Action

Converted to clofarabine triphosphate, a competitive inhibitor of DNA synthesis, and inhibitor of DNA polymerse α and ribonucleotide reductase. It is not cell cycle specific

8.4.4 Storage & Stability

Clofarabine is formulated at a concentration of 1 mg/mL in sodium chloride (9 mg/mL), United States Pharmacopeia (USP), and Water for Injection, USP, quantity sufficient (qs) to 1 mL. Clofarabine is supplied in one vial size: a 20-mL clear, glass vial with gray stopper and blue flip off seal.The 20-mL vial contains 20 mL (20 mg)of solution with a pH range of 4.5 to 7.5. The solution is sterile, clear and practically colorless, is preservative-free, and is free from foreign matter.

8.4.5 Dose Specifics

Arm B Induction Cycle I: 30 mg/m²/day IV by 1 hour infusion QD x 5 days. See Section 5.1.3.

Arm B Induction Cycle II: 20 mg/m²/day IV by 1 hour infusion QD x 5 days. This cycle should not begin before day 21. See Sections 5.1.3 and 5.1.5.

Arm D Consolidation: 20 mg/m²/day x 5 days. See Section 5.1.6.

8.4.6 Preparation

Clofarabine for injection should be filtered through a sterile 0.2 mcm syringe filter and then further diluted with 5% dextrose injection USP (D5W) or 0.9% sodium chloride injection USP (normal saline [NS]) prior to IVI. If the use of a 0.2 mcm syringe filter is not feasible then clofarabine should be either pre-filtered with a 5 mcm filter, or administered through a 0.2 or a 0.22 mcm in-line filter. Clofarabine can be further diluted in D5W or NS. Recommended total volume is up to 250 mL. The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation. The final concentration of the solution should be between 0.15 mg/mL to 0.4 mg/mL.

8.4.7 Route of Administration

Clofarabine should be administered by daily intravenous infusion over 1 hour.

8.4.8 Incompatibilities

To prevent drug incompatibilities, no other medication should be administered through the same IV line.

8.4.9 Drug Availability

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Clofarabine is distributed free of charge from Genzyme CPRS. Refer to Section 4.0 for regulatory documents required prior to study entry. Be sure to allow ample time for processing of the regulatory packet

and drug request. **Clofarabine should only be ordered for patients randomized to Arm B on Induction and Arm D on Consolidation.**

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Initial Drug Orders for Each Patient

Following submission and approval of the required regulatory documents and patient registration, a supply of Clofarabine may be ordered from Genzyme Oncology. Investigators must email a completed E2906 Clofarabine Study Drug Request Form **(See Appendix III)** to Genzyme at GTOdrugorders@genzyme.com or fax to (210) 949-8484. **Clofarabine should only be ordered for patients randomized to Arm B on Induction and Arm D on Consolidation. If needed, starter supplies may be requested. Please indicate on the drug request form that a starter supply is being ordered.**

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Clofarabine will be shipped to a responsible person (e.g., a pharmacist) at the investigator's institution, who will document the amount and condition of the drug and enter these data into forms provided by the drug distributor. A suggested initial shipment is 30 vials (1.5 cycles). Allow 5 business days from date of receipt of the E2906 Drug Request Form to the date of receipt of clofarabine at the. Please note the date by which clofarabine is needed and Genzyme will attempt to accommodate more rapid delivery as necessary. The Drug Supply Shipment Form can be downloaded from the ECOG website in WORD format.

IMPORTANT REORDER INSTRUCTIONS

Once it is determined that the patient will continue treatment, please reorder study drug immediately. Institutions should keep in mind the number of vials used per cycle, and that shipments may take 5 business days from the date Genzyme receives the request.

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Shipments will be made from Genzyme CPRS on Monday through Thursday for delivery onsite Tuesday through Friday. There will be no weekend or holiday delivery of drugs.

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Clofarabine Quality and Complaints Handling

Genzyme has requested that the pharmacist or pharmacy designee report any issues related to the quality of Clofarabine to Genzyme Clinical Pharmacy Research Services (CPRS) via fax at 508-424-4484 or 877-237-1292 (toll-free). The company's CPRS Fax is available for Clofarabine handling and complaint reporting on a 24-hour basis. Complaints are reviewed during normal business hours.

Where possible, once a complaint has been logged with CPRSP, the Clofarabine in question should be stored as instructed on the label in a limited access area until otherwise instructed by Genzyme.

Questions regarding quality issues can be addressed to CPRS by calling 800-326-7002 or e-mailing CPRSProductComplaints@genzyme.com.

Drug Destruction and Return

At the completion of the patient's treatment at your institution, all unused drugs, partially used, or empty containers must be destroyed at the site according to the institution's policy for drug destruction. Please maintain appropriate records of the disposal, including dates and quantities.

Drug Inventory Records

Investigational Product Records at Investigational Site(s): It is the responsibility of the Investigator to ensure that a current record of investigational product disposition is maintained at each study site where investigational product is inventoried.

8.4.10 Side Effects (for a more complete list of side effects see Section 4 of the IB)

1. Most frequently reported: Nausea, asthenia, diarrhea, myalgia.
2. Body as a whole: Fever, malaise, asthenia, abdominal pain, back pain, extremity pain, sepsis, pain.
3. Gastrointestinal: Nausea, vomiting, anorexia, diarrhea, stomatitis, GI disorder, GI hemorrhage, liver damage, liver function abnormalities.
4. Musculoskeletal: Myalgia, arthralgia.
5. Cardiovascular: Hemorrhage, chest pain, cardiac dysrhythmia, hypotension, heart failure, hypertension. Cardiac insufficiency and cardiomyopathy have been observed in rats treated with clofarabine.
6. Respiratory: Dyspnea, lung disorder, cough.
7. Dermatologic: Rash, urticaria.
8. Neurologic: Headache, CNS disorder.
9. Hematologic: Leukopenia, neutropenia, thrombocytopenia.

8.4.11 Interactions

Unknown; no other medication should be administered through the same IV line.

8.4.12 Nursing/Patient Implications

1. Educate patient/support person(s) about potential side effects, particularly related to myelosuppression & immunosuppression.
2. Follow protocol guidelines for discontinuing monitoring WBC, neutrophil and platelet count.
3. Administer antiemetics and analgesics as ordered/needed: Prophylactic use of antiemetics is recommended. See Section 5.3.
4. Monitor for signs of infection, bleeding, hand-foot syndrome.
5. Monitor renal and hepatic function with clofarabine administration.

8.5 Fludarabine Monophosphate (Fludara®)

8.5.1 Availability

Fludarabine monophosphate is commercially available as FLUDARA IV as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH. Store at 15-30°C (59-86°F). Please refer to the agent's package insert for additional information.

8.5.2 Storage & Stability

Reconstituted FLUDARA IV is chemically and physically stable for 24 hours at room temperature or 48 hours if refrigerated. In addition, reconstituted FLUDARA IV contains no antimicrobial preservative and thus care must be taken to assure the sterility of the prepared solutions and should be discarded eight hours after initial entry.

8.5.3 Preparation

FLUDARA IV should be prepared for parenteral use only by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, USP, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7-8.5. The product may be further diluted for intravenous administration to a concentration of 1 mg/ml in 5% Dextrose for Injection USP or in 0.9% Sodium Chloride, USP.

8.5.4 Administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine will be delivered as a piggy-back via an ongoing IV line, over a period of 30 minutes.

8.5.5 Toxicity

Myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only been rarely demonstrated at the 25-30 mg dosage of fludarabine monophosphate. Very rarely described complications include transfusion-associated graft versus host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome, complicating fludarabine monophosphate therapy has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed in both pre-treated patients receiving fludarabine and in individuals

receiving fludarabine combined with other agents (corticosteroids, mitoxantrone, and cyclophosphamide).

8.6 Tacrolimus (Prograf®)

8.6.1 Availability

Tacrolimus is a commercially available macrolide compound with potent immunosuppressant properties. Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg, or 5 mg of anhydrous tacrolimus. For IV use, tacrolimus is available as a sterile solution in 1mL ampules containing the equivalent of 5 mg of anhydrous tacrolimus per mL.

The oral absorption of tacrolimus is erratic and incomplete; absolute bioavailability is approximately 25%; peak serum levels are seen 1 to 3 hours after an oral dose, and therapeutic trough blood concentrations have ranged from 5 to 20 ng/mL; tacrolimus is extensively metabolized in the liver, with only small amounts of unchanged drug (2% or less) being recovered in the urine; the elimination half-life of tacrolimus is approximately 10 hours.

Tacrolimus suppresses both humoral (antibody) and cell-mediated immune responses. The compound is chemically distinct from cyclosporine but both agents elicit similar immunosuppressant effects. The immunosuppressive activity of tacrolimus is, however, more marked than that of cyclosporine.

Please refer to the agent's package insert for additional information.

8.6.2 Preparation -- For IV use

Tacrolimus concentrate for injection must be diluted prior to IV infusion. For IV infusion, the concentrate is diluted with 0.9% sodium chloride or 5% dextrose injection to a concentration of 4-20 µg/mL. Preparation of the solution in polyethylene or glass containers allows storage for 24 hours beyond which unused solution should be discarded. A plasticized polyvinyl chloride (PVC) container should not be used because stability of the solution is decreased and polyoxyl 60 hydrogenated castor oil contained in the formulation may leach phthalates from PVC containers. Tacrolimus concentrate for injection and diluted solutions of the drug should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

8.6.3 Administration

Tacrolimus is to be initiated on Day -2. Begin tapering between Day +90 to +120 in the absence of GVHD as tolerated with a goal of stopping by Day +150 to +180. See protocol text for tapering instructions, and for instructions for patients who are unable to take oral tracolimus.

8.6.4 Storage & Stability

Store tacrolimus capsules at controlled room temperature, 15-30°C (59-86°F) (Prod Info Prograf®, 1997). An extemporaneous suspension of tacrolimus with a final concentration of 0.5 milligrams/milliliter was stable for 56 days when it was stored at 24-26°C in glass or plastic amber prescription bottles.

8.6.5 Toxicity

In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Mild to moderate hypertension was reported in 38% to 50% of patients receiving tacrolimus. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Chest pain was reported in 19%. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%); and dizziness (19%). Tremor and headache may respond to a dosage reduction. Agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15% of tacrolimus-treated patients. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%), hypophosphatemia (49%), and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. In addition, hirsutism occurs only rarely with tacrolimus. Hyperuricemia has been reported in greater than 3% of tacrolimus-treated patients. Gastrointestinal adverse effects of tacrolimus have included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%) and diarrhea (37% to 72%). Gingival hyperplasia observed in patients treated with cyclosporine has not been reported with tacrolimus therapy. Nephrotoxicity was reported in 36% to 40% and 52% of liver and kidney transplant patients receiving tacrolimus. Overt nephrotoxicity is usually seen early after transplantation and is characterized by an increased serum creatinine and a decrease in urine output. Hematuria has been reported in greater than 3% of tacrolimus-treated patients (Prod Info Prograf®, 1997). Abnormal liver function tests have been reported in 6% to 36% of patients receiving tacrolimus; ascites was reported in 7% to 27% of these patients.

Other miscellaneous effects that have occurred in clinical trials include pain (24% to 63%), fever (19% to 48%), asthenia (11% to 52%), back pain (17% to 30%), and peripheral edema (12% to 36%). The incidence of hyperglycemia is 17% and may require therapy with insulin. Other less frequently occurring effects (greater than 3%) include abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus contains castor oil which has been associated with anaphylaxis in other drugs containing castor oil derivatives.

The incidence of bloodstream infection is 22%. Most infections are due to bacteria (81%), followed by candidemia (14%), and cryptococcemia (5%). The source of bloodstream infection was primarily intravascular catheter, accounting for 39% of cases.

8.7 Methotrexate (Amethopterin®; MTX)

8.7.1 Availability

Commercially available in 2 mL, 4 mL, 8 mL, 10 mL vials, or 1 g vials or preserved with benzyl alcohol. Please refer to the agent's package insert for additional information.

8.7.2 Preparation

The 1 gm vial may be diluted in 100 mL of saline or D_5W.

8.7.3 Compatibility

Additive incompatibility: bleomycin, prednisone.

8.7.4 Storage & Stability

Stability and compatibility of methotrexate sodium solutions depend on several factors including the formulation of methotrexate sodium used, presence of preservatives, concentration of drug, specific diluents used, resulting pH, and temperature; the manufacturer's labeling and specialized references should be consulted for specific information. Methotrexate sodium solutions should be inspected visually for particulate matter and discoloration whenever solution or container permits.

8.7.5 Administration

Administer via slow IV push. Hydrate intravenously and induce diuresis.

8.7.6 Toxicity

Hematologic including leukopenia (1.5%), thrombocytopenia (5%; nadir 5-12 days; recovery 15-27 days), anemia (nadir 6-13 days), pancytopenia (1.5%); gingivitis, glossitis, pharyngitis, stomatitis, enteritis; nausea/vomiting, anorexia, diarrhea; hematemesis, melena; acute and chronic hepatotoxicity; transaminases increase 1-3 days after administration, hepatic fibrosis and cirrhosis with long-term therapy; pulmonary toxicity including pneumonitis, pulmonary fibrosis that is not dose-dependent and may not be fully reversible; pruritis, urticaria, photosensitivity; CNS: drowsiness, blurred vision, tinnitus, malaise, seizures; nephropathy: cystitis, dysuria, azotemia, hematuria, renal failure; diabetes; when administered it may cause headache, back pain, rigidity.

8.7.7 Drug Interactions

Aminoglycosides may cause decreased absorption of methotrexate, and increased renal toxicity. Folic acid may decrease response to methotrexate. The use of NSAIDs may increase methotrexate levels. Probenecid, salicylates, sulfonamides may increase therapeutic and

toxic effect of methotrexate. Procarbazine can cause increased nephrotoxicity. Theophylline may increase plasma levels. Alcohol may result in increased hepatotoxicity. Thiazides may cause granulocytopenia. Food will delay absorption, and decreases methotrexate peak.

8.8 Filgrastim (r-met HuG-CSF, G-CSF: Granulocyte Colony-Stimulating Factor, Neupogen®)

8.8.1 Availability

r-met HuG-CSF is commercially available in 1.0 and 1.6 mL vials containing 300 µg and 480 µg G-CSF, respectively. Please refer to the agent's package insert for additional information.

8.8.2 Storage & Stability

G-CSF is available as a sterile buffered protein solution and must be stored at 2-8°C. DO NOT ALLOW THE DRUG TO FREEZE.

8.8.3 Administration

Each vial should be entered only once, and the remainder of the vial discarded and not re-entered a second time. The daily dose should be injected subcutaneously in one or two sites. Standard dosing is 5 µg/kg daily as a subcutaneous injection. Higher dosing (10 µg/kg) will be used for donors in this protocol.

8.8.4 Toxicity

Chills, nausea, anorexia, myalgias, bone pain, local injection site pain or inflammation, abnormal liver function tests, thinning of hair, and enlargement of the spleen. Rarely fluid retention and pericardial effusion. All of these are generally reversible when the drug is discontinued.

8.9 Busulfan (Busulfex®)

8.9.1 Availability

Busulfan is commercially available as 60 mg/10 mL ampuls. Please refer to the agent's package insert for additional information.

8.9.2 Preparation

Dilute busulfan injection in 0.9% sodium chloride injection or dextrose 5% in water. The dilution volume should be ten times the volume of busulfan injection, ensuring that the final concentration of busulfan is ≥ 0.5 mg/mL.

8.9.3 Storage & Stability

Store unopened ampuls under refrigeration at 2°C to 8°C. The diluted solution is stable for up to 8 hours at room temperature (25°C) but the infusion must also be completed within that 8-hour time frame. Dilution of busulfan injection in 0.9% sodium chloride is stable for up to 12 hours at refrigeration (2°C-8°C) but the infusion must also be completed within that 12-hour time frame.

8.9.4 Administration

Intravenous busulfan should be administered via a central venous catheter as a 2-hour infusion every 6 hours for 2 consecutive days for a total of 8 doses.

8.9.5 Toxicity

Severe myelosuppression with marrow ablation, alopecia, and mild nausea/vomiting are expected. Alopecia may not be completely reversible. Liver toxicity including severe or fatal veno-occlusive disease (<5%) may occur. Pulmonary toxicity is rare in this schedule. In combination with etoposide, busulfan causes severe mucositis, esophagitis, and possible enteritis. It is expected that patients will require mouth care including narcotic analgesia, and may require parenteral nutrition. In combination with etoposide, busulfan may cause skin toxicity including painful desquamation, and this may require local care and narcotic analgesia. Darkening of the skin may occur and may last several months. Seizures may occur (<5%).

Busulfan causes immunosuppression and risk of opportunistic infection even after resolution of neutropenia. Busulfan is expected to cause nearly universal infertility in the doses used, although men may occasionally father children.

8.9.6 Nursing Implications

1. GI toxicities leading to alteration in nutritional status. Patients require daily mouth care regimen which may include narcotic analgesic and potentially parenteral nutrition.
2. Painful desquamation may require local care and narcotic analgesics.

8.10 Antithymocyte Globulin (Rabbit) (Thymoglobulin®; rabbit ATG)

8.10.1 Availability

Antithymocyte globulin is commercially available as a lyophilized powder for reconstitution containing 25 mg per vial. Each vial of powder is supplied with 5 mL diluent.

8.10.2 Storage & Stability

Intact vials should be stored under refrigeration and protected from light. Do not freeze. Reconstituted solutions should be used within 4 hours. Further diluted solutions for infusion should be used immediately after dilution.

8.10.3 Preparation

Remove the ATG rabbit plus diluent from the refrigerator and allow them to reach room temperature prior to reconstitution. Reconstitute each 25 mg vial with 5 mL of the diluent provided (sterile water for injection, USP). Rotate the vial gently to dissolve the powder. The resultant solution contains 5 mg/mL of ATG rabbit. Withdraw the calculate dose and inject into D_5W or NS for IV infusion. The final

concentration should be 0.5 mg/mL. The solution should be administered through a 0.22 micron filter.

8.10.4 Administration

ATG rabbit will be administered IV at a dose of 2.5 mg/kg/day for 3 days (on Days -4, -3, and -2). The first dose should be infused over at least six hours, and subsequent doses over at least 4 hours. Infuse through a 0.22 micron in-line filter. Acetaminophen 650 mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisolone 1 mg/kg IV should be administered at the initiation and midway through each antithymocyte globulin (rabbit) infusion to minimize infusion reactions.

8.10.5 Toxicity

Infusion reactions such as fever and chills are common, occurring in more than 10% of patients. Steroids, antihistamines and acetaminophen will be given, as described above, to minimize infusion reactions. Hypersensitivity reactions, including anaphylaxis, occur less frequently and may also be minimized with steroids and antihistamines.

Immunosuppression from antithymocyte globulin (rabbit) is associated with an increase in opportunistic infections, including fungal, viral, and pneumocystis infections.

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9. Statistical Considerations

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9.1 Original Design Overview

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In this study, 747 patients will be stratified by age (60 to 69 vs. over 70), therapy-related AML, and the presence of AHD (antecedent hematologic disorder) at the time of diagnosis of AML, then randomized to receive either the standard therapy induction treatment or clofarabine induction treatment. If achieving CR or CRi, these patients will continue to receive standard or clofarabine consolidation treatments. Patients who have a matched sibling donor and who achieve a 'morphologic leukemia-free state' will undergo non-myeloablative allogeneic transplant. For the patients who do not undergo non-myeloablative allogeneic transplant, after receiving the induction and consolidation therapies, if patients are still in CR or CRi, they will be randomized to receive either decitabine maintenance treatment or observation, stratified on the induction and consolidation therapy, cytogenetics and age. We anticipate that approximately 8% of patients will undergo allogenic transplant. We assume 25% of patients who do not receive allogenic transplant go through the second randomization.

The primary objective of this trial is to determine the effect of clofarabine on overall survival (OS) in comparison with standard therapy (daunorubicin & cytarabine in induction and cytarabine in consolidation) in newly-diagnosed AML patients with age over 60 years. Since clofarabine is believed to be less toxic and also clofarabine is expected to prolong OS, a non-inferiority design with superiority alternative is preferred. If clofarabine turns out to be non-inferior to the standard therapy, the superiority of clofarabine will be tested. Another main endpoint of interest in this study is the 30-day mortality rate. It is suspected that clofarabine has a lower early mortality rate. The 30-day mortality rate will be tested after the inferiority of overall survival of clofarabine is rejected. The non-inferiority test of OS, the superiority test of OS and the mortality endpoints fit into a hierarchical framework, since the superiority test of OS and the mortality endpoint will both be tested only conditional on inferiority of OS being rejected. Therefore, non-inferiority of OS will be tested at a one-sided 0.025 level, and superiority of OS and 30 day mortality rate will both be tested at a nominal level of 0.0125 (one-sided). The overall type I error rate of all three tests is still controlled at 0.025.

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As one of secondary endpoints, the induction complete response (CR) rates between clofarabine arm and the standard treatment arm will be compared. A non-inferiority test with superiority alternative at a one-sided significance level of 0.025 will be used. If the inferiority of CR rate of clofarabine is rejected, the superiority test will be performed at a one-sided 0.025 significance level.

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At each interim analysis, the study will be monitored for early stopping in favor of either superior OS on the clofarabine arm or inferior OS on the clofarabine arm in the context of the non-inferiority test of OS. Meanwhile, the study will also be monitored for early stopping in favor of an inferior CR rate on the clofarabine arm. In the event that at an interim analysis the criterion for inferiority of CR rates is met, but there is a trend towards superiority of survival, the DMC may decide to continue the study in spite of the inferiority of the CR rate.

As other secondary endpoints, we want to determine the effect of decitabine on disease free survival (DFS) as a maintenance therapy in comparison with observation. Also in this study, we want to evaluate the impact of consolidation with non-ablative conditioning and allogeneic hematopoietic stem cell transplantation with an HLA-identical sibling on overall survival in patients who achieve a 'morphologic leukemia-free state'.

9.2 Primary Endpoint and Sample Size

The primary objective of this trial is to determine the effect of clofarabine on overall survival in comparison with standard therapy. The primary endpoint is overall survival (OS). OS is defined as the time between randomization and death from any cause. Patients last known to be alive at the time of an analysis will be censored.

The primary comparison of OS will be based on the treatment assignment received at randomization. To reduce potential bias of the primary comparison from the alloSCT arm, the patients will be censored at the time of allogeneic transplant for the primary analysis. Due to the potential confounding by maintenance therapy on the induction comparison, a weighted analysis (weighted Cox regression) (121,122,123) will be used for the primary analysis. Let π denote the actual proportion randomized to the observation arm of all patients entering on maintenance randomization. In the weighted analysis, patients who do not enter maintenance randomization get weight 1; Observation maintenance arm patients get weight $1/\pi$. Decitabine maintenance arm patients get weight 0. Thus, patients on the observed maintenance arm represent themselves as well as the response of $(1/\pi - 1)$ similar patients in the decitabine maintenance arm. A robust variance estimator that correctly accounts for the weights will be used in this setting.

Because long-term cures have been observed in previous studies with the same patient population, we assume a cure rate mixture distribution for the standard arm, with the distribution on the clofarabine arm given by a proportional hazards shift from the standard arm, to calculate power. Based on a previous ECOG study, E3999, we assume that the long-term cure rate in the standard treatment arms will be 10% and median survival among the non-cured group will be about 9 month, i.e., the survival model for the standard treatment arm $S_0(t) = 0.1+0.9 \exp(-t*\log(2)/9)$. The study team would consider clofarabine to be acceptable if the long-term cure rate in the clofarabine arms will be no lower than 8% and median survival among the non-cured group will be no less than 8 months, which is approximately a less than 12% increase in hazard rate. Therefore, our null hypothesis is that the hazard ratio for clofarabine/standard is no less than 1.12. The alternative is that the hazard ratio for clofarabine/standard is no more than 0.86. The non-inferiority of clofarabine will be tested at a one-sided significance level of 0.025.

We assume that 8% of those patients will undergo allogeneic transplant. We also assume that 25% of patients who do not receive allogeneic transplant go through the second randomization. Adjusted for the sequential monitoring described below and based on the weighted analysis previously described, with 685 patients, the study will have approximately 80% power to reject the null hypothesis of inferiority of clofarabine at the one-sided significance level of 0.025 under the alternative above, assuming 5 years of accrual and 2 years of follow-

up after the completion of accrual. The size and power of the study will not be affected even if the proportions of patients receiving maintenance are imbalanced between the induction arms. The full information needed is 507 events. This information is the expected number of deaths among the non-allo non-decitabine patients under the alternative. Deaths on the decitabine maintenance arm do not contribute information to this comparison. Since the allo transplant patients are censored at the time of transplant, they do not contribute events. Therefore, the overall sample size was increased by 1/(1-.08), around 9%, to make up for the loss of information. A total accrual of 747 patients is planned for this study.

If criteria for early stopping given in Section 9.3 are not met, the final analysis will be performed when 507 non-allo non-decitabine patients have died, which is anticipated to occur 7 years after the study begins active accrual. A two-sided 95% confidence interval on the log hazard ratio (clofarabine/standard) will be provided based on the weighted analysis as previously described, with age (60 to 69 vs. over 70), therapy-related AML, and the presence of AHD at the time of diagnosis of AML as the stratification factors.

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If the 95% confidence interval on the log hazard ratio lies entirely below log(1.12), the non-inferiority of clofarabine treatment will be accepted. This analysis will be conducted in two ways: (1) comparing the treatments based on treatment assignment received at randomization and censoring the allo transplant patients at the time of transplant; and (2) comparing the treatment based on actual treatment received and again censoring the allo transplant patients (the weighted analysis removing the effect of maintenance decitabine will be used for both). The results will be credible only if the two analyses are reasonably consistent.

If clofarabine turns out to be non-inferior to the standard therapy, a two-sided 97.5% confidence interval on the log hazard ratio (clofarabine/standard) will be provided for the superiority test of OS. If the 97.5% confidence interval lies entirely below log(1), then clofarabine will be considered superior to the standard treatment. A p-value for superiority based on the weighted analysis will be presented to allow assessment of the strength of the evidence. The superiority test will be based on the ITT (intention-to-treat) principle), including all randomized patients and censoring the allo transplant patients at the time of transplant.

9.3 Interim Analyses of Primary Endpoint

Interim analysis of the study will be performed for all semi-annual DMC meetings beginning when approximately 25% of planned full information (126 events) has occurred. Table 1 gives the expected timing of the analyses and the expected number of events under the alternative at each interim analysis. Because of delays in initiation of accrual and delays in data submission and processing, it is likely the actual analysis time will be 6-12 months later.

The study will be monitored for early stopping in favor of either the alternative hypothesis of superior OS on the clofarabine arm or the null hypothesis of inferior OS on the clofarabine arm using the repeated confidence interval (RCI) methodology similar to that described by Jennison and Turnbull (124). At each interim analysis, a two-sided 95% RCI on the log hazard ratio

(clofarabine/standard) will be computed, using the partial likelihood estimate and their truncated O'Brien-Fleming critical points listed in Table 1. The partial likelihood estimate of log hazard ratio and its robust variance estimator will be obtained using the weighted analysis described in Section 9.2. Since we do not want to stop the trial and declare the inferiority or superiority of clofarabine unless the results are convincing, a hazard ratio of 1 is pre-specified. If at any of the interim analyses, the 95% confidence interval of the log hazard ratio lies entirely below log(1), we may consider stopping the trial for the superiority of clofarabine; if the 95% confidence interval lies entirely above log(1), the study may need to be stopped due to the inferiority of clofarabine.

Table 1: The Interim Analyses for the Primary OS Comparison

Real Time (Years)	Information Time	Events Under the alternative	Truncated O-F critical points
2.2	0.25	127	3.2905
2.7	0.34	175	3.2905
3.2	0.44	224	3.2905
3.7	0.54	274	2.9491
4.2	0.64	325	2.6418
4.7	0.74	377	2.4402
5.2	0.85	429	2.2623
5.7	0.92	465	2.1928
7.0	1.00	507	2.0974

9.4 Secondary Objectives

As a secondary objectives of this trial, we will evaluate the 30-day mortality rate (all-cause mortality within 30 days of first dose) of clofarabine in comparison with standard treatment (daunorubicin & cytosine arabinoside) in newly diagnosed AML patients age > 60 years. Based on the results from E3999, we assume the 30-day mortality of standard treatment is 10%. With 747 patients in total, we will have 80% power, at the one-sided 0.0125 significance level, to detect a 6% reduction in the 30-day mortality rate in the clofarabine arm using Fisher's exact test.

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In this trial, we will also compare the induction complete response (CR) rates between the clofarabine arm and the standard treatment arm. Based on the results from E3999, we assume the CR rate of standard treatment is about 40%. The study team would consider clofarabine to be acceptable if the CR rate is no more than 10% less than the standard treatment. A non-inferiority test was used for this endpoint. Our null hypothesis is that the difference in the CR rates between the standard treatment arm and the clofarabine arm (CR rate of standard treatment – CR rate of clofarabine) is more than 10% and the alternative is that the difference of CR rates between the standard treatment arm and the clofarabine arm (CR rate of standard treatment – CR rate of clofarabine) is less than -1%. With 747 patients in total, the study will have 85% power to reject the null hypothesis of inferiority of clofarabine at the one-sided significance level of 0.025, adjusting for the sequential monitoring described below. At the

end of the study, the 95% confidence interval on the difference in the CR rates (CR rate of standard treatment – CR rate of clofarabine) will be computed based on the normal approximation to the difference of two independent binomial proportions. If the 95% confidence interval lies entirely below 0.1, the non-inferiority of clofarabine in CR rate will be accepted. If the 95% confidence interval lies entirely below 0, then clofarabine is superior to the standard treatment in CR rate.

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The induction CR rates will be monitored to allow for early stopping in favor of inferiority using the repeated confidence interval methodology described by Jennison and Turnbull (123). Interim analyses will be conducted for induction CR rates when induction response data is available on 25%, 45%, 65%, 85% and 100% of patients. At each interim analysis, the two-sided 95% repeated confidence interval on the difference of CR rate (CR rate of standard treatment – CR rate of clofarabine) will be computed. This repeated confidence interval uses the critical value from a truncated O'Brien-Fleming error spending rate function with an overall one-sided 2.5% error rate. If the confidence interval lies entirely above 0, the induction randomization may need to be stopped due to the inferiority of clofarabine and subsequent patients will directly be assigned to standard treatment arm.

Another objective of this trial is to determine the effect of decitabine on disease free survival (DFS) as a maintenance therapy in comparison with observation. DFS is defined as the time from the second randomization to relapse or death without relapse. If 25% of patients who do not receive allogeneic transplant go through the second randomization, there will be 172 patients = randomized to either the decitabine arm or the observation arm.

We assume that the long-term cure rate for the observation arm is 10% and the median DFS in the non-cured group is 7 months. The study will have 90% power to detect approximately a 36% reduction in the DFS hazard rate in the decitabine maintenance therapy arm. This power calculation is based on a one-sided log-rank test at the significance level of 0.1 and assumes 5 years of accrual and 2 years of follow-up after the completion of the accrual. The number of DFS events needed is 140. The primary analysis of DFS will be an intention-to-treat analysis and will use a one-sided log rank test stratified on the induction and consolidation therapy and on patient age and cytogenetics.

Another secondary objective of this study is to evaluate the impact of consolidation with non-ablative conditioning and allogeneic hematopoietic stem cell transplantation with an HLA-identical sibling on overall survival in patients who achieve a 'morphologic leukemia-free state'.

The intention–to-treat approach will be used in this analysis. Patients with donors who choose not to have AlloSCT (or are unable due to insurance issues) need to be included in the AlloSCT arm for analysis. Assuming that 50% of patients receiving chemotherapy in induction and consolidation achieve a 'morphologic leukemia-free state' and 25% of those patients have a donor, 94 patients from the allogeneic transplant arm and 280 patients from the chemotherapy arms will be available for this OS comparison.

For the chemotherapy arms, we assume that the long-term cure rate will be 10% and median survival among the non-cured group will be about 9 months. The

study will have 82% power to detect approximately a 32% reduction in the OS hazard rate in the allogenic transplant arm using one-sided log-rank test at the significance level of 0.025. This power calculation assumes 5 years of accrual and 2 years of follow-up after the completion of accrual. Full information needed is 292 deaths. The analysis of this secondary objective will use a one-sided log rank test stratified on age, therapy-related AML, the presence of AHD at the time of diagnosis of AML, and the induction therapy they received.

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9.5 Design Changes

Following a pre-planned interim analysis of primary endpoint OS, accrual to Step 1 of this study was suspended on February 23, 2015 based on DSMC's recommendation, due to differences in overall survival favoring standard daunorubicin and cytarabine compared to clofarabine for induction and consolidation. By that time, 727 patients had been accrued to this study.

Among those 727 patients, 120 (16.5%) entered the second randomization. The original protocol design requires 172 patients to be randomized to either the decitabine arm or the observation arm (number of DFS events needed 140), in order to have 90% power to detect approximately a 36% reduction in the DFS hazard rate in the decitabine maintenance therapy arm at the significance level of 0.1.

We plan to reopen the study to accrue additional 52 patients to the second randomization, to finish the accrual goal of 172 patients. This requires about 74 CR/CRi Patients to be pre-registered after their induction therapy. Patients will receive consolidation while on study and then will be randomized to receive either decitabine maintenance treatment or observation, stratified on the induction therapy, cytogenetics and age. Based on the accrual rate of the first 120 randomized patients, we anticipate the additional accrual could be completed within 1.8 years. The final analysis will be performed at 2 years of follow-up after the completion of the accrual. Based on the event rate observed, at that time, 125 DFS events could occur. The study will have approximately 90% power to detect a 37% reduction in the DFS hazard rate in the decitabine maintenance therapy arm, using a one-sided log-rank test at the significance level of 0.1. The half width of two-sided 80% confidence interval on the log hazard ratio (decitabine /observation) will be about 0.23. The half width of two-sided 95% confidence interval on the log hazard ratio (decitabine /observation) will be about 0.35.

Prior to the study reopen, as the study has reached 60% information for the maintenance comparison (Arms E vs. F), an interim analysis will be performed and the maintenance randomization will be monitored for early stopping in favor of null hypothesis by computing the conditional power. The conditional power will be calculated given the current data, assuming the study will continue until 125 events of the maintenance comparison is reached. If the conditional power is higher than 0.7, the study will be reopen to pre-register 74 CR/CRi patients and accrue additional 52 patients to the second randomization. The cutoff of conditional power is set high, as we want to reopen the study only if there is a good chance of a positive result.

If the conditional power is lower than 0.7, the study will be closed permanently. No more patients will be accrued. Based on the current event rate of the 120

patients, the events are anticipated to be accumulated very slowly. Therefore, the final analysis of the maintenance comparison will be performed at six months after the interim analysis. Based on the current event rate, at that time, we assume 90 DFS events could be observed. With 90 events, the study will have approximately 85% power to detect a 36% reduction in the DFS hazard rate in the decitabine maintenance therapy arm, using a one-sided log-rank test at the significance level of 0.15. The half width of two-sided 70% confidence interval on the log hazard ratio (decitabine /observation) will be about 0.22. The half width of two-sided 95% confidence interval on the log hazard ratio (decitabine /observation) will be about 0.41.

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9.6 AML Clinical Epidemiology

We assume that approximately 600 (82% of all 727 registered to Step 1) patients will complete the ALESE questionnaire. The proportions of obese patients and patients who had benzene exposure, ever smoked in their lifetime, took aspirin regularly, took acetaminophen regularly, ever lived in rural/farm environments, or had other exposures or lifestyle factors will be calculated separately, along with their 95% CIs. With 600 patients, the width of the 95% CI will be no wider than 0.08.

We will examine the impact of smoking, obesity, regular acetaminophen use, regular aspirin use, benzene exposure, and living in a rural/farm environment on OS. The impact of some other exposures or lifestyle factors on OS will also be explored. Assume 50% patients ever smoked in lifetime and assume for those patients a 10% long-term cure rate and 8-month median overall survival in the non-cured group. With treatment arms combined, the study will have approximately 80% power to detect a 22% reduction in the hazard rate in non-smoking patients, using a one-sided log rank test at the significance level of 0.025 and assuming 2 years of follow-up. The number of events needed is 502. Assume 20% patients had benzene exposure and assume those patients have a 10% long-term cure rate and 7.5 -month median overall survival in the non-cured group. With treatment arms combined, the study will have approximately 80% power to detect a 27% reduction in the hazard rate in non- benzene- exposure patients, using a one-sided log rank test at the significance level of 0.025 and assuming 2 years of follow-up. The number of events needed is 482. When data are available, a one-sided log-rank test stratified by induction treatment effect will be used to evaluate the impact of those exposures or lifestyle factors on OS. Moreover, the weighted analysis as described in Section 9.2 will also be used in order to reduce the potential bias from the maintenance therapy. Patients receiving alloSCT will be included in this analysis.

9.7 Quality of Life

9.7.1 Primary Endpoint

Quality of life (QOL) will be assessed using the FACT-Leukemia and FACIT Fatigue instruments as well as the Geriatric Assessment Battery. The FACT-Leukemia and FACIT –Fatigue instruments will be administered at the following time points: at the time of randomization, two weeks after beginning induction therapy (at the time of nadir), at 28 – 30 days after beginning induction therapy, at the time of beginning consolidation therapy, at the time of randomization to

maintenance therapy or observation, and also at the time the patient discontinues treatment. The Geriatric Assessment Battery will be administered at the time of induction randomization for a baseline assessment. The primary objective of the QOL study is to compare health-related QOL in elderly AML patients receiving standard induction therapy with those receiving clofarabine. Of primary interest are the scores on the FACT-Leu TOI (combines Physical Well-Being + Functional Well-Being components of FACT-G and leukemia-specific subscales) and FACIT Fatigue score. The FACT-Leu TOI has 31 items and the score ranges from 0-124. The FACIT Fatigue has 13 items and the score ranges from 0-52. The primary endpoints will be defined as the change of the FACT-Leu TOI score and change of FACIT Fatigue scores from time of randomization to day 28 – 30 after beginning induction therapy. The changes of the FACT-Leu TOI score and changes of FACIT Fatigue scores will both be tested at a nominal level of 0.025 (two-sided) to adjust for multiple comparisons.

The FACT-Leu TOI is reported to have a standard deviation (SD) ranging from 18.1-21.7. Differences in FACT-Leu TOI mean change scores between the standard treatment arm and the clofarabine arm from time of randomization to day 28 – 30 after beginning induction therapy that can be detected with 80% power at two-sided significance level of 0.025 are presented in the following table. The expected survival models for each treatment arm specified in Section 9.2 were used to establish the estimated number of patients alive at day 28 – 30 after beginning induction therapy. A sensitivity analysis for the % of patients assumed alive to complete the questionnaire at day 28 – 30 after beginning induction therapy (65%, 80%), allowing for slightly less and more variability in the instrument (18.0, 20.0, 22.0) and assuming different correlation (0.4, 0.6) between repeated measures was performed. For example, assuming a SD of 22.0 for the FACT-Leu TOI, a correlation between repeated measures of 0.6 and using a two-sided t-test with a 0.025 significance level, there is sufficient power to detect a true difference in the FACT-Leu TOI mean change score between the two arms of 5.1-5.7.

Table 2: Differences in FACT-Leu TOI Mean Change Scores

Standard deviation (SD)	Correlation	SD of change	Difference between treatment arms in mean change score from time of randomization to day 28 – 30 after beginning induction	
			65% compliance (n=456)	80% compliance (n=561)
18.0	0.4	19.7	5.7	5.1
	0.6	16.1	4.7	4.2
20.0	0.4	21.9	6.3	5.7
	0.6	17.9	5.2	4.7
22.0	0.4	24.1	7.0	6.3
	0.6	19.7	5.7	5.1

The FACIT Fatigue scores are reported to have a standard deviation (SD) ranging from 9.4-13.6. Differences in FACIT Fatigue mean

change scores between the treatment arms from time of randomization to day 28 – 30 after beginning induction therapy that can be detected with 80% power at two-sided significance level of 0.025 are presented in following table. For example, assuming a SD of 12.5 for the FACIT Fatigue, a correlation between repeated measures of 0.6 and using a two-sided t-test with a 0.025 significance level, there is sufficient power to detect a true difference in the FACIT Fatigue mean change score between the two arms of 2.9-3.2.

Table 3: Differences in FACIT Fatigue Mean Change Scores

Standard deviation (SD)	Correlation	SD of change	Difference between treatment arms in mean change score from time of randomization to day 28 – 30 after beginning induction	
			65% compliance (n=456)	80% compliance (n=561)
9.5	0.4	10.4	3.0	2.7
	0.6	8.5	2.5	2.2
11.0	0.4	12.0	3.5	3.1
	0.6	9.8	2.8	2.6
12.5	0.4	13.7	4.0	3.6
	0.6	11.2	3.2	2.9
14.0	0.4	15.3	4.4	4.0
	0.6	12.5	3.6	3.3

9.7.2 Secondary Endpoints

For the FACT-G, leukemia-specific, FACIT –Fatigue and Geriatric Assessment Battery subscales, we will provide descriptive statistics and calculate Cronbach's alpha at each time point to assess reliability. Repeated measures analysis techniques will also be utilized to examine the treatment effect and time effect on FACT-Leu TOI score and FACIT Fatigue score. For patients randomized to the induction treatments, the longitudinal scores collected from time of randomization, two weeks after beginning induction therapy (at the time of nadir), and at 28 – 30 days after beginning induction therapy, will be analyzed according to the methods described in Schluchter[5] and Schluchter, Greene and Beck[6]. These methods take into account the possibility of informative missingness by jointly modeling the longitudinal response (QOL scores) and the time to dropout.

We will also examine whether components of a comprehensive geriatric assessment or QOL scale could predict ability to complete AML treatment. For patients who received induction therapy, logistic regression models on whether patients complete induction treatment will be fitted with all those scores (FACT-Leu TOI, FACIT Fatigue, and each component of Geriatric Assessment Battery at time of induction randomization) as covariates and treatment effect as a factor. For patients who received consolidation therapy, logistic regression models on whether patients complete consolidation treatment will be

fitted with FACT-Leu TOI, and FACIT Fatigue scores at beginning of consolidation therapy as covariates and treatment effect as a factor. For patients who received decitabine maintenance therapy, logistic regression models on whether patients complete maintenance treatment will be fitted with FACT-Leu TOI and FACIT Fatigue scores at the time of maintenance randomization as covariates.

For those patients who go to allo transplant, the FACT-Leukemia and FACT Fatigue instruments will be administered at the following time points: at the beginning of the conditioning regimen and 100 days (± 14 days) post transplant. We will provide descriptive statistics of FACT-Leukemia and FACT Fatigue subscales at each time point. Paired t-tests will be used to examine the impact of allo transplant on FACT-Leu TOI and FACIT Fatigue.

9.8 Correlative Studies

The first goal of the correlative studies is to perform expression and methylation profiling on all patients receiving decitabine and to correlate their integrated epigenetic signatures with response to decitabine. The second goal is to examine the epigenetic profiles of remission marrow in patients randomized to observation vs. decitabine in E2906 to determine whether the epigenetic signature of apparently morphologically normal bone marrow is predictive of relapse or response to decitabine maintenance. These analyses will be exploratory in nature. Assume that 86 patients will receive decitabine maintenance treatment and 86 patients will be randomized to the observation arm. Among them, 45%-50% will submit samples and have sufficient material for expression and methylation profiling. Genomic microarrays will be used to study DNA methylation, and gene expression arrays will be used to study expression. Both types of arrays are made by the NimbleGen company. The assay for DNA methylation is HELP (HpaII tiny fragment enrichment by ligation-mediated PCR). DNA is digested in parallel with MspI (resistant to DNA methylation), and then the HpaII and MspI products are amplified by ligation-mediated PCR and hybridized using separate fluorochromes to a customized array. The analysis of HELP data involves quality analysis and normalization. The methods described in Thompson et.al (127) will be used to identify epigenetics signatures which are different between patients who relapsed and patients who did not relapse. Signatures identified by epigenetic analysis will be correlated with DFS using a LARS/LASSO approach (128,129) for each maintenance arm. For the gene expression data, we will use Dchip software to normalize the expression levels and generate model-based expression intensities. Genes will be filtered by examining ratios of standard deviation to the mean. Genes will be hierarchically clustered to find genes with high/low fold change/differences. Supervised analyses will be used to identify genes that are differentially expressed between patients who relapsed and patients who did not relapsed. Genes or epigenetic signatures identified by gene expression or epigenetic analysis will be correlated with DFS using LARS/LASSO approach (128,129).

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A third goal is to study how genetic lesions cooperate to induce the resistant phenotype and to confer an adverse prognosis to elderly patients with AML. These analyses aim at elucidating in detail the spectrum of somatic alterations in elderly AML and at determining which alterations or pathways are the major

determinants of chemoresistance in this disease. While it is possible that thousands of coding and non-coding genes are affected by mutations or epigenetic lesions, these genes often have in common the perturbation of common pathways, such as DNA repair, RNA splicing, etc. This goal also aims at determining the biological functions perturbed by genetic lesions, using bio-assays and animal models. The E2906 patient cohort will allow a comparison between somatic mutations in leukemia oncogenes and tumor suppressor genes previously identified in younger AML patients accrued to E1900 with those dominating in elderly AML.

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We assume that 635 patients (87% of 727 patients) will submit samples and have sufficient material for mutation analysis. We estimate about 20 mutations will be examined. For a mutation with 50% prevalence, we assume for those mutated patients a 10% long-term cure rate and 7.5-month median overall survival in the non-cured group. With treatment arms combined, the study will have approximately 81% power to detect a 29% reduction in the hazard rate in wild-type patients, assuming 2 years of follow-up. The number of events needed is 528. For a mutation with 20% prevalence, we assume those mutated patients have a 10% long-term cure rate and 6.3-month median overall survival in the non-cured group. With treatment arms combined, the study will have approximately 81% power to detect a 35% reduction in the hazard rate in wild-type patients, assuming 2 years of follow-up. The number of events needed is 501. Those power calculations were based on a two-sided log rank test with the family-wise type I error rate controlled at 0.05 using the Bonferroni correction. For each mutation we will perform a stratified log-rank test (using the strata at randomization) adjusted for multiple testing using resampling and the min-p method of Westfall and Young (1993) as implemented in the R package multtest. In addition we will perform tests for interaction between treatment assignment and mutational status to identify potential predictive mutations. We will begin these analyses by assessing the prognostic relevance of known mutations on overall survival and response to therapy, including FLT3, DNMT3A, IDH1, IDH2, TET2, ASXL1, WT1, and MLL, and we will also include novel mutations identified in our discovery studies and by other efforts (including the AML TCGA project) to determine if novel mutations affect outcomes in this cohort of elderly patients with AML.

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We will also identify prognostic risk groups by using recursive partitioning and logistic regression, both of which are highly suitable for binary predictors. We plan to use a randomly chosen subsample of 423 (2/3 of 635) patients for training these models and the remaining 212 for validation.

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Another goal of the correlative studies is to explore the possible association of response to clofarabine with ABC-transporter P-glycoprotein (Pgp). We hypothesize that the relative expression of Pgp will determine differential activity of clofarabine in subsets of patients who overexpress Pgp, and will manifest itself in the CR rate. An aliquot of bone marrow or peripheral blood from pre-treatment samples will be evaluated for Pgp protein expression by flow cytometric assessment of binding of anti-Pgp antibody, MRK-16. In E3999, MRK-16 binding significantly correlated with P-glycoprotein function as a drug-efflux pump. We assume that 360 patients will receive clofarabine and 87% of those patients will submit samples and will have sufficient material for analysis. We assume about 20% of patients will overexpress Pgp and the CR rate of the patients who

overexpress Pgp is 30%. With 63 patients who overexpress Pgp the study will have 80% power to detect an effect size of 0.80 at the two-sided significance level of 0.05 using a Wilcoxon rank sum test. Effect size is defined as the difference of the expression of Pgp between CR patients and non-CR patients divided by the common standard deviation of the two groups. Table 4 presents the effect size to be detected with 80% power under various percentages of patients overexpress Pgp and various CR rates of those patients.

Table 4: Effect Size of the Expression of Pgp between CR Patients and non-CR Patients (two-sided α =0.05, power=80%)

CR rate of the patients who overexpress transporter	Percentages of patients overexpress transporter	Effect size to be detected
30%	40%	0.56
	30%	0.65
	20%	0.80
35%	40%	0.54
	30%	0.62
	20%	0.76

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In addition, expression of CXCR4, will be assessed in this study by flow cytometric assay to correlate the intensity of its expression with other established prognostic factors (in particular with cytogenetics) in patients receiving induction treatments. Expression intensity of CXCR4 will be divided into three groups (low, intermediate, high). Based on results of Spoo, et al [87], it is anticipated that high/intermediate CXCR4 expression is associated with inferior clinical outcomes of response or survival. Therefore, the grading will be further simplified as high/intermediate vs. low. Data will be first analyzed by induction treatment arms. We assume 315 patients will receive induction therapy in each arm and have sufficient material for analysis, and among them, 35% of patients will have low expression of CXCR4[87]. Assuming 40% overall CR rate in that treatment arm, there will be 85% power to detect a difference in CR rate if the CR rate in the low expression of CXCR4 group is 52% and 34% in the high/intermediate expression of CXCR4 group. Alternatively, assuming 45% overall CR rate in that treatment arm, there will be 81% power if the CR rate in the low expression of CXCR4 group is 56% and 39% in the high/intermediate expression of CXCR4 group. This power calculation is based on Fisher's exact test at two-sided significance level of 0.05. When data are available, logistic and Cox regression analysis, adjusted by treatment effect and prognostic factors (including cytogenetics), will be used to assess whether CXCR4 expression level is an independent predictor for response or survival.

9.9 Accrual

If the interim analysis of the maintenance comparison (described in Section 9.5) demonstrate the study should reopen, we will reopen the study to accrue an additional 52 patients to the second randomization, to finish the accrual goal of 172 patients. This requires about 74 CR/CRi patients to be pre-registered after their induction therapy. Based on the accrual rate of the first 120 randomized patients, we anticipate the additional accrual could be completed with 1.8 years.

9.10 Anticipated Accrual by Gender and Ethnicity

Based on previous data from E3999, the anticipated accrual in subgroups defined by gender and race is:

Ethnic Category	Sex/Gender		Total
	Females	Males	
Hispanic or Latino	11	7	18
Not Hispanic or Latino	335	448	783
Ethnic Category: Total of all subjects	346	455	801
Racial Category			
American Indian or Alaskan Native	0	0	0
Asian	2	2	4
Black or African American	17	18	35
Native Hawaiian or other Pacific Islander	0	0	0
White	327	435	762
Racial Category: Total of all subjects	346	455	801

9.11 Study Monitoring

This study will be monitored by the ECOG-ACRIN Data Monitoring Committee (DMC). The DMC meets twice each year. For each meeting, all monitored studies are reviewed for safety and progress toward completion. When appropriate, the DMC will also review interim analyses of outcome data. Copies of the toxicity reports prepared for the DMC meetings are included in the study reports prepared for the ECOG-ACRIN group meeting (except that for double blind studies, the DMC may review unblinded toxicity data, while only pooled or blinded data will be made public). These group meeting reports are made available to the local investigators, who may provide them to their IRBs. Only the study statistician and the DMC members will have access to interim analyses of outcome data. Prior to completion of this study, any use of outcome data will require approval of the DMC. Any DMC recommendations for changes to this study will be circulated to the local investigators in the form of addenda to this protocol document. A complete copy of the ECOG-ACRIN DMC Policy can be obtained from the ECOG-ACRIN Operations Office - Boston.

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10. Correlative Studies

NOTE: ECOG-ACRIN requires that all biological samples submitted be entered and tracked via the online ECOG-ACRIN Sample Tracking System. An STS shipping manifest form must be generated and shipped with the sample submissions. See Section 10.8.

10.1 Immunophenotype and Molecular Genetics

Immunophenotyping has become an essential part of the diagnostic work-up of all leukemia patients. In fact, the diagnosis of leukemia without immunophenotypic characterization is no longer acceptable. ECOG-ACRIN has, therefore, developed a model system for antigenic data collection that requests specimens from all patients entered on ECOG-ACRIN leukemia treatment trials be studied by ECOG-ACRIN's Leukemia Translational Studies Laboratory (LTSL). In addition to establishing the leukemia subtype, this centralized testing and data collection has allowed that research questions of clinical relevance be applied to a growing database (e.g., definition of prognostically significant antigen expression levels to eventually yield specific treatment subcategories). Depending on the study protocol and tissue availability, anti-coagulated (heparin, EDTA, ACD) peripheral blood or bone marrow or both are to be submitted to the LTSL. The dual function of the LTSL in eligibility determination and sample processing/banking (ECOG-ACRIN's Leukemia Tissue Bank), facilitates the distribution of fresh or adequately processed specimens to other laboratories involved in protocol-embedded correlative studies, such as the analyses of epigenetic and genetic lesions.

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In addition to the study of abnormal hematopoietic cells, the focus of research on circulating serum factors in patients with leukemia or myelodysplasia has increased. Two tubes of coagulated peripheral blood (red top tubes) are requested for future research studies that may aim at identifying pathogenetic, diagnostic, or prognostic factors associated with leukemia or myelodysplasia.

Serum and cells from peripheral blood or bone marrow from patients entered on studies of hematologic malignancies are stored in ECOG-ACRIN's Leukemia Tissue Bank for future laboratory studies. The bank provides the scientific community a source of leukemia specimens that are collected, processed, and maintained following quality control and quality assurance guidelines. The bank will accommodate requests from investigators within and outside ECOG-ACRIN in a timely and efficient manner, with respect to tissue type, tissue preparation, and most importantly, biologic characteristics of specimens.

10.1.1 Sample Collection and Submission Schedule

Bone marrow, peripheral blood, and smears **must** be submitted at the following time points **(Mandatory Submissions)**:

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- Pre-Registration
  - Notification to the submitting institution:

    The ECOG-ACRIN Leukemia Translational Studies Laboratory will notify the submitting institution of the results of the centralized immunophenotyping within 4-6 hours of receipt of the samples.

- Time of Outcome Following Induction – Prior to Consolidation (at time of response assessment or off-study, whichever occurs first)
- End of Consolidation (Prior to 2nd Randomization - Step 3)
- Relapse

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Bone marrow and peripheral blood will also be used for the optional correlative studies per patient consent.

Peripheral blood and buccal rinse [preferred] or swabs (baseline only) are to be submitted at the above time points for banking per patient consent.

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Samples are to be shipped on the day they are drawn. If this is not possible, call the LTSL.

If you have questions, contact the LTSL at (718) 920-9992.

10.1.2 Sample Preparation Guidelines

NOTE: All cooperative groups should send specimens to the ECOG-ACRIN Leukemia Translational Studies Laboratory.

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Dr. Paietta's institutional regulations require that she receive a copy of the patient's consent and a copy of the HIPAA authorization at time of or prior to submission of the pre-study samples.

All samples must be clearly labeled with the ECOG-ACRIN protocol number (E2906), ECOG-ACRIN patient sequence number, patient's initials, date of collection, and sample type.

10.1.2.1 The following are to be submitted:

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1. MANDATORY AT ALL TIME POINTS: Heparinized bone marrow aspirate. The laboratory will accept any amount as long as it represents a first pull. It is imperative that aspirate from a separate first pull be submitted. DO NOT SUBMIT THE SECOND OR THIRD PULL OF ASPIRATE FROM THE SAME ASPIRATION SITE. With every pull from the same aspiration site, the blast count decreases due to hemodilution. Ideally, 2-3 mL of aspirate from a separate aspiration site should be submitted

   For patients with an inaspirable bone marrow ("dry tap"), or if a bone marrow has been done previously and the patient refuses to have another aspiration done, call Dr. Paietta's laboratory at (718) 920-9992 to discuss the case and the possibility for submitting peripheral blood only. Be prepared to report the WBC count and the blast count in the peripheral blood at the time of the call.

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2. MANDATORY AT ALL TIME POINTS: Heparinized or EDTA peripheral blood (four (4) green or purple top tubes, 30-40 mL).

3. MANDATORY AT ALL TIME POINTS: At least two (2) Wright-Giemsa stained bone marrow smears and one (1) Wright-Giemsa stained peripheral blood smear.

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NOTE: These smears will be forwarded to Dr. Daniel Arber at Stanford University for morphologic review, if necessary.

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4. MANDATORY AT ALL TIME POINTS: A copy of the institutional pathology report on the bone marrow must be submitted. If the pathology report is not available at the time the samples are mailed to the ECOG-ACRIN Leukemia Translational Studies Laboratory, the report must be FAXED at a later time to: (718) 920-1161. The pathology report must include cytogenetic results and any results from fluorescence-in-situ (FISH) hybridization and/or molecular studies done at the submitting institution.

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5. Two (2) red top serum tubes of peripheral blood (15-20mLno anticoagulent)

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- Submit from patients who answer "YES" to *"I agree to provide additional specimens for research."*

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NOTE: If samples designated for banking only are not submitted, please note the reason in the comments section of the Sample Tracking System.

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6. Buccal Cell Samples

Most commonly, institutions will have buccal swab kits for the collection of cells for HLA-typing. Alternatively, Scope, a commercial brand mouthwash, or normal saline in a small sealed bottle can be given to the patient for a mouthwash.

Aseptic techniques must be used to collect buccal cells from patients on-site and buccal cells must not be contaminated with cells from any other source. Patients should not brush their teeth or consume food prior to buccal cell collection.

If a cytobrush is used, the collection end should not be touched and the patient should not scrape his/her cheek too vigorously. The inside of the cheek should be scraped 6 times. Several models of cytobrushes are available, such as the Omni swab or Bio-Swab from Arrowhead Forensics or the Cyto-Pak CytoSoft Brush from Medical Packaging Corp.

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- If mouthwash (e.g. Scope) or normal saline is used, the patient should pour approximately 10cc of mouthwash or saline into his/her mouth and

vigorously swish it against the cheeks for 10 seconds and deliver the solution into a labeled 15cc polypropylene test tube or a sterile urine cup. Among mouthwashes, the Scope brand fares best in collecting buccal cells for the preparation of high-quality DNA in high yield.

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- It is important that buccal cells do not dry out during shipping. Institutions are advised to seal the container containing the buccal cells tightly. Ship containers on ice-packs, together with the patient's peripheral blood and bone marrow specimens.

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- Submit from patients who answer "YES" to *"I agree to provide additional specimens for research."*

  NOTE: Buccal rinse (preferred) or swabs are strongly encouraged to be collected at baseline, but can be collected at any other time during the study if necessary.

10.1.3 Shipping Procedures

Log the shipment into the ECOG-ACRIN STS the day of shipment. If the STS is unavailable, an Generic Specimen Submission Form (#2981) must be submitted with the samples. Once STS is available, retroactively log the shipment into STS, using the actual collection and shipping dates.

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The LTSL must be notified by telephone the day of shipment.

Fax or Email to Dr. Paietta is NOT Acceptable.

Telephone: (718) 920-9992

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During off hours, all information regarding the shipment should be left on the answering machine in the LTSL including:

E2906 Sequence Number

Patient's Initials

Type of Specimen Shipped

Name, Telephone Number, and Institution

Follow the directions given in the phone message.

For questions regarding the shipment, Dr. Paietta and her staff can be reached at the cell phone numbers provided on the recorded message. Please always try Dr. Paietta first. Questions can also be addressed to Dr. Paietta via e-mail (epaietta@earthlink.net), however, please do not use e-mail for shipment notifications.

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Heparinized bone marrow and peripheral blood samples or EDTA peripheral blood samples, coagulated blood in red top tubes and buccal cells must be sent fresh (on the day of collection) on **cool**

packs (do not freeze and do not use ice cubes) by overnight courier (preferably Federal Express) to arrive within 24 hours to:

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Elisabeth Paietta, Ph.D.
Montefiore Medical Center-North Division (Wakefield)
600 East 233rd Street
6th Floor, Immunology Laboratory
Bronx, New York 10466-2697
Tel: (718) 920-9992
FAX: (718) 920-1161

An STS shipping manifest form must be generated and shipped with all sample submissions.

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Please enter all information into the STS, including time and date of specimen collection and peripheral blood WBC count and blast count.

The LTSL is open to receive shipments Monday through Saturday. Shipments on Fridays for Saturday delivery must have "Saturday Delivery" marked on the overnight courier slip.

If samples need to be drawn late at night, on Sunday, or on a holiday when Federal Express does not operate, keep the samples in a refrigerator between 10 and 15 degrees Celsius until the next day when it can be shipped.

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In general, always ship specimens the day before a holiday for delivery the day after the holiday.

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10.1.3.1 Sample Processing and Routing

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Samples will be processed in ECOG-ACRIN's Leukemia Translational Studies Laboratory and sent to Dr. Ari Melnick's laboratory as frozen viable cells or isolated DNA if viable cells are not available. Dr. Melnick will perform his studies (Section 10.3) and/or forward DNA to Dr. Ross Levine for Dr. Levine's studies (Section 10.4). From the remaining specimen, an aliquot will be sent to GenPath for Array CGH studies.

The priority of work is as follows: 1. Melnick 2. Levine 3. Array CGH at GenPath. In some cases, RNA may not be available for epigenetic studies (Dr. Melnick), although DNA may be available for mutation analysis (Dr. Levine) and Array CGH (GenPath).Bone marrow and peripheral blood smears may be forwarded to Dr. Daniel Arber at Stanford University for morphology review.

Dr. Elisabeth Paietta's laboratory will perform the P-glycoprotein and CXCR4 expression studies as outlined in Section 10.5.

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10.2 Cytogenetic Review

The cytogenetic review will be performed at the Mayo Clinic Cytogenetic Laboratory.

10.2.1 Sample Submission Schedule and Preparation Guidelines

Karyotypes must be collected and submitted at the following time points:

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- Enrollment/Baseline

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- Time of Outcome Following Induction (Prior to Consolidation) [at time of response assessment or off-study, whichever occurs first]
- End of Consolidation (Prior to 2nd Randomization - Step 3)
- Relapse

NOTE: Karyotypes are required at all time points.

10.2.1.1 Karyotypes (MANDATORY AT ALL TIME POINTS)

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Within 30 days of specimen collection at each time point, investigators must send the two (2) original karyotypes per clone, FISH results, institution's cytogenetic laboratory report, the Leukemia Cytogenetic Form (#365R) to the ECOG-ACRIN Cytogenetic Committee.

NOTE: If cytogenetic studies are not successful, submit the laboratory report and #365R form to Gary Hicks by either mail or fax.

Original karyotypes will be returned, upon written request, when the review and analysis are complete.

10.2.1.2 Shipping Guidelines

Log the shipment into the ECOG-ACRIN STS the day of shipment. If the STS is unavailable, an Generic Specimen Submission Form (#2981) must be submitted with the karyotypes. Once STS is available, retroactively log the shipment into STS, using the actual collection and shipping dates.

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Direct questions to Gary Hicks at Tel: (507) 284-2950 or Fax: (507) 284-0043.

The forms and karyotypes are to be shipped to:

Gary Hicks
Mayo Clinic Cytogenetic Laboratory
970 Hilton
200 First Street, S.W.
Rochester, MN 55905
Tel: (507) 284-2950
Fax: (507) 284-0043

An STS shipping manifest form must be generated and shipped with all submissions.

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10.3 Epigenetics

E2906 incorporates a randomization to maintenance therapy with decitabine, a methyltransferase inhibitor, and affords a unique opportunity to study the epigenetic phenotype in AML at presentation and in remission. The following clinical & basic hypotheses will be studied in Dr. Ari Melnick's laboratory at Weill Cornell Medical College, New York, NY.

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10.3.1 Clinical

Our preliminary data show that AML comes in different epigenetic subtypes, and that a phenotype of epigenetic instability seems to correlate with responsiveness with DNA methylransferase inhibitors. In addition to the epigenetic unstable phenotype, many of the more defined AML epigenetic subtypes had different medical outcomes in retrospective studies and so may be highly clinically relevant. Interestingly, gene expression profiling failed to identify several of these subtypes alone, demonstrating the power of epigenetic analysis to capture important biological differences in AML. Finally, the integration of gene expression and epigenetic signatures were shown to synergistically capture biological differences between patients with leukemia.

Based on these data we predict that patients who present with AML and epigenetic instability will be the better candidates to respond to decitabine maintenance. Therefore we propose to perform expression and methylation profiling on all patients enrolled in 2906 and correlate their integrated epigenetic signatures with response to decitabine. Of equal importance we also predict that specific integrated epigenetic subtypes associated with differential clinical outcomes will be confirmed in this prospective study and could lead to an important advance in leukemia classification. The integrated signatures captured upon enrollment of these patients will be used to prospectively validate, confirm and possibly expand our ability to accurately segregate patients into biologically distinct cohorts. We plan to use these data to direct the design of future ECOG-ACRIN clinical trials.

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10.3.2 Basic

Data from the published literature and our laboratory indicate that progressive alteration of epigenetic signatures in bone marrow cells occurs during normal aging. We hypothesize that certain epigenetic signatures associated with aging might constitute an epigenetic field defect that could result in leukemogenesis. We will examine the epigenetic profiles of remission marrow in patients randomized to observation vs decitabine in E2906 to determine whether epigenetic signature of apparently morphologically normal bone marrow is predictive of relapse or response to decitabine maintenance. Our data performed in fractionated vs unfractionated marrow from the same individuals indicate that epigenetic signatures indicative of aberrant epigenetic programming are equally readily evident in either case, and

are readily distinguishable between normal and abnormal samples. Therefore, we expect our experimental approach to successfully capture indications of epigenetic field defects in this patient cohort.

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10.4 Somatic Mutation Analysis

Following the model applied to E1900, a study in younger AML, E2906 will delineate the full spectrum of genetic lesions occurring in elderly patients with AML to discover potential differences dependent on age. The following clinical & basic hypotheses will be studied in Dr. Ross Levine's laboratory at Memorial Sloan Kettering Cancer Center, New York, NY.

10.4.1 Clinical

High throughput mutational profiling of patients enrolled in E1900 allowed us to delineate the somatic mutational frequencies of all known leukemia oncogenes and tumor suppressors in a large patient cohort who presented with de novo AML <60 years of age. Specifically, we found that mutations in ASXL1 and PHF6 are associated with an adverse outcome in the entire E1900 cohort and with primary induction failure. On the other hand, the IDH R140Q mutation was associated with an improved outcome in younger AML. In addition, we found that TET2, ASXL1, PHF6, DNMT3A, and WT1 mutations are associated with an adverse outcome in intermediate risk AML, including in patients without FLT3 mutations. Moreover, we found that patients with mutated DNMT3A, which occurs in 30% of younger AMLs, benefited from randomization to 90mg daunorubicin. By contrast, there was no benefit of higher dose chemotherapy for patients with wild-type DNMT3A, suggesting that DNMT3A mutations represent a novel biomarker with immediate therapeutic relevance. Analogous studies are currently underway to determine somatic genetic lesions in elderly AML from ECOG trial, E3999.

10.4.2 Methodology

Samples from E2906 will be analyzed as patients are accrued to the trial. High quality genomic DNA will be extracted from mononuclear cells provided by the LTSL, as done previously for E1900 and E3999 specimens. Control DNA will be isolated from normal T-cell contaminating the leukemia specimens or from remission samples. Genomic DNA from blasts and control cells will be subjected to Agilent SureSelect sequence capture followed by bar-coding and sequencing on our HiSeq2000. Genomic DNA will also be processed for reduced representation bisulfate sequencing and examined on the HiSeq2000. Excess DNA will be stored and made available to ECOG-ACRIN for distribution to other approved investigators. Validation will be done using amplicon based sequencing on our existing resequencing platform (used for E1900 and E3999 mutational studies) to facilitate higher throughput candidate gene mutational analysis of genomic DNA. Wherever possible, mutations will be analyzed in remission or T-cell DNA to ascertain whether specific alterations are somatic or in the germline. We will use our suite of computational biostatistical tools for identifying candidate somatic mutations. The data set will be evaluated for genetic signatures that distinguish

patient subtypes using unsupervised methods such as hierarchical clustering, principal component analysis, and consensus clustering. Signatures of co-occurring lesions will be determined using supervised methods including moderated T tests with correction for multiple testing, LEMMA, random forest, etc. The biological connectivity between gene sets and their genomic features will be derived using tools including iPAGE, FIRE, IPA, etc. We will use a series of statistical approaches to determine whether lesions in specific recurrently affected genes or pathways indicate risk of chemotherapy failure, disease recurrence or survival. In addition to univariate and multivariate analyses, we will use machine-learning algorithms like SuperPC and BDVAL to identify genetic, epigenetic, and integrated classifiers. All mutations detected will be validated by repeat PCR and sequencing on the unamplified diagnostic sample. Wherever possible, matched control DNA (remission or T-lymphocyte DNA) will be resequenced to determine if candidate disease alleles are somatic or present in germline.

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10.5 AML Prognostic Factors & Mechanisms of Resistance

Clofarabine (like cytarabine) requires active cellular uptake by equilibrative or concentrative nucleoside transporters, but is not a substrate for P-glycoprotein (Pgp). We hypothesize that the relative expression of Pgp will determine differential activity of clofarabine in subsets of patients who overexpress these transporters, and manifest in the CR rate. An aliquot of BM from pre-treatment will be evaluated for Pgp expression by flow cytometric assessment of the binding of antibody MRK-16[86], an antibody directed against a cell-surface epitope of Pgp. In addition, expression of CXCR4, which has been associated with inferior prognosis in retrospective studies in younger patients, will be assessed prospectively in this study by flow cytometry in the LTSL to correlate the intensity of its expression with other established prognostic factors (in particular with cytogenetics)[87].

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10.6 Array CGH Studies

Cytogenetic analysis provides valuable diagnostic and prognostic information for the evaluation of hematologic malignancies and solid tumors. Traditional and new techniques have been applied in clinical oncology with a variable range of resolution and sensitivity including chromosome analysis, fluorescence *in situ* hybridization (FISH) and more recently DNA array-based technologies.

Karyotypic analysis in metaphase cells is considered a routine test for the detection of chromosomal aberrations associated with the diagnosis, prognosis and treatment of the malignant disorders. However, many genomic abnormalities remain undetected by karyotyping due to its relatively low resolution (~5 Mb) and inadequate sensitivity (it depends on the sample cell distribution and proliferation). Alternatively, FISH has been applied for the detection of diagnostic/prognostic relevant chromosomal abnormalities in interphase nuclei. Although this technique increases the sensitivity for the identification of targeted lesions (~1 Mb), the resolution is still low since the detection of unbalanced rearrangements is not well defined. Recently, the application of DNA array-based technologies such as array comparative genomic hybridization (aCGH) and single nucleotide polymorphism arrays (SNP-A) have

enabled the detection of previously undetected copy number changes (CNC) and the more precise determination of genomic break points of regions that are gain or loss with an increased resolution and sensitivity.

The use of aCGH in the identification of CNC and correct mapping of genomic aberrations breakpoints is becoming the gold standard for the diagnosis of hematological malignancies such as lymphoid, plasma cell and myeloid disorders. The used of aCGH has uncovered the complexity of the CNC observed in those malignancies and suggests the need for further investigation. Several of these genomic alterations are known to affect oncogenes or tumor suppressor genes. Classical examples include amplification of ERBB2 and MYC oncogenes and deletions of RB1, PTEN and CDKN2A tumor suppressor genes.

Considering the high resolution and sensitivity of aCGH in addition to the flexibility of using DNA instead of cultured cells, we developed and clinically validated a custom microarray using the Agilent platform. GenArray™ - Molecular Karyotyping is a non-FDA approved test based in aCGH technology for the detection of genomic imbalances of diagnostic, prognostic and therapeutic significance in hematological malignancies. The microarray contains approximately 64,000 DNA oligonucleotide probes. The design of the probes is based on the human genome sequence build hg18 (NCBI build 36). The probes are spaced at approximately 50 kb intervals across the genome and there is a higher probe density in some cancer-relevant regions (1 probe every 10 kb). The current array cannot detect balanced chromosomal rearrangements (reciprocal translocations, Robertsonian translocations, balanced inversions and insertions) and imbalances of areas not covered by the microarray. In addition, the oligonucleotide probes in this microarray are not designed to detect point mutations, epigenetic effects or loss of heterozygosity (LOH) events.

In order to detect genomic gains and losses, 500ng of genomic DNA from the patient and a normal reference DNA are differentially labeled with fluorophores (Cy3 and Cy5) and competitively hybridized to the array (40 hours). Normal reference DNA is promega male and female reference DNA which have been optimized extensively and CLIA certified for GenPath's Array CGH assay. The relative fluorescence intensities of the hybridized DNA from each sample (at each oligonucleotide probe) is measured by a high-resolution scanner and presented as a log (base 2) ratio of the sample/reference intensity signal. A plot of these ratios across the genome sequence displays any variations between the patient and reference DNA sample. The array has a resolution of 250 kb (5 consecutive probes with a minimum absolute ratio value of 0.25). The detection limit/sensitivity for chromosomal alterations is approximately 20-30% (can be detected is present in at least 20-30% of the DNA) with 100% specificity. DNA gains or losses greater than 1 Mb will be reported and alterations less than 1 Mb in size will be interpreted at the discretion of the geneticist.

GenPath's team of experts in the microarray laboratory has accumulated significant experience in Array CGH testing. GenPath has run the GenArray in more than 3,000 cases in hematological malignancies with an overall failure rate of less than 1%, primarily due to the poor quality of DNA. GenPath currently runs 300 cases a month and the volume continues to grow. The group has discovered novel and recurrent alterations not detected by cytogenetics of FISH analyses in myeloid, lymphoid and plasma cell neoplasms with important clinical significance. This methodology has the potential to enrich the understanding on

tumor biology, clarify the cancer classification systems, and help in the identification of useful molecular markers of prognostic or therapeutic value.

All samples will be cross validated by Dr. Melnick's next-generation sequencing and Q-PCR platforms for focused lesions that are of particular interest. In addition, germline DNA will be sequenced as part of Dr. Melnick's correlative study and in cases where germline copy number abnormalities by sequencing are detected validation by PCR will be completed. GenPath may provide further validation by running repeat arrays.

The number of samples tested is contingent upon the number of patient samples with sufficient testing material. Ideally, all patients in E2906 will have Array CGH studies completed but that is most likely not possible given specimen constraints.

10.7 ECOG-ACRIN Sample Tracking System

It is **required** that all samples submitted on this trial be entered and tracked using the ECOG-ACRIN Sample Tracking System (STS). The software will allow the use of either 1) an ECOG-ACRIN user-name and password previously assigned (for those already using STS), or 2) a CTSU username and password.

When you are ready to log the collection and/or shipment of the samples required for this study, please access the Sample Tracking System software by clicking https://webapps.ecog.org/Tst

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Important: Please note that the STS software creates pop-up windows, so you will need to enable pop-ups within your web browser while using the software. A user manual and interactive demo are available by clicking this link: http://www.ecog.org/general/stsinfo.html Please take a moment to familiarize yourself with the software prior to using the system.

A shipping manifest form must be generated and shipped with all sample submissions.

Please direct your questions or comments pertaining to the STS to ecog.tst@jimmy.harvard.edu

10.7.1 Study Specific Notes

Generic Specimen Submission Form (#2981) will be required only if STS is unavailable at time of sample submission. Indicate the appropriate Lab ID# on the submission form:

- 0002 = ECOG-ACRIN Leukemia Translational Studies Laboratory
- 0003 = ECOG-ACRIN Cytogenetic Laboratory

Retroactively enter all specimen collection and shipping information when STS is available.

10.8 Sample Inventory Submission Guidelines

Inventories of all samples collected, aliquoted, and used on the above mentioned laboratory correlative studies will be submitted to the ECOG-ACRIN Operations Office - Boston on a monthly basis. Inventories will be submitted electronically by any laboratory holding and/or using any specimens associated with this study. All other correspondence should be addressed to the attention of the Translational Science Team.

10.9 Lab Data Transfer Guidelines

The data collected on the above mentioned laboratory correlative studies will be submitted to the ECOG-ACRIN Operations Office - Boston by the central laboratory on a quarterly basis. The quarterly cut-off dates are March 31, June 30, September 30 and December 31. Data is due at the ECOG-ACRIN Operations Office - Boston 1 week after these cut-off dates.

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10.10 Banking

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The residuals and/or derivatives of samples collected for this study will be retained at the LTSL/ECOG-ACRIN Leukemia Tissue Bank for possible use in ECOG-ACRIN approved future studies. If future use is denied or withdrawn by the patient, the samples will be removed from consideration for use in any future study.

11. Records to Be Kept

Please refer to the E2906 Forms Packet for the forms submission schedule and copies of all forms. The E2906 Forms Packet may be downloaded by accessing the ECOG World Wide Web Home Page (http://www.ecog.org). Forms must be submitted to the ECOG-ACRIN Operations Office - Boston, FSTRF, 900 Commonwealth Avenue, Boston, MA 02215 (ATTN: DATA).

This study will be monitored by the CTEP Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly from the ECOG-ACRIN Operations Office - Boston to CTEP by electronic means.

11.1 Records Retention

This study is being conducted under an IND exemption and is not intended to support any FDA-related filings. However, ECOG-ACRIN requires clinical investigators to retain all trial-related documentation, including source documents, for at least one year from the posting of the final technical report of the outcome of this trial to support any publication of the data.

12. Please contact the ECOG-ACRIN Operations Office - Boston prior to destroying any source documents.Patient Consent and Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

13. References

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Phase III Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)

Appendix I

Informed Consent Template for Cancer Treatment Trials (English Language)
[Deleted in Addendum #5]

INFORMED CONSENT INTENTIONALLY REMOVED FROM PROTOCOL DOCUMENT

Appendix I was removed from the protocol document in Addendum #5 and is posted as a separate document on the ECOG website. This was removed from the protocol to comply with NCI formatting guidelines.

Phase III Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)

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Appendix II

Patient Thank You Letter

We ask that the physician use the template contained in this appendix to prepare a letter thanking the patient for enrolling in this trial. The template is intended as a guide and can be downloaded from the ECOG web site at http://www.ecog.org. As this is a personal letter, physicians may elect to further tailor the text to their situation.

This small gesture is a part of a broader program being undertaken by ECOG-ACRIN and the NCI to increase awareness of the importance of clinical trials and improve accrual and follow-through. We appreciate your help in this effort.

PATIENT NAME] [DATE]

[PATIENT ADDRESS]

Dear [PATIENT SALUTATION],

Thank you for agreeing to take part in this important research study. Many questions remain unanswered in cancer. With the participation of people like you in clinical trials, we will improve treatment and quality of life for those with your type of cancer.

We believe you will receive high quality, complete care. I and my research staff will maintain very close contact with you. This will allow me to provide you with the best care while learning as much as possible to help you and other patients.

On behalf of ***[INSTITUTION]*** and the ECOG-ACRIN Cancer Research Group, we thank you again and look forward to helping you.

Sincerely,

[PHYSICIAN NAME]

Phase III Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)

Appendix III

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E2906 Clofarabine Study Drug Request Form

Study Drug Request Form: Investigator-Sponsored Trials (ISTs)

Date of Request:	**Date Needed By:**	**Protocol No. / Title:**	
Attention:			
Ship to Address:			
Phone #:		**PI:**	**Site #:**
Fax #:			
Email:		**Institution:**	

Qty	Units	Study Drug Requested

Comments:

Please email the completed form to: **GTOdrugorders@genzyme.com** or fax to: 908-635-5941.
Allow 5-7 business days for initial drug orders and 3-5 business days for drug re-orders from the acknowledgement of order receipt to the date of delivery. There are no weekend or Monday deliveries.
For questions regarding shipments, please contact Jay Tiña @ 908-981-4535 / jay.tina@sanofi.com or Joseph Silverman @ 908-981-3981 / joseph.silverman@sanofi.com.

Do Not Write Below this Line - for Genzyme Use Only

Medical Affairs

The following documents are required for initial shipments:

1. IRB Approval Letter……☐ Y ☐ N ☐ N/A
2. Signed Research Agreement…… ☐ Y ☐ N ☐ N/A
3. IND Status document……☐ Y ☐ N ☐ N/A
4. clinicaltrials.gov listing……☐ Y ☐ N ☐ N/A
5. IACUC Approval Letter (Pre-clinical only)……☐ Y ☐ N ☐ N/A

Comments:

Signature: _____ Date: _____
Fax this form to CPRS: 508-424-4484

CPRS

Processed by: _____ Date: _____
Reviewed by: _____ Date: _____

Comments:

POR#: _____

Phase III Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)

Appendix IV

E2906 Decitabine Study Drug Request Form

Site Instructions: All shaded areas must be completed before forwarding the drug request to the ECOG-ACRIN Operations Office - Boston.

Email completed form as an attachment to 900.drugorder@jimmy.harvard.edu or fax to 617-632-2063. The ECOG-ACRIN Drug Team will approve and email the order to Fisher Clinical Services

Secondary Contact (Research Nurse/Study Coordinator/Pharmacist):			
Name:		Title:	
Telephone:		Email:	
Investigator Address (Please include contact information):		**Ship Supplies To (If different from Investigator Address):**	
Institution Name:		Institution Name:	
Address:		Address:	
City, State, Zip:		City, State, Zip:	
Telephone:		Telephone:	
Email:		Email:	

Date Requested: (MM/DD/YY)	
Study Drug:	Decitabine (50 mg vials)
Shipment Must Reach Destination By: (MM/DD/YY) - Deliveries are not made on Mondays	

ECOG-ACRIN Patient Sequence Number:	
# Vials Needed: ***(3 vials needed per cycle, please order 2 cycles at a time)*** ***NOTE: Vial packages contain 5 vials per package***	
Materials ID#:	

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To Be Completed By The ECOG-ACRIN Drug Team	
Cgroup/Inst/Affil: **CTEP ID #:** **IRB Approval Date:** **Regulatory Docs:**	**Completed By:** **Date:**

Phase III Trial of Clofarabine as Induction and Post-Remission Therapy vs. Standard Daunorubicin & Cytarabine Induction and Intermediate Dose Cytarabine Post-Remission Therapy, Followed by Decitabine Maintenance vs. Observation in Newly-Diagnosed Acute Myeloid Leukemia in Older Adults (Age ≥ 60 Years)

Appendix V

ALESE Baseline Questionnaire

Rev. 2/13

Acute Leukemia Epidemiology & Survival In ECOG (Alese Study)

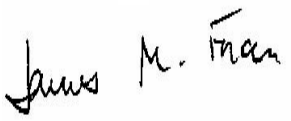
Dear Patient,

Thank you for taking part in the **A**cute **L**eukemia **E**pidemiology & **S**urvival i**n** **E**COG study being done by Leukemia researchers as part of the E2906 trial. The purpose of this study is to identify environmental, lifestyle, and medical factors related to the development of acute myeloid leukemia (AML) in adults and assess the potential impact of those factors on outcomes after treatment.

The study consists of filling out this survey. This survey asks for general information about you as well as information about your personal habits, such as smoking and alcohol use, medical history, and environmental and chemical exposures you may have had on the job or in your everyday life. It will take about 20-30 minutes to fill out. Please answer every question as best you can. If you do not know the answer to a question, please provide your best guess.

In order to obtain the most valid information possible, **it is important that this survey is answered by the person it was given to**. If you are not able to fill in the survey, you can discuss with your physician or research nurse who will assist you. If you have any other questions as you fill out this survey, please discuss this with the physician or research nurse looking after you. You or they may also contact the Study Chair (Dr. James Foran) with questions at foran.james@mayo.edu.

Warm regards,



James M. Foran, MD
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About You

Thank you for helping with this survey. Your answers are important.

Please take the time to read and answer each question carefully.

For some questions, you will **PUT AN X OR ✓ IN THE BOX** that goes with your answer, like this:

EXAMPLE 1:

1. Are you? 1 ☒ Male 2 ☐ Female or 1 ☑ Male 2 ☐ Female

You will sometimes be told to skip over some questions in this survey. When this happens, you will see an arrow with a note that tells you what questions to answer next, like this:

EXAMPLE 2:

☐ YES ⟶ **Go to Question 13**

For some questions, you will enter letters or numbers in boxes. Please enter one letter or number per box and stay within the box, like this:

EXAMPLE 3:

A	B	2	8

Today's date: ☐☐ ☐☐ ☐☐☐☐

1. How old are you? ☐☐ Years old

2. Are you?

1 ☐ Male
2 ☐ Female

3. Are you of Hispanic or Latino origin?

1 ☐ No
2 ☐ Yes

4. Which best describes your racial background (choose the **one** you most identify with)?

1 ☐ White
2 ☐ Black or African American
3 ☐ Native Hawaiian or other Pacific Islander
4 ☐ Asian
5 ☐ American Indian/Alaska Native
6 ☐ Other, please specify: _____

5. What is your marital status?

1 ☐ Married or living in a marriage-like relationship
2 ☐ Widowed
3 ☐ Divorced
4 ☐ Separated
5 ☐ Never married

6. What is the **highest** level of schooling you have completed? (Mark one.)

1 ☐ 8th grade or less
2 ☐ Some high school
3 ☐ High school graduate
4 ☐ GED (high school equivalency)
5 ☐ 1 to 3 years vocational education beyond high school
6 ☐ Some college
7 ☐ College graduate
8 ☐ One or more years of graduate or professional school
9 ☐ Other, please specify:_____

7. Thinking back to two years ago, which of the following best describes your **total** household income?

1 ☐ Up to $10,000
2 ☐ More than $10,000 up to $20,000
3 ☐ More than $20,000 up to $40,000
4 ☐ More than $40,000 up to $60,000
5 ☐ More than $60,000 up to $80,000
6 ☐ More than $80,000 up to $100,000
7 ☐ More than $100,000

8. Which State do you live in, and how many years have you lived there?

☐☐ State

☐☐ Years

9. How many years have you lived in your current residence?

☐☐ Years

Lifestyle

10. Have you ever used **any** tobacco products for six months or longer? (Please include cigarettes, cigars, pipes, snuff, and chewing tobacco.)

1 ☐ No → **Go to question 18.**
2 ☐ Yes

11. At any time in your life have you smoked cigarettes for six months or longer?

1 ☐ No → **Go to question 17.**
2 ☐ Yes
↓

12. If yes, at what age did you start smoking cigarettes?

☐☐ Years old

13. Do you currently smoke cigarettes?

1 ☐ No → 15. Before stopping, how many cigarettes did you usually smoke per day?

2 ☐ Yes

14. How many cigarettes do you usually smoke per day?

☐☐ Cigarettes per day

☐☐ Cigarettes per day

16. How old were you when you stopped smoking cigarettes?

☐☐ Years old

17. Going back two years ago and thinking about your life up until that point, did you use any of these tobacco products for 12 months or longer?

a. Cigar	1 ☐ No	2 ☐ Yes	For how many years? ☐☐ Years
b. Pipe	1 ☐ No	2 ☐ Yes	For how many years? ☐☐ Years
c. Snuff	1 ☐ No	2 ☐ Yes	For how many years? ☐☐ Years
d. Chewing Tobacco	1 ☐ No	2 ☐ Yes	For how many years? ☐☐ Years

18. Not including the past two years, have you ever lived with someone who regularly smoked cigarettes around you?

1 ☐ No

2 ☐ Yes

19. How many years did they smoke cigarettes around you regularly?

☐☐ Years

20. Not including the past two years, have you ever worked with someone who regularly smoked cigarettes around you?

1 ☐ No
2 ☐ Yes

21. How many years did they smoke cigarettes around you regularly?

☐☐ Years

22. During your entire life, have you had 12 drinks or more of any kind of alcohol? (One drink of alcohol is equal to one can of beer, one glass of wine, or one shot of liquor, such as whiskey, brandy, or gin.)

1 ☐ No → **Go to question 24.**
2 ☐ Yes

23. If yes, for each age group below, how many drinks of alcohol did you usually have?

	None	Less than 1 each month	1 to 3 each month	1 to 2 each week	3 to 6 each week	1 to 2 each day	3 or more each day	
a. Less than 18 years old	a.☐	b.☐	c.☐	d.☐	e.☐	f. ☐	g.☐	
b. 18-22 years old	a.☐	b.☐	c.☐	d.☐	e.☐	f. ☐	g.☐	
c. 23-30 years old	a.☐	b.☐	c.☐	d.☐	e.☐	f. ☐	g.☐	
d. 31-49 years old	a.☐	b.☐	c.☐	d.☐	e.☐	f. ☐	g.☐	
e. 50-65 years old	a.☐	b.☐	c.☐	d.☐	e.☐	f. ☐	g.☐	
f. 66+ years old	a.☐	b.☐	c.☐	d.☐	e.☐	f. ☐	g.☐	☐ Not that age yet

Physical Activity

24. In the past 10 years, thinking about the job you held the longest, which of the following categories best describes the activity level at that job:

1 ☐ Sedentary occupation (You spend most of your time sitting. Examples: office worker, bus/cab driver, etc.)

2 ☐ Standing occupation (You spend most of your time standing or walking. However, your work does not require intense physical effort. Examples: waitress/waiter, flight attendant, shop assistant, hairdresser, guard, etc.)

3 ☐ Physical work (This involves some physical effort including handling of heavy objects and use of tools. Examples: plumber, cleaner, nurse, sports instructor, electrician, carpenter, etc.)

4 ☐ Heavy manual work (This involves vigorous physical activity including handling of very heavy objects. Examples: docker, miner, bricklayer, construction worker, etc.)

25. During most of your adult life, when walking outside of your home, how often did you walk for more than 10 minutes without stopping?

1 ☐ Rarely or never ⟶ **Go to question 28.**
2 ☐ 1 to 3 times each month
3 ☐ 1 time each week
4 ☐ 2 to 3 times each week
5 ☐ 4 to 6 times each week
6 ☐ 7 or more times each week

26. How many minutes did you usually walk?

☐☐☐ Minutes

27. What was your usual speed (check the **one** which applies most often)?

1 ☐ Casual (less than 2 miles an hour)
2 ☐ Average or normal (2 to 3 miles an hour)
3 ☐ Fairly fast (3 to 4 miles an hour)
4 ☐ Very fast (more than 4 miles an hour)
5 ☐ Don't know

28. During most of your adult life, how often did you usually do **strenuous or very hard exercise**? (Exercise where you work up a sweat and your heart beats fast. Do not include walking outside of your home or any physical activity associated with any jobs.)

1 ☐ Rarely or never
2 ☐ 1 to 3 days per month
3 ☐ 1 day per week
4 ☐ 2 days per week
5 ☐ 3 to 4 days per week
6 ☐ 5 or more days per week

Examples:
aerobics
aerobic dancing
jogging
tennis
swimming laps
vigorous yard or housework

29. During most of your adult life, how often did you usually do **moderate exercise**? (Exercise that is not exhausting, but your breathing and heart rate are above resting levels. Do not include walking outside of your home or any physical activity associated with any jobs.)

1 ☐ Rarely or never
2 ☐ 1 to 3 days per month
3 ☐ 1 day per week
4 ☐ 2 days per week
5 ☐ 3 to 4 days per week
6 ☐ 5 or more days per week

Examples:
biking outdoors
using an exercise machine like a stationary bike or treadmill
calisthenics
easy swimming
popular or folk dancing
golfing without a cart
moderate yard or housework

30. During most of your adult life, how often did you usually do **mild exercise**? (Exercise that is not exhausting. Do not include walking outside of your home or any physical activity associated with any jobs.)

1 ☐ Rarely or never
2 ☐ 1 to 3 days per month
3 ☐ 1 day per week
4 ☐ 2 days per week
5 ☐ 3 to 4 days per week
6 ☐ 5 or more days per week

Examples:
slow dancing
bowling
golfing with a cart
hunting
gardening
light housework

Medical History

31. Not including the past two years, did you regularly take any of the following medications? (Do not include occasional use of less than once per month)

Medication	Average days per month used	On days used, number of pills taken	Total number of years taken
a. **Aspirin** (regular or extra strength, 163 mg or more, e.g., Bufferin, Anacin, Bayer, Excedrin, Ecotrin, etc.) 1 ☐ No 2 ☐ Yes → 3 ☐ Not sure	☐☐ days per month	☐☐ number of pills	☐☐ years
b. **Aspirin** (baby or low dose, 162 mg or less) 1 ☐ No 2 ☐ Yes → 3 ☐ Not sure	☐☐ days per month	☐☐ number of pills	☐☐ years
c. **Ibuprofen** (e.g., Motrin, Advil, Nuprin, Mediprin, etc.) 1 ☐ No 2 ☐ Yes → 3 ☐ Not sure	☐☐ days per month	☐☐ number of pills	☐☐ years

What was your main reason for using this?

_____

What was your main reason for using this?

_____

What was your main reason for using this?

_____

(Question 31 continued)

Medication	Average days per month used	On days used, number of pills taken	Total number of years taken

d. **Acetaminophen** (e.g., Tylenol, Phenaphen, etc.)

1 ☐ No
2 ☐ Yes → ☐☐ days per month ☐☐ number of pills ☐☐ years
3 ☐ Not sure

What was your main reason for using this?

_____

e. **Other anti-inflammatory analgesics** (e.g., Naprosyn, Anaprox, Aleve, Voltaren, Feldene, Toradol, Indocin, etc.) ☐☐ days per month ☐☐ number of pills ☐☐ years

1 ☐ No
2 ☐ Yes →
3 ☐ Not sure

What was your main reason for using this?

_____

f. **COX-2 inhibitors** (e.g., Celebrex, Vioxx, Bextra, etc.)

1 ☐ No
2 ☐ Yes → ☐☐ days per month ☐☐ number of pills ☐☐ years
3 ☐ Not sure

What was your main reason for using this?

_____

The following questions are about your height and weight at different ages. If you don't remember exactly what they were, please give your BEST GUESS. (Women, if you were pregnant at any of these ages, please provide your weight when you were not pregnant.)

Height

32. How tall are you (without shoes on)? (Round up to nearest inch)

☐ Feet ☐☐ Inches

33. How tall were you (without shoes on) at about **age 18**? (Round up to nearest inch)

☐ Feet ☐☐ Inches

Weight

34. How much do you currently weigh? ☐☐☐ Pounds

35. What was your weight 2 years ago? ☐☐☐ Pounds

36. What was your weight

a. at about **age 18**? ☐☐☐ Pounds

b. at about **age 35**? ☐☐☐ Pounds

c. at about **age 50**? ☐☐☐ Pounds

d. at about **age 65**? ☐☐☐ Pounds ☐ Not that age yet

37. What is your maximum adult weight (the most you ever weighed since you were 18 years old)? (Remember, do not include pregnancy weight.) ☐☐☐ Pounds

38. Not including the past two years, did you ever have a blood transfusion?

1 ☐ No ⟶ **Go to question 42.**

2 ☐ Yes

39. If yes, please list the condition (e.g., neonatal jaundice, anemia, childbirth, trauma) or surgical procedure (e.g., heart surgery or hip surgery) for which you had the blood transfusion. (Start with the one when you were the youngest first and fill in as many details as you remember. Please check "Don't know" if you don't know.)

Condition or surgical procedure	Age at transfusion	Number of transfusions	How much of this blood was your own?
a._____	☐☐ Years old	☐☐ Number	1 ☐ None 2 ☐ Some 3 ☐ All 4 ☐ Don't know
b._____	☐☐ Years old	☐☐ Number	1 ☐ None 2 ☐ Some 3 ☐ All 4 ☐ Don't know
c._____	☐☐ Years old	☐☐ Number	1 ☐ None 2 ☐ Some 3 ☐ All 4 ☐ Don't know

40. Were you transfused for more than three conditions or surgical procedures?

1 ☐ No ⟶ **Go to question 42.**
2 ☐ Yes

41. If yes, please provide details on the last condition or surgical procedure for which you were transfused, excluding the last 12 months.

Condition or surgical procedure	Age at transfusion	Number of transfusions	How much of this blood was your own?
			1 ☐ None
_____	☐☐ Years old	☐☐ Number	2 ☐ Some
			3 ☐ All
			4 ☐ Don't know

42. Did you ever have an organ transplant (including a bone marrow transplant)?

1 ☐ No **Go to question 44.**
2 ☐ Yes ⟶

43. If yes, what organs were transplanted and what year did you receive them?

	Organ	**Year received**
a.	_____	☐☐☐☐
b.	_____	☐☐☐☐

44. Were you told by a doctor or other health professional that you had any of the following conditions? (Please mark a box even if you have never had the condition.)

Condition	No	Yes	Not Sure	If yes, age you were first diagnosed
A. Hyperthyroidism	☐	☐	☐	
B, Hypothyroidism	☐	☐	☐	
C. Peptic ulcer	☐	☐	☐	
D. Ankylosing spondylitis	☐	☐	☐	
E. Heart disease, angina or heart attack	☐	☐	☐	
F. High blood pressure	☐	☐	☐	
G. Diabetes mellitus (sugar diabetes not associated with pregnancy)	☐	☐	☐	
H. Rheumatoid arthritis	☐	☐	☐	
I. Osteoarthritis (degenerative arthritis)	☐	☐	☐	
J. Crohn's disease	☐	☐	☐	
K. Ulcerative colitis	☐	☐	☐	

(Question 44 continued)

Were you told by a doctor or other health professional that you had any of the following conditions? (Please mark a box even if you have never had the condition.)

	Condition	No	Not Sure	Yes			If yes, age you were first diagnosed
l	Celiac disease	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
m	Sjögren's disease or sicca syndrome	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
n	Lupus or Systemic lupus erthyomatosis	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
o	Polymyositis, dermatomyositis, or polymyalgia rheumatica	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
p	Eczema	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
q	Contact dermatitis	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
r	Cirrhosis of the liver or liver damage	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
s	Infectious mononucleosis ("mono")	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
t	Chronic fatigue syndrome	1 ☐	2 ☐	3 ☐	→	☐☐	Years old
u	Epilepsy (convulsions or seizures not related to high fever)	1 ☐	2 ☐	3 ☐	→	☐☐	Years old

45. Have you ever been diagnosed with cancer? (Please do not include your recent leukemia diagnosis.)

1 ☐ No → **Go to question 47.**

2 ☐ Yes

46. If yes, please provide:

	Type of cancer	**Age you were first diagnosed**	**Treatments received** (Mark all that apply)
a.	_____	☐☐ Years old	☐ Surgery ☐ Radiation ☐ Chemotherapy ☐ Other, specify: _____ ☐ None ☐ Don't know
b.	_____	☐☐ Years old	☐ Surgery ☐ Radiation ☐ Chemotherapy ☐ Other, specify: _____ ☐ None ☐ Don't know
c.	_____	☐☐ Years old	☐ Surgery ☐ Radiation ☐ Chemotherapy ☐ Other, specify: _____ ☐ None ☐ Don't know

Rev. 12/13

If male ⟶ **Go to question 62.**

If female please continue below.

Rev. 12/13 **Female History**

47. At what age did you start having menstrual periods?

☐☐ Years old

48. Have you **ever** taken birth control pills for any reason?

1 ☐ No ⟶ **Go to question 50.**
2 ☐ Yes

49. Please mark the total number of years you took the pills.

1 ☐ Less than 1 year
2 ☐ 1 to 4 years
3 ☐ 5 to 9 years
4 ☐ 10 to 14 years
5 ☐ 15 to 19 years
6 ☐ 20 to 24 years
7 ☐ 25 or more years

50. Have you gone through menopause (the change of life) or had surgery that caused you to completely stop having menstrual periods?

1 ☐ No ⟶ **Go to question 53.**
2 ☐ Yes
3 ☐ Not Sure

51. At what age did you have your last menstrual period?

☐☐ Years old

52. What type of menopause did you have?

1 ☐ Natural menopause
2 ☐ Hysterectomy with uterus and both ovaries removed
3 ☐ Hysterectomy with uterus and one or neither ovary removed
4 ☐ Only ovaries removed
5 ☐ Surgery, but not sure what type
6 ☐ Other, please specify: _____

53. Have you ever taken any hormones/estrogen, such as Premarin, for symptoms or conditions related to menopause (to treat hot flashes, to prevent bone loss, or for the change of life)? (Include pills, skin patches, implants, creams, suppositories, and shots.)

1 ☐ No
2 ☐ Yes

54. How old were you when you first used hormones/estrogen for symptoms or conditions related to menopause?

☐☐ Years old

55. Are you currently taking hormones/estrogen for symptoms or conditions related to menopause?

1 ☐ No
2 ☐ Yes

56. How many years altogether did you take hormones/estrogen for symptoms or conditions associated with menopause? (Do not count any time when you stopped.)

1 ☐ Less than 1 year
2 ☐ 1 to 3 years
3 ☐ 4 to 5 years
4 ☐ 6 to 10 years
5 ☐ 11 to 15 years
6 ☐ 16 or more years

57. Not including the past two years, did you ever take oral progestins (such as Provera) in combination with estrogens for symptoms or conditions related to menopause?

1 ☐ No
2 ☐ Yes

58. Have you ever been pregnant? (This includes live births, stillbirths, miscarriages, tubals [ectopics], and abortions.)

1 ☐ No
2 ☐ Yes

59. How many pregnancies led to live births?

☐☐ Number of pregnancies

60. How old were you at the birth of your first child?

☐☐ Years old

61. How old were you at the birth of your last child?

☐☐ Years old

Family History

Family health history is an important part of learning about the cause and prevention of diseases like myeloid leukemia. We also know that people may not know their complete family health history. As you read these questions, please know that we understand this and we hope that you just do the best you can.

62. Were you adopted?

1 ☐ No
2 ☐ Yes

Most people in the United States have ancestors who came from other parts of the world. Many people have ancestors who came from more than one country.

63. What country or countries did your biological father's ancestors come from?

_____

64. What country or countries did your biological mother's ancestors come from?

_____

65. Counting only persons related to you by blood (including siblings who are now deceased and half siblings), please provide numbers for each of the following.

a. How many brothers, including half brothers, do/did you have? ☐☐ Brothers ☐ Don't know

b. How many sisters, including half sisters, do/did you have? ☐☐ Sisters ☐ Don't know

c. How many of your children are male? Include any of those who may have died. ☐☐ Sons

d. How many of your children are female? Include any of those who may have died. ☐☐ Daughters

66. Have your parents, brothers, sisters, sons, or daughters related by blood (include half brothers and sisters) ever been diagnosed as having cancer? (Please include leukemia, lymphoma, breast cancer, and non-melanoma skin cancer, as well as any other cancer.)

1 ☐ No **Go to question 67.**
2 ☐ Yes
2 ☐ Not Sure

For each cancer, please mark which relative was diagnosed and their age at diagnosis.

A. Leukemia

	Age at diagnosis
☐ Father	☐☐
☐ Mother	☐☐
☐ Brother	☐☐
☐ Sister	☐☐
☐ Son	☐☐
☐ Daughter	☐☐
☐ Other Blood Relative: _____	☐☐
☐ Other Blood Relative: _____	☐☐

B. Lymphoma

	Age at diagnosis
☐ Father	☐☐
☐ Mother	☐☐
☐ Brother	☐☐
☐ Sister	☐☐
☐ Son	☐☐
☐ Daughter	☐☐
☐ Other Blood Relative: _____	☐☐
☐ Other Blood Relative: _____	☐☐

C. Breast Cancer

	Age at diagnosis
☐ Mother	☐☐
☐ Sister	☐☐
☐ Daughter	☐☐
☐ Other Blood Relative: _____	☐☐
☐ Other Blood Relative: _____	☐☐

D. Non-melanoma Skin Cancer

(includes basal cell and squamous cell carcinomas)

	Age at diagnosis
☐ Father	☐☐
☐ Mother	☐☐
☐ Brother	☐☐
☐ Sister	☐☐
☐ Son	☐☐
☐ Daughter	☐☐
☐ Other Blood Relative: _____	☐☐
☐ Other Blood Relative: _____	☐☐

E. Other cancer, please specify:

_____ →

	Age at diagnosis
☐ Father	☐☐
☐ Mother	☐☐
☐ Brother	☐☐
☐ Sister	☐☐
☐ Son	☐☐
☐ Daughter	☐☐
☐ Other Blood Relative: _____	☐☐
☐ Other Blood Relative: _____	☐☐

F. Other cancer, please specify:

_____

	Age at diagnosis
☐ Father	☐☐
☐ Mother	☐☐
☐ Brother	☐☐
☐ Sister	☐☐
☐ Son	☐☐
☐ Daughter	☐☐
☐ Other Blood Relative: _____	☐☐
☐ Other Blood Relative: _____	☐☐

Farm/Rural Living

67. Have you **ever** lived on a farm or in a rural area (a community of 1,000 people or less)?

1 ☐ No **Go to question 70 below.**
2 ☐ Yes

68. For how many years have you lived on a farm or in rural area?

☐☐ Years

69. While you lived on a farm or in a rural area, what was the main source of your drinking water? (Check the one that applied most often.)

1 ☐ Municipal water supply
2 ☐ Private well
3 ☐ Spring
4 ☐ Bottled water
5 ☐ Other, please specify: _____

70. Have you ever worked or lived on a farm at least half time for more than 1 year?

1 ☐ No ⟶ **Go to question 78.**
2 ☐ Yes

71. At any time you worked or lived on a farm, what were the major income-producing animals that you raised? (Mark all that apply)

☐ No animals
☐ Beef/non-dairy cattle
☐ Dairy cattle
☐ Hogs/swine
☐ Poultry
☐ Sheep
☐ Eggs
☐ Other farm animals: _____

72. At any time you worked or lived on a farm, what were the major income-producing crops that you raised? (Mark all that apply)

☐ No crops
☐ Apples
☐ Alfalfa
☐ Blueberries
☐ Cabbage
☐ Corn, popcorn
☐ Corn, field corn
☐ Corn, seed corn
☐ Corn, sweet corn
☐ Cucumbers
☐ Grapes
☐ Green peppers
☐ Hay
☐ Oats
☐ Peas
☐ Potatoes
☐ Snapbeans
☐ Sorghum
☐ Soybeans
☐ Sugar beets
☐ Strawberries
☐ Sweet potatoes
☐ Tomatoes
☐ Trees
☐ Watermelon
☐ Wheat
☐ Other fruit: _____
☐ Other vegetables: _____
☐ Other small grains: _____

On the Job Pesticide Exposure

73. Did a veterinarian ever tell you that any livestock on any farm you may have worked on had leukemia or lymphoma caused by a virus?
    1 ☐ No **Go to question 78.**
    2 ☐ Yes

74. Was it cattle?
    1 ☐ No
    2 ☐ Yes

75. When was the first time you recall this happening?
    ☐☐ Years ago

76. Was it chickens?
    1 ☐ No
    2 ☐ Yes

77. When was the first time you recall this happening?
    ☐☐ Years ago

78. As part of any job, have you ever personally mixed or applied any pesticides, crop, livestock, or structural insecticides, herbicides, fungicides, or fumigants? Include pesticides used for farm use, commercial application, or other workplace that uses these chemicals. (We will ask about your home exposure near the end of the survey.)

    1 ☐ No **Go to question 83.**
    2 ☐ Yes

79. How many years did you personally mix or apply these products?

    1 ☐ 1 year or less
    2 ☐ 2 to 5 years
    3 ☐ 6 to 10 years
    4 ☐ 11 to 20 years
    5 ☐ 21 to 30 years
    6 ☐ 31 years or more

80. When you personally mixed these products, what additives did you generally use?

1 ☐ Solvents (like diesel fuel)
2 ☐ Fertilizers
3 ☐ Other pesticides
4 ☐ Surfactants, crop oil concentrates
5 ☐ Other, please specify: _____
6 ☐ Don't usually use additives

81. As part of any job, have you personally mixed or applied any of the following pesticides? (Mark all that you have used)

Crop, Nursery, Lawn, and Garden Insecticides

☐ Ambush, Pounce, Asana, or other permethrin or pyrethroid products
☐ Counter or other terbufos products
☐ Dyfonate or other fonofos products
☐ Dylox or other trichlorfon products
☐ Furadan, Curaterr, or other carbofuran products

Crop/Poultry/Livestock/Animal Confinement Area Insecticides

☐ Lorsban, Dursban, or other chlorpyrifos products
☐ Co-Ral or other coumaphos products
☐ Ectiban, Atroban, Permetrina or other permethrin products
☐ Vapona, Duravos, or other dichlorvos or DDVP products
☐ Forlin, Gamaphex, or other lindane products
☐ Malathion
☐ Parathion (ethyl or methyl)
☐ Sevin, Carbamine, or other carbaryl products
☐ Spectricide, Dianon, or other diazinon products
☐ Temik or other aldicarb products
☐ Thimet, Rampart, or other phorate products
☐ Aldrin (no longer on the market)
☐ Chlordane (no longer on the market)
☐ Dieldrin (no longer on the market)
☐ DDT (no longer on the market)
☐ Heptachlor (no longer on the market)
☐ Toxaphene (no longer on the market)

Herbicides

☐ AAtrex, Atranex, or other atrazine products
☐ Prowl or other pendimethalin products

- ☐ Banvel, Metambane, or other dicamba products
- ☐ Bladex, Match, or other cyanazine products
- ☐ Dual, Cycle, or other metolachlor products
- ☐ Eradicane, Eptam, or other EPTC products
- ☐ Lasso, Chimiclor, or other alachlor products
- ☐ Pursuit or other imazethapyr products
- ☐ Roundup, Jury, or other glyphosate products
- ☐ Treflan, Trilin, Commence, or other trifluralin products
- ☐ 2, 4-D
- ☐ Classic or other chlorimuron ethyl products
- ☐ Lexone, Sencor, or other metribuzin products
- ☐ Paraquat
- ☐ Silvex or other 2,4,5 T P products (no longer on the market)
- ☐ 2,4,5 T (no longer on the market)

(Question 81 continued)

As part of any job, have you personally mixed or applied any of the following pesticides? (Mark all that you have used)

Fungicides

- ☐ Bravo, Evade, Daconil 2787, or other chlorothalonil products
- ☐ Orthocide, Clomitane, or other captan products
- ☐ Benlate, Tersan, or other benomyl products
- ☐ Manex, Manzate, Dithane Z-78, or other maneb or mancozeb products
- ☐ Ridomil, Subdue, or other metalaxyl products
- ☐ Zirex, Corozate, or other ziram products

Fumigants (gases or liquids that turn to gas when released, used in enclosed spaces, or to treat soil)

- ☐ Broom-O-Gas, Brom-O-Sol, or other methyl bromide products
- ☐ Phostoxin, Gastoxin, or other aluminum phosphide products
- ☐ Carbon tetrachloride/carbon disulfide (80/20 mix) (no longer on the market)
- ☐ EDB, E-D-Bee, Bromofume, or other ethylene dibromide products (no longer on the market)

82. Are there any other pesticides you were exposed to?

1 ☐ No

2 ☐ Yes (If yes, please list them below and include reason for use.)

_____

_____

_____

83. What has been your usual job during most of your adult life, that is, the job or type of job you have held the **longest**? (Please record your job title. For example: gasoline engine assembler, grinder operator, farmer, homemaker, sales manager, registered nurse, etc. If military – please include description of job duties and highest rank.)

Job Title: _____

Description: _____

_____

84. For how many years did you work at this job?

☐☐ Years

85. Have you ever been exposed to any of the following substances listed below, for at least 8 hours per week for 1 year or more, either on a job or working on a hobby? (Check no or yes beside each substance and if yes, write in approximately how many years you were exposed in the boxes provided.)

	No	Yes	Number of Years
a. Cutting oils, motor vehicle oils	1 ☐	2 ☐ →	☐☐
b. Asphalt, tar or pitch	1 ☐	2 ☐ →	☐☐
c. Benzene or other solvents	1 ☐	2 ☐ →	☐☐
d. Gasoline	1 ☐	2 ☐ →	☐☐
e. Pesticides	1 ☐	2 ☐ →	☐☐
f. Herbicides	1 ☐	2 ☐ →	☐☐

(Question 85 continued)

Have you ever been exposed to any of the following substances listed below, for at least 8 hours per week for 1 year or more, either on a job or working on a hobby? (Check no or yes beside each substance and if yes, write in approximately how many years you were exposed.)

	No	Yes		Number of Years
g. Fertilizers	1 ☐	2 ☐	→	☐☐
h. Arsenic	1 ☐	2 ☐	→	☐☐
i. Mineral oils	1 ☐	2 ☐	→	☐☐
j. Soot	1 ☐	2 ☐	→	☐☐
k. Creosote	1 ☐	2 ☐	→	☐☐
l. Inks, dyes, tanning solutions	1 ☐	2 ☐	→	☐☐
m. Dry cleaning agents	1 ☐	2 ☐	→	☐☐
n. Rubber and rubber products	1 ☐	2 ☐	→	☐☐
o. Vinyl chloride, plastics	1 ☐	2 ☐	→	☐☐
p. Acrylic and oil-based paints	1 ☐	2 ☐	→	☐☐
q. Varnish, lacquers, or glues	1 ☐	2 ☐	→	☐☐
r. Paraffin waxes	1 ☐	2 ☐	→	☐☐
s. Coal dust	1 ☐	2 ☐	→	☐☐
t. Metals (lead, nickel, zinc)	1 ☐	2 ☐	→	☐☐
u. Radioactive materials	1 ☐	2 ☐	→	☐☐
v. X-ray machines	1 ☐	2 ☐	→	☐☐

Information About Your Home

We are interested in chemicals that may have been used in places where you lived, including inside homes, on lawns/yards, or on family gardens. Do not include use of these chemicals if they were used as part of your job (such as farming activities, work at a nursery, or other workplace that uses these chemicals).

86. Have insecticides (chemicals that kill insects) ever been used around your home, lawn/yard, or family garden at any of the residences where you have lived?

    1 ☐ No ⟶ **Go to question 89.**
    2 ☐ Yes

87. If yes, approximately how many years (total) were these products used?

    1 ☐ 4 years or less
    2 ☐ 5 to 15 years
    3 ☐ 16 to 30 years
    4 ☐ 31 years or more

88. Did you personally handle any of these products?

    1 ☐ No
    2 ☐ Yes

89. Have fertilizers (not including pesticides) ever been used on lawns/yards at any of the residences where you have lived?

    1 ☐ No ⟶ **Go to question 92.**
    2 ☐ Yes

90. Approximately how many years (total) were these products used?

    1 ☐ 4 years or less
    2 ☐ 5 to 15 years
    3 ☐ 16 to 30 years
    4 ☐ 31 years or more

91. Did you personally handle any of these products?

    1 ☐ No
    2 ☐ Yes

92. Have herbicides (chemicals that kill weeds) ever been used around your home, lawn/yard, or family garden at any of the residences where you have lived?

1 ☐ No ⟶ **Go to question 95.**
2 ☐ Yes

93. If yes, approximately how many years (total) were these products used?

1 ☐ 4 years or less
2 ☐ 5 to 15 years
3 ☐ 16 to 30 years
4 ☐ 31 years or more

94. Did you personally handle any of these products?

1 ☐ No
2 ☐ Yes

95. Have pesticides or chemicals to control or prevent termites ever been used at any of the residences where you have lived?

1 ☐ No ⟶ **Go to question 98.**
2 ☐ Yes

96. If yes, approximately how many years (total) were these products used?

1 ☐ 4 years or less
2 ☐ 5 to 15 years
3 ☐ 16 to 30 years
4 ☐ 31 years or more

97. Did you personally handle any of these products?

1 ☐ No
2 ☐ Yes

98. Have you ever had cats as pets?

1 ☐ No ⟶ **Go to comments below.**
2 ☐ Yes

99. If yes, did a veterinarian ever tell you that any of your cats had feline leukemia or lymphoma?

1 ☐ No ⟶ **Go to comments below.**
2 ☐ Yes

100. If yes, how many of your cats had feline leukemia or lymphoma?

☐☐ Cats

Comments:

Please provide any comments or additional information below.

ALESE Study
Future Contact

To maintain your confidentiality, this page will be stored separately from your responses to the survey.

A. How was the survey completed?

1 ☐ I filled it out myself

2 ☐ Someone helped me fill it out

3 ☐ Spouse

4 ☐ Child

5 ☐ Other, please specify: _____

B. What is your date of birth? ☐☐ ☐☐ ☐☐☐☐
Month Day Year

C. Would you like to receive any updates or study results?

1 ☐ No

2 ☐ Yes

D. We would like to know if it would be okay to contact you in the future to see if you have learned anything more about your family health history and to record any changes. This would probably be about 3 to 5 years from now. Would this be okay?

1 ☐ No

2 ☐ Yes

Please turn to the next page.

Please record your name and address so that we may contact you in the future:

Name _____

Street Address _____

City, State Zip _____

Phone (__ __ __) __ __ __ - __ __ __ __

In case you move and we cannot find you, please provide the names, addresses, and phone numbers for two people that will always know where you are (such as your spouse, siblings, or children).

Person #1

Name _____

Relationship to you _____

Street Address _____

City, State Zip _____

Phone (__ __ __) __ __ __ - __ __ __ __

Person #2

Name _____

Relationship to you _____

Street Address _____

City, State Zip _____

Phone (__ __ __) __ __ __ - __ __ __ __

Thank you for your time!

Return Instructions for Sites - ALESE Questionnaire

Optimally, the ALESE Questionnaire will be completed by the patient within the first week of the beginning of their treatment on E2906.

*Please add the Pt. ID number to the top of each page of the questionnaire prior to mailing.

Please mail the questionnaire to the investigator after patient has completed it:

James M. Foran, M.D.
Attn: ALESE
Mayo Clinic, Florida
Division of Hematology & Medical Oncology
4500 San Pablo Road
Jacksonville, FL 32224

The questionnaire may be scanned and sent directly as pdf to the investigator's e-mail:

foran.james@mayo.edu