Protocol #: LCI-HEM-AML-SCD-001

TITLE: LEUKEMIA STEM CELL DETECTION IN ACUTE MYELOID LEUKEMIA

LAY TITLE: STEM CELL DETECTION FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA

Coordinating Site:
Levine Cancer Institute (LCI)
Research and Academic Headquarters
1021 Morehead Medical Drive
Charlotte, NC 28204

Sponsor-Investigator:
Michael R. Grunwald, M.D.
1021 Morehead Medical Drive, Suite 5300
Charlotte, NC 28204
Phone: (980) 442-5125
Email: Michael.Grunwald@carolinashealthcare.org

Statistician:
James Symanowski, PhD
1100 Blythe Blvd, Suite 1166
Charlotte, NC 28203
Telephone: (980) 442-2348
Email: James.Symanowski@carolinashealthcare.org

Myra Robinson, MSPH
1100 Blythe Blvd, Suite 1166
Charlotte, NC 28203
Telephone: (980) 442-2390
Email: Myra.Robinson@carolinashealthcare.org

Investigational drug/device:
N/A

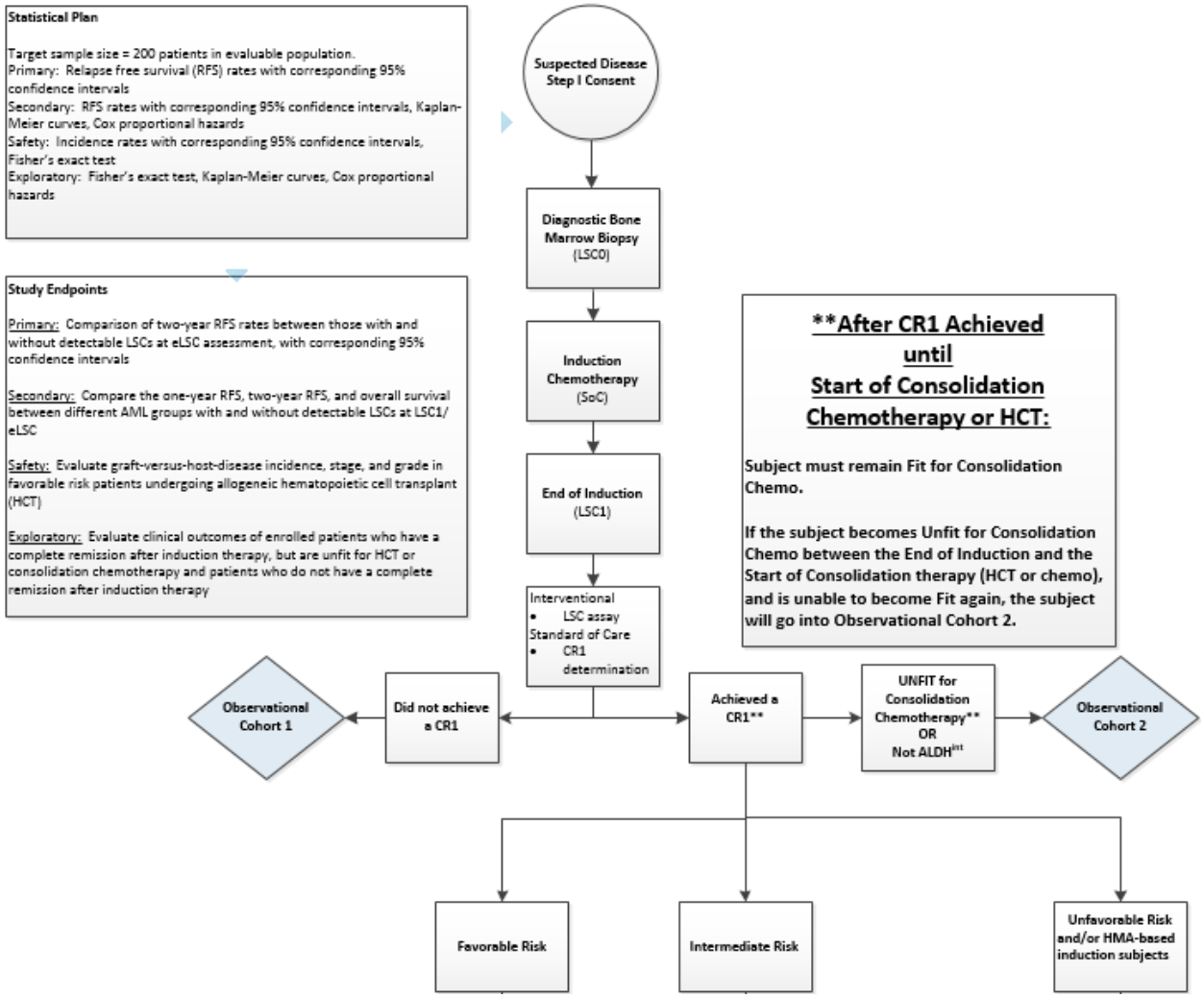
The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

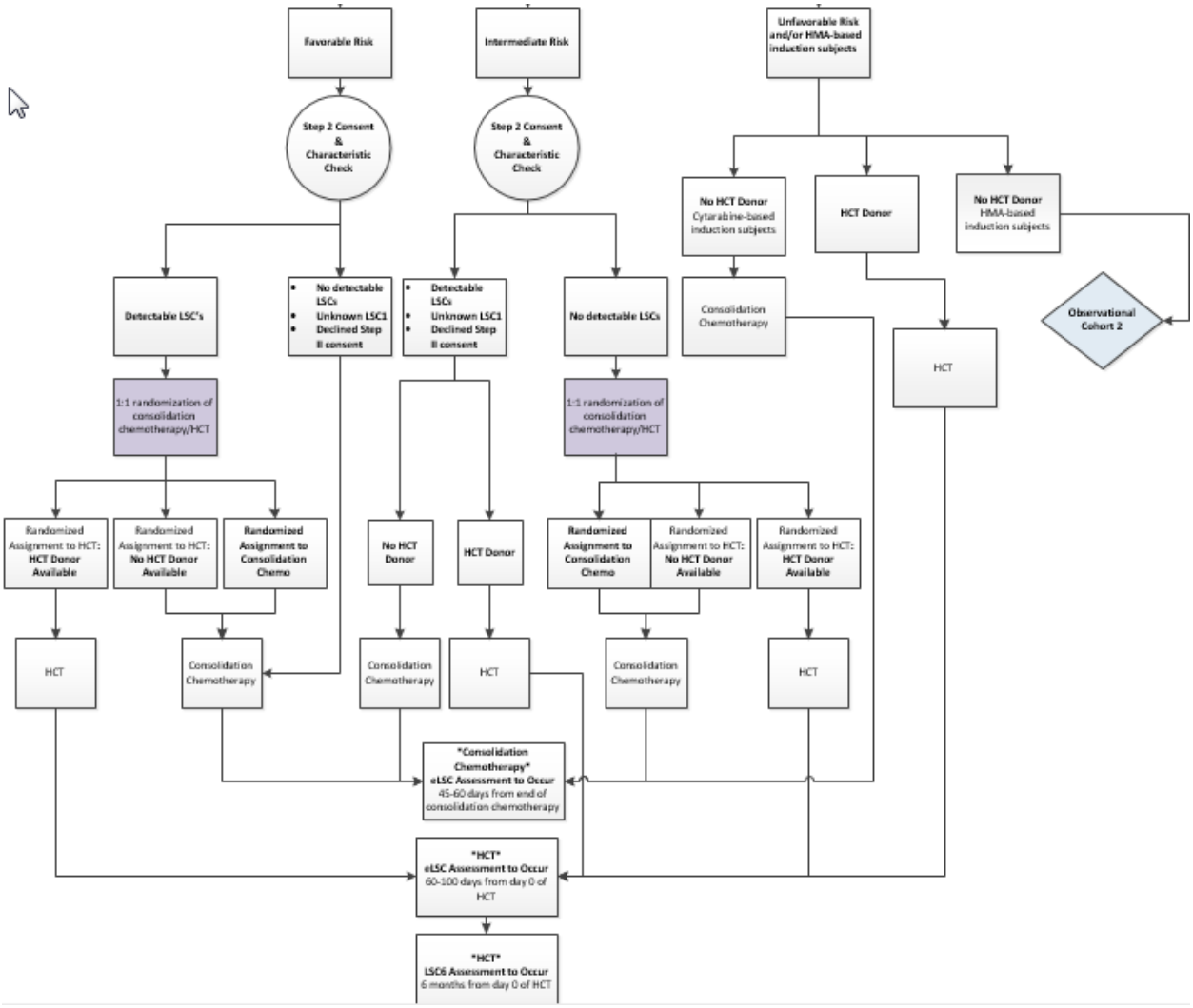
Original Phase 3 / Version 2 / February 27, 2017

PROTOCOL SUMMARY	
A. Study Title	Leukemia stem cell detection in acute myeloid leukemia
B. Indication	Acute myeloid leukemia
C. Clinical Phase	III
D. Summary of Rationale	Most patients with AML achieve CR following induction chemotherapy. However, a large majority subsequently relapse and succumb to the disease. Currently, cytogenetics and molecular aberrations are the best prognostic indicators; however, these factors cannot prognosticate accurately for individual patients. Overall, the majority of patients with favorable or intermediate-risk AML will experience relapse. Prognosis after relapse is dismal with a five-year overall survival rate of less than 10%. A LSC paradigm may explain this failure of CR to reliably translate into cure.
E. Study Objectives	The primary endpoint is to compare the two-year relapse-free survival (RFS) rates of those with and without detectable LSCs at eLSC assessment. The secondary endpoints are to compare the one-year RFS, two-year RFS, and overall survival (OS) between different AML groups with and without detectable LSCs at LSC1/eLSC. Safety objectives include the evaluation of graft-versus-host-disease timing (acute vs chronic), incidence, and grade/severity in subjects undergoing allogeneic hematopoietic cell transplant (HCT). Exploratory objectives include evaluation of clinical outcomes of subjects who are enrolled to the study but do not meet the criteria to be included in the evaluable population. These subjects will be included in one of the two following observational cohorts: Observational cohort 1 (OC1): enrolled subjects who do not achieve a CR to induction therapy Observational cohort 2 (OC2): enrolled subjects who achieve a CR to induction therapy but meet one or more of the following criteria: • Lack the immunophenotype of interest ($CD34^{+}CD38^{-}ALDH^{int}$) by flow cytometry at the diagnostic LSC assay (LSC0) • Cytarabine based induction subjects: Are candidates for (as determined by the investigator) and do not receive consolidation therapy

	HMA-based induction subjects: Are not candidates for (as determined by the investigator) do not receive HCT
F. Sample	200 evaluable (for the primary objective) subjects and estimated 300-500 total including observational subjects.
G. Inclusion/ Exclusion	Inclusion: • At least 18 years of age • New diagnosis of AML, other than APL, confirmed by bone marrow aspirate/biopsy • Have completed induction therapy and post-induction bone marrow biopsy. Exclusion: • Any illness that would preclude ability to follow study procedures • Indeterminate results at LSC0
I. Statistical Analysis	The primary endpoint of this study is two-year relapse-free survival (RFS) of subjects with acute myeloid leukemia (AML). Subjects with detectable leukemia stem cells (LSCs) in their bone marrow at end of consolidation (eLSC+) following the time of achieving first complete remission (CR1) will be compared to the two-year RFS of patients without detectable LSCs (eLSC-). RFS is defined as the duration of time from the initial CR date (enrollment date) to the date of relapse, as determined by the Cheson criteria.

SCHEMA


Statistical Plan
Target sample size = 200 patients in evaluable population.
Primary: Relapse free survival (RFS) rates with corresponding 95% confidence intervals
Secondary: RFS rates with corresponding 95% confidence intervals, Kaplan-Meier curves, Cox proportional hazards
Safety: Incidence rates with corresponding 95% confidence intervals, Fisher's exact test
Exploratory: Fisher's exact test, Kaplan-Meier curves, Cox proportional hazards
Study Endpoints
Primary: Comparison of two-year RFS rates between those with and without detectable LSCs at eLSC assessment, with corresponding 95% confidence intervals
Secondary: Compare the one-year RFS, two-year RFS, and overall survival between different AML groups with and without detectable LSCs at LSC1/ eLSC
Safety: Evaluate graft-versus-host-disease incidence, stage, and grade in favorable risk patients undergoing allogeneic hematopoietic cell transplant (HCT)
Exploratory: Evaluate clinical outcomes of enrolled patients who have a complete remission after induction therapy, but are unfit for HCT or consolidation chemotherapy and patients who do not have a complete remission after induction therapy
Suspected Disease Step I Consent
Diagnostic Bone Marrow Biopsy (LSC0)
Induction Chemotherapy (SoC)
End of Induction (LSC1)
Interventional
LSC assay
Standard of Care
CR1 determination
**After CR1 Achieved until Start of Consolidation Chemotherapy or HCT:
Subject must remain Fit for Consolidation Chemo.
If the subject becomes Unfit for Consolidation Chemo between the End of Induction and the Start of Consolidation therapy (HCT or chemo), and is unable to become Fit again, the subject will go into Observational Cohort 2.
Observational Cohort 1
Did not achieve a CR1
Achieved a CR1**
UNFIT for Consolidation Chemotherapy** OR Not ALDHint
Observational Cohort 2
Favorable Risk
Intermediate Risk
Unfavorable Risk and/or HMA-based induction subjects


Favorable Risk
Intermediate Risk
Unfavorable Risk and/or HMA-based induction subjects
Step 2 Consent & Characteristic Check
Step 2 Consent & Characteristic Check
No HCT Donor Cytarabine-based induction subjects
HCT Donor
No HCT Donor HMA-based induction subjects
Detectable LSC's
No detectable LSCs
Unknown LSC1
Declined Step II consent
Detectable LSCs
Unknown LSC1
Declined Step II consent
No detectable LSCs
Consolidation Chemotherapy
Observational Cohort 2
HCT
1:1 randomization of consolidation chemotherapy/HCT
1:1 randomization of consolidation chemotherapy/HCT
Randomized Assignment to HCT: HCT Donor Available
Randomized Assignment to HCT: No HCT Donor Available
Randomized Assignment to Consolidation Chemo
No HCT Donor
HCT Donor
Randomized Assignment to Consolidation Chemo
Randomized Assignment to HCT: No HCT Donor Available
Randomized Assignment to HCT: HCT Donor Available
HCT
Consolidation Chemotherapy
Consolidation Chemotherapy
HCT
Consolidation Chemotherapy
HCT
Consolidation Chemotherapy eLSC Assessment to Occur 45-60 days from end of consolidation chemotherapy
HCT eLSC Assessment to Occur 60-100 days from day 0 of HCT
HCT LSC6 Assessment to Occur 6 months from day 0 of HCT

LIST OF ABBREVIATIONS

AE	Adverse event
ALDH	Aldehyde dehydrogenase
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
CBF	Core-binding factor
CFR	Code of Federal Regulations
CML	Chronic myeloid leukemia
CR	Complete response
CR(1)	First complete response
CR(2)	Second complete response
CRF	Case report form
CSC	Cancer stem cell
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	Clinical trial management system
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
eCRF	Electronic case report form
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
GCP	Good Clinical Practice
GVHD	Graft versus host disease
HCT	Hematopoietic cell transplant
HMA	Hypomethylating agent
IRB	Institutional Review Board
LCI	Levine Cancer Institute
LSC	Leukemic stem cell
LSC0	Leukemic stem cell status pre induction therapy
LSC1	Leukemic stem cell status post induction therapy
eLSC	Leukemic stem cell status at end of consolidation therapy
LSC6	Leukemic stem cell status at six months post day 0 of HCT
MDS	Myelodysplastic syndrome
MRD	Minimum residual disease
MPN	Myeloproliferative neoplasm
MUD	Matched unrelated donor
OS	Overall survival
RFS	Relapse-free survival
PCR	Polymerase chain reaction
SAE	Serious adverse event
SI	Sponsor-Investigator
SOP	Standard operating procedure
NCI	National Cancer Institute

t-AML	Therapy-related acute myeloid leukemia
VOD	Veno-occlusive disease

GLOSSARY

LSC0: Diagnostic bone marrow sample or peripheral blood sample that the leukemia stem cell assay is performed on. LSC0 occurs prior to induction therapy for diagnostic purposes.

LSC1: Bone marrow sample that the post-induction leukemia stem cell assay is performed on. LSC1 occurs following induction chemotherapy at the time of complete remission assessment (End of Induction visit).

eLSC: Bone marrow sample that the leukemia stem cell assay is performed on, collected on evaluable subjects at the end of consolidation therapy. For those undergoing treatment with consolidation chemotherapy, eLSC occurs 45-60 days from day 1 of the last consolidation chemotherapy cycle. For those undergoing treatment with consolidation HCT, eLSC occurs 60-100 days from day 0 (day cells are transplanted).

LSC6: This sample only applies to HCT subjects. This is the fourth sample that the leukemia stem cell assay is performed in HCT subjects. For those undergoing treatment with consolidation HCT, LSC6 occurs 6 months (180 days +/- 30 days) from day 0 (day cells are transplanted).

Cytarabine-based induction subjects: Subjects who receive induction therapy that is primarily cytarabine-based. Some of these subjects may receive a cycle of hypomethylating agent (HMA)-based chemotherapy prior to cytarabine-based induction.

Hypomethylating agent (HMA) based induction subjects: Subjects who receive induction therapy that is primarily HMA-based. These subjects will follow the same treatment algorithm as the cytarabine-based induction subjects with unfavorable risk except they will not receive cytarabine-based consolidation chemotherapy if they are unable to have a HCT.

Start of treatment: For those undergoing treatment with consolidation chemotherapy, start of treatment is day 1, cycle 1. For those undergoing treatment with consolidation HCT, start of treatment is day 0 (day cells are transplanted).

End of treatment: For those undergoing treatment with consolidation chemotherapy, end of treatment is defined as 30 days from last administration of consolidation chemotherapy. For those undergoing treatment with consolidation HCT, end of treatment is 30 days from day 0 (day cells are transplanted).

Observational Cohort 1 (OC1): enrolled subjects who do not achieve a CR (CR1) to standard cytarabine or HMA-based induction therapy per standard clinical criteria (See Appendix 1).

Observational Cohort 2 (OC2): enrolled subjects who achieve a CR

(CR1) from standard cytarabine or HMA-based induction therapy per standard clinical criteria (See Appendix 1) and meet one or more of the following criteria:

- Lack the immunophenotype of interest ($CD34^{+}CD38^{-}ALDH^{int}$) by flow cytometry at the diagnostic LSC assay (LSC0)
- Cytarabine based induction subjects: Are not candidates for [as determined by the investigator (e.g. unfit or refusal)] and do not receive consolidation therapy (cytarabine-based chemotherapy or HCT)
  HMA-based induction subjects: Are not candidates for [as determined by the investigator (e.g. unfit, lack of donor, refusal)] and do not receive HCT

Evaluable Cohort: enrolled subjects who achieve a CR (CR1) from standard cytarabine or HMA-based induction therapy per standard clinical criteria (see Appendix 1) and meet all of the following criteria:

- Have confirmed presence of $CD34^{+}CD38^{-}ALDH^{int}$ population by flow cytometry at the diagnostic LSC assay (LSC0)
- Cytarabine-based induction subjects: Are candidates for (as determined by the investigator) and receive consolidation therapy (cytarabine-based chemotherapy or HCT)
- HMA-based induction subjects: Are candidates for (as determined by the investigator) and receive HCT

TABLE OF CONTENTS

1. OBJECTIVES

1.1. Primary Objective

The primary objective is to compare the two-year relapse-free survival (RFS) of subjects with acute myeloid leukemia (AML) with detectable leukemia stem cells (LSCs) at end of consolidation (eLSC+) to the two-year RFS of subjects without detectable LSCs (eLSC-). Before determination of end of consolidation LSC (eLSC) status, subjects will have completed either standard cytarabine-based consolidation chemotherapy or allogeneic stem cell transplantation.

1.2. Secondary Objectives

a. Compare the one-year RFS of AML subjects with detectable LSCs at eLSC to the one-year RFS of AML subjects without detectable LSCs at eLSC.
b. Compare the two-year RFS of favorable risk AML subjects without detectable LSCs at the time of achieving CR1 (LSC1 time point) who are treated with standard cytarabine-based consolidation to that of favorable risk AML subjects with detectable LSCs at LSC1 who are also treated with standard cytarabine-based consolidation.
c. Compare the two-year RFS of intermediate risk AML subjects with detectable LSCs at LSC1 who are treated with allogeneic hematopoietic cell transplant (HCT) to that of intermediate risk AML subjects without detectable LSCs at LSC1 who are treated with allogeneic HCT.
d. Compare the two-year RFS of favorable risk AML subjects with detectable LSCs at LSC1 who undergo allogeneic HCT to the two-year RFS of favorable risk AML subjects with detectable LSCs at LSC1 who receive standard cytarabine-based consolidation chemotherapy.
e. Compare the two-year RFS of intermediate risk AML subjects without detectable LSCs at LSC1 who undergo allogeneic HCT to the two-year RFS of intermediate risk AML subjects without detectable LSCs at LSC1 who receive cytarabine-based consolidation chemotherapy.
f. Compare the overall survival (OS) of AML subjects with detectable LSCs at eLSC to the OS of AML subjects without detectable LSCs at eLSC. Compare the two-year RFS of subjects with AML with detectable LSCs at LSC1 to subjects without detectable LSCs at LSC1.
g. Compare the one-year RFS of subjects with AML with detectable LSCs at LSC1 to subjects without detectable LSCs at LSC1.
h. Compare the two-year RFS of unfavorable risk AML subjects without detectable LSCs at LSC1 who receive standard cytarabine-based consolidation to the two-year

RFS of unfavorable risk AML subjects without detectable LSCs at LSC1 who undergo allogeneic HCT.

i. Compare the two-year RFS of subjects with detectable LSCs at LSC6 in HCT subjects and detectable LSCs at eLSC in chemotherapy subjects to the two-year RFS of HCT/chemotherapy subjects without detectable LSCs at LSC6/eLSC, respectively.
j. In the subjects that undergo allogeneic HCT, evaluate the change in LSC status from eLSC (end of consolidation) to LSC6 (6 months after day 0 of transplant).

1.3. Safety Objective

Evaluate the graft-versus-host disease (GVHD) classification (acute vs chronic), incidence, and grade/severity in AML subjects undergoing allogeneic HCT.

1.4. Exploratory Objective

Evaluate the clinical outcomes of the population of enrolled subjects who are not included in the evaluable population (as defined in section 8.3). This will involve the following observational cohorts:

- Observational cohort 1 (OC1): enrolled subjects who do not achieve a CR (CR1) to standard cytarabine or HMA-based induction therapy per standard clinical criteria (see Appendix 1).
- Observational cohort 2 (OC2): enrolled subjects who achieve a CR (CR1) to standard cytarabine or HMA-based induction therapy per standard clinical criteria (see Appendix 1) and meet one or more of the following criteria:
  - Lack the immunophenotype of interest ($CD34^{+}CD38^{-}ALDH^{int}$) by flow cytometry at the diagnostic LSC assay (LSC0)
  - Cytarabine based induction subjects: Are not candidates for [as determined by the investigator (e.g. unfit or refusal)] and do not receive consolidation therapy (cytarabine-based chemotherapy or HCT)
    HMA-based induction subjects: Are not candidates for [as determined by the investigator (e.g. unfit, lack of donor, refusal)] and do not receive HCT

2. BACKGROUND AND RATIONALE

2.1. Introduction

Although most patients with AML achieve CR following standard induction chemotherapy, a large majority subsequently relapse and succumb to the disease.[1-3]

Most patients who receive HMA treatment for AML also die of their leukemia. Currently, cytogenetic and molecular aberrations are the best prognostic indicators[3-5]; however, these factors predict primarily for groups of patients and cannot prognosticate accurately for individual patients. For example, core-binding factor (CBF) cytogenetic abnormalities are considered favorable, yet roughly half of these patients relapse.[6] Overall, the majority of patients with favorable or intermediate-risk AML will experience relapse. Prognosis after relapse is dismal with a five-year overall survival rate of less than 10%.[7] A LSC paradigm may explain this failure of CR to reliably translate into cure. Leukemia appears to retain the normal hematopoietic hierarchical structure: rare stem cells with self-renewal capacity give rise to partially differentiated progeny that comprise the bulk of the leukemia but possess only limited proliferative potential.[8] Although existing therapies are highly active against the leukemic bulk, it appears they spare the hardier LSCs, which are then responsible for relapse.[9-10] OS and RFS of HCT in CR2 is much worse than in CR1.[11] Therefore, detection of LSCs in CR1 may provide a more precise method to predict outcome and a valuable window of opportunity to guide treatment decisions. In addition, the detection of LSCs at the end of AML treatment may provide prognostic information and also an opportunity for intervention.

2.2. Leukemia Stem Cells

Since 1994 when Dick and colleagues reported that only rare AML cells, characterized by a classical $CD34^+CD38^-$ normal hematopoietic stem cell (HSC) phenotype, were capable of generating leukemia in immunodeficient mice,[12] these putative LSCs have been the focus of considerable research. Although it is generally accepted that $CD34^+CD38^-$ cells are enriched for LSCs,[12] this population is heterogeneous and includes both normal and leukemic cells. Moreover, recent data have challenged the $CD34^+CD38^-$ phenotype of LSCs in AML, leading many investigators to advocate for a functional definition of LSCs: those leukemic cells capable of engrafting immunodeficient mice.[13-16] However, even with this current gold standard, the identification of LSCs has proven elusive.[8,12] A significant portion of AML patient samples will not engraft mice, and the assay is cumbersome and often indeterminate.[17] More importantly, the clinical implications of this assay are unclear.[18] Furthermore, even in leukemia, where the cancer stem cell (CSC) model is perhaps best established, there is a paucity of data demonstrating that LSCs are responsible for disease resistance or relapse.

Relapse after CR is due to minimal residual disease (MRD): residual leukemia cells persistent in quantities undetectable by conventional assays used to evaluate remission. If LSCs are indeed clinically relevant, then their persistence should correlate with

recurrence, and MRD should be enriched for these cells. Exploiting the similarities between LSCs and their normal counterparts,[19] we recently employed strategies established for the isolation of normal HSCs[20-22] in chronic myeloid leukemia (CML). We found that the fraction of the $CD34^+CD38^-$ cells with high activity of Aldefluor, as determined by aldehyde dehydrogenase (ALDH) levels, was highly enriched for leukemic cells capable of engrafting immunodeficient mice.[23] Therefore, we assessed ALDH activity as a marker for clinically significant MRD in AML.

2.3. ALDH Activity

When analyzed for ALDH activity, the normal bone marrow $CD34^+CD38^-$ cells consistently exhibited two, non-overlapping populations: one expressing low ALDH activity ($CD34^+CD38^-ALDH^{low}$) and the second expressing high activity ($CD34^+CD38^-ALDH^{high}$) (Fig 1a). The normal marrow $CD34^+CD38^-ALDH^{high}$ cells comprised an average of 10% (range 9-12%) of the total $CD34^+$ cells and 76% (range 61-85%) of the $CD34^+CD38^-$ cells. As few as 1000 of these $CD34^+CD38^-ALDH^{high}$ cells engrafted NOG mice.

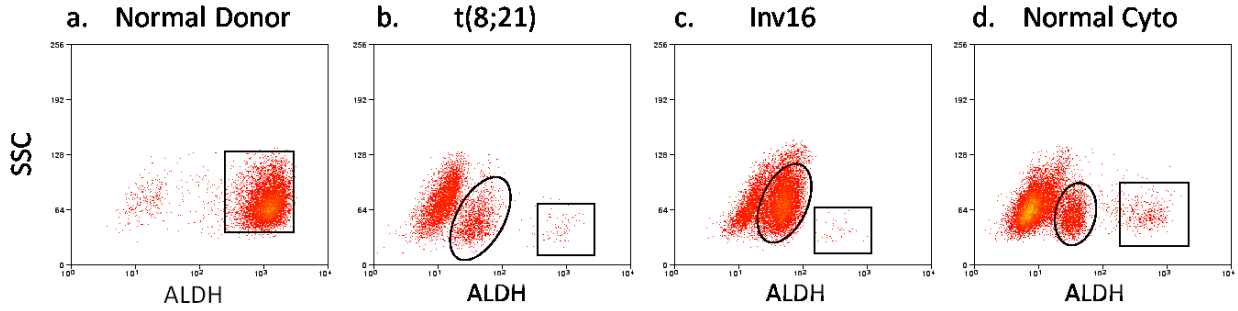


Figure 1. Expression of ALDH in the $CD34^+CD38^-$ population of bone marrow from a normal donor(a), and three leukemia patients with cytogenetic profiles of t(8;21), inv16, and 46xy profiles(b,c,d). Populations with high (boxed) and low ALDH expression are apparent in normal marrow(a). The AML samples contained a discrete third population of ALDH intermediate cells (circled). In the patients with CBF AML this $CD34^+CD38^-ALDH^{int}$ population was essentially completely leukemic by FISH, as was the $CD34^+CD38^-ALDH^{low}$ population. In contrast, the small $CD34^+CD38^-ALDH^{high}$ populations from CBF AML patients lacked the leukemia-specific FISH marker.

Initial analyses focused on patients with newly diagnosed CBF AML, as the FISH-detectable abnormalities enabled quantification of leukemic cells in isolated populations. In contrast to the normal samples, the $CD34^+CD38^-$ cells from all CBF AML patients exhibited three well-defined populations by ALDH expression. They contained a population with intermediate ALDH expression ($CD34^+CD38^-ALDH^{int}$) (Fig 1b-c), in addition to high and low ALDH populations observed in normal marrow. The $CD34^+CD38^-ALDH^{high}$ cells were rare in the newly-diagnosed CBF AML patients, constituting an average of only 0.12% of the total $CD34^+$ cells (range 0.005-0.5%, $p<0.001$ vs. the normal samples) and 1.24% of the $CD34^+CD38^-$ cells (range 0.03-4.3%, $p<0.001$ vs. the normal samples). This $CD34^+CD38^-ALDH^{high}$ population was essentially devoid of cells with the leukemia-specific cytogenetic abnormality.

Similar to those isolated from normal donors, as few as 1000 of these cells yielded normal human hematopoietic engraftment of NOG mice. Conversely, the intermediate and low ALDH fractions were both virtually entirely leukemic by FISH. As few as 1000 of the $CD34^+CD38^-ALDH^{int}$ cells produced leukemic engraftment of NOG mice, whereas, the $CD34^+CD38^-ALDH^{low}$ cells did not engraft. The $CD34^+CD38^-$ cells from other cytogenetic variants of AML, including those with normal cytogenetics, also demonstrated three ALDH populations (Fig 1d) and similar FISH results (when applicable). The $CD34^+CD38^-ALDH^{int}$ population appears to contain a putative LSC population, distinguishable from the normal HSC population.

Only four of 20 newly-diagnosed AML patients did not have this characteristic flow cytometric pattern containing a $CD34^+CD38^-ALDH^{int}$ population. In two of these patients, most of the $CD34^+CD38^-$ leukemic cells exhibited high ALDH activity, with no discernible separate population of normal $CD34^+CD38^-ALDH^{high}$ cells by FISH or PCR. One patient had normal cytogenetics with a FLT3 internal tandem duplication, and the second had complex cytogenetics, including deletions of chromosomes 5 and 7. Notably, both patients had primary refractory disease, which ultimately proved fatal. In the two additional patients, the diagnostic leukemic cytogenetic marker was present only in the $CD34^-$ cells, as has been previously described in a minority of AML cases.[24] One of these patients had an 11q23 abnormality and has since relapsed within a year of diagnosis; the other has remained in CR for more than one year since diagnosis and nine months since allogeneic transplantation.

We hypothesized that, because this $CD34^+CD38^-ALDH^{int}$ population represents putative LSCs, if it is present during CR it would correlate with future relapse, and therefore define a clinically relevant MRD population. Therefore, we evaluated AML patient samples at various stages of treatment to determine if their flow cytometric pattern predicted relapse.

2.4. Presence of LSCs Following Treatment

Of the 20 AML patients analyzed at diagnosis, three had primary refractory disease, one died during induction chemotherapy, one did not receive full induction or consolidation chemotherapy, and two others had $CD34^-$ leukemia. Of the 13 patients with $CD34^+$ leukemia analyzed at diagnosis who achieved morphologic CR after induction, follow-up samples were available in nine. An additional seven AML patients who achieved CR, but in whom diagnostic samples were not available, were also followed: two starting after induction and five after consolidation therapy, for a total of 16 evaluable patients.

Of those 16 patients, eight were analyzed in CR1 prior to consolidation. The CR samples exhibited two general patterns: two populations, with a predominant $CD34^+CD38^-ALDH^{high}$ population and a smaller $CD34^+CD38^-ALDH^{low}$ population, as seen in normal samples (Figure 2A); three populations including a $CD34^+CD38^-$

ALDH^{int} population (Figure 2B). Five patients exhibited the normal pattern, and both of the cell populations were normal by FISH. The three patients who have consistently exhibited this normal pattern remain in CR, with an average follow-up of 293 (range 185-370) days since diagnosis. In the other two patients, the CD34^{+}CD38^{-}ALDH^{int} population was detected at follow-up while still in CR after consolidation, and both ultimately relapsed. The remaining three patients exhibited an MRD pattern in their initial CR1 marrow (Figure 2B) and the CD34^{+}CD38^{-}ALDH^{int} population was ≥85% leukemic by FISH. Two of these patients relapsed within 33 days of detection of the MRD pattern and subsequently died. The third patient underwent allogeneic HCT in CR1 due to adverse risk cytogenetics (complex karyotype, including deletion 7q) and has remained in CR for over 17 months.

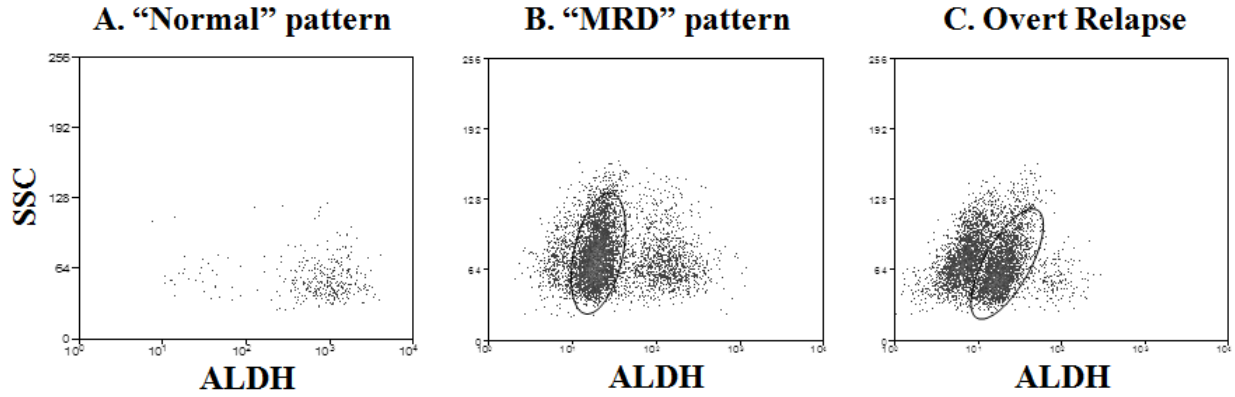


Figure 2. Aldeflour expression in the CD34^{+}CD38^{-} bone marrow cells. Representative examples of staining patterns in CR1: (A) normal, (B) MRD, (C) overt clinical relapse.

Eleven AML patients who achieved morphologic CR with induction chemotherapy have been followed since completion of consolidation. Of the seven patients with a consistently normal flow pattern after consolidation, none has yet relapsed, with an average duration of follow-up of 509 days since diagnosis (range 185-810). However, all four of the patients with the MRD pattern after consolidation relapsed at an average of 53 days (range 32-81) after detection of the MRD pattern (p=0.003 when compared to those patients with a consistently normal pattern). The CD34^{+}CD38^{-}ALDH^{int} population was overwhelmingly leukemic (≥95%) by FISH. One of the relapsed patients converted to a normal pattern after re-induction and allogeneic transplantation and has remained in second CR for over one year. The MRD pattern persisted in two other patients, who achieved a second CR after re-induction, both of whom have subsequently relapsed. Of note, the CD34^{+}CD38^{-}ALDH^{int} population was not detected in an additional two patients who have been in CR for more than three years, but have been followed only since their second year post-consolidation. The CD34^{+}CD38^{-} ALDH^{int} population was present in all six analyzed cases of overt clinical relapse (Figure 2C).

2.5. Leukemic Burden After Chemotherapy

According to the LSC paradigm, LSCs should constitute a small fraction of the leukemic burden during active disease, but should comprise a substantially larger portion of the leukemic population during clinical remission. Although three patients in CR after induction who exhibited the MRD pattern had no morphologic, karyotypic, or FISH evidence of disease in the unfractionated marrow cells, the cytogenetic abnormality was still detectable in an average of 90% of the $CD34^{+}CD38^{-}ALDH^{int}$ cells (range 85-95%) and in an average of 22% of the $CD34^{+}$ cells (range 8-36%). This leukemic $CD34^{+}CD38^{-}ALDH^{int}$ population comprised an average of 34% of the total leukemic burden by FISH (range 9-51%). In contrast, this population constituted only 2% of the total leukemic burden at diagnosis (range 0.3-7%, $p<0.001$ vs. cytogenetic CR). Moreover, although the total leukemic burden decreased by >200-fold from diagnosis to CR ($p<0.001$), the $CD34^{+}CD38^{-}ALDH^{int}$ population decreased by only 13-fold ($p=0.4$).

Three different patients who had cytogenetic but no morphologic evidence of disease, had an average of 8% leukemic cells (range 4.5-9.5%) in their unfractionated marrow as compared to 96% in the $CD34^{+}CD38^{-}ALDH^{int}$ cells (range 94-99%, $p<0.001$). Furthermore, the proportion of the $CD34^{+}CD38^{-}ALDH^{int}$ fraction represented an average of 8% of the total leukemic clone (range 2-12%). At overt relapse, as at initial diagnosis, this population again comprised only a small fraction (average 1%, range 0.5-2%) of the total leukemic burden. These data demonstrated that this $CD34^{+}CD38^{-}ALDH^{int}$ population behaves as a putative LSC population.

Further, these data suggest that the presence of this $CD34^{+}CD38^{-}ALDH^{int}$ population predicts relapse, as expected of an LSC population. This group of cells would define a clinically relevant MRD population that indicates a high risk of AML relapse. Patients with this population, despite lacking the standard poor-risk prognostic markers, would be at a high risk of relapse and would likely benefit from more intensive therapy in first complete remission, due to the very poor prognosis of AML in relapse: five-year overall-survival rates are less than 10% after relapse.[7] On the contrary, patients lacking the $CD34^{+}CD38^{-}ALDH^{int}$ population in CR1 may not benefit from more intensive therapy in first complete remission.

Therefore, we propose a prospective study of AML subjects to evaluate the prognostic character of the assay for $CD34^{+}CD38^{-}ALDH^{int}$ LSCs. In addition, cytarabine-based induction subjects with favorable and intermediate risk AML, upon achieving a CR after induction therapy, will receive consolidation treatment assignment guided by the presence or absence of LSCs in CR1. This approach contrasts with the present

standard strategy using cytogenetic and molecular markers present at diagnosis to determine post-remission treatment.

This will be a prospective, randomized, clinical trial, in which favorable and intermediate risk evaluable AML subjects who have the $CD34^{+}CD38^{-}ALDH^{int}$ population at diagnosis will be assigned (based on LSC1 status and/or randomization) to either standard chemotherapy or HCT for consolidation.

$CD34^{+}CD38^{-}$ALDHint LSCs must comprise at least 0.001% of total mononuclear cells to be considered detectable.

HMA-based induction AML subjects (regardless of risk):
For HMA-based induction AML subjects who are candidates for transplant, the standard of care is allogeneic HCT in CR1.

- AML subjects who received HMA-based induction who are transplant candidates and have an available donor will undergo transplant. However, not all transplant candidates have donors available.
- An HMA-based induction subject who is unable to receive HCT for any reason will be included in Observational Cohort 2 (OC2).

Favorable risk AML cytarabine-based induction subjects

- without detectable LSCs in CR1 or who decline Step 2 ICF will receive standard consolidation chemotherapy
- with detectable LSCs in CR1 who accept Step 2 ICF will be randomized to receive standard consolidation chemotherapy or allogeneic HCT

Intermediate risk AML cytarabine-based induction subjects

- with detectable LSCs in CR1 or who decline Step 2 ICF will receive allogeneic HCT
- without detectable LSCs in CR1 who accept Step 2 ICF will be randomized to receive standard consolidation chemotherapy or allogeneic HCT

Unfavorable risk AML cytarabine-based induction subjects:
For unfavorable risk AML subjects who are candidates for transplant, the standard of care is allogeneic HCT in CR1.

- Unfavorable risk subjects on this study who are transplant candidates and have an available donor will undergo transplant. However, not all transplant candidates have donors available.
- An unfavorable risk subject without an available donor will be recommended to receive consolidation chemotherapy per standard of care. We will gather

information regarding the detectability of LSC in CR1 in this population and correlate the detectability of LSCs with outcomes.

3. SUBJECT SELECTION AND ENROLLMENT

3.1. Accrual

Subjects must meet all of the following criteria to be pre-screened for the study:

- 18 years and older
- Newly diagnosed or suspected AML
- Planned induction therapy (either cytarabine-based or HMA-based)

Subjects who are consented with the Step 1 consent and meet the eligibility criteria after induction chemotherapy will be enrolled on the trial. Subjects will continue to be accrued until 200 subjects meeting the criteria for the evaluable cohort as defined in Section 4.2.3 with successful and interpretable eLSC evaluations are obtained This will constitute the evaluable population (see Section 8.3) for the primary efficacy analyses. Subjects who do not meet the criteria for the evaluable cohort will be enrolled but followed only in an observational manner for outcomes. An estimated total of 300 to 500 subjects will be enrolled under the treatment and observational portion of the study. Subjects that are enrolled, but followed in an exploratory manner are not considered to be part of the evaluable population.

The original diagnosis of AML must have been confirmed by bone marrow aspirate and/or biopsy review by an institutional hematopathologist. Subjects are eligible if they have AML that is not classified as APL and meet all other eligibility criteria (see Section 3.2).

The study is open to both adult men and women, and to all racial/ethnic subgroups. There is no explicit mention of different treatment effects in male and female subjects and in different racial/ethnic subgroups in the literature. Therefore, this study will not have separate accrual targets for these groups.

3.2. Eligibility Criteria

3.2.1. Inclusion Criteria

Subjects must meet all the following criteria to be enrolled into the study:

a. Must have previously signed the Step 1 informed consent document prior to LSC0 (Step 2 informed consent is not required for enrollment)

b. Age 18 years and older
c. New diagnosis of AML, other than APL, confirmed by bone marrow aspirate/biopsy and reviewed by an institutional hematopathologist
d. Have completed induction, as defined by the Investigator and post-induction bone marrow biopsy.

3.2.2. Exclusion Criteria

Subjects must **not** meet the following criterion in order to be enrolled to the study:

a. Any debilitating medical or psychiatric illness that would preclude ability to follow study procedures.
b. Indeterminate results at LSC0

Donor eligibility will be determined per standard HCT criteria**.**

4. INVESTIGATIONAL PLAN

4.1. Milestone Date Definitions

Entry Date: the date the subject signs the Step 1 informed consent document
Eligibility Date: the date of the last documented criterion that confirmed post-induction subject eligibility for enrollment to the study
Enrollment Date: the date that the post-induction bone marrow aspirate was obtained. Note: this is the sample that determines post-induction remission status.
Off Study Date: the date on which the subject is determined to have completed, withdrawn, or been withdrawn by the investigator from the study (see section 4.10 Off Study).

4.2. Overall Study Design

This is a prospective cohort study evaluating the prognostic value of eLSC. This study also includes randomization aspects enabling the comparison of the two consolidation schemas (standard cytarabine-based consolidation chemotherapy and allogeneic stem cell transplantation (HCT)) in AML subjects. Subjects will be enrolled and included in one of three cohorts after induction therapy if they meet the post-induction eligibility criteria. These cohorts are defined as follows:

4.2.1. Observational Cohort 1 (OC1)

: enrolled subjects who do not achieve a CR to induction therapy, regardless of diagnostic phenotype.

Following completion of induction therapy and remission bone marrow aspirate, if a subject is determined to not have achieved a complete remission to induction therapy, he or she would be included in observational cohort 1.

4.2.2. Observational Cohort 2 (OC2): enrolled subjects who achieve a CR to induction therapy but meet one or more of the following criteria:

- Lack the immunophenotype of interest,
- Cytarabine based induction subjects: Are not candidates for [as determined by the investigator (e.g. unfit or refusal)] and do not receive consolidation therapy (cytarabine-based chemotherapy or HCT)
- HMA-based induction subjects: Are not candidates for [as determined by the investigator (e.g. unfit, lack of donor, refusal)] and do not receive HCT

Final investigator determination of fit-ness can occur at any time until the start of consolidation therapy. Being unfit for consolidation therapy could be a result of any of the following reasons:

i. Any debilitating medical or psychiatric illness that would preclude ability to receive optimal treatment.
    1. Includes ECOG $\geq$ 3
ii. Poor renal function: creatinine $\geq$ 2.5mg/dL or requiring hemodialysis
iii. Poor liver function: bilirubin $\geq$ 2.5mg/dL, excluding Gilbert's Syndrome
iv. Breastfeeding or known pregnancy
v. Receiving any other investigational agents.
vi. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements per the Investigator.

HMA-based induction subjects will not receive consolidation cytarabine-based chemotherapy as part of the evaluable cohort if they do not receive HCT.

Note the subjects enrolled and included in any of the observational cohorts will not contribute to the 200 evaluable subjects required for the primary objective. An estimated total of 300 to 500 subjects will be enrolled to the study under the treatment and observational portions of the study.

4.2.3. Evaluable Cohort: enrolled subjects that will contribute to the population of subjects who are evaluable for the primary and secondary objectives. This will not include any subjects who end up in either observational cohort. To be included in the evaluable cohort, the subject must meet the following requirements:

a. Complete remission (CR1) from standard cytarabine or HMA-based induction therapy per standard clinical criteria (see Appendix 1)
b. Have confirmed presence of $CD34^{+}CD38^{-}ALDH^{int}$ population by flow cytometry at the diagnostic LSC assay (LSC0)
c. Cytarabine-based induction subjects: Are candidates for (as determined by the investigator) and receive consolidation therapy (cytarabine-based chemotherapy or HCT)
   HMA-based induction subjects: Are candidates for(as determined by the investigator) and receive HCT

   To be considered a candidate for consolidation therapy, a subject must be considered fit. Criteria for fitness may include, but is not limited to the following:
   i. Good renal function (creatinine <2.5mg/dL)
   ii. Good liver function (bilirubin <2.5mg/dL)
   iii. ECOG < 3
   iv. Not receiving any other investigational agents
   v. Not breastfeeding or known pregnancy
   vi. No intercurrent illness that would limit compliance with study requirements per the Investigator.

Subjects will be assigned to the evaluable cohort based on the criteria above and will then be assigned or randomized to either consolidation chemotherapy or HCT. Subjects who receive consolidation therapy will be included in the evaluable cohort.
Enrollment to the study will continue until 200 subjects evaluable for the primary objective (i.e. with known eLSC status) are enrolled (Section 10.3).

4.3. Informed Consent

The applicable informed consent(s) must be obtained and signed prior to any study related procedures.

There are multiple informed consents for this study. Patients at Levine Cancer Institute with a suspected or confirmed diagnosis of AML will be presented with information about this study. All interested subjects will first be consented with the Step 1 consent before starting induction chemotherapy for exploratory/observational participation on this study.

Once induction chemotherapy is completed, entered subjects must meet the following criteria in order to be presented with the Step 2 consent:

- Received cytarabine-based induction
- Determined to be a candidate for consolidation chemotherapy
- Confirmed presence of $CD34^{+}CD38^{-}ALDH^{int}$ population by flow cytometry at diagnosis (LSC0)
- Favorable or intermediate risk. The risk level is determined prior to induction chemotherapy and determines which subjects may have treatment decisions informed by their LSC status.

4.4. Registration/Study Entry (Pre-Screening)

Following Step 1 informed consent, subjects will be considered entered into the study, and eligibility check per standard operating procedures begins. Subjects will have a bone marrow aspirate/biopsy for diagnostic and clinical care purposes. This bone marrow sample may be used for the diagnostic leukemia stem cell assay (LSC0). Alternatively, a peripheral blood sample may be used for LSC0. Shortly after the diagnostic bone marrow sample, planned induction per standard of care will begin.

4.5. End of Induction (EOI)

4.5.1. Procedures and Assessments

At the EOI, eligibility checks will be completed, subject's remission status will be assessed, and other assessments/procedures will occur according to the Study Calendar (Section 5.0). A bone marrow aspirate/biopsy evaluating remission status will be performed and documented in the electronic medical record. EOI assessments/procedures, including bone marrow aspirate/biopsy, may occur on different days but must be performed within 30 days prior to the start of

consolidation therapy. An LSC assay (LSC1) will also be performed during this time frame.

4.5.2. Enrollment

After completion of induction therapy, subjects will be evaluated for any remaining eligibility criteria. All eligible subjects will be considered enrolled to the study as of the date of obtaining the post-induction bone marrow aspirate. Subjects meeting the requirements described in Section 4.2.3 (achieved CR after induction, fit for consolidation chemotherapy) will be included in the evaluable cohort. Subjects not meeting the requirements described in section 4.2.3 will fall into one of two observational cohorts and will be followed in an exploratory manner evaluating LSC status and outcomes as they receive standard of care.

For evaluable cytarabine-based induction subjects, treatment assignment will be informed by the subjects's clinical risk status (favorable, intermediate, or unfavorable) based on cytogenetic and/or molecular abnormalities from the diagnostic bone marrow biopsy. If their risk level is intermediate or favorable, those subjects will be presented with Step 2 informed consent. Subject's refusing Step 2 consent will be recommended for standard of care treatment according to their clinical risk level. Subjects who sign and agree to Step 2 consent will proceed through a treatment algorithm by which the subject's treatment assignment is informed by their risk level and LSC1 status (detected/positive, not detected/negative, or unknown).

Evaluable HMA-based induction subjects will be assigned to HCT regardless of cytogenetic and/or molecular abnormalities from the diagnostic bone marrow biopsy.

The treatment algorithm assignment will be either deterministic or accomplished by randomization in a 1:1 fashion, as described in Section 4.4.3 (Randomization), to either the HCT arm or the consolidation chemotherapy arm based on several strata.

In order to evaluate the putative LSC population, cells from subject bone marrow aspirate(s) will be studied. Peripheral blood may be used at the LSC0 timepoint. A minimum of 100,000 $CD34^+$ cells is required for complete analysis. The $CD34^+$ cells will be isolated by Ficoll density centrifugation separation, followed by selection using anti-CD34 magnetic beads. Cells will be analyzed and sorted based on expression of CD34, CD38, and Aldeflour. FISH analysis for cytogenetic abnormalities will be performed on sorted cells. If there are insufficient $CD34^+$ cells on remission bone marrow aspirate (up to 2 attempts) and/or the hematopathologist is unable to render a definitive LSC status, the LSC status will be considered unknown.

All enrolled subjects will be assigned a Study ID as determined by the Levine Cancer Institute Biostatistics Core. This will be accomplished by utilizing a list of Study IDs and, where applicable, associated treatment arm assignments generated prior to study activation. These will be given by a member of the Levine Cancer Institute Biostatistics Core. The Study ID will be a four digit randomly generated ID number, ranging from 0001 to 9999.

4.5.3. Randomization

Evaluable subjects, who meet the requirements for certain treatment pathways within the treatment algorithm, will be randomized to one of two standard treatment groups (HCT versus cytarabine-based consolidation).

Population eligible for Randomization; 1:1 HCT or consolidation chemotherapy

- Favorable risk
    - Signed Step 2 ICF
    - Achieved CR with cytarabine-based induction therapy
    - Detectable LSCs at LSC1 (LSC1+)
    - Fit for consolidation chemotherapy.
- Intermediate risk,
    - Signed Step 2 ICF
    - Achieved CR with cytarabine-based induction therapy
    - No detectable LSCs at LSC1 (LSC1-)
    - Fit for consolidation chemotherapy.

This randomization will be provided by the Levine Cancer Institute Biostatistics Core. A stratified block randomization will be utilized including the following stratification factors to reduce confounding of comparisons between the HCT and the conventional therapy arms within each of the selected pathways involving randomization:

- Age (<55 versus ≥ 55)
- Gender (Female versus Male)

Randomization or assignment to a specific treatment type does not guarantee that the subject will ultimately receive that type of therapy. Subject must meet, or continue to meet, clinically required criteria to receive that treatment type. Criteria includes, but is not limited to, being fit per lab values and vital signs, and availability of a donor (for HCT). Cytarabine-based subjects who do not ultimately

receive their assigned treatment, but do receive consolidation therapy per the study will still be considered part of the evaluable cohort.

Examples of this include, but are not limited to:

- Cytarabine-based induction, assigned to HCT, donor not available, subject fit for and received consolidation chemo
- Cytarabine-based induction, assigned to, but not fit for HCT, subject fit for and received consolidation chemo

4.5.4. Consolidation Therapy

Consolidation chemotherapy and HCT are both approved procedures and accepted clinic standards for treating patients with AML. Any assessments or tests run while the subject receives their consolidation therapy will be per investigator discretion and are considered to be standard of care.

Procedures required for subjects before and after consolidation therapy are described in the Study Calendar (Section 5.0).

Cytarabine-based subjects:
Subjects who receive cytarabine-based induction will be categorized according to risk (from the diagnostic sample) as defined below:

AML Risk Status Definitions
Subjects with any one or more of the following cytogenetic and/or molecular abnormalities will be categorized as having **unfavorable** risk AML:

- complex karyotype (≥3 clonal chromosomal abnormalities)
- monosomal karyotype
- -5
- 5q-
- -7
- 7q-
- 11q23 [non t(9;11)]
- inv(3) or t(3;3)
- t(6;9)
- t(9;22)
- normal cytogenetics with the FLT3-ITD mutation.

Subjects will also be categorized as **unfavorable** risk:

- Therapy-related AML (t-AML)

- AML arising from previously diagnosed myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN).

Subjects with any one or more of the following cytogenetic and/or molecular abnormalities will be categorized as having favorable risk AML:

- t(8;21)
- inv(16) and/or t(16;16)
- normal cytogenetics with the NPM1 and/or dual CEBPA mutations without the FLT3-ITD mutation.

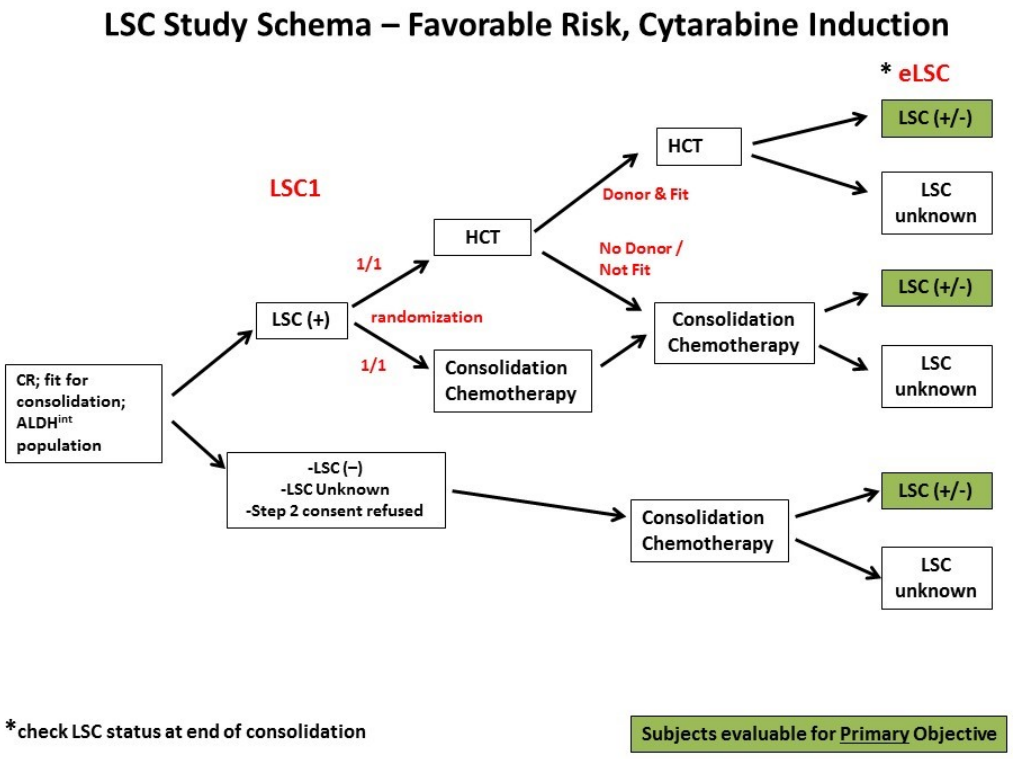
Cytarabine-based induction subjects who cannot be categorized as unfavorable risk or favorable risk will be categorized as **intermediate** risk.

HMA-based induction subjects:
HMA-based induction subjects will not be categorized according to a risk category from the diagnostic sample.

Treatment Algorithms:
Cytarabine-based induction subjects with favorable risk AML will be treated as follows:

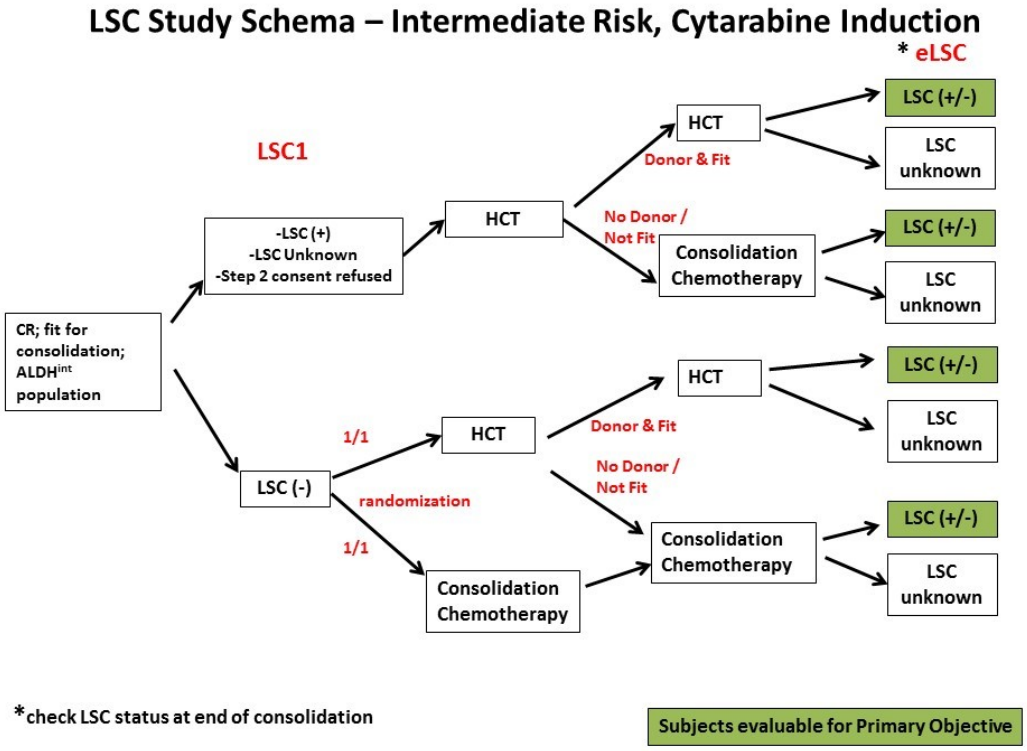
- Subjects without detectable LSCs at LSC1 by flow cytometry will be assigned to standard cytarabine-based chemotherapy as per institutional standards.
- Subjects with detectable LSCs at LSC1 by flow cytometry will be randomized in a 1:1 ratio to undergo allogenic HCT versus cytarabine-based consolidation. Favorable risk subjects who are randomized to HCT but are subsequently found to not be fit for HCT and/or who do not have a donor (matched related, haploidentical, or matched unrelated donor) will receive consolidation chemotherapy.

Note: Subjects with unknown LSC status at LSC1 or who refuse Step 2 consent for entering the treatment algorithm will be assigned to standard cytarabine-based chemotherapy as per institutional standards. The study schema for favorable risk AML subjects is as follows:



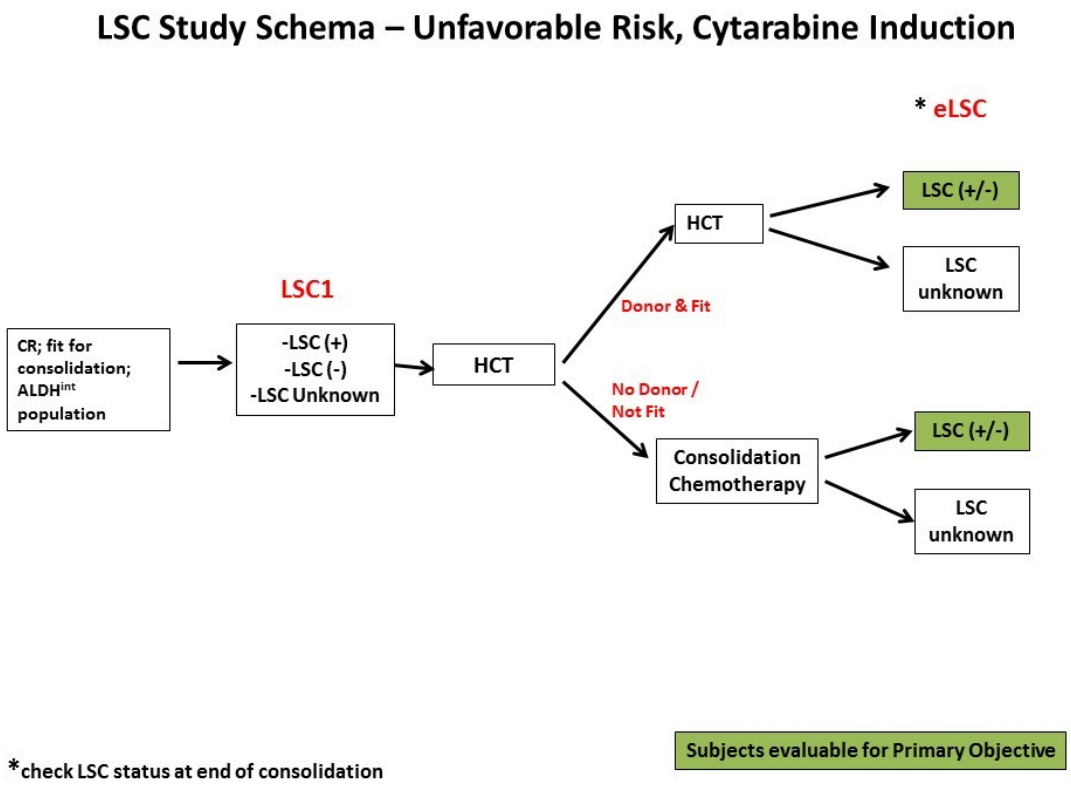
Cytarabine-based induction subjects with intermediate risk AML will be treated as follows:

- Subjects with detectable LSCs at LSC1 by flow cytometry will be assigned to allogeneic HCT as per institutional standards.
- Subjects without detectable LSCs at LSC1 by flow cytometry will be randomized in a 1:1 ratio to undergo allogeneic HCT versus cytarabine-based consolidation therapy. Intermediate risk subjects who are assigned or randomized to HCT who are found to not be candidates for HCT and/or who do not have a donor (matched related, haploidentical, or matched unrelated donor) will be treated with standard cytarabine-based consolidation. Note: Subjects with unknown LSC status at LSC1 or who refuse Step 2 consent for entering the treatment algorithm will be assigned to allogeneic HCT (donor and fit-ness for HCT permitting) as per institutional standard. The study schema for intermediate risk AML subjects is as follows:



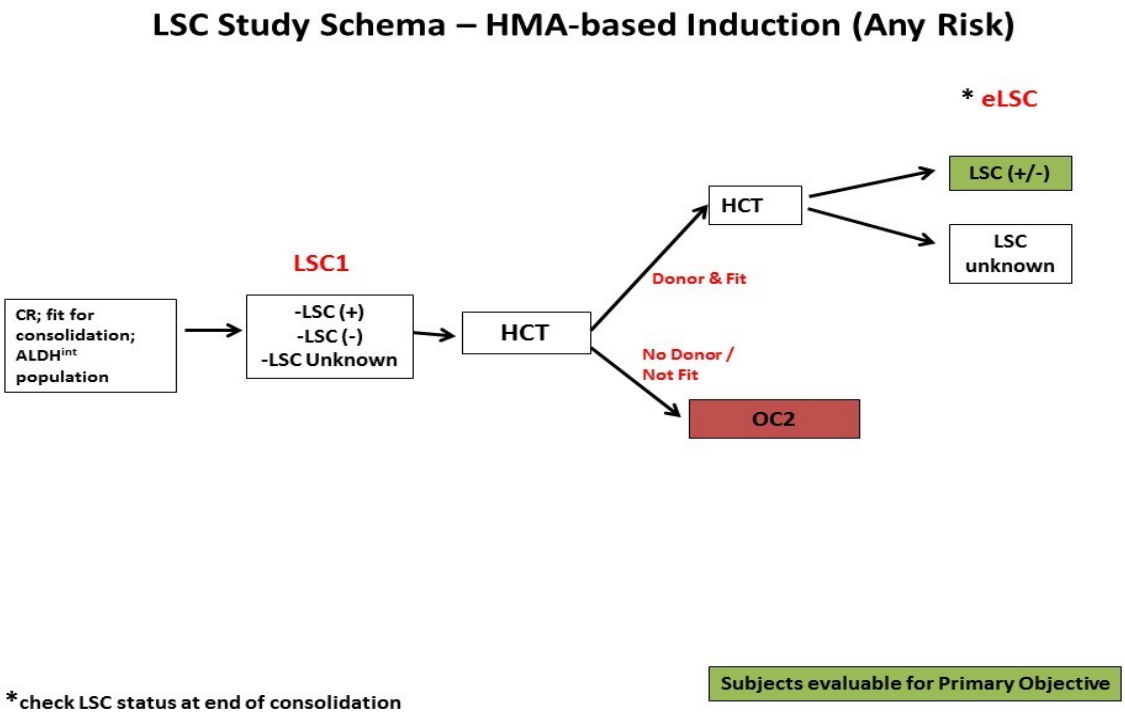
- **<u>Cytarabine-based induction subjects with unfavorable risk AML will be treated as follows:</u>** All unfavorable risk subjects who are candidates for HCT and who have an available matched related, matched unrelated, or haploidentical donor will be assigned to undergo HCT. Those subjects who lack an available donor or who are not fit for HCT will be treated with standard cytarabine-based consolidation chemotherapy.

Note: The treatment assignment applies even if the subject has unknown LSC status. The study schema for unfavorable risk AML subjects is as follows:



HMA-based induction subjects (regardless of risk) will be treated as follows:

- All HMA-based induction subjects who are candidates for HCT and who have an available matched related, matched unrelated, or haploidentical donor will be assigned to undergo HCT. HMA-based induction subjects who do not receive HCT (for any reason) will be enrolled and included in the Observational Cohort 2. Note: The treatment assignment applies even if the subject has unknown LSC status. The study schema for HMA-based induction subjects is as follows:



HCT

Subjects and their first degree relatives will undergo HLA typing as soon as possible after diagnosis according to standard of care. A matched unrelated donor (MUD) search will also be initiated if a related donor is not found. Umbilical cord transplants will not be performed on this trial. Among multiple suitable donors, selection of the optimal donor will use institutional priority extant at the time. Subjects eligible for myeloablative conditioning will be prioritized to receive it; subjects not eligible for myeloablative conditioning will receive non-myeloablative conditioning.

Subjects undergoing HCT will be permitted to receive up to four cycles of consolidation cytarabine or HMA-based therapy prior to undergoing HCT.

4.5.4.1. Study Parameters

Institutional guidelines for consolidation therapy with HCT or standard chemotherapy will be followed per routine.

4.5.4.2. Supportive Care

Supportive care, including infection management and transfusion support, will follow good medical practice and institutional standard guidelines.

4.6. End-of-Consolidation (EOC)

4.6.1. End of treatment

For evaluable subjects undergoing treatment with consolidation chemotherapy, end of treatment is defined as 30 days from day 1 of last cycle of chemotherapy. For subjects undergoing treatment with consolidation HCT, end of treatment is defined as 30 days from day 0 of HCT.

4.6.2. End-of-Consolidation Visit

This visit occurs after completion of consolidation chemotherapy or HCT. For evaluable subjects who received consolidation chemotherapy, the EOC visit should occur within 60 days of day 1 of the last cycle of chemotherapy. Last cycle of chemotherapy may be cycle 3 or cycle 4. For subjects undergoing treatment with consolidation HCT, the EOC visit should occur within 100 days from day 0 (day cells are transplanted).

4.6.3. Procedures and Assessments

The following procedures and assessments will occur at the end of consolidation (EOC) according to the Study Calendar (Section 5.0): bone marrow aspirate/biopsy, remission assessment, and maintenance therapy status. For subjects undergoing treatment with consolidation HCT, GVHD is assessed.

4.7. Short and Long-Term Follow-up Visits

Subjects will be followed for up to five years from enrollment to the study according to the Study Calendar (Section 5). For Year 1, follow-up visits will occur at 6 and 12 months from the enrollment date. If a follow-up visit timepoint occurs while the subject is still receiving therapy (induction or consolidation), that follow-up visit will not be required. For all subjects, the following procedures and/or assessments will be recorded during follow-up: remission assessment, maintenance therapy status, survival. For subjects who underwent consolidation HCT, GVHD will also be assessed.

For subjects who underwent HCT and who have not relapsed, a bone marrow biopsy is required at 6 months (± 30 days) and 12 months (± 30 days) from Day 0, and the 6 month timepoint will include an LSC assay (LSC6) . For subjects who underwent consolidation chemotherapy and have not relapsed, bone marrow biopsies are optional per investigator discretion during follow-up.

During Years 2-5, subjects will complete follow-up annually for relapse and survival status from the date of the Year 1, 12-month follow-up visit. Methods of follow-up contact will include standard of care office visits, medical records review, phone calls, and/or postal mail. Bone marrow biopsies during long-term follow-up (years 2-5) are optional, per investigator discretion.

4.8. Off-Study

Subjects will be considered Off Study following the final follow-up assessment at year 5. Subjects may come off-study earlier for the following reasons:

- Study Consent withdrawal (see Section 4.10)
- Death
- Lost to follow-up, defined as no successful attempts to contact subject over the course of three months
- Study termination
- Other, as determined by the Investigator

4.9. Subject Replacements

Enrolled subjects who have discontinued participation in the study after enrollment (the time of post-induction bone marrow aspirate) will not be replaced.
A subject who, for any reason (e.g. failure to satisfy the eligibility criteria or withdraws consent), terminates participation in the study before enrollment (the time of post-induction bone marrow aspirate) is regarded as a "screen failure". Screen failures may be replaced.

4.10. Subject Withdrawal

Subjects are defined as withdrawn from the study if they revoke consent(s) for study procedures, or if the Investigator withdraws them from the study. Subjects **must** be withdrawn for the following reasons:

- Subject withdraws consent(s) from study procedures. A subject must be removed from the study at his/her own request. At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Subject is lost to follow-up after no successful attempts at contact over three consecutive months.

Subjects **may be** withdrawn from the study by the Investigator for the following reasons:

- The subject is non-compliant with study procedures (as determined by the Investigator); including the use of anti-cancer therapy not prescribed by the study protocol.
- Development of an intercurrent illness or situation which would, in the judgment of the Investigator, significantly affect assessments of clinical status and trial endpoints.

Any subject who withdraws themselves or is withdrawn from the study by the Investigator will remain under medical supervision until discharge or transfer is medically acceptable.

In all cases, the reason for withdrawal must be recorded in the subject's medical records and/or research chart. Withdrawn subjects are considered to be Off Study.

Details for premature termination of the study as a whole (or components thereof [e.g. investigational sites, treatment arms,]) are provided in Section 11.2.

5. STUDY CALENDAR

Visit Name	Pre-Screening	End of Induction	End of Consolidation[g]	Short Term (Year 1) Follow-Up[j]	Long Term (Years 2-5) Follow-Up
Visit Occurrence	**Occurs at suspected AML diagnosis**	**Occurs at completion of induction therapy**	**Occurs at completion of consolidation therapy**	**Occurs 6, 12 months from enrollment date**	**Occurs annually from date of Year 1 Follow-Up**
Visit Window	**Prior to start of induction chemotherapy**	**Within 30 days prior to the start of consolidation therapy**	**Within 60 days from day 1 of last chemotherapy cycle or within 100 days from Day 0 (HCT)**	**+/- 30 days**	**+/- 30 days**
Step 1 Consent	X				
LSC0 Assay	X				
LSC1 Assay		X			
eLSC Assay			X		
LSC6 Assay				X[f,g]	
Step 1 Eligibility Check		X			
Step 2 Consent[i]		X			
Registration		X			
Physical Exam		X[a]			
Laboratory Tests		X[a,h]			
ECOG Performance Status		X[a]			
Bone Marrow Aspirate/Biopsy	X	X	X	X[b,g]	X[c,g]
GVHD Assessment[e]			X	X[g]	X[g]
Remission Evaluation[d]		X	X	X	X
Maintenance Therapy Evaluation			X	X	X
Survival Status				X	X

a. Required for the purposes of the study.
b. Required at 6 months (± 30 days) and 12 months (± 30 days) from Day 0 for evaluable subjects who received HCT**;** Optional for evaluable subjects who received consolidation chemotherapy, or per investigator discretion.
c. Optional for all evaluable subjects at each long-term follow-up visit, per investigator discretion.
d. Per Cheson Criteria for Leukemia. Also includes reporting first relapse in evaluable and OC2 cohort subjects.
e. For evaluable subjects undergoing consolidation therapy with HCT.
f. For evaluable subjects undergoing HCT, an LSC Assay will be performed 6 months (+/- 30 days) following HCT.
g. Only required for evaluable subjects
h. Required lab tests include at a minimum: Creatinine, bilirubin
i. Favorable and intermediate risk subjects only.
j. If a follow-up visit timepoint occurs while the subject is still receiving therapy (induction or consolidation), that follow-up visit will not be required.

6. TREATMENT EVALUATION

Response criteria and measurement of effect will be per the Cheson criteria for AML (see Appendix A)[1].

Relapse following complete remission is defined as:

1. Peripheral Blood Counts: reappearance of blasts in the blood.
2. Bone Marrow Aspirate and Biopsy: Presence of > 5% blasts, not attributable to another cause (e.g., bone marrow regeneration) or dysplasia in greater than 10% of any lineage with cytopenias in one or more lineages or presence of a cytogenetic abnormality consistent with MDS or AML.
3. Development of extramedullary disease.

7. TREATMENT-RELATED ADVERSE EVENTS

All adverse events will be managed per the Investigator according to standard of care. Adverse events can be unpredictable in nature and severity, although all care will be taken to minimize them. If subjects suffer any physical injury as a result of participating in this study, immediate medical treatment is available at the treatment center. Frequent blood samples will be taken to monitor side effects.

8. DATA AND SAFETY MONITORING PLAN

8.1. Safety Monitoring

In addition to the early stopping rules for safety described for GVHD in Section 10.8.1, this protocol will be monitored according to the processes in effect for all Levine Cancer Institute Investigator-Initiated Trials and will abide by standard operating procedures set forth by both the Carolinas HealthCare System Office of Clinical and Translational Research and the Levine Cancer Institute Clinical Trials Office. It is the responsibility of the Sponsor-Investigator to monitor the safety data for this study. The Sponsor-Investigator will monitor the study accrual data every month and will evaluate laboratory correlative data on a subject-by-subject basis. The Sponsor-Investigator, Statistician, and other team members as needed will meet regularly to monitor subject consents, enrollment and retention, safety data for all subjects, and validity/integrity of the data. Documentation of these meetings will be kept with study records.

SAEs will be reported to the IRB and LCI Data and Safety Monitoring Committee (DSMC) per their requirements. Major protocol deviations that result in a threat to subject safety or the integrity of the study will be reported to IRB per their requirements. The Sponsor-Investigator will submit data to the DSMC according to the overarching LCI Data and Safety Monitoring Plan (DSMP).

8.2. Data Quality Assurance

This study will be organized, performed, and reported in compliance with the study protocol, standard operating procedures (SOPs) of the Levine Cancer Institute and Carolinas HealthCare System Office of Clinical and Translational Research, and other applicable regulations and guidelines (e.g. GCP).

Data will be collected on electronic Case Report Forms (eCRFs).

A subset of subjects will be monitored by LCI Research Monitors routinely for data quality. This monitoring will be done by comparing source documentation to the eCRFs.

The study database will be reviewed and checked for omissions, apparent errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification will be generated and addressed by the appropriate research personnel. Only authorized personnel will make corrections to the study database and all corrections will be documented in an electronic audit trail.

Any variation between the two data sets will be discussed with the appropriate research personnel.

8.3. Communication Between Investigational Sites

Investigational sites will be required to report any problem that could affect the validity/integrity of the study data to the Sponsor-Investigator. Any problem should be communicated to the Sponsor-Investigator by email as soon as possible but within 2 business days of the treating Investigator learning of the event.

9. SAFETY DATA COLLECTION, RECORDING AND REPORTING

For the purposes of the study, safety variables include instances of GVHD in evaluable subjects who have undergone HCT only. Safety assessments should be performed according to the study calendar and as clinically necessary.

9.1. Unanticipated Problem Definition

A UAP is any incidence, experience or outcome that is unexpected, given the information provided in research-related documentation (e.g., Investigator's brochure,

informed consent) and the study population characteristics that is related or possibly related to participation in the research study and places the participant at an increased risk.

9.2. Study Adverse Event (AE) Definition

An adverse event is any untoward medical occurrence associated with the use of a drug/device in humans, whether or not considered drug/device related. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study treatment, whether or not considered related to the study treatment.

Both forms of treatment on this study (cytarabine-based consolidation chemotherapy and HCT) are approved treatments for this disease population, and are considered standard of care. Therefore, with the exception of GVHD, which is an event of clinical interest, adverse events experienced by the subjects will not be recorded in the eCRFs or included in the analysis for the purposes of this study. However, such safety events will still be managed according to standard of care and recorded in the subject's medical record per routine clinical standard.

9.3. "Unexpected" Definition

An AE or SAR is to be considered unexpected if the event is not listed in the current Investigator Brochure or is not listed in the severity or specificity observed. Investigators should refer to the Safety Information section of the current IB for the therapy given, including the DCSI (development core safety information), for the expected side effects of the given therapy. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions.

9.4. Study Serious Adverse Event (SAE) Definition

A serious adverse event is one that results in any of the following:

- Death
- Life-threatening illness
- Inpatient hospitalization or prolongation of existing hospitalization.
    - NOTE: In general, hospitalization means that the subject has been detained at the hospital or emergency ward for observation or treatment that would not have been appropriate in the physician's office or outpatient setting. **For this study, hospitalization that is part of planned therapy is not considered an SAE.**
- Persistent or significant disability or incapacity.

  - NOTE: Disability is defined as a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
- A congenital anomaly or birth defect
- An important medical event.
  - NOTE: An event may be considered an important medical event when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- Pregnancy

As previously described, each of the treatments in this study (cytarabine-based consolidation chemotherapy and HCT) are FDA approved and are considered standard of care in this disease population. Therefore, with the exception of GVHD, which is an event of clinical interest, safety events experienced by the subjects will not be recorded in the eCRF. However, such safety events will still be **managed according to standard of care** and recorded in the subject's medical record per routine clinical standard**.**

9.5. Event of Clinical Interest – Graft Versus Host Disease (GVHD)

For the purposes of the study, we will only be collecting and analyzing events of GVHD in evaluable subjects who received HCT. This data will be recorded in a study eCRF and be analyzed with the study dataset. We will collect data for confirmed incidents of GVHD from the time of consolidation HCT through long term follow-up.

The Investigator is responsible for verifying and providing source documentation for all incidents of GVHD that occur in evaluable subjects who received HCT, and assigning the classification and grade for each incident.

Occurrences of GVHD should be coded using an internationally recognized dictionary. Because the CTCAE for toxicity cannot be applied to GVHD, the Investigator will classify the toxicity based on the timing as defined below.

- **Acute GVHD**: Confirmed GVHD within 100 days of HCT.

- **Chronic GVHD**: Confirmed GVHD 100 days or more after HCT.

Each occurrence of GVHD in evaluable subjects who received HCT, will be further graded by the investigator according to accepted institutional practice which is informed by either CIBMTR grading for Acute GVHD or NIH Consensus criteria for Chronic GVHD.

9.6. SAE Reporting Requirements

SAEs meeting each of the following criteria must be reported to the Sponsor-Investigator within 24 hours of awareness via the CTMS.

- Subject must be in the evaluable cohort
- Event meets the definition of SAE per Section 9.4.
- Event is considered unexpected to the study intervention
- Event is assigned an attribution of at least possibly related to the study intervention

SAEs will be assigned toxicity scores using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

If the SAE is GVHD, the Investigator will quantify the toxicity based on intensity and stage per Section 9.5.

Reportable SAEs will be captured from the time of enrollment through 30 days after the date of last consolidation chemotherapy administration or 30 days from Day 0 of HCT in subjects who receive consolidation HCT. SAEs will be followed until clinical recovery is complete, laboratory tests have returned to baseline, or until there has been acceptable resolution of the event. This may at times cause the follow-up period for SAEs to be greater than 30 days. The above referenced 30-day time period applies even if the subject begins subsequent anti-cancer therapy during this time period. Similarly, the treating investigator is responsible for following the subject during the required follow-up period even if the subject lives elsewhere or has been released from his or her care and is being treated under another service at LCI.

Confirmed Events of Clinical Interest, as defined in Section 9.5, will be reported in evaluable subjects from date of Consolidation HCT (Day 0) through long term follow-up which is 5 years from enrollment.

9.7. Safety Reporting to the IRB

All events occurring during the conduct of a protocol and meeting the definition of a UAP or SAE will be reported to the IRB per IRB reporting requirements.

Protocol deviations will be reported promptly to the IRB per IRB reporting requirements.

10. STATISTICAL METHODS

10.1. Sample Size

The primary analysis will compare two-year RFS probabilities for subjects with detectable LSCs at eLSC (eLSC+) and subjects without detectable LSCs at eLSC (eLSC-). The targeted sample size is 200 subjects who are evaluable for the primary objective (Section 10.3). Assuming a prevalence of 63% for eLSC+ subjects and 37% for eLSC- subjects based on the subject distribution shown in the probability diagram (Figure 4), we anticipate that there will be approximately 125 subjects with detectable LSCs and 75 subjects without detectable LSCs at end of consolidation. This assumed prevalence of having detectable LSCs at end of consolidation (eLSC+) subjects was determined using the joint probabilities for each arm shown in the probability tree diagram (Figure 4). The probabilities for each of the conditional events (e.g. the probability of detectable LSCs post induction given that the subject is determined to be intermediate risk is P(LSC1+|IR) = 0.7) are assumed based on previous study data. The joint probabilities for each path are determined by multiplying the conditional event probabilities along that path. For example, the probability that a favorable risk subject who was positive for LSCs post induction (LSC1+) and who underwent HCT will have no detectable LSCs at end of consolidation (eLSC-) is $P(FR \cap LSC1+ \cap HCT \cap eLSC-) = 0.1*0.5*0.45*0.5 = 0.01125$. The marginal probabilities for eLSC+ and eLSC- are then found by summing all the joint probabilities that have the final outcome of eLSC+ and eLSC-, respectively. From Figure 4, the resulting probability of having detectable LSCs at end of consolidation is P(eLSC+) = 0.62705, while the probability of not having detectable LSCs at end of consolidation is P(eLSC-) = 0.37295. This assumed distribution of eLSC+ and eLSC- subjects will provide, at minimum, 90% power to detect at least a 25% improvement in 2-year recurrence free survival probabilities between the eLSC+ and eLSC- groups, assuming two year relapse free survival rates ranging from 10% to 40% in the eLSC+ group. These calculations were made to preserve a two-sided significance level of alpha = 0.05. Note that, since the assumptions about the various pathways may not reflect the subject population accrued for the study, the power calculations will be updated according to the true prevalence of having

detectable LSCs at end of consolidation.

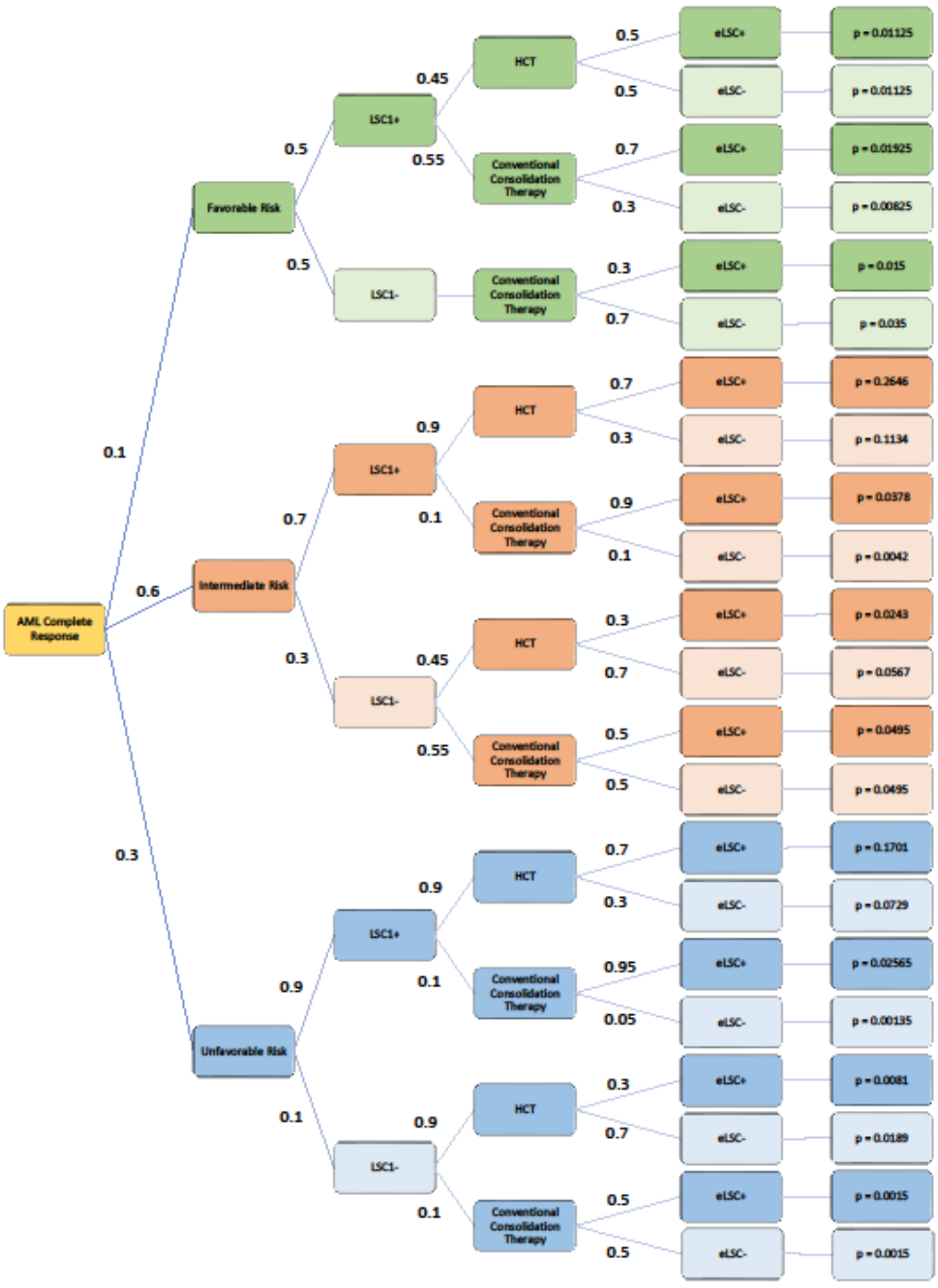


Figure 4. Probability Tree

10.2. Endpoint Definitions

10.2.1. 2-Year and 1-Year Relapse Free Survival

In this study, 2-year relapse free survival will be calculated from a recorded binary variable determined for each subject indicating whether or not the subject experienced any disease relapse or death from any cause within two years from CR1. Relapse will be classified according to Cheson criteria. The binary variable described above will also be calculated at one year for the secondary objective of one-year RFS.

10.2.2. Overall Survival

Overall survival (OS) is defined as the duration of time from the enrollment date (date of the post-induction LSC1 assay) to the date of death from any cause. Subjects who are alive or lost to follow-up at the time of the analysis will be censored at the last known date they were alive.

10.2.3. Relapse Free Survival

Relapse free survival (RFS) is defined as the duration of time from the enrollment date (date of the post-induction LSC1 assay) to the date of relapse or death from any cause. Relapse will be classified according to Cheson criteria. For surviving subjects who have not experienced a relapse or are lost to follow-up at the time of analysis, RFS will be censored at the date of last objective disease assessment.

10.2.4. Safety Endpoints

The graft-versus-host disease (GVHD) classification (acute vs chronic), incidence, and grade/severity will be the primary safety endpoint.

10.3. Analysis Populations

The primary efficacy analyses will be conducted on the subset of subjects who were included in the evaluable cohort (defined in Section 4.2) and who also had a successful/interpretable end of consolidationeLSC assay. This population of subjects will be evaluable for the primary objective. The evaluable populations used for the secondary efficacy analyses will be determined by those subjects in the evaluable cohort who have successful and interpretable LSC1, eLSC, and LSC6 assays, depending on the objective. Additionally, analyses of the safety endpoints will be conducted on the applicable treatment cohort of subjects (Section 4.2). It is noted, however, that the evaluation of GVHD will only be applicable to the subjects undergoing HCT. In

addition to evaluating outcomes across all enrolled subjects, exploratory assessments will be conducted on the following two observational cohorts:

Observational cohort 1 (OC1): enrolled subjects who do not achieve a CR to induction therapy

Observational cohort 2 (OC2): enrolled subjects who achieve a CR to induction therapy but meet one or more of the following criteria:

- Lack the immunophenotype of interest,
- Cytarabine based induction subjects: Are not a candidate for [(as determined by the investigator (e.g. unfit or refusal)] and do not receive consolidation therapy (cytarabine-based chemotherapy or HCT)
- HMA-based induction subjects: Are not a candidate for [as determined by the investigator (e.g. unfit, lack of donor, refusal)] and do not receive HCT

10.4. Analysis Methods

10.4.1. Timing of Analysis

The analyses for the primary objective will occur when either all subjects in the study have experienced a relapse or else when they have been followed for the 2-year period. Subject disposition, baseline characteristics, treatment compliance, will also be assessed at that time. Additionally, secondary analyses involving two-year and one-year RFS rates will be assessed along with observational cohorts.

For the purposes of overall survival and relapse free survival, initial analysis will take place at the time of the primary analysis. However, subjects will continue to be followed until either all subjects have died from any cause or when they have been followed for 5 years from the date of the post-induction LSC1 assay. Whenever these criteria are met, final survival analysis methods will be implemented for OS and RFS, and the study will be closed to IRB.

10.4.2. Subject Disposition

An accounting of all consented subjects will be provided at the end of the study. This will include a breakdown of subjects who consented, enrolled, achieved CR1, were included in the evaluable cohort, , received HCT (versus conventional consolidation therapy), completed 5 years of follow-up, were discontinued early, and those who were lost to follow-up or withdrew consent.

A summary of subject demographics and disease-related characteristics will be completed.

10.5. Efficacy Analysis

10.5.1. Primary Analysis

The primary analysis will be to compare the relapse free rate at two years between the subjects who have detectable LSCs at end of consolidation (eLSC+) and those who do not have detectable LSCs at end of consolidation (eLSC-). The relapse free survival rates for each group will be calculated and the corresponding 95% confidence intervals will be estimated using the Clopper-Pearson method for binomial confidence intervals. The relapse free survival rates will also be compared between the groups in a stratified logistic regression model. The model will be stratified for each of the possible treatment pathways shown in Figure 4 and will include a covariate for eLSC group. In the event that any pathway is too sparse, the stratified design will be collapsed as needed. The group difference will be tested at a 2-sided alpha = 0.05 significance level.

10.5.2. Secondary Analyses

For each of the secondary objectives, a two-sided alpha = 0.05 level of significance will be used for testing. Most of the secondary objectives involve comparing RFS rates calculated for selected subgroups (e.g. LSC1+ versus LSC1- groups in the subgroup of favorable risk subjects who received consolidation) and at the appropriate point in time. The specific group comparisons are described in Section 1.2. The RFS rates for the selected subgroups will be analyzed in a similar fashion as described above for the 2-year RFS rate primary analysis.

Overall survival and relapse free survival in the AML subjects will also be assessed using Kaplan-Meier techniques. A log-rank test will be used to compare the eLSC+ group to the eLSC- group. Cox proportional hazards models will be used to evaluate baseline and disease characteristics in addition to the eLSC group effect. These methods will also be repeated for all relevant objectives using temporally comparable LSC sample measurements (LSC6 samples for HCT patients and eLSC samples for the chemo subjects), comparing the LSC6+ (or eLSC+, respectively) group versus the LSC6- (or eLSC-) group, as these measurements are closer chronologically than the end of consolidation samples between the chemotherapy and transplant groups.

Additional RFS rate comparisons will be explored among other groups. This includes comparing the treatment effects (HCT versus consolidation therapy) on two-year RFS in unfavorable subjects without detectable LSCs at LSC1, as well as broader comparison of the LSC1 groups (LSC1+ versus LSC1-) and their impact on the two-year and one-year RFS rates. Additionally, the impact of

baseline and disease characteristics on some outcomes will be evaluated. Univariate models will be used to identify independent prognostic factors. The treatment group term will be added to the models to obtain adjusted group effects.

In the subjects that undergo allogeneic HCT, descriptive statistics will be used to evaluate the change in LSC status from eLSC (end of consolidation) to LSC6 (6 months after day 0 of transplant). A breakdown will be provided, summarizing the frequency and proportion of subjects in each of the following LSC status categories: eLSC+/LSC6+; eLSC+/LSC6-; eLSC-/LSC6+; eLSC-/LSC6-.

10.6. Exploratory Analyses

Clinical outcomes of the two observational cohorts (Section 10.3) of subjects who are enrolled in the study but do not become a part of the evaluable population will be analyzed. Selected comparisons of interest will be evaluated using Fisher's Exact test. Additionally, survival outcomes will be evaluated as necessary using Kaplan Meier methods and Cox proportional hazards regression.

10.7. Interim Analyses

A planned interim analysis of the primary endpoint, 2-year relapse free survival, at 50% information time (n=100 subjects evaluable for the primary objective with 2 years of follow up from CR1) will be conducted. This will be a group sequential analysis utilizing the O'Brien-Fleming boundaries shown in Table 1. The corresponding nominal 2-sided significance level at 50% information time is 0.003, and at 100% information time, 0.049, to achieve at total 2-sided alpha of 0.05. If the actual interim analysis does not occur at exactly 50% information time, then the O'Brien-Fleming boundaries will be updated accordingly.

Table 1. O'Brien-Fleming boundaries for group sequential interim analysis at 50% information time.

Analysis	% Information Time	Nominal Alpha	Lower Boundary	Upper Boundary
Interim	50%	0.003	-2.96259	2.96259
Final	100%	0.049	-1.96857	1.96857

10.8. Safety Analyses

Incidence rates for GVHD collected from the time of HCT until the off study date will be summarized as counts and proportions. For selected events, 95%

confidence intervals will be calculated using the Clopper-Pearson method. Selected comparisons of interest will be evaluated using Fisher's Exact test.

10.8.1. Early Stopping Rules for Safety

This study will monitor acute grade III/IV GVHD in the transplant arm in the favorable risk cohort. If it becomes evident that the proportion of acute grade III/IV GVHD convincingly exceeds 10%, the study will be halted for a safety consultation. The stopping rule will hold enrollment if the posterior probability of toxicity risk exceeding 0.10 is 75% or higher in the favorable risk cohort. The prior for this monitoring rule is beta (1,9). This means that our prior guess at the proportion of acute grade III/IV GVHD is 10%, and there is 90% probability that this proportion is between 0.57% and 28.3%. The operating characteristics of the stopping rule are given in the Table 3 and are based on 5000 simulations:

Table 2. Early stopping rule cutoffs for numbers of GVHD events.

Number of GVHD Events	Max. Denominator Cutoff for Early Stopping	Posterior Probability Pr(Risk>0.1| Data)
2	8	0.7556
3	16	0.7744
4	25	0.7552
5	34	0.7520
6	43	0.7528
7	50	0.7738

Table 3. Operating characteristics of stopping rule based on 5000 simulations.

True Tolerability Risk	Prob. Declare Treatment Not Tolerable	Average Sample Size
0.02	1.2%	39.6
0.05	9.4%	37.3
0.10	39.8%	29.9
0.15	72.9%	21.0
0.20	91.1%	14.4
0.25	98.0%	10.1
0.30	99.6%	7.7

11. STUDY COMPLETION

11.1. Completion

The study will be considered complete when one or more of the following conditions is met:

- All subjects have completed all study visits.
- All subjects have discontinued from the study.
- The IRB, LCI DSMC, or Sponsor-Investigator discontinues the study.
- The Sponsor-Investigator defines an administrative or clinical cut-off date.

11.2. Termination

The study will be terminated when one or more of the following conditions occur:

If risk-benefit ratio becomes unacceptable owing to, for example:

- Safety findings from this study (e.g. SAEs)
- Results of any interim analysis
- Results of parallel clinical studies
- Results of parallel animal studies (e.g. toxicity, teratogenicity, carcinogenicity or reproduction toxicity).
- If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the study within a reasonable time frame.

The Sponsor-Investigator has the right to close the study at any site and at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions must be informed as applicable according to local law.
- In case of a partial study closure, ongoing subjects, including those in follow-up, must be taken care of in an ethical manner.

Details for individual subject's withdrawal can be found in Section 4.11.

12. RETENTION OF RECORDS

Essential documentation including all IRB correspondence, will be retained for at least 2 years after the investigation is completed. Documentation will be readily available upon request.

13. ETHICAL AND LEGAL ISSUES

13.1. Ethical and Legal Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Investigators abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate agencies (IRB) will be obtained before the start of the study, according to GCP, Declaration of Helsinki, local laws, regulations and organizations. When necessary, an extension, amendment, or renewal of IRB approval must be obtained and kept on file.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the Investigators may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the Sponsor-Investigator without IRB approval. However, the Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior approval from applicable agencies. As soon as possible, the implemented deviation or change, the reasons for it, and if appropriate, the proposed protocol amendment should be submitted to the appropriate agencies. Any deviations from the protocol must be explained and documented by the Investigator.

The Sponsor-Investigator is responsible for the conduct of the clinical trial at the sites in accordance with Good Clinical Practice (GCP) and the Declaration of Helsinki. The Sponsor-Investigator is responsible for overseeing the treatment of all study subjects. The Sponsor-Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all applicable regulations and guidelines regarding clinical trials both during and after study completion.

The Sponsor-Investigator will be responsible for assuring that all the required data will be collected and properly documented.

13.2. Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

14. CLINICAL TRIAL REGISTRATION AND RESULTS REPORTING

The Sponsor-Investigator will ensure that the information and results regarding the study will be made publicly available on the internet at www.clinicaltrials.gov.

REFERENCES

1. Cheson BD, Bennett JM, Kopecky KJ et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin.Oncol. 2003;21:4642-4649.
2. Jemal A, Clegg LX, Ward E et al. Annual report to the nation on the status of cancer, 1975-2001, with a special feature regarding survival. Cancer 2004;101:3-27.
3. Dohner H, Estey EH, Amadori S et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010;115:453-474.
4. Grimwade D, Walker H, Oliver F et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 1998;92:2322-2333.
5. Byrd JC, Mrozek K, Dodge RK et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002;100:4325-4336.
6. Estey E, Dohner H. Acute myeloid leukaemia. Lancet 2006;368:1894-1907.
7. Rowe JM, Li X, Cassileth PA et al. Very Poor Survival of Patients with AML Who Relapse after Achieving a First Complete Remission: The Eastern Cooperative Oncology Group Experience. ASH Annual Meeting Abstracts 2005;106:546.
8. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat.Med. 1997;3:730-737.
9. Jones RJ, Matsui WH, Smith BD. Cancer stem cells: are we missing the target? J.Natl.Cancer Inst. 2004;96:583-585.
10. Huff CA, Matsui W, Smith BD, Jones RJ. The paradox of response and survival in cancer therapeutics. Blood 2006;107:431-434.
11. Villela LM, Bolanos-Meade J. Blood and Bone Marrow Transplantation for Acute Myeloid Leukemia. Clinical Leukemia 2009;3:E11-E21.
12. Lapidot T, Sirard C, Vormoor J et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645-648.
13. Taussig DC, Miraki-Moud F, njos-Afonso F et al. Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood 2008;112:568-575.
14. Taussig DC, Vargaftig J, Miraki-Moud F et al. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. Blood 2010;115:1976-1984.

15. Sarry JE, Murphy K, Perry R et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rgammac-deficient mice. J.Clin.Invest 2011;121:384-395.
16. Eppert K, Takenaka K, Lechman ER et al. Stem cell gene expression programs influence clinical outcome in human leukemia. Nat.Med. 2011;17:1086-1093.
17. Pearce DJ, Taussig D, Zibara K et al. AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. Blood 2006;107:1166-1173.
18. Rombouts WJ, Martens AC, Ploemacher RE. Identification of variables determining the engraftment potential of human acute myeloid leukemia in the immunodeficient NOD/SCID human chimera model. Leukemia 2000;14:889-897.
19. Wang JC, Dick JE. Cancer stem cells: lessons from leukemia. Trends Cell Biol. 2005;15:494-501.
20. Christ O, Lucke K, Imren S et al. Improved purification of hematopoietic stem cells based on their elevated aldehyde dehydrogenase activity. Haematologica 2007;92:1165-1172.
21. Pearce DJ, Taussig D, Simpson C et al. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. Stem Cells 2005;23:752-760.
22. Pearce DJ, Bonnet D. The combined use of Hoechst efflux ability and aldehyde dehydrogenase activity to identify murine and human hematopoietic stem cells. Exp.Hematol. 2007;35:1437-1446.
23. Gerber JM, Qin L, Kowalski J et al. Characterization of chronic myeloid leukemia stem cells. Am.J.Hematol. 2011;86:31-37.
24. van der Pol MA, Feller N, Roseboom M et al. Assessment of the normal or leukemic nature of CD34+ cells in acute myeloid leukemia with low percentages of CD34 cells. Haematologica 2003;88:983-993.
25. Slovak ML, Kopecky KJ, Cassileth PA et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood 2000;96:4075-4083.
26. Cassileth PA, Harrington DP, Appelbaum FR et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N.Engl.J Med. 1998;339:1649-1656.

APPENDICES

Appendix A
Cheson Criteria for Remission

Response Criteria in AML

Response Criterion	Time of Assessment	Neutrophils (µL)	Platelets (µL)	Bone Marrow Blasts (%)	Other
Early treatment assessment	7-10 days after therapy	NA	NA	<5	
Morphologic leukemia-free state	Varies by protocol	NA	NA	<5	Flow cytometry[1]; no EMD
Morphologic CR	Varies by protocol	1,000	100,000	< 5	Transfusion independent; no EMD
Cytogenetic CR	Varies by protocol	1,000	100,000	< 5	Cytogenetics—normal, no EMD
Molecular CR	Varies by protocol	1,000	100,000	< 5	Molecular—negative, no EMD
Partial remission	Varies by protocol	1,000	100,000	50 or decrease to 5-25	Blasts< 5% if Auer rod positive

[1] Flow cytometry may also be useful to distinguish between leukemia and a regenerating bone marrow

Abbreviations: AML, acute myelogenous leukemia; EMD, extramedullary disease; CR, complete remission.

Cheson et al: Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia ; J Clin Oncol 2003