**CLINICAL RESEARCH PROJECT Protocol # PXXXXX**

**IND or IDE? No**

|  |  |
| --- | --- |
| **Date:** | April 2015 |

|  |  |
| --- | --- |
| **Title:** | A pilot study of biomarkers for personalized early assessment of response during salvage chemotherapy in patients with relapsed or refractory acute myeloid leukemia |

|  |  |
| --- | --- |
| **Short Title:** | PEARL15 |

|  |  |
| --- | --- |
| **Other Identifying Words:** | Measurable residual disease, MRD, relapsed/refractory AML, salvage chemotherapy |

Principal Investigator:

|  |  |  |
| --- | --- | --- |
| \*Christopher Hourigan, M.D., D.Phil., HB, NHLBI (E) | 451-0257 | Bldg 10, 6C-103C |

Lead Associate Investigator:

|  |  |  |
| --- | --- | --- |
| \*Nestor R. Ramos, M.D., HB, NHLBI | 402-9181 | Bldg 10, 6C-103C |

Medically Advisory and Accountable Investigator:

|  |  |  |
| --- | --- | --- |
| \* Christopher Hourigan, M.D., D.Phil., HB, NHLBI (E) | 451-0257 | Bldg 10, 6C-103C |

Associate Investigators:

\*Catherine Lai M.D., OD, NCI (E) 451-1898 Bldg 10, 12N226

Neal S. Young, M.D., Chief, HB, NHLBI (E) 496-5093 Bldg 10, CRC 3-5142

A. John Barrett M.D., HB, NHLBI (E) 402-4170 Bldg 10, CRC 3-5322

Richard Childs, M.D., HB, NHLBI (E) 594-8008 Bldg 10, CRC 3-5332

Cynthia E. Dunbar, M.D., NHLBI, HB (E) 496-1434 Bldg 10, CRC 4-5132

Adrian Wiestner, M.D., HB, NHLBI (E) 594-6855 Bldg 10, CRC 3-5140

Andre Larochelle M.D., HB, NHLBI (E) 451-7139 Bldg 10, CRC 5-5130

Minoo Battiwalla, M.D., M.S. HB, NHLBI (E) 827-0939 Bldg 10, CRC 5-3581

Danielle Townsley, M.D., M.Sc., HB, NHLBI (E) 222-7625 Bldg 10,CRC 3-5140

James Cooper M.D., HB, NHLBI (E) Bldg 10,CRC 3-5140

Jingrong Tang M.D. Ph.D., HB, NHLBI (E) 594-0507 Bldg 10, 6C/104

Meghali Goswami B.S. (V) Bldg 10, 6C/104

Katherine R. Calvo, M.D, Ph.D, DLM, CC, NIH (E) 594-9578 Bldg 10, 2C416A

Raul C. Braylan M.D., DLM, CC, NIH (E) 594-9556 Bldg 10, 2C416A

Judith E. Karp M.D., (V) 451-0257 Bldg 10, 6C/104

Nirali N Shah M.D., POB, NCI (F) 451-0390 Bldg 10, CRC 1-1621

Bogdan Dumitriu, M.D., HB, NHLBI (F) 594-7041 Bldg 10, CRC 3-5140

Roger Kurlander, M.D., DLM, CC, NIH (E) 496-2779 Bldg 10, 2C390

Sawa Ito M.D., HB, NHLBI (E)

Monica Schmitt, HB, NHLBI (E)

Dennis Hickstein M.D., ETIB, NCI (E)

Kirsten Williams M.D., ETIB, NCI (V)

Ronald Gress M.D., ETIB, NCI [E]

Christopher Kanakry M.D., ETIB, NCI [E]

Edward W. Cowen, M.D., M.H.Sc., DB, NCI (E) 496-4299 Bldg 10, Rm12N238

M. Jennifer Cheng M.D., PPCS, CC, NIH (E)

Nicole Gormley M.D. HB, NHLBI (V)

Pawel Muranski M.D. HB, NHLBI (E)

*The \* indicates investigators allowed to obtain informed consent for this protocol.*

Biostatisticians/Associate Investigators

Lauren Kunz Ph.D., OBR, NHLBI (C) 435-1287 6701 Rockledge Drive, Room 9199

Dong-Yun Kim Ph.D., OBR, NHLBI (E) 480-0436 6701 Rockledge Drive, Room 9200

Research Contact:

|  |  |  |
| --- | --- | --- |
|  |  |  |

*To be assigned by NHLBI OCD.*

|  |  |
| --- | --- |
| Estimated Duration of Study: | 5 years |
| Estimated Completion Date of Study: | April 2020 |

|  |  |  |  |
| --- | --- | --- | --- |
| Subjects in study: | Number | Gender | Age range |
|  | Up to 36 | M/F | 18-70 |

|  |  |
| --- | --- |
| Multicenter trial: | No |
| Ionizing Radiation for Research: | No (Medically indicated only) |
| Off-Site Project: | No |
| DSMB Involvement: | No |
| Tech Transfer: | No |
| IND: | No |
|  |  |
|  |  |

|  |  |
| --- | --- |
| Version | 1.0 |

Table of Contents

[1. Objectives 5](#_Toc415646837)

[2. Background and Scientific Justification 5](#_Toc415646838)

[2.1 Epidemiology of AML 5](#_Toc415646839)

[2.2 Response criteria in AML therapy 5](#_Toc415646840)

[2.4 Rationale for treatment selection 7](#_Toc415646841)

[2.5 Scientific and clinical justification of the protocol 8](#_Toc415646842)

[3. Study Design 8](#_Toc415646843)

[4. Eligibility Assessment 10](#_Toc415646844)

[4.1 Inclusion criteria 10](#_Toc415646845)

[4.2 Exclusion criteria 10](#_Toc415646846)

[5. Treatment Plan 11](#_Toc415646847)

[5.1 Administration of salvage induction chemotherapy 11](#_Toc415646848)

[5.2 Dose delays, modifications or discontinuation for non-hematologic side effects 11](#_Toc415646849)

[5.3 Dose delays, modifications or discontinuation for hematologic side effects 11](#_Toc415646854)

[5.4 Infectious prophylaxis: 11](#_Toc415646855)

[5.5 Tumor lysis prophylaxis and management 12](#_Toc415646856)

[5.6 Supportive care 12](#_Toc415646862)

[5.7 Transfusion support 12](#_Toc415646863)

[6. Clinical Evaluation 13](#_Toc415646864)

[6.1 Pre-enrollment assessment 13](#_Toc415646865)

[6.2 Inpatient monitoring 14](#_Toc415646866)

[6.3 Outpatient monitoring 14](#_Toc415646867)

[6.4 Off study criteria 15](#_Toc415646868)

[7. Laboratory Research Studies 16](#_Toc415646869)

[7.1 Research sample collection 16](#_Toc415646870)

[7.2 Primary Laboratory Research Measurement 16](#_Toc415646871)

[7.3 Auxiliary Laboratory Research Studies 17](#_Toc415646872)

[7.4 Storage 18](#_Toc415646873)

[8. Biostatistical Considerations 19](#_Toc415646874)

[8.1 Primary Outcome 19](#_Toc415646875)

[8.2 Statistical Hypotheses 19](#_Toc415646876)

[8.3 Statistical Test 19](#_Toc415646877)

[8.4 Sample Size 19](#_Toc415646878)

[8.5 Power Calculation 19](#_Toc415646879)

[8.6 Secondary Analysis 19](#_Toc415646880)

[9. Data and Safety Monitoring 20](#_Toc415646881)

[9.1 Safety monitoring 20](#_Toc415646882)

[9.2 Event Characterization and Reporting 20](#_Toc415646883)

[9.3 Data management 24](#_Toc415646885)

[10. Human Subject Protection 25](#_Toc415646886)

[10.1 Rationale for subject selection 25](#_Toc415646887)

[10.2 Participation of pediatric patients 25](#_Toc415646888)

[10.3 Risks and Discomforts: 26](#_Toc415646889)

[10.4 Risks in Relation to Benefit 26](#_Toc415646890)

[10.5 Informed Consent Processes and Procedures 27](#_Toc415646893)

[10.6 Conflict of Interest 27](#_Toc415646898)

[11. Pharmaceuticals 27](#_Toc415646899)

12. Reference list 28

[APPENDIX A: AML Diagnostic and Treatment Response Criteria (per SWOG) 30](#_Toc415646900)

[APPENDIX B: Examples of conventional cytotoxic salvage chemotherapy regimens studied and utilized in patients with relapsed/refractory AML 31](#_Toc415646901)

[APPENDIX C Anthracycline Equivalent Dosing 32](#_Toc415646902)

[APPENDIX D NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES 33](#_Toc415646903)

# 1. Objectives

The ***primary objective*** is to determine if an abnormal peripheral blood gene expression signature on day four of chemotherapy is predictive of failure to achieve complete remission in patients with relapsed/refractory acute myeloid leukemia.

***Secondary objectives*** are to:

* determine relationship between day 4 peripheral blood based testing and conventional day 14 bone marrow assessment,
* investigate relationship between kinetics of gene expression changes and response to chemotherapy,
* determine proportion of patients for whom results of day 4 peripheral blood gene expression testing are available before day 8 of therapy,
* determine proportion of patients for whom results from pre-treatment molecular and genetic testing are available before day 8 of therapy,
* assess patient and disease specific factors associated with failure to achieve a complete remission,
* determine feasibility of measuring AML residual disease burden using alternative technologies and alternative tissue sources
* determine proportion of patients who receive allogeneic hematopoietic stem cell transplant after completion of therapy.
* to determine incidence of infectious complications with the use of modern prophylactic antimicrobial agents compared with historical comparisons *and*
* determine feasibility of recruiting patients with refractory and relapsed acute myeloid leukemia to the NIH Clinical Center.

# 2. Background and Scientific Justification

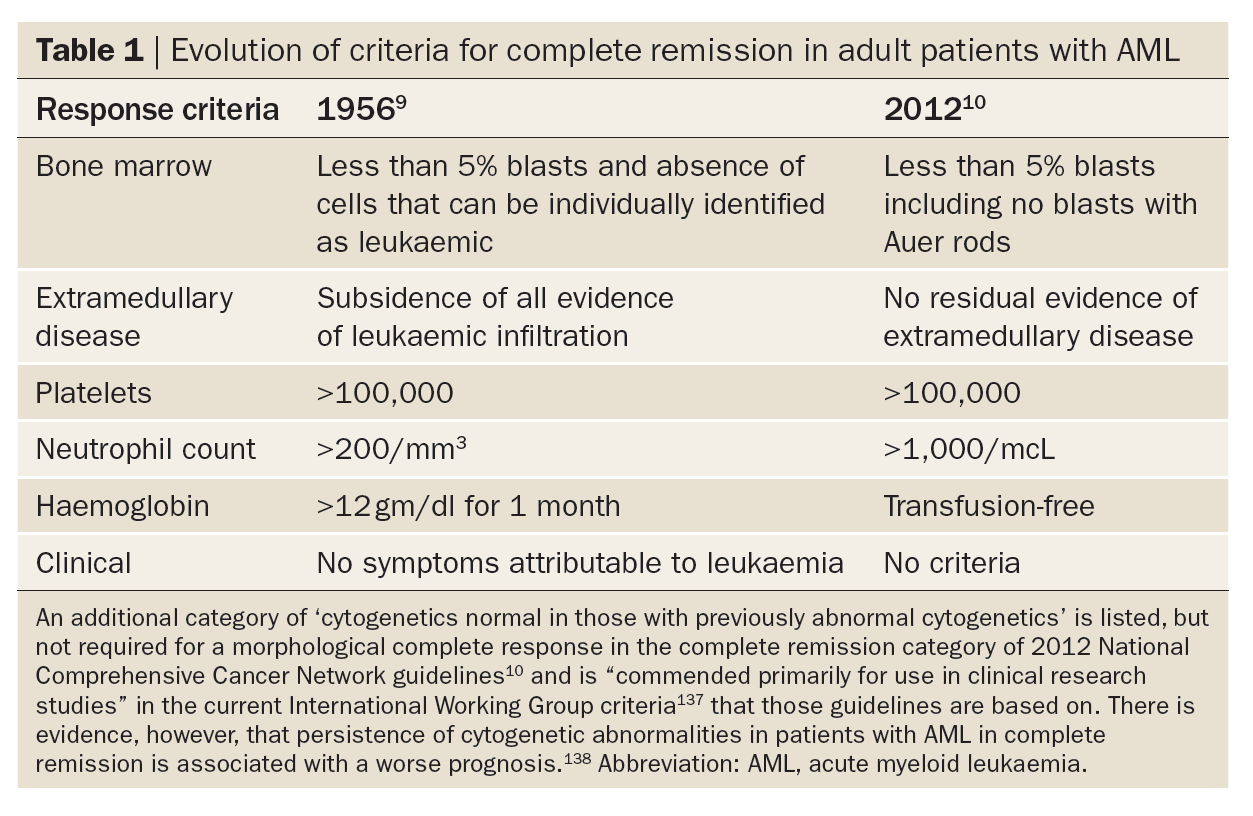
## 2.1 Epidemiology of AML

Approximately 20,800 patients will be diagnosed with acute myeloid leukemia (AML), with greater than 10,000 AML patient deaths in the United States during 2015 [[1](#_ENREF_1)]. Although complete response (CR) rates can be as high as 80% in patients undergoing initial induction cytotoxic chemotherapy, the majority of AML patients will ultimately be diagnosed with relapsed or refractory disease (RR-AML), which generally portends a poor prognosis [[2-4](#_ENREF_2)]. While a variety of different treatment regimens have been studied in an effort to improve outcomes of patients with RR-AML, there appears to be no single superior approach (Appendix A). Unfortunately the outcomes for untreated and refractory AML are dire, and those for relapsed AML are only slightly better with less than 30% of patients surviving 12 months after relapse [[3](#_ENREF_3)].

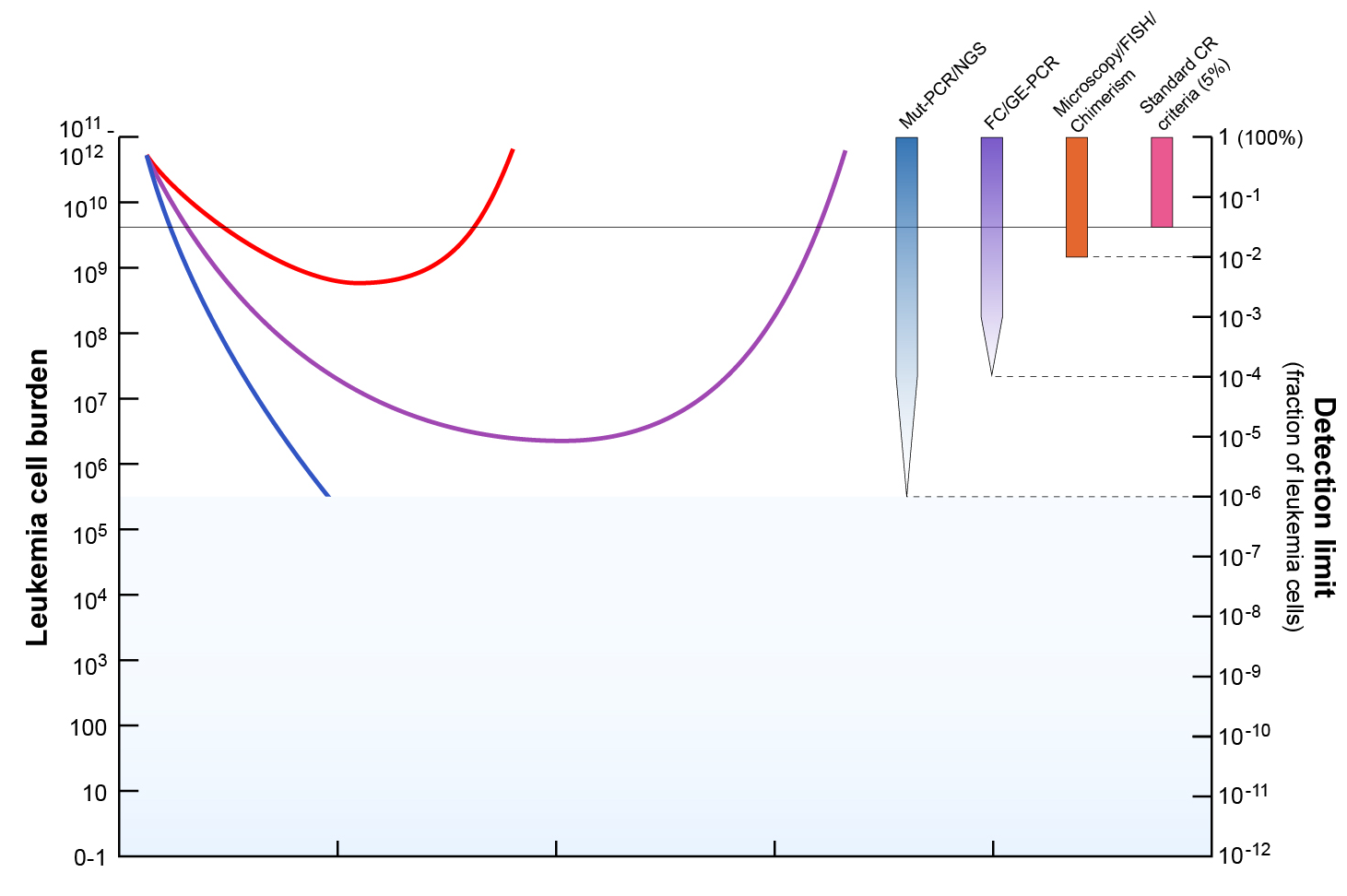
It is likely that many patients experiencing AML “relapse” are in fact manifesting the clinical outgrowth of a refractory clone that has persisted despite apparently successful initial therapy [9]. We would argue, at least conceptually, that the main problem of AML relapse is not that we cannot adequately prevent or treat relapse, but rather that our apparently successful initial treatment was not as effective as we had hoped. Relapse is therefore not a sign that an initial successful treatment has now failed, but rather simply that it was not a successful treatment. It is in this context that the modest success of the various second-line therapies for relapsed AML should be judged and, in the absence of any obvious standard of care, we suggest that all patients with refractory or relapsed AML be offered a referral to an appropriate clinical trial whenever possible.

## 2.2 Response criteria in AML therapy

The development of increasingly sensitive minimal or measurable residual disease (MRD) assays has demonstrated that current remission criteria, originally proposed in 1956 (Table 1), do not provide a sensitive assessment of AML disease burden, as evidenced by the disconnect between the apparent success of current induction therapy in achieving complete remission in most patients and the stark reality of median overall survival times of less than two years [[5](#_ENREF_5)]. Furthermore, studies evaluating remission status and subsequent relapse risk have shown traditional morphologic assessment is inferior when compared with newer methods such as flow cytometry [[6-8](#_ENREF_6)] or detection of AML associated mutations [[9](#_ENREF_9)] or gene over-expression[[10-12](#_ENREF_10)]. Nevertheless, while it is clear that clinically evident relapsed AML represents an end-stage, advanced process that could potentially be detected at an earlier time utilizing a sensitive MRD assay (Figure 1), the role for MRD measurement in the treatment algorithm for patients with RR-AML has yet to be defined.

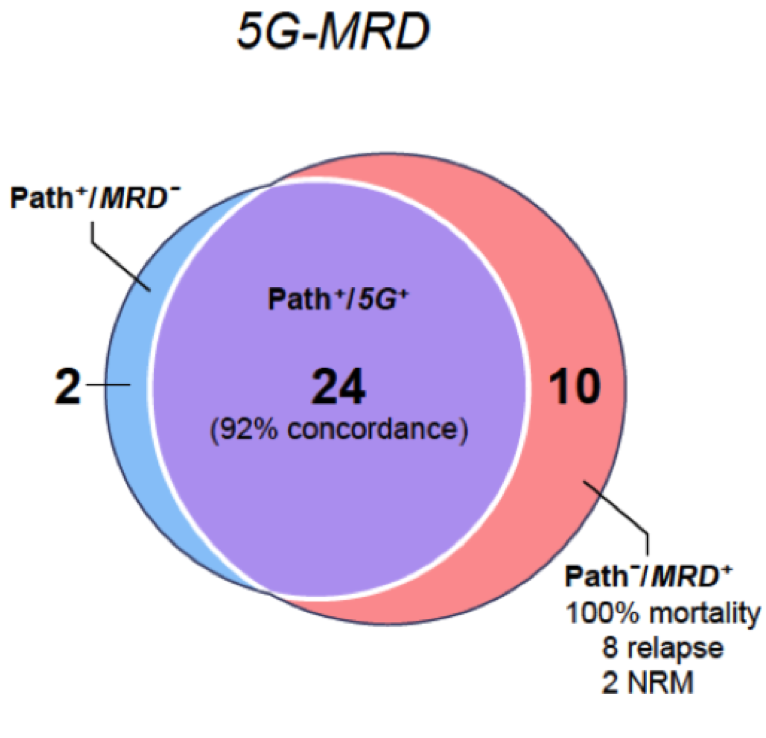


**Table 1: CR criteria in adult AML patients.** Adapted from Hourigan and Karp, Nature Reviews Clinical Oncology, 2013 [5].

Several studies have confirmed a prognostic value for MRD detection after completion of induction chemotherapy. As an example, a study by Terwijn et al showed that MRD positivity predicts adverse clinical outcome in AML after induction treatment as well as after consolidation therapy and propose its application as an improved patient-tailored risk-stratification tool [[13](#_ENREF_13)]. A study evaluating assessment of MRD by flow cytometry found that MRD positivity during aplasia predicts poor outcome (5-year RFS of 16% versus 43% in patients with negative MRD, P=0.009) [[14](#_ENREF_14)]. **We propose that normalization of MRD on day 4 is a surrogate for response to salvage chemotherapy in those patients with RR-AML.**

**Figure 1: Higher sensitivity measurements of AML disease burden allows for better understanding of response to treatment and greater ability to predict subsequent clinical relapse.** Morphological examination (Pink bar, >5% blasts), FISH/chimerism (orange bar, ~10-2 sensitivity), flow cytometry/gene expression (purple bar, ~10-4 sensitivity) or PCR for re-arranged&mutated sequence/next generation sequencing (blue bar, ~10-6 sensitivity). Adapted from Hourigan and Karp, Nature Reviews Clinical Oncology, 2013 [5].

**2.3 Rationale for research molecular testing:**

AML has historically been approached as a homogeneous diagnostic entity with a resulting “one size fits all” treatment strategy when, in reality, the acute myeloid leukemias are a heterogeneous group of diseases with distinct molecular and phenotypic characteristics. While factors such as patient age, secondary AML, WBC count at presentation, and cytogenetic and molecular markers all are *associated* with treatment resistance, they offer suboptimal predictive power for the individual patient [[2](#_ENREF_2)].

The ***primary objective*** of this study is to determine if an abnormal peripheral blood gene expression signature on day four of chemotherapy (*“measurable residual disease”, “MRD”)* is predictive of failure to achieve complete remission in patients with relapsed/refractory acute myeloid leukemia. Our section recently described a five-gene expression panel (5G-MRD) for the highly sensitive detection of acute myeloid disease burden [11]. This work demonstrated that this MG-MRD assay *tested on peripheral blood samples* had good concordance with pathologist diagnosis of relapsed/refractory disease based on assessment of *bone marrow examination* (Figure 2). The ***central hypothesis*** therefore is that those patients with continued over-expression of genes associated with AML MRD on day 4 of treatment (most AML chemotherapy regimens include approximately 7-8 days of therapy) will not achieve a complete remission when assessed by standard criteria (typically at day 28-42 of therapy). Such early assessment on day 4 of the failure of therapy may in future protocols allow for personalization of therapy either by escalation, switching to alternative treatment modalities (eg: immunotherapy) or by tailoring therapy on the basis of information regarding specific aberrant pathways that could be targeted.

**Figure 2: Relationship between blood based MRD test and pathologist diagnosis.** Peripheral blood 5G-MRD testing (after chemotherapy prior to transplantation) identified 24 of 26 patients determined to have RR-AML by conventional bone marrow examination. From Goswami *et. al., BMT,* 2015 [11]

Advances in genome sequencing technology have allowed for increased understanding of AML biology, which in turn has led to the identification of deregulated pathways that drive blast proliferation which may allow for timely and economically feasible personalized therapy based on molecular profiling and perhaps *ex vivo* drug sensitivity and resistance testing [[15-17](#_ENREF_15)]. The turn-around time for genomic sequencing will be a major constraint in such personalization, as results should ideally be available prior to completion of induction chemotherapy. As a *secondary objective* we will evaluate if samples sent for molecular and genetic testing at the time of initiation of salvage therapy can result in potentially actionable information being available by day 8.

## 2.4 Rationale for treatment selection

This clinical study is designed to test the hypothesis that blood based biomarkers can be used for personalized early assessment of treatment response in relapsed or refractory AML, rather than to evaluate any particular treatment. We will therefore only use “standard of care treatment”, that is drugs that have been FDA approved for the treatment of AML alone or in combinations previously tested in published clinical trials. Examples of such chemotherapy regimens are listed in Appendix B.

## 2.5 Scientific and clinical justification of the protocol

The mandate of the NHLBI Hematology Branch Myeloid Malignancies Section is to investigate “the detection, prevention and treatment of acute myeloid leukemia relapse” not only through the evaluation of novel therapeutic interventions but also by use of novel laboratory assays geared towards disease monitoring and personalized therapy.

This protocol will evaluate the possible predictive capability of sensitive AML disease-burden monitoring assays in patients with RR-AML undergoing treatment with intensive salvage chemotherapy. This protocol will also serve several goals, as it will also help determine the feasibility of obtaining pre-treatment molecular and genetic results and day 4 gene expression results before day 8 of therapy, allow investigation of the relationship between kinetics of gene expression changes and response to chemotherapy, to determine feasibility of measuring AML residual disease burden using alternative technologies and alternative tissue sources and to determine feasibility of recruiting patients with RR-AML to the NIH Clinical Center.

# 3. Study Design

This study is a feasibility study to evaluate if peripheral blood-based molecular assays for monitoring of AML disease burden on or before day 4 can provide early prediction of therapy failure in patients with RR-AML.

Eligible patients (Section 4) will undergo screening of adequate organ function and undergo a bone marrow examination to confirm the diagnosis of AML (Section 6). Research samples will be collected as described (Section 7). Patients will then be treated as per current standard of care ie: with agents FDA approved for use in AML given either alone or in combinations described in previously published studies (please refer to Appendix B for examples of salvage therapy regimens).

A research peripheral blood sample (20 mL whole blood) will be collected daily on days 1-14 and at the time of subsequent bone marrow examinations for response assessment subject to NIH clinical center limits (section 8).

A clinical bone marrow examination may be performed on day 14-20 of treatment to document marrow aplasia. If results from this interim bone marrow assessment demonstrate progression of disease (as defined by a >50% increase in the amount of malignant cells or blasts when compared to pre-treatment bone marrow examination) the subject may initiate a second course of induction treatment prior to completion of final assessment (early second induction), at the discretion of the principal investigator.

A clinical bone marrow examination for end-point response assessment will be performed at day 28-35 of treatment. In the event that bone marrow hypo-cellularity (<20%) prevents interpretation repeat bone marrow examinations may be performed every 7 to 14 days. Responses will be assessed according to Appendix A.

Patients with ECOG performance status of 0-2 who fail to achieve a CR or CRi as defined in Appendix A will be eligible to receive a second course of therapy if no other contraindications to further treatment. Patients undergoing such a second induction (either after final response assessment or early second induction) will follow steps as previously outlined, to include research peripheral blood sample (10 mL whole blood) collection daily on days 1-14 and at the time of subsequent bone marrow examinations for response assessment. The first day of administration of the latest treatment regimen will be designated as day 1B.

Day

1

14

Patients with RR-AML

Response Assessment (Primary Endpoint)

Inadequate responsec

CR or CRi

Salvage therapy

Off Study

Good PSd

Poor PSd

AML burden assessmentb

**Figure 3: Study Design.**  a) Bone marrow examination is performed 1) prior to treatment, 2) between day 14-20, 3) for response assessment at day 28-31 (if BM cellularity <20% may be repeated every 7-14 days) b) Peripheral blood samples for research taken daily on days 1 to 14 and at time of subsequent bone marrow assessments; c) Refractory disease, partial response, progression of disease; d) Eastern cooperative group performance status. Good = 0-2. Poor = 3 or greater. A second cycle of salvage therapy may be offered to those with good performance status and no other contraindications with inadequate response to the first cycle.

Max 1x

# 4. Eligibility Assessment

## 4.1 Inclusion criteria

4.1.1 Unequivocal diagnosis of relapsed or refractory acute myeloid leukemia (AML) according to WHO criteria confirmed by bone marrow evaluation within 30 days prior to study enrollment

4.1.2 Age 18-70 years inclusive

4.1.3 ECOG performance status of 0 to 2

4.1.4 Must have received at least one prior AML therapy before study enrollment

4.1.5 Ability to comprehend the investigational nature of the study and provide informed consent

4.1.6 Availability of a physician willing to assume clinical care after completion of this research study.

4.1.7 Subject agreement to use a medically-approved method of contraception to avoid pregnancy throughout the study if a woman of childbearing potential or a male subject with partner of childbearing potential.

## 4.2 Exclusion criteria

4.2.1 Diagnosis of acute promyelocytic leukemia

4.2.2 Lifetime anthracycline dose greater than equivalent of 450mg/m2 DAUNOrubicin (Appendix C)

4.2.3 Ejection fraction less than 40% by Echocardiogram or MUGA

4.2.4 Calculated or measured creatinine clearance less 50 milliliters per minute

4.2.5 Serum bilirubin greater than 1.5 times upper limit of normal (excluding unconjugated hyperbilirubinemia in those with known Gilbert syndrome)

4.2.6 Aspartate aminotransferase (AST) or Alanine aminotransferase (ALT) greater than 3 times upper limit

normal

4.2.7 Decreased oxygen saturation at rest (e.g. pulse oximeter less than 88% or PaO2 less than or equal to 55 millimeters of mercury)

4.2.8 Clinically significant active infection not responding adequately to therapy

4.2.9 Known positive for HIV; active Hepatitis B or C infection

4.2.10 Prior allogeneic hematopoietic stem cell transplant

4.2.11 Additional malignancy requiring concurrent treatment

4.2.12 Uncontrolled hepatic, renal, cardiac, gastrointestinal, pulmonary, neurologic, infectious, or metabolic disease of such severity, which in the opinion of the PI, would preclude ability to tolerate protocol.

4.2.13 Severe psychiatric illness or complex social situations that would limit the patient’s ability to tolerate and/or comply with study requirements.

4.2.14 Active central nervous system (CNS) leukemic infiltration

4.2.15 Current pregnancy or breastfeeding

4.2.16 Prolonged QTc interval prior to therapy (>470ms)

# Treatment Plan

## 5.1 Administration of salvage induction chemotherapy

Although the primary objective for this study is to evaluate the predictive capability of sensitive AML disease-burden monitoring assays in patients with RR-AML, subjects participating in this protocol will receive standard re-induction (“salvage”) chemotherapy. Treatment will consist of FDA approved agents alone in or combinations previously published as having activity in patients with RR-AML. Some examples of salvage chemotherapy regimens are listed in Appendix B. Details and final selection of treatment regimen will be determined by the principal investigator (PI).

## 5.2 Dose delays, modifications or discontinuation for non-hematologic side effects

## 5.2.1 Cardiotoxicity:

## Patients receiving an anthracycline as part of induction therapy who develop new cardiotoxicity confirmed via echocardiogram or MUGA scan will have the offending agent discontinued. The other concurrent antineoplastic agents can be continued as long as patient remains clinically stable and with adequate performance status to tolerate intensive therapy.

## 

## 5.2.2 Cerebellar toxicity:

## Subjects who develop cerebellar toxicity while receiving cytarabine will have this therapeutic agent discontinued. Other antineoplastic agents may be continued at the discretion of the PI.

## 5.3 Dose delays, modifications or discontinuation for hematologic side effects

**5.3.1 Pancytopenia:**

Subjects receiving induction therapy are expected to develop anemia, thrombocytopenia, and neutropenia during their course of treatment. Therapy will not be discontinued or modified for pancytopenia.

## 5.4 Infectious prophylaxis:

Subjects with AML are considered to be at high risk for the development of infectious complications due to their disease as well as a projected prolonged period of neutropenia as consequence from their treatment. Antimicrobial prophylaxis will be provided based on the National Comprehensive Cancer Center guidelines for prevention and treatment of cancer-related infections (Version 1.2015, www.nccn.org).

**5.4.1 Antibacterial prophylaxis:**

Levofloxacin at prophylaxis dose will be started on day 1 of therapy and continued until resolution of neutropenia (typically ANC > 500). If subject is unable to tolerate fluoroquinolones, an alternate agent or intervention will be administered at the discretion of the PI.

**5.4.2 Antifungal prophylaxis:**

Posaconazole at prophylaxis dose will be started 24 hours after completion of chemotherapy regimen administration and will be continued until resolution of neutropenia (typically ANC > 500). Subjects may receive voriconazole, fluconazole, or an alternate antifungal agent at prophylaxis dose at the discretion of the PI.

**5.4.3 Antiviral prophylaxis:**

Acyclovir at prophylaxis dose will be started on day 1 of therapy and will be continued until resolution of neutropenia. Subjects may receive valacyclovir or an alternate antiviral agent at prophylaxis dose the discretion of the PI.

## 5.5 Tumor lysis prophylaxis and management

Subjects with RR-AML are at increased risk of developing tumor lysis syndrome (TLS). Subjects enrolled into this protocol will receive TLS prophylaxis based on their risk category: *low risk* (WBC less than 25,000/mm3 and serum LDH level less than twice the upper limit of normal), *intermediate risk* (WBC equal-to or greater-than 25,000/mm3 but less than 100,000/mm3 OR WBC less than 25,000/mm3 and LDH greater-than or equal-to twice the upper limit of normal), *high risk* (WBC greater-than or equal-to 100,000/mm3). Strict urine output measurements will be documented throughout duration of therapy administration with goal urine output of 100mL/hr during period of highest risk for TLS (circulating blasts, WBC count greater than 10,000/mm3).

## 5.5.1 Low risk for TLS prophylaxis:

## Adequate hydration and allopurinol 200 mg orally twice daily will be started on the day of initiation of therapy.

## 5.5.2 Intermediate risk for TLS prophylaxis:

Intravenous hydration and allopurinol 200mg orally three times daily will be initiated on day of therapy initiation. Laboratory testing every 8-12 hours.

## 5.5.3 High risk for TLS prophylaxis:

Intravenous hydration and allopurinol 200mg orally three times daily will be initiated as soon as possible. One dose of rasburicase 0.2 mg/kg IV will be administered at least four hours before chemotherapyunless contraindicated (G6PD deficiency or history of hypersensitivity to the medication). Laboratory testing every 6-8 hours.

**5.5.4 Indications for Rasburicase:**

## One dose of rasburicase (up to 0.2 mg/kg IV) may be given in all subjects when uric acid is greater than 7.5 mg/dL. Subsequent doses of rasburicase will be given daily for up to 5 days only if clinically indicated. Patients will be monitored for evidence of hemolysis.

## 5.6 Supportive care

In particular multi-agent antiemetic regimens, gastritis prevention, diet modifications, phosphate binders and steroid eye drops may be indicated. Where possible established institutional supportive care guidelines will be followed (http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml). The determination of supportive agents to be utilized will be agent specific at the discretion of the PI in consultation with the pharmacist and palliative care service where necessary.

## 5.7 Transfusion support

Subjects participating in this protocol will likely require transfusion support. Leukocyte depleted and

irradiated blood products will be transfused when necessary.

**6. Clinical Evaluation**

Lab tests not performed at NIH will be sent to the Research Nurse. The PI will review outside test results and selected will be filed as hard copies in the research charts. All charts will be stored in a secure room.

For the evaluation timeframes that describe the timing of tests/procedures below in the term of month(s), a month is defined as 30 days.

**6.1 Pre-enrollment assessment**

Patients will be screened for participation by signing consent to participate in a Hematology Branch screening protocol or another NIH clinical center protocol. Their eligibility to participate in this protocol will be determined based on the Inclusion and Exclusion criteria (Section 4) and data collected from other NIH protocols, when available. Baseline status will be evaluated as follows:

* A complete medical history.
* Physical examination including vital signs, height and weight.
* Medication and allergy review (prescribed, non-prescription).
* A digital photograph (if the patient gives permission) will be taken (passport type snap shot) and kept in the research records. The patient will sign a separate consent form specifically for digital photography. The purpose of this is to facilitate review of all registered patients.
* Review of prior diagnostic records including pertinent laboratory tests, pathology samples (e.g., bone marrow biopsies, soft tissue biopsies, etc.), and radiology studies relevant to patient’s diagnosis of relapsed or refractory AML. May also request and re-evaluation of results from non-NIH facilities as necessary (e.g., if diagnosis or extent of disease involvement unclear) and re-testing or interpretation of archived pathology specimens if appropriate.
* Baseline laboratory studies (peripheral blood), as follows:
  + Complete blood count with differential
  + Reticulocyte count
  + Peripheral blood smear
  + Acute care (Na, K, Cl, CO2, Creatinine, Glucose, and Urea Nitrogen), mineral (Calcium, Phosphorus, Magnesium), and hepatic panels (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
  + CK, LDH, total protein, albumin, uric acid,
  + Coagulation and thrombosis screens (PT, PTT, D-dimer, Fibrinogen)
  + Pregnancy test (urine or blood HCG) in women of child bearing potential.
  + Thyroid function tests
  + Viral serologies for HIV, hepatitis B, hepatitis C, HSV, EBV and CMV
  + HLA typing (if not already available)
  + DAT (direct antiglobulin test)
  + Type and screen
  + Urinalysis
  + Lymphocyte profiling (T/B/NK analysis)
  + G6PD testing (as clinically indicated)
* Baseline bone marrow aspiration and biopsy.
  + Morphology and immunohistochemistry
  + Cytogenetics (karyotype) and/or FISH.
  + AML molecular testing panel (e.g.: c-Kit, FLT3-ITD, NPM1 or CEBPA mutation) as described in NCCN guidelines.
  + Flow cytometry if clinically indicated in judgment of the pathologist.
* Evaluation of myocardial function (via echocardiogram or MUGA scan) if not already performed in the 30 days immediately prior to study enrollment.
* Electrocardiogram (12 lead).
* Baseline non-contrast computerized tomography scans of chest and sinus will be performed prior to, or in first five days after treatment initiation. Further baseline imaging will not be routinely performed except for those with a history of extra-medullary myeloid disease (also known as granulocytic sarcoma, myeloid sarcoma, or chloroma), another malignancy or disease where imaging represents the clinical standard of care, or suspicion for extra-medullary myeloid disease or ongoing infection.
* Placement of central venous access under guidance (unless suitable access already in place).

## 6.2 Inpatient monitoring

Subjects undergoing salvage induction chemotherapy will be admitted no later than day 1 of treatment initiation and will remain inpatient until completion of final response assessment and confirmation of CR/CRi or disenrollment from the protocol. However, exceptions will be considered on a case by case basis and will be at the discretion of the PI. Typically:

* Weight (prefer standing, twice daily during days 0-12, then daily)
* Strict fluid intake and output monitoring, vital signs including pain.
* CBC with differential (typically daily\*)
* Acute care, hepatic and mineral panels (typically daily\*)
* Uric Acid (typically daily\*)
* LDH (typically daily\*)
* CRP (typically daily)
* Protein, Total and Prealbumin (twice weekly)
* PT (Prothrombin time) and PTT (Partial thromboplastin time)
* D-dimer, Fibrinogen, FDP, Peripheral Blood Smear (DIC panel)
* Other disease-specific molecular testing
* Bone marrow aspirate and aspiration if concern for progression of disease (as needed)
* Drug levels when appropriate (e.g., gentamicin, vancomycin, posaconazole, voriconazole) (frequency variable)

*\*More frequent testing may be clinically indicated in medium and high risk tumor lysis subjects, and those with active bleeding.*

## 6.3 Outpatient monitoring

This is predominantly an inpatient protocol. Follow up outpatient visits at the NIH clinical center are optional, will be arranged for the purposes of supportive care and facilitation of safe transition of care in the immediate three months following treatment. Follow up visits may include:

* History and physical examination
* Laboratory Testing:
  + Complete blood count with differential
  + Reticulocyte count
  + Peripheral blood smear
  + Acute care, mineral and hepatic panels.
  + Type and screen (as clinically indicated)
* Marrow aspiration and biopsy may be performed at if peripheral smear examination is abnormal, if cytopenias develop or worsen, or for other clinical suspicion of relapse.
* Post treatment evaluation of myocardial function (echocardiogram or MUGA scan).

Protocol participants who elect to undergo treatment and/or surveillance visits elsewhere will be offered the opportunity of sending research samples (as detailed in section 8) and clinical information to the NIH. Subjects who are inpatient in the NIH clinical center for treatment or evaluation on a different clinical protocol are still eligible for repeat evaluations. Current and previous protocol participants and/or their nominated surrogate will be contacted typically at least annually (no more frequently every three months) to determine clinical remission and survival status.

**6.4 Off study criteria**

**6.4.1 Per subject choice**

Subjects may withdraw from study at their request.

**6.4.2 Principal Investigator decision**

Patients may be removed from the study at the discretion of the principal investigator or his proxy.

**6.4.3 Completion of study**

Subjects attaining CR or CRi will be taken off study. Subjects who receive additional therapy not associated with this protocol (eg: allogeneic hematopoietic stem cell transplant) will be taken off study. Subjects who fail to respond at endpoint assessment to a second cycle of induction therapy will be taken off study. Subjects with inadequate response to first cycle but ineligible for a second cycle will be taken off study.

**6.4.4 Off-Study Procedure**

A subject who goes off study will have an off study note placed in the medical record. Appropriate transitional support will be provided to allow patient to re-establish care with their own physician.

**7. Laboratory Research Studies**

**7.1 Research sample collection**

Research samples for translational studies will be obtained in strict compliance with clinical center guidelines. Samples will be obtained at the time of diagnostic procedures wherever possible.

**7.1.1 Venipuncture**

As per NIH guidelines, the amount of blood that may be drawn from adult patients and volunteers for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

**7.1.2 Apheresis for AML blast collection (optional)**

An apheresis procedure may be performed for the purpose of obtaining cells for in vitro studies related to the biology of AML or to test and develop new therapies. Apheresis will be performed in the NIH Department of Transfusion Medicine; a 2-4 liter apheresis harvest will be obtained, which will take approximately 2 hours. Apheresis will typically be performed using the one arm intravenous technique. Apheresis can be performed using peripheral venous access; a central line will not be placed specifically for research apheresis. Adverse reactions to apheresis are rare, and are generally mild. They include pain and bruising at the insertion site of the intravenous line, and a temporary decrease in the platelet count and/or hemoglobin level. Peripheral sensory changes caused by apheresis-induced hypocalcemia rarely occurs; this reaction can be reversed by interrupting the procedure. To be eligible for apheresis, patients must have a hematocrit of greater that 28%, and a platelet count of greater than 75,000/mm3. The WBC count is not an eligibility criterion for apheresis (apheresis can be performed with any WBC count). Patients may be asked to undergo repeat apheresis after at least three months, with no more than two apheresis procedures in a one year period. Patients presenting with a WBC >100,000/mm3 may undergo emergent apheresis or chemotherapy for leukocyte reduction.

**7.1.3 Bone marrow aspiration and biopsy**

Bone marrow examinations will be performed for clinical purposes prior to initiation of treatment, for interim assessment between day 14-20 and for final response assessment at day 28-35 of treatment (in the event that bone marrow hypo-cellularity prevents interpretation repeat bone marrow examinations may be performed every 7 to 14 days). Additional bone marrow aspirate and biopsies may be performed as clinically indicated (i.e. concern for refractory disease based on clinical examination and/or peripheral blood-based laboratory tests). An extra volume of bone marrow aspirate (up to 25 ml) will be collected during each procedure, when possible, for research.

**7.1.4 Non-invasive biological sample collection**

Samples of urine, stool and saliva may be requested for research purposes.

**7.1.5 Biological samples collected elsewhere**

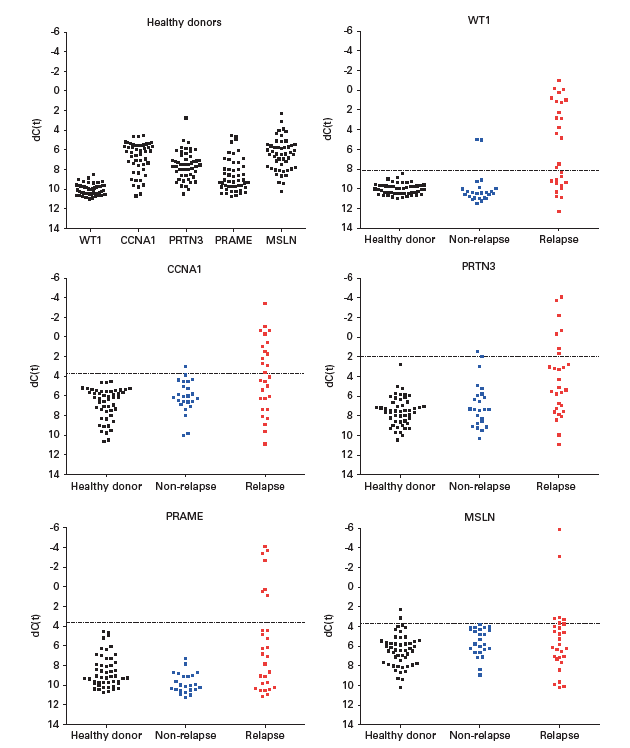
Patients will be asked to consent that the PI may request a release of material concerning any biological samples available for research that were previously collected at an institution other than NHLBI.

**7.2 Primary Laboratory Research Measurement**

**7.2.1 Justification of assay and timing**

We have reported the use of a real-time quantitative PCR (RQ-PCR) using an array of multiple genes potentially overexpressed in AML (5G-MRD) as a highly sensitive measure to residual AML burden [11]. We propose that patients who have an abnormal blood test on day 4 of chemotherapy (ie: typically half way through induction therapy) are unlikely to achieve a complete remission. **This laboratory research test is not CLIA certified, therefore no clinical decisions will be made on the basis of results and no information will be returned to patients.** This is a feasibility study to see if, in subsequent clinical protocols, if a 5G-MRD test sent at the approximately half-way point of treatment (most AML induction therapies include 7-8 days of treatment) would return actionable information within 96 hours therefore allowing treatment modification decisions to be made in real-time.

**7.2.2 Laboratory testing and thresholds for analysis.**

The details of this test have been published elsewhere [11] but in brief RNA is isolated from peripheral blood samples, and one microgram of total RNA is reverse-transcribed into cDNA. Custom RT2 Profiler PCR array plates including controls for human genomic DNA contamination, reverse transcription and PCR efficacy (SABiosciences, Qiagen) are used for RQ–PCR reactions performed using RT2 SYBR Green ROX qPCR Mastermix (SABiosciences, Valencia, CA, USA) according to the manufacturer’s instructions on an thermal cycler using a program of 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. ΔC(t) is calculated as the C(t) of target gene ‘X’ minus ABL1 housekeeping gene. Thresholds for positive tests are set based on the upper expression limits seen in a cohort of fifty healthy adult donor baseline controls (for WT1, CCNA1, PRTN3, PRAME) or in non-relapsing AML patients (for MSLN) as previously published [11, see also Figure 4]

**Figure 4: Thresholds setting based on 5G-MRD levels seen in healthy donor and AML patients prior to allo-HSCT.** From Goswami *et. al., BMT,* 2015 [11]

**7.3 Auxiliary Laboratory Research Studies:**

All or a selection of the following investigations will also be performed:

* Detection, quantification and characterization of protein, carbohydrate and/or lipid biomarkers.
* Evaluation of gene expression in unsorted and sorted biologic samples (e.g.: blood and marrow).
* Evaluation of protein expression in unsorted and sorted biologic samples.
* Evaluation of gene variants and mutations in unsorted and sorted biologic samples.
* Determination of epigenetic, histone and chromatin modifications.
* Evaluation of cellular phenotype in unsorted and sorted biologic samples.
* Evaluation of cellular function in unsorted and sorted biologic samples.
* Use of biologic samples to develop, test, and validate novel therapeutic agents.
* Use of biologic samples to develop, test, and validate surrogate predictive and prognostic markers of disease behavior.

The above laboratory studies are all investigational. None of the resulting information will be returned to the patient or used in clinical decision making unless performed in a CLIA certified laboratory and ordered for a clinical indication as described in Section 6. Studies described in Appendix D: NHLBI Hematology Branch Laboratory Research Studies may also be performed.

**7.4 Storage**

**7.4.1 Procedures for stored specimens**

All samples will be stored under the direction of the PI of the study. All laboratory personnel with access to patient information will complete the NIH online course in Protection of Human Subjects. Laboratory personnel are assessed for competency prior to being allowed to work with patient samples. Efforts to ensure protection of patient information include:

* The laboratory is located in a controlled access building.
* Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
* An electronic database is used to store information related to patient samples processed.
* Upon specimen receipt each sample is assigned a unique number.
* Vials holding patient samples are labeled with the sequential laboratory accession ID number that does not contain any personal identifier information.

**7.4.2 Procedures for collecting, processing and storing of research samples**

* Blood will be collected in the phlebotomy suite, on a clinical ward, or in an outpatient clinic of the CRC, NIH.
* Bone Marrow will be collected in the 3SWN procedure area, on a clinical ward, or in an outpatient clinic of the CRC, NIH.
* Orders for apheresis collections should be placed in CRIS (Clinical Research Information System, Clinical Research Center, NIH, Bethesda, MD). Apheresis is performed in the Department of Transfusion Medicine.
* Samples will be transferred to the NHLBI research laboratory at room temperature. Cells will be separated by Ficoll density gradient centrifugation and only mononuclear cells will be harvested, processed, analyzed, and stored.
* Tumor and normal blood cells may be viably frozen, typically at concentrations of 20-100x106/mL in FCS with 10% DMSO using a temperature controlled freezing process to optimize sample viability. Samples will be transferred to nitrogen tanks for long term storage.
* Processed biologic material (DNA, RNA, protein) is stored at -80C in a temperature controlled, alarm secured -80C freezer.
* All research samples are assigned a unique number, cataloged and will be stored in the laboratory of Dr. Christopher Hourigan.
* Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

**7.4.3 Tracking**

Samples will be ordered and tracked through CRIS. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB and the NHLBI Clinical Director will be notified. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside the NIH without IRB notification and an executed MTA.

# 8. Biostatistical Considerations

**8.1 Primary Outcome:**

The primary outcome of this protocol are the outcomes of the blood test performed on day 4 during chemotherapy, and the standard response criteria including morphological bone marrow examination following chemotherapy.

**8.2 Statistical Hypotheses:**

Let P4 denote the proportion of patients who are found to be having the disease by the blood test performed on day 4 (T4). Likewise, let P30 denote the proportion of patients having disease at count recovery, where P4 is determined by MRD blood test and P30 by conventional bone marrow examination based response assessment test (T30). T30 is the standard to which other tests are comparedand the underestimation of disease by T4 is preferable to overestimation because the latter leads to unnecessary exposure to toxic treatments. Taking this into account, we propose to test the statistical hypotheses: H0: P4 ≥ P30 vs. H1: P4 < P30.

**8.3 Statistical Test:**

Since P4 and P30 are correlated quantities based on same patients, and the outcomes for each patient are binary, the McNemar test (1947) is used. The test is available in standard statistical software packages including SAS and R.

**8.4 Sample Size:**

Assuming the expected maximum recruitment during the time period of this study to be 36, with the 30-day mortality 15%, approximately 31 patients' test data will be available. It is also assumed that the patient dropout rate is negligible due to the severity of the disease.

**8.5 Power Calculation:**

The power under the alternative hypothesis depends on the choice of parameters for P4, P30, and the proportions (1) p01=Prob(T4-negative and T30-positive), and (2) p10=Prob(T4-positive and T30-negative). Considering the direction of alternative hypothesis, we have more flexibility in statistical design with respect to p01 over p10, allowing p01> p10. Assuming P30 of 0.6 (=1-CR), a value of P4=0.4 (the proportion difference 0.2) with p01=0.27 and p10=0.07 gives at least 80% power for the sample size 31. If P30=0.6 and P4=0.3, then 90% power can be achieved with the same sample size, p01=0.42 and p10=0.12. Depending on data, the actual values of p01 and p10 can vary considerably, but as long as they remain below those levels specified in the respective scenarios, the McNemar test holds at least 80% power or better. The power calculation is based on equation (5.6) in Miettinen (1968).

**8.6 Secondary Analysis:**

#1: The diagnostic results of T4 as well as patients' covariate information, e.g., age, gender, disease category, clinical status, genetic information, baseline blood count, may be combined to build statistical models to predict T30 test outcome. Multiple logistic regression models or other generalized linear models (GLM) will be considered. Exploratory data analysis (EDA) such as subgroup analysis will be utilized to identify which patient group is more suitable to the MRD test.

#2. T4 and the bone marrow test on day 14 (TB14) will be compared, also using the McNemar test. The statistical hypotheses are: H0: P4≥P14 vs. H1: P4 < P14, where P14 is the proportion of positive diagnosis by TB14. Since this portion of statistical analysis is not a part of the primary analysis, it is intended for hypothesis-generating purpose only.

#3. Patient peripheral blood will be drawn daily from day 1 to day 14 for MRD tests T1,..., T14, respectively. The test results will be analyzed to investigate the possibility of finding an optimal test or a combination of tests that closely match the results of T30.

**9. Data and Safety Monitoring**

## 9.1 Safety monitoring

Relapsed and refractory acute myeloid leukemia is universally fatal without treatment. Unfortunately best available standard of care treatment itself is associated with significant morbidity and mortality. **The investigational component of this study is feasibility of biomarker data collection and attempts to associate of such testing with clinical outcome. No investigational new drugs will be tested**.

*Principal Investigator:* All data will be collected in a timely manner and reviewed by the PI and/or lead associate investigator for toxicity. In the event that unacceptable events occur, the IRB will be informed and appropriate measures as outlined in the study will be taken.

***NHLBI IRB:*** Accrual and safety data will be monitored and reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed patient consent will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to Title 45 CFR 46. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

***DSMB****:* This protocol tests potential utility of a biomarker measurement during standard of care therapy. As no new drugs or combinations of drugs will be tested in this non-blinded, small single site biomarker trial Data Safety and Monitoring Board (DSMB) monitoring is not indicated.

**9.2 Event Characterization and Reporting**

Events include adverse events (AE), serious adverse events (SAE), protocol deviations (PD), unanticipated problems (UP), and non-compliance.

**9.2.1 Definitions**

*Adverse Event (AE):* Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s participation in the research, whether or not considered related to the research. An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

* results in discontinuation from the study
* is associated with clinical signs or symptoms
* requires treatment or any other therapeutic intervention
* is associated with death or any other serious adverse event, including hospitalization
* is judged by the investigator to be of significant clinical impact
* if any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect of the test drug and about the patient’s outcome

*Serious Adverse Event (SAE):* A serious adverse event that:

* results in death
* is life-threatening (places the subject at immediate risk of death from the event as it occurred)
* results in in-patient hospitalization or prolongation of existing hospitalization
* results in a persistent or significant incapacity
* results in a congenital anomaly/birth defect
* based upon appropriate medical judgment, may jeopardize the subject’s health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

*Suspected adverse reaction:* Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

*Serious event:* An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

*Unexpected adverse reaction:* An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected”, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

*Unanticipated Problem (UP):* Any incident, experience, or outcome that meets all of the following criteria:

* unexpected in terms of nature, severity, or frequency in relation to:
  + the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator’s Brochure or other study documents; and
  + the characteristics of the subject population being studied; and
* related or possibly related to participation in the research; and
* places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

*Unanticipated Problem that is not an Adverse Event:* An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

*Protocol Deviation (PD):* Any change, divergence, or departure from the IRB approved research protocol.

*Non-compliance:* The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:

a. Increases risks, or causes harm, to participants.

b. Decreases potential benefits to participants.

c. Compromises the integrity of the NIH HRPP.

d. Invalidates the study data.

2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. Minor (non-serious) non-compliance: Non-compliance that is neither serious nor continuing.

**9.2.2 Adverse events Management:**

AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to the investigational study procedure (peripheral blood based monitoring of AML disease burden) and graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at <http://ctep.cancer.gov/reporting/ctc.html>.

**Grading of Adverse Events:**

|  |  |  |
| --- | --- | --- |
| **1** | Mild | Symptom barely noticeable to subject; does not influence performance or functioning. Prescription drug not ordinarily needed for relief of symptom but may be given because of personality of subject. |
| **2** | Moderate | Symptom of a sufficient severity to make subject uncomfortable; performance of daily activities influenced; subject is able to continue in study; treatment for symptom may be needed. |
| **3** | Severe | Symptom causes severe discomfort. May be of such severity that subject cannot continue. Severity may cause cessation of treatment with test drug; treatment for symptom may be given and/or subject hospitalized. |
| **4** | Life-threatening | Symptom(s) place the patient at immediate risk of death from the reaction as it occurred; it does not include a reaction that, had it occurred in a more serious form, might have caused death. |

**Attribution of Adverse Events:**

|  |  |  |
| --- | --- | --- |
| **Relationship** | **Attribution** | **Description** |
| Unrelated to investigational  agent/intervention1 | Unrelated | The AE *is clearly* ***NOT*** *related* to the intervention |
| Unlikely | The AE *is* ***doubtfully related*** to the intervention |
| Related to investigational  agent/intervention1 | Possibly | The AE ***may be related*** to the  intervention |
| Probably | The AE ***is likely related*** to theintervention |
| Definitely | The AE ***is clearly related*** to the intervention |

## 

## 9.2.3 Serious Adverse Events Management

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clearly not related to the research).

**Treatment related SAEs (TRSAEs)** are those attributed as definitely, probably, or possibly related that will be monitored. These include death and any grade IV non-hematologic toxicity considered to be probably or definitely related to investigational agent/invention.

Hospitalizations for administrative issues (to receive a transfusion) or upgrading to ICU for routine monitoring will not be reported as an SAE.

**Duration of serious adverse event collecting and reporting:** The collection of SAEs will begin on the first day of treatment and will continue along as the subject is on the study. SAE reporting will continue as long as the subject remains on study.

**9.2.4 Reporting events**

All events will be reported to Principal Investigator of this study.

**9.2.4.1 Reporting Timeframes to IRB Chair, Clinical Director, and/or NHLBI IRB**

**Serious Events**

*Reports to the IRB and CD:* The PI must report Serious UPs, and Serious PDs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event via iRIS using the NIH Problem Report Form.

*Reports to the IRB Chair and CD:* The PI must report all SAEs that do not meet the definition of UP to the IRB chair and CD not more than 14 days after the PI first learns of the event via iRIS, using the NIH Problem Report Form.

**Non-serious Events**

*Reports to the IRB and CD:* The PI must report all UPs that are not serious to the IRB and CD, and PDs that are not serious to the IRB, not more than 14 days after the PI first learns of the event via iRIS using the NIH Problem Report Form.

**Deaths**

The PI must report all deaths (that are not UPs) to the CD as soon as possible, but not more than 7 days after the PI first learns of the event.

**9.2.4.2 At continuing review, the PI will provide to the IRB a summary of:**

* All UPs
* All PDs
* All AEs (except for those granted a waiver of reporting)
* If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or published literature, these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

**Exclusions to data reporting:**

The following adverse events will be captured only in the source documents and will not be reported to the IRB at the time of continuing review.

* Given the intended therapeutic action of standard of care induction therapy, and the underlying illness of AML, severe blood count abnormalities (up to grade 4 levels in leukocytes, neutrophils, lymphocytes, red blood cells and platelets) are expected. Thus we will collect hematologic laboratory values in the subject’s source documents, but will not record or report these abnormalities as adverse or serious adverse events. If the myelosuppressive event is fatal (Grade 5), or if the Investigator finds the event to be medically important, then it will be collected as an SAE.

In addition, the following non-hematologic AEs will be captured only in the source documents and will not be reported to the IRB at the time of continuing review:

* **This clinical trial is a study to assess the feasibility of biomarker use. No new drugs or any new combinations of existing drugs will be investigated. All treatment given will be in accordance with the standard of care and associated with known toxicity profiles** (Appendix B)**.** Therefore any observed or volunteered adverse events will not be reported for standard of care treatment unless the adverse event is previously unknown (not on the package insert or published clinical trials for combination therapy); or (2) the adverse event is more severe or frequent than previously reported. The collection of AE information will begin on the first day of initiation of therapy and conclude thirty days after completion of therapy.

## 9.3 Data management

**9.3.1 Data collection and distribution**

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

**9.3.2 End of study procedures**

Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

**9.3.3 Loss or destruction of data**

Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Data will not be distributed outside NIH without IRB notification and an executed MTA or CTA.

**9.3.4 Publication policy**

Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protection (OHSRP).

# 10. Human Subject Protection

## 10.1 Rationale for subject selection

The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. No subjects will be excluded from participation based on gender, race or ethnicity.

**Recruitment efforts:** The study will be listed on the clinicaltrials.gov, Clinical Center research studies, the Leukemia and Lymphoma Society, and the National Heart, Lung and Blood Institute patient recruitment websites. Posters, flyers and electronic communications may be created and distributed following review by the IRB. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment.

**Reimbursement for protocol participation, travel, food, and lodging** will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

*Payment for participation:* $0. Study participants will not be reimbursed for their time and inconvenience.

*Travel (air/train/bus):* Travel from home for the first NIH visit will not be reimbursable. If the patient consents to the research protocol, travel home following the first visit will be reimbursable. Subjects will be reimbursed 100% of government rate for travel to additional protocol-related visits as deemed clinically-indicated by the PI once the subject has been determined eligible to participate and signs consent.

*Local Travel (< 50 miles) (car/taxi/shuttle/train/bus):* Subjects will be reimbursed for local train/bus and/or shuttle costs. Car mileage will be reimbursed $0.41/mile when the distance from home is greater than 50 miles. Reimbursement for mileage less than 50 miles from home is not provided. Subjects will not be reimbursed for rental car cost beyond the car mileage rate. Taxi will be paid only when medically necessary and authorized by the PI.

*Meals:* Subjects will not be reimbursed for meals.

*Lodging:* Subjects will be reimbursed for hotel/motel lodging at a rate of $60/night for a maximum of 7 days after which the reimbursement is $30/night. If space is available, the Children’s Inn or the Family Lodge will be paid directly.

*Guardian coverage:* Subjects will be reimbursed for guardian travel (100% of government rate) and lodging ($15.00/night) provided the services of a guardian are medically indicated and pre-approved.

**Competition between Branch protocols:** There are no competing Branch protocols for this patient population. 12-H-0146 is a study of clofarabine followed by lenalidomide for treatment of high risk myelodysplastic syndromes and acute myeloid leukemia. The eligibility criteria for 12-H-0146 however states that eligible “participants must not be candidates for standard treatments” such as those given on this study. Patients with RR-AML at the NIH clinical center are currently treated under “standard of care” or allogeneic transplant protocols.

## 10.2 Participation of pediatric patients

Patients under the age of 18 years are excluded from this study, as inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this study.

## 10.3 Risks and Discomforts:

**10.3.1 Related to blood draws**

No major risks are involved with blood draws. Minor complications include bleeding, pain, and hematoma formation at the site of blood draws. Rarely an infection may occur.

**10.3.2 Related to chemotherapy**

Subjects participating in this protocol will receive standard of care therapy including only FDA approved chemotherapy agents given only in previously published combinations for treatment of RR-AML (Annex B). Common complications from such salvage induction chemotherapy include: abnormal liver enzymes (up to 90% of patients), nausea/vomiting (88%), diarrhea (70%), rash (70%), infection (69%), alopecia (62%), mucositis (60%), hemorrhage (50%), hand-foot syndrome (50%), febrile neutropenia (47%), pulmonary toxicity (46%), cardiotoxicity (9%).

**10.3.3 Related to the apheresis procedure**

The apheresis procedures will be performed in accordance with standard apheresis donation policies and procedures operative in DTM and will be in compliance with the Blood Donor Standards of the American Association of Blood Banks and the rules and regulations of the Food and Drug Administration. Adverse reactions to apheresis procedures are rare, but include:

* Pain and hematoma at the needle placement site.
* Vasovagal episodes, characterized by transient hypotension, dizziness, nausea and rarely, syncope are seen in less than 2% of the procedures. Hypotension secondary to volume depletion is known for the rare potential for a cerebrovascular or cardiovascular event.
* Cutaneous or circumoral parasthesias, chills, nausea, heartburn and rarely muscle spasms may result from the use of citrate anticoagulant used to prevent clotting in the extracorporeal circuit. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets or with intravenous calcium gluconate.

Prior to each apheresis, the potential risks associated with the procedure will be explained to the patient and a separate informed consent obtained.

**10.3.4 Related to bone marrow aspirate and biopsy**

Aspirates are typically obtained from the posterior superior iliac crest. A sterile technique is utilized and the skin is cleaned utilizing alcohol and betadine. Local anesthesia utilizing 1% xylocaine or 1% lidocaine is administered to the epidermis and periosteal layers. A small incision is performed utilizing a scalpel, after which two to eight individual aspirates are obtained through a 16 gauge Illinois or Jamshidi needle, ideally yielding 0.5-2 x 109 nucleated cells. Generally, a maximum of 7 aspirates are obtained from each site; the needle is advanced about 0.5 cm between each aspiration. Bone marrow is aspirated into a heparinized syringe or into a syringe containing anticoagulated culture medium, depending on the use for which it is intended. A core biopsy may also be obtained. Overall, the risks associated with this procedure are minimal, but include bleeding, hematoma formation, pain, and infection. If the procedure is performed under conscious sedation, risks would also include hypersensitivity to conscious sedation medications and over-sedation leading to respiratory compromise. Conscious sedation will be performed with anesthesiology service support.

**10.4 Risks in Relation to Benefit**

With the exception of collection of extra research samples during clinical indicated procedures (with exception of research apheresis see 10.3.3), the tests, procedures and therapies performed in this protocol are common clinical practice as suggested in the AML NCCN national consensus guidelines (http://www.nccn.org/professionals/physician\_gls/PDF/aml.pdf). As such, participation in this protocol poses only a minor increase in risk, especially when balanced again their medical situation and standard treatment.

## Untreated relapsed AML and untreated refractory AML is fatal without treatment. Patients derive benefit by having their disease treated and closely monitored by medical experts during treatment. In addition, the knowledge gained from the completion of this study could potentially lead to changes in the way we treat RR-AML.

## Therefore for adult patients, participation in this protocol involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102).

## 10.5 Informed Consent Processes and Procedures

## The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient during the initial evaluation. The PI, Dr. Hourigan or an associate investigator on this protocol identified with an asterix on the cover page will lead this discussion and obtain the informed consent. The consent form will be signed in the presence of the investigator and a witness prior to commencement of the treatment plan. The treatment plan and risks will be discussed again and in detail during their hospital visit for treatment.

## If at any time during participation in the protocol new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

## Non-English speaking research participants: We anticipate enrolling non-English speaking research participants into this study. The IRB approved full consent document will be translated into the subject's native language in accordance with the Clinical MAS Policy M77-2. If there is an unexpected enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use a short form oral consent process as described in MAS Policy M77-2, 45CFR46.117(b)(2) and 21CFR50.27(b)(a). The summary that will be used is the English version of the extant IRB approved consent document.

## We request prospective IRB approval of the use of the short form for up to a maximum of 5 participants and we will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

## 10.6 Conflict of Interest

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH’s Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added.

# 11. Pharmaceuticals

No new drugs or any new combinations of existing drugs will be investigated by this protocol. All treatment given will be in accordance with the standard of care and as such all medications will be FDA approved and available at the NIH Pharmacy. A copy of prescribing information is available upon request.

**12.** **Reference List**

1. *American Cancer Society. Cancer Facts & Figures 2015*. Atlanta, American Cancer Society, 2015. p. 4.

2. Walter, R.B., et al., *Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center.* Leukemia, 2014.

3. Breems, D.A., et al., *Prognostic index for adult patients with acute myeloid leukemia in first relapse.* J Clin Oncol, 2005. **23**(9): p. 1969-78.

4. Lowenberg, B., J.R. Downing, and A. Burnett, *Acute Myeloid Leukemia.* New England Journal of Medicine, 1999. **341**(14): p. 1051-1062.

5. Hourigan, C.S. and J.E. Karp, *Minimal residual disease in acute myeloid leukaemia.* Nat Rev Clin Oncol, 2013. **10**(8): p. 460-71.

6. Freeman, S.D., et al., *Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia.* J Clin Oncol, 2013. **31**(32): p. 4123-31.

7. Grimwade, D. and S.D. Freeman, *Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"?* Blood, 2014. **124**(23): p. 3345-55.

8. Inaba, H., et al., *Comparative analysis of different approaches to measure treatment response in acute myeloid leukemia.* J Clin Oncol, 2012. **30**(29): p. 3625-32.

9. Ivey, A., et al., *Molecular Detection of Minimal Residual Disease Provides the Most Powerful Independent Prognostic Factor Irrespective of Clonal Architecture in Nucleophosmin (NPM1) Mutant Acute Myeloid Leukemia*. Vol. 124. 2014. 70-70.

10. Cilloni, D., et al., *Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study.* J Clin Oncol, 2009. **27**(31): p. 5195-201.

11. Goswami, M., et al., *A multigene array for measurable residual disease detection in AML patients undergoing SCT.* Bone Marrow Transplant, 2015.

12. Steinbach, D., et al., *Prospective validation of a new method of monitoring minimal residual disease in childhood acute myelogenous leukemia.* Clin Cancer Res, 2015. **21**(6): p. 1353-9.

13. Terwijn, M., et al., *High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study.* J Clin Oncol, 2013. **31**(31): p. 3889-97.

14. Kohnke, T., et al., *Early assessment of minimal residual disease in AML by flow cytometry during aplasia identifies patients at increased risk of relapse.* Leukemia, 2015. **29**(2): p. 377-86.

15. Pemovska, T., et al., *Individualized systems medicine strategy to tailor treatments for patients with chemorefractory acute myeloid leukemia.* Cancer Discov, 2013. **3**(12): p. 1416-29.

16. Becker, P.S., et al., *Feasibility Trial of Individualized Therapy for Relapsed or Refractory Acute Myeloid Leukemia Based on a High Throughput in Vitro Drug Sensitivity Assay.* Blood, 2014. **124**(21): p. 3748-3748.

17. Hourigan, C.S. and J.E. Karp, *Personalized therapy for acute myeloid leukemia.* Cancer Discov, 2013. **3**(12): p. 1336-8.

18. Archimbaud, E., et al., *Timed sequential chemotherapy for previously treated patients with acute myeloid leukemia: long-term follow-up of the etoposide, mitoxantrone, and cytarabine-86 trial.* J Clin Oncol, 1995. **13**(1): p. 11-8.

19. Amadori, S., et al., *Mitoxantrone, etoposide, and intermediate-dose cytarabine: an effective and tolerable regimen for the treatment of refractory acute myeloid leukemia.* J Clin Oncol, 1991. **9**(7): p. 1210-4.

20. Trifilio, S.M., et al., *Mitoxantrone and etoposide with or without intermediate dose cytarabine for the treatment of primary induction failure or relapsed acute myeloid leukemia.* Leuk Res, 2012. **36**(4): p. 394-6.

21. Price, S.L., et al., *Salvage chemotherapy regimens for acute myeloid leukemia: Is one better? Efficacy comparison between CLAG and MEC regimens.* Leuk Res, 2011. **35**(3): p. 301-4.

22. Greenberg, P.L., et al., *Mitoxantrone, etoposide, and cytarabine with or without valspodar in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome: a phase III trial (E2995).* J Clin Oncol, 2004. **22**(6): p. 1078-86.

23. Kohrt, H.E., et al., *Second-line mitoxantrone, etoposide, and cytarabine for acute myeloid leukemia: a single-center experience.* Am J Hematol, 2010. **85**(11): p. 877-81.

24. Link, H., et al., *Mitoxantrone, cytosine arabinoside, and VP-16 in 36 patients with relapsed and refractory acute myeloid leukemia.* Haematol Blood Transfus, 1990. **33**: p. 322-5.

25. Lee, S.R., et al., *The clinical outcome of FLAG chemotherapy without idarubicin in patients with relapsed or refractory acute myeloid leukemia.* J Korean Med Sci, 2009. **24**(3): p. 498-503.

26. Montillo, M., et al., *Fludarabine, cytarabine, and G-CSF (FLAG) for the treatment of poor risk acute myeloid leukemia.* Am J Hematol, 1998. **58**(2): p. 105-9.

27. Virchis, A., et al., *Fludarabine, cytosine arabinoside, granulocyte-colony stimulating factor with or without idarubicin in the treatment of high risk acute leukaemia or myelodysplastic syndromes.* Br J Haematol, 2004. **124**(1): p. 26-32.

28. Milligan, D.W., et al., *Fludarabine and cytosine are less effective than standard ADE chemotherapy in high-risk acute myeloid leukemia, and addition of G-CSF and ATRA are not beneficial: results of the MRC AML-HR randomized trial.* Blood, 2006. **107**(12): p. 4614-22.

29. Wrzesien-Kus, A., et al., *A multicenter, open, non-comparative, phase II study of the combination of cladribine (2-chlorodeoxyadenosine), cytarabine, and G-CSF as induction therapy in refractory acute myeloid leukemia - a report of the Polish Adult Leukemia Group (PALG).* Eur J Haematol, 2003. **71**(3): p. 155-62.

30. Martin, M.G., et al., *Cladribine in the treatment of acute myeloid leukemia: a single-institution experience.* Clin Lymphoma Myeloma, 2009. **9**(4): p. 298-301.

31. Wierzbowska, A., et al., *Cladribine combined with high doses of arabinoside cytosine, mitoxantrone, and G-CSF (CLAG-M) is a highly effective salvage regimen in patients with refractory and relapsed acute myeloid leukemia of the poor risk: a final report of the Polish Adult Leukemia Group.* Eur J Haematol, 2008. **80**(2): p. 115-26.

32. Faderl, S., et al., *Results of a phase 1-2 study of clofarabine in combination with cytarabine (ara-C) in relapsed and refractory acute leukemias.* Blood, 2005. **105**(3): p. 940-7.

33. Faderl, S., et al., *Clofarabine plus cytarabine compared with cytarabine alone in older patients with relapsed or refractory acute myelogenous leukemia: results from the CLASSIC I Trial.* J Clin Oncol, 2012. **30**(20): p. 2492-9.

34. Scappini, B., et al., *Cytarabine and clofarabine after high-dose cytarabine in relapsed or refractory AML patients.* Am J Hematol, 2012. **87**(12): p. 1047-51.

35. Becker, P.S., et al., *Clofarabine with high dose cytarabine and granulocyte colony-stimulating factor (G-CSF) priming for relapsed and refractory acute myeloid leukaemia.* Br J Haematol, 2011. **155**(2): p. 182-9.

36. Becker, P.S., et al., *Retrospective comparison of clofarabine versus fludarabine in combination with high-dose cytarabine with or without granulocyte colony-stimulating factor as salvage therapies for acute myeloid leukemia.* Haematologica, 2013. **98**(1): p. 114-8.

37 McNemar, Q., Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika, 1947. 12(2): p. 153-7.

38. Miettinen, O. S. On the matched-pairs design in the case of all-or-none responses. Biometrics, 1968. 24: p. 339-52.

## APPENDIX A: AML Diagnostic and Treatment Response Criteria (per SWOG)

Acute Myeloid Leukemia (AML) is a clonal expansion of myeloid blasts in bone marrow, blood or other tissue, ICD-O code 9861/3. The most significant change from the FAB classification is that the requisite blast percentage for a diagnosis of acute myeloid leukemia be ≥ 20% myeloblasts in the blood or marrow.

There are two exceptions to this rule. Acute erythroleukemia (erythroid/myeloid subtype) is defined by the presence in the bone marrow of greater than or equal to 50% erythroid precursors in the entire nucleated cell population and greater than or equal to 20% myeloblasts in the non-erythroid cell population. Pure erythroid leukemia is defined as a neoplastic proliferation of immature cells committed exclusively to the erythroid lineage (> 80% of the marrow nucleated cells) with no evidence of a significant myeloblastic component.

Diagnostic and Staging Criteria

Definitions:

1. Bone marrow cellularity: The volume of hematopoietic nucleated cells, expressed as a percentage of marrow volume less volume of fibrosis.
2. Blasts: For AML, the following cell types are considered equivalent to blasts and are included in the calculation of blast percentages. Note that erythroblasts are not counted as blasts in calculating blast percentages.
3. Myeloblasts include both agranular and granular variants.
4. Monoblasts and promonocytes for Acute Monoblastic and Monocytic Leukemia.
5. Megakaryoblasts for Acute Megakaryoblastic Leukemia.

3. Bone Marrow Blast Percentage is calculated as the percent of blasts among all nucleated marrow cells.

Response Definitions for AML:

1. **Morphologic complete remission (CR):** ANC ≥ 1,000/mcl, platelet count ≥ 100,000/mcl, < 5% bone marrow blasts, no Auer rods, no evidence of extramedullary disease. (No requirements for marrow cellularity, hemoglobin concentration).
2. **Morphologic complete remission with incomplete blood count recovery (CRi):** Same as CR but ANC may be < 1,000/mcl and/or platelet count < 100,000/mcl.
3. **Partial remission (PR):** ANC ≥ 1,000/mcl, platelet count > 100,000/mcl, and at least a 50% decrease in the percentage of marrow aspirate blasts to 5-25%, or marrow blasts < 5% with persistent Auer rods.
4. **Resistant Disease (RD):** The bone marrow shows persistent AML, and patient survives at least 7 days beyond end of treatment course.
5. **Not Evaluable:** Data incomplete or inadequate for time-point or overall assessment.

## APPENDIX B: Examples of conventional cytotoxic salvage chemotherapy regimens studied and utilized in patients with relapsed/refractory AML

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Regimen | Agents | CR | TRM or 30 day mortality | Common toxicities (non-hematologic) | Reference |
| EMA-86 | Mitoxantrone 12mg/m2 days 1-3  Cytarabine 500mg/m2 CI days 1-3 & 8-10  Etoposide 200mg/m2 CI days 8-10 | 60% | 11% | WHO Grade >2: infection (54%), oral mucositis (23%), vomiting (9%), hyperbilirubinemia (8%), bleeding (6%), cutaneous rash (5%), diarrhea (3%), complex metabolic disorders (2%), cerebellar syndrome (1%) | [[18](#_ENREF_18)] |
| MEC | Mitoxantrone 6mg/m2 days 1-6  Etoposide 80mg/m2 days 1-6  Cytarabine 1g/m2 days 1-6 | 59% - 66% | 3% - 6% | Nausea/vomiting (81%), mucositis (60%), infection (53%), hemorrhage (50%), FUO (47%), cardiotoxicity (9%), liver toxicity (6%) | [[19](#_ENREF_19), [20](#_ENREF_20)] |
| Mitoxantrone 8mg/m2 days 1-5  Etoposide 100mg/m2 days 1-5  Cytarabine 1mg/m2 days 1-5 | 18% - 24% | 7% - 11% | Grade III/IV: hepatic (38%), neurologic (16%) | [[21-23](#_ENREF_21)] |
| MAV | Mitoxantrone 10mg/m2 days 4-8  Cytarabine 100mg/m2 CI days 1-8  Etoposide 100-120mg/m2 days 4-8 | 58% | 11% | WHO grade III/IV: nausea/vomiting (26%), mucositis (22%), septicemia (20%), FUO (13%), diarrhea (11%), hemorrhage (9%), CHF (7%), bilirubinemia (6%), localized infection (6%), cardiac arrhythmia (2%) | [[24](#_ENREF_24)] |
| FLAG  FLAG-IDA | Fludarabine 30mg/m2 days 1-5  Cytarabine 2g/m2 days 1-5  G-CSF 5mcg/kg day 0 until ANC recovery | 48% - 55% | 10% -11% | Infections (26% - 44%), FUO (44%), nausea/vomiting (11%), diarrhea (3% - 8%), mucositis (10%), hepatic dysfunction (8%), rash (2%), lethargy (2%) | [[25](#_ENREF_25), [26](#_ENREF_26)] |
| Fludarabine 30mg/m2 days 1-5  Cytarabine 2 g/m2 days 1-5  G-CSF 300mcg day 0 until ANC recovery  Idarubicin 8mg/m2 days 1-3 | 63% | 17% | FUO (54%), nausea (28%), bacteremia (18%), aspergillus (15%), vomiting (13%), rash (10%), diarrhea (4%), mucositis (2%) | [[27](#_ENREF_27)] |
| FLA | Fludarabine 30mg/m2 days 1-5  Cytarabine 2g/m2 days 1-5 | 61% | 7% | Mean WHO grade after 1 course of treatment: alopecia 2.4, nausea/vomiting 1.3, oral 1.1, diarrhea 0.8, cardiac 0.3 | [[28](#_ENREF_28)] |
| CLAG  CLAG-M | Cladribine 5mg/m2 on days 2-6  Cytarabine 2g/m2 days 2-6  G-CSF 300mcg days 1-6 | 38% - 50% | 0% - 17% | Alopecia (62%), infection/FUO (40% - 60%), diarrhea (12%), nausea/vomiting (10%), hemorrhage (0% - 10%), mucositis/stomatitis (9%), cardiotoxicity (9%), hepatotoxicity (2%), nephrotoxicity (2%), allergy (2%) | [[21](#_ENREF_21), [29](#_ENREF_29), [30](#_ENREF_30)] |
| Cladribine 5mg/m2 days 1-5  Cytarabine 2g/m2 days 1-5  G-CSF 300mcg days 0-5  Mitoxantrone 10mg/m2 days 1-3 | 50% - 58% (53% after first course) | 0% - 7% | Grade III-IV toxicity: infection (45%), nausea/vomiting (20%), diarrhea (14%), mucositis (10%), bleeding (10%), rash (6%), hyperbilirubinemia (2%), seizure 1%), MI (1%, died) | [[30](#_ENREF_30), [31](#_ENREF_31)] |
| Clofarabine Cytarabine | Clofarabine 40mg/m2 days 2-6  Cytarabine 1g/m2 days 1-5 | 28% - 51% | 6.2% - 13% | All grades: Hepatic dysfunction (21% - 90%), nausea/vomiting (45% - 70%), rash (36% - 70%), diarrhea (38% - 70%), infection (69%), hand-foot syndrome (50%), mucositis (13% - 35%), headaches (35%), FUO (31% - 47%), facial flushing (15%), fatigue (10%), palpitations/arrhythmias (10%), drug fever (5%), multiorgan failure (2%)  Grade III/IV: Febrile neutropenia (47%), hypokalemia (18%), pneumonia (14%), increased AST (11%), increased ALT (10%), bacteremia (9%), hypertension (9%), diarrhea (8%), enterococcal bacteremia (8%), fatigue (6%), hyperglycemia (6%), hyponatremia (6%), hypotension (6%), acute renal failure (5%), increased bilirubin (5%), increased lipase (5%), hypocalcemia (5%) | [[32](#_ENREF_32)] |
| Clofarabine 40mg/m2 days 1-5  Cytarabine 1g/m2 days 1-5 | [[33](#_ENREF_33), [34](#_ENREF_34)] |
| Clofarabine 22.5 mg/m2 days 1-5  Cytarabine 1g/m2 days 1-5 |
| GCLAC | Clofarabine 25mg/m2 days 1-5  Cytarabine 2g/m2 days 1-5  G-CSF 5mcg/kg day 0 until ANC recovery | 46%, (CR+CRp 61%) | 13% | Pulmonary toxicity (46%), infection (40%), elevated transaminases (16%), gastrointestinal toxicity (12%), rash (10%), hyperbilirubinemia (8%), renal dysfunction (4%), neuropathy (2%), tumor lysis (2%), pain (2%) | [[35](#_ENREF_35), [36](#_ENREF_36)] |

## APPENDIX C Anthracycline Equivalent Dosing

Cumulative doses should be calculated using the following table, taking into account all previous anthracyclines or anthracenediones received during the patient’s lifetime.

|  |  |  |
| --- | --- | --- |
| **AGENT** | **SUGGESTED CONVERSION**  **FACTOR TO DOXORUBICIN DOSE\*** | **SUGGESTED MONITORING THRESHOLD\*\*** |
| DAUNOrubicin | x 0.5-0.83 | 450 mg/m2 |
| DOXOrubicin | x 1 | 300 mg/m2 |
| epirubicin | x 0.5-0.67 | 600 mg/m2 |
| IDArubicin | x 2-5 | 150 mg/m2 |
| mitoXANTRONE | x 2.2-4 | not defined |

* based on relative hematological toxicities
* \*\* Treatment may continue beyond these doses in selected patients, if the clinician has considered the potential risks and benefits. The addition of dexrazoxane may be considered, and monitoring should be increased. Maximum tolerated doses are variable; some patients may tolerate DOXOrubicin equivalent doses exceeding 1000 mg/m2 while other patients exhibit symptomatic CHF at DOXOrubicin equivalent doses doses less than 300 mg/m2.

*From: BC Cancer Agency Cancer Drug Manual*

*Mitoxantrone Revised: 1 August 2013*

*http://www.bccancer.bc.ca/HPI/DrugDatabase/DrugIndexPro/Mitoxantrone.htm (accessed April 1st 2015)*

## APPENDIX D NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES

-2/5/2013

|  | **DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION** | **Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?** | **Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?** |
| --- | --- | --- | --- |
|  |  |  |  |
| **A** | **Stem Cell Allotransplantation Section (Dr. A. John Barrett)** |  |  |
| **A.1** | Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques. | No | No |
| **A.2** | Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples. | No | No |
| **A.3** | Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses. | No | No |
| **A.4** | Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies. | No | No |
| **A.5** | Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions. | No | No |
| **A.6** | Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application. | No | No |
| **A.7** | Identification of individual T cell clones by their T cell receptor sequence. | No | No |
| **A.8** | Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids. | No | No |
| **A.9** | Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes. | No | No |
| **A.10** | DNA and RNA typing of genes that control immune responses in lymphocytes. | No | No |
| **A.11** | Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions. | No | No |
|  |  |  |  |
| **B** | **Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)** |  |  |
| **B.1** | Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells. | No | No |
| **B.2** | Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function. | No | No |
| **B.3** | Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro. | No | No |
| **B.4** | Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro. | No | No |
|  |  |  |  |
| **C** | **Cell Biology Section (Dr. Neal Young)** |  |  |
| **C.1** | Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion. | No | No |
| **C.2** | Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests. | No | No |
| **C.3** | Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins. | No | No |
| **C.4** | Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow. | No | No |
| **C.5** | Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained. | No | No |
| **C.6** | Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations. | No | No |
| **C.7** | Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations. | No | No |
| **C.8** | Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells. | No | No |
| **C.9** | Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells. | No | No |
| **C.10** | Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology). | No | No |
| **C.11** | Mitochondrial DNA (mtDNA) sequence heterogeneity. | No | No |
| **C.12** | Measurement of EBV viral load. | No | No |
| **C.13** | Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1. | No | No |
| **C.14** | Outgrowth assay of EBV transformed B cells. | No | No |
| **C.15** | Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12). | No | No |
| **C.16** | Quantification of EBV cytotoxic T cells (tetramerstaining). | No | No |
| **C.17** | Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA | No | No |
| **C.18** | Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2.* | No | No |
| **C.19** | Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation. | No | No |
| **C.20** | Confocal microscopic imaging of bone marrow. | No | No |
| **C.21** | Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots. | No | No |
| **C.22** | Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques. | No | No |
| **C.23** | Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection. | No | No |
|  |  |  |  |
| **D** | **Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS** |  |  |
| **D.1** | Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays. | No | N/A |
| **D.2** | Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements. | No | N/A |
| **D.3** | Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2). | No | N/A |
| **D.4** | Spectra-typing of blood cells to determine response to known or putative viral infections. | No | N/A |
| **D.5** | HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies. | No | N/A |
| **D.6** | Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity. | No | N/A |
|  |  |  |  |
| **E** | **Solid Tumor Section (Dr. Richard Childs)** |  |  |
| **E.1** | Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells. | No | No |
| **E.2** | ELISA for IL-12 maturity of DC's made from subjects monocytes. | No | No |
| **E.3** | ELISA for IFN ã to evaluate specificity of CTL clones. | No | No |
| **E.4** | H thymidine uptake to evaluate proliferation potential of antigen specific T-cells. | No | No |
| **E.5** | PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant. | No | No |
| **E.6** | Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets. | No | No |
| **E.7** | Surface marker analysis of peripheral blood mononuclear cells using flow cytometry. | No | No |
| **E.8** | cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect. | No | No |
| **E.9** | Geno typing of tumor or tissue samples by high density cDNA arrays. | No | No |
| **E.10** | VHL mutation analysis on kidney cancer tissue. | No | No |
| **E.11** | Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions. | No | No |
| **E.12** | Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient). | No | No |
| **E.13** | Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples. | No | No |
| **E.14** | Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples. | No | No |
| **E.15** | Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant. | No | No |
| **E.16** | Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts. | No | No |
| **E.17** | Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts. | No | No |
| **E.18** | Determination of etiology of membraneous nephropathy using serum from subjects. | No | No |
| **E.19** | Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation. | No | No |
| **E.20** | Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH. | No | No |
|  |  |  |  |
| **F** | **Lymphoid Malignancies Section (Dr. Adrian Wiestner)** |  |  |
| **F.1** | Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions. | No | No |
| **F.2** | Generation of stable cell lines for the study of hematologic malignancies. | No | No |
| **F.3** | Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions. |  |  |
| **F.4** | Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression. | No | No |
| **F.5** | Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays. | No | No |
| **F.6** | Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing. | No | No |
| **F.7** | Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes. | No | No |
| **F.8** | Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens. | No | No |
| **F.9** | Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy. | No | No |
| **F.10** | Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma. | No | No |