A Phase I/II study of lenalidomide maintenance after autologous stem cell transplant for elderly patients with Acute Myeloid Leukemia (AML).

Principal Investigator: Ira Braunschweig, M.D.
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474
Email : ibraunsc@montefiore.org

Co- Investigator: Olga Derman, M.D.
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474
Email : oderman@montefiore.org

Co- Investigator: Ramakrishna Battini, MD
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474
Email :RABATTIN@montefiore.org

Co-Investigator: Amit K. Verma, M.B.B.S.
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus,
Chanin Building, Room 302B
1300 Morris Park Avenue
Bronx, New York 10461
Phone 718-930-8761
Email: amit.verma@einstein.yu.edu

Co-Investigator: Noah Kornblum, M.D.
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474
Email : nkornblu@montefiore.org

Co-Investigator: Ulrich Steidl, MD PhD
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus,
Chanin Building, Room 306
1300 Morris Park Avenue
Bronx, New York 10461
Phone 718-430-3437
Email: ulrich.steidl@einstein.yu.edu

Co-Investigator: Murali Janakiram, M.D.
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474
Email : MJANAKIR@montefiore.org

Co-Investigator: Ioannis Mantzaris, MD, MS
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474
Email : imantzar@montefiore.org

Research Nurse: Michelly Abreu, NP
Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer Pavilion, Room 100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-4826/Fax 718-798-7474

Email : mabreu@montefiore.org

Statistician:
Nan Xue, PhD
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus
1300 Morris Park Avenue
Belfer Building, Room 1303C
Bronx, NY 10461
Phone: 718-430-2431 Pager: 917-218-6906
Email: Xiaonan.xue@einstein.yu.edu

Study Coordinator/Data Management:
Lawrence Almanzar
Montefiore Medical Center-Moses Division
Depart. of Oncology, Hofheimer Pavilion 100
Bronx, NY 10467
Phone 718- 920-2006/718-405-8544
Fax 718-798-7474/718-405-8544
Email: lalmanzar@montefiore.org

PARTICIPATING INSTITUTION	PI's NAME	Telephone	Email
Comprehensive Cancer Center Wake Forest University	David Hurd, MD	(336)-713-5440	dhurd@wakehealth.edu

SYNOPSIS

BACKGROUND: AML in patients 60 and over has a very poor prognosis. With standard induction chemotherapy and consolidation with autologous stem cell transplant a substantial proportion of patients will be able to achieve remission. Unfortunately, virtually all of those that achieve remission will relapse and die of their disease. Recently, lenalidomide has been shown to be an active agent for AML. In addition, lenalidomide has been shown to be a feasible and effective maintenance strategy for myeloma post autologous transplant. We therefore propose to study the feasibility and efficacy of lenalidomide maintenance after autologous transplant in AML.

The primary objective is to determine the safety and efficacy of maintenance lenalidomide after autologous stem cell transplant in this setting.
Secondary Objectives are to determine the progression free survival and overall survival for patients treated with this approach.

TREATMENTElderly patients with AML who have achieved morphologic remission will be treated with escalating doses of lenalidomide (starting at 10mg once daily 21 days each month) in groups of 3 until a maximum dose of 25mg once daily 21 days each month has been reached.

Key Eligibility Criteria: Patients must have a diagnosis of Acute Myeloid Leukemia excluding Acute Promyelocytic Leukemia (M3). Age 60-80, No uncontrolled CHF. Adequate renal and liver function. ECOG performance status ≤ 2, and a life expectancy of > 12 months. Patients will be excluded if they have an untransfused platelet count of < 75, are growth factor dependent, have uncontrolled infection, have HIV or HTLV-1 infection, have active CNS disease, or prior malignancy other than adequately treated non-melanoma skin cancer.

Statistical Considerations: The aim of this proposal is to provide an accurate estimate of the two-year relapse free survival rate for elderly AML patients treated with lenalidomide maintenance after autologous stem cell transplantation. With approximately 30 patients, the study will be able to estimate the two year relapse free survival rate with not more than 9% standard error(i.e., if the observed rate is 40%, the 95% CI will be (23%, 56%). In addition, our study will have over 80% power to conclude that the two year relapse rate is significantly reduced when patients are treated with lenalidomide, assuming 60% and 90% patients will relapse with and without lenalidomide, respectively. The observed relapse free survival rate will be calculated along with its 95% CI. A one sample test on proportion will be used to detect if the relapse free survival rate with lenalidomide is significantly higher than that without the treatment (relapse rate is expected to be >95%).Secondary analysis including Kaplan-Meier analysis on overall survival.

TABLE OF CONTENTS

APPENDICES

1. OBJECTIVES

1.1. Primary Objectives

To determine the safety and efficacy of maintenance lenalidomide post autologous peripheral blood stem cell transplantation (PBSCT) for elderly patients with AML.

1.2. Secondary Objectives

1.2.1To define maximum tolerated dose (MTD) and establish therapeutic dose level (TDL) of lenalidomide given post autologous transplant for AML.

1.2.2 To determine the progression free survival for patients treated with this approach.

1.2.3To determine the overall survival for patients treated with this approach.

1.2.4 To determine the role of residual AML stem cells on efficacy of lenalidomide maintenance after autologous PBSCT.

2. BACKGROUND and RATIONALE

2.1 Induction Therapy for Elderly AML Patients

AML is mainly a disease of the elderly with a median age of 69 years in the white US population, and prognosis worsens every decade beginning at age 30 to 40.[1] A report by the German Acute Myeloid Leukemia Cooperative Group studied patients 16 to 85 years

of age enrolled in two consecutive trials who had AML. In a multivariate analysis of prognostic factors, age ≥ 60 years was a statistically significant poor prognostic factor for complete remission, overall survival, remission duration, and relapse free survival.[2] Population based studies have reported 3- and 5-year survival rates of only 9 to 10% and 3 to 8%, respectively, in patients over age 60, compared with 5-year survival rates of up to 50% for younger patients.[3]Poorer outcome has traditionally been considered at least in part due to reluctance of physicians to treat this population aggressively. As such, lower levels of aggressive treatments may compound underlying prognostic differences associated with patient factors and disease biology.[4] Recent studies have therefore focused on dose intensification of anthracyclines and/or postremission therapy. Lowenberg and colleagues compared induction therapy with daunorubicin (DNR) at a dose of 45 mg/m^2or 90mg/m2 in 813 patients over age 60 with previously untreated AML. Although complete response (CR) rates were improved in the group that received the higher dose of DNR (64% vs 54%, P=.002) there was no significant difference in the two induction groups with respect to overall survival (OS) and event-free survival (EFS). Thirty-day mortality was 11% to 12% in both groups. Two year probabilities of relapse after CR for the standard versus intensified DNR groups were 61% versus 54%. Of note, dose reduced anthracycline led to inferior CR rates and survival was not as good in certain subgroups of patients.[5] As such, although the superiority of higher dose anthracycline as induction is not established in AML patients over 60 there 1) seems to be no increased toxicities with these regimens and 2) there is certainly no reason to attenuate doses of anthracycline in AML patients over 60. Doses of DNR at 60mg/m^2 or Idarubicin at 12mg/m^2 for 3 days together with cytarabine would be appropriate to use.[4]

2.2Autologous Stem Cell Transplantation as Consolidation Therapy for AML

The antileukemic efficacy **of** autologous stem cell transplantation for AML has been well established for some time. Zittoun and colleagues randomized 623 AML patients in first remission to either further chemotherapy, allogeneic stem cell transplant or autologous stem cell transplant. Disease free survival at four years was 55% for allogeneic transplantation, 48% for autologous transplantation and 30% for intensive chemotherapy. The difference between autologous transplantation and chemotherapy was statistically significant.[6]More recently, Vellenga and colleagues randomized over 500 patients with AML in first CR to either further intensive chemotherapy or autologous peripheral blood stem cell transplant. The transplant group showed a markedly reduced relapse rate (58% vs. 70%, P=.02) and better relapse free survival at 5 years (38% vs 29%, P=.065) Overall survival was similar because of more opportunities for salvage with second-line chemotherapy and stem cell transplantation in patients relapsing on the chemotherapy arm.[7]

Autologous stem cell transplantation has also been studied in the elderly patient with AML. Lashkari and colleagues studied 27 patients aged 60-71 years with newly diagnosed AML in first CR. They underwent high dose chemoradiotherapy followed by

peripheral blood stem cell transplant after a single cycle of cytarabine-based consolidation therapy. Leukemia free survival and overall survival at 3 years was 25% and 28% respectively. There was one death from transplant related mortality. The authors concluded that autologous transplantation of peripheral blood stem cells is well tolerated and feasible for patients ≥ 60 years of age with AML in first CR.[8] Ferrara and colleagues studied sixty three patients with non-M3 AML. The median age was 69 with range between 61 and 81. Included were patients with AML secondary to myelodysplastic syndrome. The transplant related mortality was 0%. Those patients that received a transplant (n=17)had a significantly better overall survival and disease free survival when compared to those that did not undergo transplant(n=10).[9] Oriol and colleagues evaluated the feasibility and results of autologous stem cell transplant in 258 AML patients in first CR who were over 60. The probability of 2 year leukemia-free survival was 39% for those candidate patients who underwent transplant and 22% for those that did not (p=.07). The authors concluded that autologous transplantation has a tolerable toxicity and may have a positive impact on leukemia-free survival.[10]

In summary then a few conclusions are reasonable to make for the patient with AML: 1) Standard induction chemotherapy is feasible ,and certainly there is no role to reduce the dose of anthracycline for a fit elderly patient 2) Autologous stem cell transplant may have superior anti-leukemic efficacy in the young AML patient when compared with standard consolidation chemotherapy 3) There is some suggestion that the superior antileukemic efficacy of autologous transplant may translate to benefit the elderly patient with AML as well 4) Autologous stem cell transplant is feasible in the elderly patient with AML vis a vis toxicities 5) While remissions in the elderly patient with AML are attainable, relapse is still the major obstacle to reach the goal of long term leukemia-free survival.

As such, new approaches are needed to enable those that achieve remission to remain in remission for the long term.

2.3Lenalidomide as Treatment of AML

Lenalidomide is a Food and Drug Administration (FDA) approved antineoplastic drug indicated for the treatment of myeloma in combination with dexamethasone, and for low or intermediate-1 risk myelodysplastic syndrome associated with a deletion 5q cytogenetic abnormality. A recent study has also shown that lenalidomide is an active agent for AML. Fehniger and colleagues evaluated the efficacy of lenalidomide as frontline therapy for older patients with AML. Patients 60 years of age or older with untreated AML received high-dose lenalidomide at 50mg daily for up to two twenty-eight day cycles. If patients achieved a CR or a CR with incomplete blood count recovery(Cri) or did not progress after 2 cycles of high dose lenalidomide, they received low dose lenalidomide (10mg daily) until disease progression, an unacceptable adverse event, or completion of 12 cycles. Thirty-three patients were enrolled. Overall CR/Cri

rate was 30% and 53% in patients completing high dose lenalidomide. The median duration of CR/CRi was 10 months. The authors concluded that high dose lenalidomide has evidence of clinical activity as initial therapy for older AML patients.[11]

2.4 Lenalidomide as Maintenance Treatment post-Transplant

The CALGB randomized 460 myeloma patients who underwent autologous stem cell transplantation and had stable disease or better to either lenalidomide maintenance or placebo. Starting dose was 10 mg/day, and escalated to 15mg/day after 3 months and continued until disease progression. Drug was stopped and dose reduced according to the development of toxicity. Drug was held for ≥ grade 3 toxicity, restarted at resolution to ≤ grade 2, and de-escalated by 5 mg or maintained as tolerated at 15, 10, or 5 mg daily for 21 of 28 days per month. The number of events (progression or death) in patients randomized to lenalidomide was 44 compared to 91 among patients randomized to placebo. The median time to progression was 42 months in the lenalidomide group vs. 22 months in the placebo group. Patients receiving lenalidomide experienced a 61% reduction in the risk of disease progression or death when compared to patients receiving placebo. A minority of patients discontinued therapy due to adverse events (12%, 28 of 231 on lenalidomide vs. 2%, 5 of 229 on placebo). The authors concluded that long term administration of lenalidomide is feasible in this setting and that lenalidomide significantly delays time to progression when given post-transplant.[12]

French investigators randomized patients who underwent an autologous stem cell transplant for myeloma to receive lenalidomide consolidation (25mg/day, 21 days/month, for 2 months) followed by maintenance with either placebo or lenalidomide (10 to 15 mg /day) until relapse. Lenalidomide maintenance improved the progression free survival(median 24 months from randomization in the placebo arm vs. 42 months from randomization in the lenalidomide arm) and the treatment was well tolerated. The definitive interruption rate for serious adverse events during maintenance was similar in both arms.[13]

We therefore propose to study the feasibility and efficacy of lenalidomide maintenance therapy for elderly patients with AML who have achieved a morphologic remission after induction and consolidation with autologous stem cell transplantation. The starting dose of 10mg/day is based on the tolerability of this dose in the post-transplant setting for myeloma.

2.5 Background Drug Information

Lenalidomide is a proprietary IMiD® compound of Celgene Corporation. IMiD® compounds have both immunomodulatory and anti-angiogenic properties which could

confer antitumor and antimetastatic effects. Lenalidomide has been demonstrated to possess anti-angiogenic activity through inhibition of bFGF, VEGF and TNF-alpha induced endothelial cell migration, due at least in part to inhibition of Akt phosphorylation response to bFGF. In addition, lenalidomide has a variety of immunomodulatory effects. Lenalidomide stimulates T cell proliferation, and the production of IL-2, IL-10 and IFN-gamma, inhibits IL-1 beta and IL-6 and modulates IL-12 production. Upregulation of T cell derived IL-2 production is achieved at least in part through increased AP-1 activity.

Although the exact antitumor mechanism of action of lenalidomide is unknown, a number of mechanisms are postulated to be responsible for lenalidomide's activity against multiple myeloma. Lenalidomide has been shown to increase T cell proliferation, which leads to an increase in IL-2 and IFN-gamma secretion. The increased level of these circulating cytokines augment natural killer cell number and function, and enhance natural killer cell activity to yield an increase in multiple myeloma cell lysis. In addition, lenalidomide has direct activity against multiple myeloma and induces apoptosis or G1growth arrest in multiple myeloma cell lines and in multiple myeloma cells of patients resistant to melphalan, doxorubicin and dexamethasone.

Indications and Usage:

Revlimid® (lenalidomide) is indicated for the treatment of patients with transfusion-dependent anemia due to Low- or Intermediate-1-risk myelodysplastic syndromes associated with a deletion 5q cytogenetic abnormality with or without additional cytogenetic abnormalities. Revlimid® is also approved in combination with dexamethasone for the treatment of patients with multiple myeloma that have received at least one prior therapy.

Lenalidomide Description

REVLIMID® (lenalidomide), a thalidomide analogue, is an immunomodulatory agent with anti-angiogenic properties. The chemical name is 3-(4-amino-1-oxo 1,3-dihydro -2H-isoindol-2-yl) piperidine-2,6-dione and it has the following chemical structure:

Chemical Structure of Lenalidomide

3-(4-amino-1-oxo 1,3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione. The empirical formula for lenalidomide is C13H13N3O3, and the gram molecular weight is 259.3.

Lenalidomide is an off-white to pale-yellow solid powder. It is soluble in organic solvent/watermixtures, and buffered aqueous solvents. Lenalidomide is more soluble in organic solvents and low pH solutions. Solubility was significantly lower in less acidic buffers, ranging from about 0.4 to 0.5 mg/ml. Lenalidomide has an asymmetric carbon atom and can exist as the optically active forms S(-) and R(+), and is produced as a

racemic mixture with a net optical rotation of zero.

CLINICAL PHARMACOLOGY

Mechanism of Action:

The mechanism of action of lenalidomide remains to be fully characterized. Lenalidomide possesses immunomodulatory and antiangiogenic properties. Lenalidomide inhibited the secretion of pro-inflammatory cytokines and increased the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. Lenalidomide inhibited cell proliferation with varying effectiveness (IC50s) in some but not all cell lines. Of cell lines tested, lenalidomide was effective in inhibiting growth of Namalwa cells (a human B cell lymphoma cell line with a deletion of one chromosome 5) but was much less effective in inhibiting growth of KG-1 cells (human myeloblastic cell line, also with a deletion of one chromosome 5) and other cell lines without chromosome 5 deletions. Lenalidomide inhibited the expression of cyclooxygenase-2 (COX-2) but not COX-1 in vitro.

Pharmacokinetics and Drug Metabolism:

Absorption:
Lenalidomide, in healthy volunteers, is rapidly absorbed following oral administration with maximum plasma concentrations occurring between 0.625 and 1.5 hours post-dose. Co administration with food does not alter the extent of absorption (AUC) but does reduce the maximal plasma concentration (Cmax) by 36%. The pharmacokinetic disposition of lenalidomide is linear. Cmax and AUC increase proportionately with increases in dose. Multiple dosing at the recommended dose-regimen does not result in drug accumulation.

Pharmacokinetic analyses were performed on 15 multiple myeloma patients treated in the phase I studies. Absorption was found to be rapid on both Day 1 and Day 28 with time to maximum blood levels ranging from 0.7 to 2.0 hours at all dose levels (5mg, 10mg, 25mg, and 50mg). No plasma accumulation was observed with multiple daily dosing. Plasma lenalidomide declined in a monophasic manner with elimination half-life ranging from 2.8 to 6.1 hours on both Day 1 and 28 at all 4 doses. Peak and overall plasma concentrations were dose proportional over the dosing range of 5mg to 50mg. Exposure (AUC) in multiple myeloma patients is 57% higher than in healthy male volunteers.

Distribution:
In vitro (14°C)-lenalidomide binding to plasma proteins is approximately 30%.

Metabolism and Excretion:

The metabolic profile of lenalidomide in humans has not been studied. In healthy volunteers, approximately two-thirds of lenalidomide is eliminated unchanged through urinary excretion. The process exceeds the glomerular filtration rate and therefore is partially or entirely active. Half-life of elimination is approximately 3 hours.

Supplier(s)
Celgene Corporation will supply Revlimid® (lenalidomide) to study participants at no charge through the RevAssist® program.

Dosage form
Lenalidomide will be supplied as capsules for oral administration.

Packaging
Lenalidomide will be shipped directly to patients. Bottles will contain a sufficient number of capsules for one cycle of dosing.

Storage
Lenalidomide should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

Special Handling Instructions
Females of childbearing potential should not handle or administer lenalidomide unless they are wearing gloves.

Prescribing Information
Lenalidomide (Revlimid®) will be provided to research subjects for the duration of their participation in this trial at no charge to them or their insurance providers. Lenalidomide will be provided in accordance with the RevAssist® program of Celgene Corporation. Per standard RevAssist® requirements all physicians who prescribe lenalidomide for research subjects enrolled into this trial, and all research subjects enrolled into this trial, must be registered in and must comply with all requirements of the RevAssist® program. Prescriptions must be filled within 7 days. Only enough lenalidomide for one cycle of therapy will be supplied to the patient each cycle.

Pregnancy Testing
Pregnancy testing is required, and must be performed within 24 hours prior to prescribing lenalidomide. Risks of Fetal Exposure, Pregnancy Testing Guidelines, Acceptable Birth Control Methods and other Precautions are presented in Appendix F.

Adverse Events
Most frequently reported adverse events reported during clinical studies with lenalidomide in oncologic and non-oncologic indications, regardless of presumed relationship to study medication include: anemia, neutropenia, thrombocytopenia and pancytopenia, abdominal pain, nausea, vomiting and diarrhea, dehydration, rash,

itching, infections, sepsis, pneumonia, Urinary Tract Infections (UTI) , Upper respiratory infection, atrial fibrillation, congestive heart failure, myocardial infarction, chest pain, weakness, hypotension, hypercalcemia, hyperglycemia, back pain, bone pain, generalized pain, dizziness, mental status changes, syncope, renal failure, dyspnea, pleural effusion, pulmonary embolism, deep vein thrombosis, Cerebrovascular attacks (CVA), convulsions, dizziness, spinal cord compression, syncope, disease progression, death not specified and fractures.

Lenalidomide has been studied in healthy volunteers and in patients with cancer of the blood and other organs of the body as well as in patients with other diseases. As with any other experimental treatment there may be side effects or risks associated with lenalidomide, some of which are not yet known.

The following is a list of the most medically significant or most common side effects reported in completed and ongoing studies considered to be related to lenalidomide. In some cases, side effects can be serious, long-lasting, may never go away, or can cause death.

<u>Common (between a 1-10% chance that this will happen):</u>
Anemia; Decrease in cells that help your blood clot; Low white blood cells with or without fever; Pneumonia or other infections; Fever; Abnormal heart beats; Shortness of breath; Blood clots in lower extremities, lungs, heart, brain or other organs; Abnormal kidney function; Diarrhea with or without bleeding; Loss of fluid; Other cancers; Pain including muscles, joints and non-heart pain; Altered sense of taste.

<u>Uncommon (between a 0.1-1% chance that this will happen):</u>

Heart attack; Heart and lungs stop working; Bleeding including bleeding in individual organs; Stroke; Seizure; Dizziness; Fainting; Diabetes; Depression; Confusion; Worsening of general condition/ disease; Feeling tired, weak and unwell; Chills; Constipation; Blockage of intestine; Nausea; Vomiting; Decreased appetite; Weight loss; Swelling including swelling of individual organs; Gall bladder problem; Chemical imbalance; Abnormal liver lab tests; Allergic reactions including serious allergic skin reactions including involvement of the lining of the nose, mouth, stomach and intestines or rash leading to the separation of the top layer of skin; Skin irritation; Fracture; Problems urinating; Cough; Breathing disorder; Abnormal blood pressure; Low oxygen to tissues of the body including heart; Absence of blood cells due to defective development; Rapid death of cancer cells where the accumulating contents of dying cancer cells cause an imbalance in the chemistry of the body which can lead to kidney damage; Sudden increase in tumor size.

<u>Rare (less than a 0.1% chance that this will happen):</u>
Abnormal lymph gland; Heart pain; Shock; Weak heart muscle; Deafness; Blindness;

Abnormal eye pressure; Vision changes; Tear in intestine; Decreased action of intestine; Food poisoning; Indigestion; Stomach ulcer with or without bleeding; Problem with thyroid; Problem with adrenal gland; Liver failure; Abnormal liver function; Destruction of red blood cells; Abnormal bone marrow test result; Blood vessel narrowing; Lack of blood supply leading to tissue damage; Arthritis with infection; Worsening of chronic lung disease with infection; Too much fluid in body; Trouble speaking; Trouble walking; Muscular inflammation; Coma; Abnormal sense of touch; Lowered level of consciousness, with drowsiness, listlessness and apathy; Headache; Neurologic problem; Pain and decreased sensation in nerves; Parkinson's disease; Repetitive speech; Sleepiness; Shaking; Irritable; Excited; Hallucination; Not able to sleep; Moody; Fluid in lungs; Runny nose; Sore throat; Blister; Change in skin color; Skin ulcer; Blood not getting to extremities; Itching; Destruction of muscle that can lead to kidney damage.

2.6 Correlative Studies

To determine the role of residual AML stem cells on efficacy of lenalidomide maintenance after autotransplant.

Myeloid malignancies can arise from a small population of quiescent cancer-initiating cells that are not eliminated by conventional cytotoxic therapies. Eliminating leukemia stem cells can reduce the rates of relapse and increase leukemia free survival. Thus it is important to evaluate the role of persistent leukemia stem cells in the efficacy of lenalidomide maintenance regimens.

Flow cytometry will be done on bone marrow aspirates to estimate the persistence of leukemic stem cells. We have established protocols to isolate rigorously defined long-term hematopoietic stem cells (LT-HSC, Lin-, CD34+, CD38-, CD90+), short-term hematopoietic stem cells (ST-HSC, Lin-, CD34+, CD38-, CD90-), common myeloid progenitors (CMP, Lin-, CD34+, CD38+, CD123+, CD45RA-), granulocyte- monocyte progenitors (GMP, Lin-, CD34+, CD38+, CD123+, CD45RA+), and megakaryocyte-erythrocyte progenitors (MEP, Lin-, CD34+, CD38+, CD123-, CD45RA-) from primary bone marrow aspirates.[14] Sorted cells will have FISH studies done on them to estimate the number of stem cells with clonal cytogenetic alterations (Leukemic stem cells). Functional assays will also be done on these sorted cells to demonstrate clonogenic potential in vitro, and leukemia-initiating potential in vivo. A recently discovered marker of AML stem cells, IL1RAP[15], will also be evaluated on AML stem cells and correlated with clinical response.

Whole transcriptome sequencing, exome sequencing, and global methylome analysis on sorted cells will also be done and correlated with response. This will allow us to correlate molecular markers with relapse or remission.

Detailed procedures for correlative studies are discussed in Section 7.

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1. Patients must have a Confirmed diagnosis of non-M3 AML. Antecedent MDS is acceptable.
3.1.2. Post autologous Stem Cell Transplant Bone marrow biopsy core that is consistent with morphologic remission.
3.1.3. Age between 60 and 80years old.
3.1.4. Must have received induction and consolidation chemotherapy, and autologous stem cell transplant for AML.
3.1.5. Life expectancy of greater than 12 months.
3.1.6. Karnofsky Performance Status 70 or greater (see Appendix A)
3.1.7. Patients must have normal organ and marrow function as defined below:

- Leukocytes ≥2,000/mcL
- Absolute neutrophil count ≥ 1,000/mcL
- Platelets ≥ 75,000/mcL
- Total bilirubin ≤4 X institutional upper limit of normal unless 2nd to Gilbert's disease
- AST(SGOT) and ALT(SGPT) ≤ 4 X institutional upper limit of normal
- Creatinine <1.5 X institutional upper limit of normal OR creatinine clearance ≥ 30 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.8 Able to take aspirin, or warfarin, or low molecular weight heparin as prophylactic anticoagulation.
3.1.9. Ability to understand and the willingness to sign a written informed consent document.
3.1.10 Must be registered into the mandatory RevAssist® program and be willing and able to comply with the requirement of RevAssist®.

3.2 Exclusion Criteria

3.2.1. Patient received chemotherapy or radiotherapy within 2 weeks prior to entering the study or has not recovered from adverse events due to agents administered more than 4 weeks earlier.
3.2.2 Patient received another investigational agent after post autologous stem cell transplant.
3.2.3 Patient who will be receiving another investigational product during the study.

3.2.4 Patient who is growth factor or transfusion dependent.
3.2.5 Patient has CNS leukemia.
3.2.6 History of allergic reactions attributed to thalidomide or lenalidomide.
3.2.7 History of erythema nodosum, characterized by a desquamating rash while taking thalidomide or similar drugs.
3.2.8 Prior history of metastatic malignancy.
3.2.9 Uncontrolled illness including, but not limited to ongoing or active infection, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Patients must not have suffered recent (< 6 months) myocardial infarction, unstable angina, uncontrolled hypertension, or difficult to control cardiac arrhythmias.
3.2.10 Evidence of uncontrolled Congestive Heart Failure (CHF).
3.2.11 Active Hepatitis B as defined by Hepatitis B surface antigen positivity, unless able to start dual anti-HepB therapy, or already on dual anti-HepB therapy
3.2.12 Patients who are positive for Hepatitis B core antibody, but negative for the hepatitis B surface antigen, should be on lamivudine 100 mg daily until at least 3 months post-transplant
3.2.13 Patient is positive for HIV or HTLV-1.
3.2.14 Women of childbearing potential (defined as a sexually mature woman who has not undergone a hysterectomy or who has had menses at any time in the preceding 24 consecutive months).
3.2.15 Men who did not agree not to father a child and who refused to use a latex condom during any sexual contact with women of childbearing potential while taking lenalidomide and for 4 weeks after therapy is stopped, even if they have undergone a successful vasectomy.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines

The **coordinating center** is:

Montefiore-Einstein Cancer Center
Montefiore Medical Center-Moses Division
Department of Oncology, Hofheimer100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-2006 or 718-405-8544
Fax 718-798-7474 or 718-405-4712

Coordinator: Lawrence Almanzar
Email: lalmanzar@montefiore.org

Eligible patients will be entered on study centrally at the Montefiore-Einstein Cancer Center Coordinating Center by the Study Coordinator. All sites should call the Study Coordinator Lawrence Almanzar; phone 718- 920-2006 to verify eligibility. The required forms (eligibility and registration form; form 1) can be found in the Appendix (Appendix B).

Following registration, patients should begin protocol treatment within 96 hours. Issues that would cause treatment delays should be discussed with the Principal Investigator (PI). If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed or e-mailed to the Study Coordinator at the Montefiore-Einstein Cancer Center Coordinating Center Lawrence Almanzar; phone 718-920-6642, fax 718-798-7474 or email: lalmanzar@montefiore.org

- Form 1 (Appendix B)
- Copy of required laboratory tests and procedures
- Signed patient consent form
- HIPAA authorization form

The research nurse or data manager at the participating site will then call the Study Coordinator at the Montefiore-Einstein Cancer Center Coordinating Center Lawrence Almanzar, phone 718- 920-2006, fax 718-798-7474 or email: lalmanzar@montefiore.org to verify eligibility. To complete the registration process, the Coordinator will - after discussion with the PI,Dr. Ira Braunschweig:

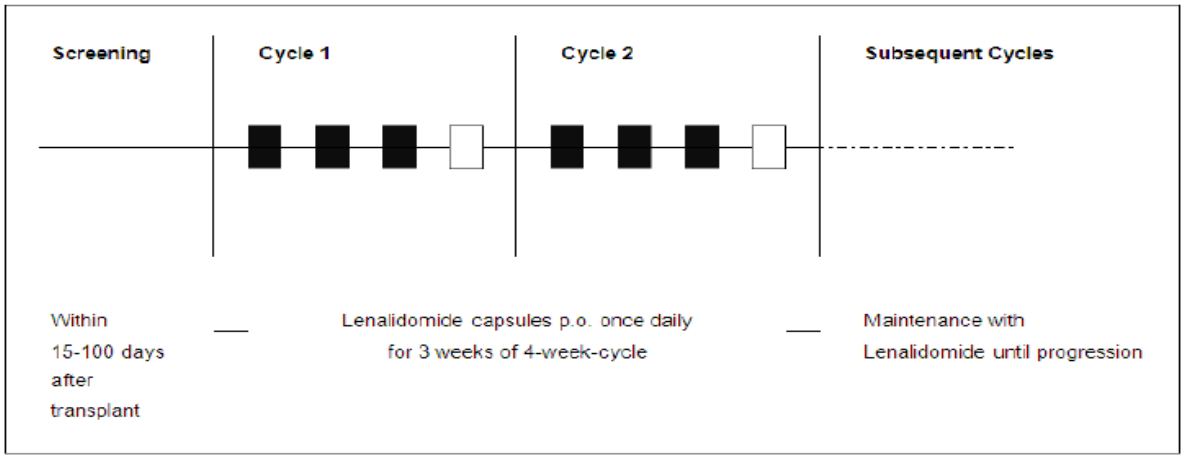
- Assign a patient study number
- Register the patient on the study
- Fax or e-mail the patient study number to the participating site
- Call the research nurse or data manager at the participating site and verbally confirm registration.

Once patients are found eligible for the study, they will be registered with the Montefiore-Einstein Cancer Center Coordinating Center. A patient number will be assigned, and a copy of appendix B including the patient number will be faxed back to the participating center/investigator.

5. TREATMENT PLAN

Treatment will be administered on an outpatient basis. Lenalidomide 2.5 mg, 5 mg, 10 mg, 15 mg and 25 mg capsules are to be taken orally (PO). In Phase I, the starting dose of Lenalidomide is 10 mg taken PO daily for 3 three consecutive weeks (Days 1-21) following one week off therapy (Days 22-28). One cycle is 4 weeks (Figure 1).

Figure 1: Phase 1 and Phase 2 Lenalidomide Dosing Schema



In phase 1, it is anticipated that 6 - 18 evaluable patients will be treated. Three to six patients will be treated at each dose level of three dose levels of Lenalidomide (10 mg, 15 mg, 25 mg) daily doses (Table 1). The initial dose is 10 mg. Patients will receive repeated cycles of Lenalidomide until disease progression or unacceptable toxicity. If no Lenalidomide related Dose Limited Toxicity (DLT) is seen in three patients at a dose level during first cycle (by day 28), the escalation to the next dose level is allowed. Each cohort will be monitored for a DLT during the first cycle before deciding to escalate the dose for the next cohort.

Table 1: Phase 1 Dose ranging

Dose Level	Daily Dose (once a day)	Number of Patients	Escalation Rule *
-1	5 mg	3 – 6	If 0/3 DLT or 1/3 DLT add 3 patients
1 (Starting Dose)	10 mg	3 – 6	If 0/3 DLT escalate 1/3 DLT add 3 patients
2	15 mg	3 – 6	If 0/3 DLT escalate 1/3 DLT add 3 patients
3	25 mg	3 – 6	If 0/3 DLT *or* 1/3 DLT add 3 patients

* Dose escalation rules are described in details in Table 2.

Dose escalation determination is summarized in Table 2. If one DLT occurs at a dose level, up to 3 additional patients will be added to that cohort and if one or more of this group suffers DLT, then dose escalation is stopped, and this dose is declared the Maximally Tolerated Dose (MTD). Three additional patients will be entered at the next lower dose level if only three patients were treated previously at that dose.

Table 2: Determination of Lenalidomide Capsules Maximum Tolerated Dose (MTD)

Number of Patients with DLT at Given Dose Level	Dose Escalation Rule
0 out of 3	Enter three patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared MTD. Three additional patients will be recruited at the next lower dose level if only three patients were treated previously at that dose.
1 out of 3	Recruit at least three more patients at this dose level. • If none of these patients experience with DLT, proceed to the next higher dose level • If one or more of this group suffer DLT, then dose escalation is stopped and this dose is declared MTD. Three additional patients will be entered at the next lower dose level if only three patients were treated previously at that dose.
≤ 1 out of 6 at highest dose level below the MTD	This will be the recommended Phase 2 dose. At least six patients must be recruited at the recommended Phase 2 dose.

If no DLT is observed at the final 25 mg dose level in the first three patients, three additional patients at the 25 mg dose level will be recruited. In Phase 1, in order to declare Therapeutic Dose Level (TDL), a minimum of six patients must be recruited at that dose level. Therapeutic

Dose Level (TDL) is defined as the dose level where DLT occurred in less or equal to one out of six patients below the MaximallyTolerated Dose level.TDL will be the recommended dose for Phase 2.

If a DLT is observed in two or more patients at the first dose level (10 mg), then dose level -1 (5 mg) will be tested with three patients first. Three additional patients will be enrolled at the 5 mg dose, if one or less DLT is observed. If one or more of this group suffers DLT, then the study is stopped and lenalidomide maintenance for elderly AML patients post autologous transplant is not deemed feasible (same as if ≥2 had a DLT to initial cohort of 5 mg).

Decisions about DLTs, expansion of a cohort to six subjects, escalation to the next dose level, or reduction to the next lower level will be made by Principle Investigator.

In Phase 1, subjects will continue study drug, lenalidomide until disease progression or unacceptable toxicity. Reported adverse events and potential risks for lenalidomide are described in Section 2.5. Dose modifications are described in Sections 5.1 and in Table 3. Subjects whoare withdrawn without a DLT before completing Cycle 1 will not be used to evaluate theMTD or TDL, but will be included in the overall safety analysis.

In Phase 2, patientswill receive the lenalidomide dose recommended in the Phase 1 portion. 30 patients will be enrolled in Phase 2. Study procedures are the same as the Phase 1 portion. Subjects will be able to continue the study treatment until disease progression or unacceptable toxicity. Detailed discontinuation information is provided in Section 5.4.

A new course of treatment may begin on the scheduled day 1 of a new cycle if;

- Absolute neutrophil count ≥ 1,000 / mcL
- Platelets count ≥ 50,000
- Resolution of adverse events to ≤ grade 1 (Details in Section 5.1).

5.1 Dose modifications

At Phase 1, subjects who develop any DLT during cycle 1 will be taken off the study. During cycle 2 and onward, dose reductions are allowed. Dose reduction rules are the same for subjects enrolled in Phase 2 (Table 3). Reduced doses will not be re-escalated for any subject during the study. Subjects may not continue Lenalidomide after the third dose reduction or beyond dose of 2.5 mg.

Table 3: Lenalidomide Capsules Dose Modifications

Original Daily Dose (mg)	First Dose Reduction (mg)	Second Dose Reduction* (mg)	Third Dose Reduction* (mg)
5	2.5	Discontinue	N/A
10	5	2.5	Discontinue
15	10	5	2.5
25	15	10	5

* Subjects may not continue Lenalidomide after third dose reduction or beyond dose of 2.5 mg.

A non-hematologic DLT is defined as a National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Events (CTCAE) Version 4 (Oct/09) Grade 3 or 4 adverse events (AE) considered related to the study drug. Grade 3 anorexia and Grade 3 fatigue are excluded since it is very common to develop anorexia and fatigue during any chemotherapy.

A hematologic DLT is defined as a grade 4 hematologic toxicity complicated by infection, severe hemorrhage, or marrow aplasia persisting greater than 2 weeks considered related to the study drug.

5.1.1 Dose Modification for hematologic toxicities after cycle 1 (for Phase 1) and during allcycles (for Phase 2):

If the ANC is less than 500/µL or the platelet count is less than 30,000/µL, then the study drug may be held for up to 8 weeks. Study drug may be re-instituted at the next lower level (i.e. from 15mg to 10 mg) if ANC is ≥ 500/ µL or the platelet count is ≥ 30,000/µL. If however, after an 8 week treatment delay, the ANC remains <500/µL or the platelet count <30,000/ µL, the patient will be removed from protocol therapy.

5.1.2 Dose Modification for Non-Hematologic Toxicities after cycle 1 (for Phase 1) and during all cycles (for Phase 2):

Neurologic Toxicity

If a patient experiences ≥ 3 neurologic toxicity, then the study drug may be held for as many as 8 weeks. If toxicity resolves to ≤ 1, then the study drug may be reinstituted at the next lower dose. If, however, after an 8 week treatment delay, the toxicity does not resolve to ≤ 1 the patient will be removed from protocol therapy.

Cardiac Toxicity

If a patient experiences ≥ 2 cardiac toxicity, then the study drug may be held for as many

as 8 weeks. If toxicity resolves to ≤ 1, then the study drug may be reinstituted at the next lower dose. If, however, after an 8 week treatment delay, the toxicity does not resolve to ≤ 1 the patient will be removed from protocol therapy.

Venous Thrombosis
Patients who develop signs or symptoms suggestive of thrombosis should be evaluated and treated as clinically indicated. Lenalidomide should be held for patients with venous thrombosis. Lenalidomide may resume when patient are adequately anti-coagulated. Patients with recurrent thrombosis despite adequate anticoagulation should be removed from protocol therapy.

Renal Toxicity
For creatinine clearance (CrCI) < 30 mL/min skip lenalidomide and reassess in four weeks. If CrCI remains < 30 mL/min after four weeks, then protocol therapy should be terminated.

For CrCI < 60 mL/min but 2: 30 mL/min:
Decrease lenalidomide to next lower dose level. Reassess after four weeks and attempt to re-escalate lenalidomide.

Other Non-Hematologic Toxicity
For other grade 3 non-hematologic toxicity,lenalidomideshould be held until toxicity resolves to ≤ grade 2, then the study drug should be resumed at the next lower dose level. Subjects are not allowed to continue study treatment if toxicity persists more than 8 weeks.

For patients who develop grade ≤ 2 non-hematologic toxicity, an attempt will be made to maintain the patient at that dose level. If the patient cannot tolerate this dose level, the treating physician should decrease the dose to the next lower dose level.

For other grade 4 non-hematologic toxicity, discontinue study drug and contact the Study Chair or Co-Chair.

In the event of any grade 1 or 2 toxicity that the patient finds intolerable, the study drug may be held until the toxicity resolves and the study drug resumed at the next lower dose level. Alternatively, the study drug may be continued at the next lower dose level without cessation of study drug. However, study drug should be held for the occurrence of a rash consistent with evolving Stevens-Johnson syndrome or toxic epidermal necrolysis (bullous, blistering that is purpuric in nature) until appropriate evaluation is made. Study drug may be held for up to 8 weeks. Contact the Study Chair or Co-Chair for consultation.

5.2 Concomitant Medications

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Participants who receive radiation therapy or chemotherapy after transplantation areconsidered to have treatment failure, unless the radiation was pre-planned consolidation and that decision was made before transplantation.

Any other treatments (including drugs, biological products, and blood products) the patient isreceiving at the time of screening or has received within one tofour weeks prior to registration andduring therapy should be recorded on the concomitant medications page of the case reportforms (CRFs). Use of colony stimulating factors (G-CSF or GM-CSF) and Erythropoietin are prohibited during the study. Subjects who need colony stimulating factors or erythropoietin should be taken off from the study.

All concomitant medications and supportive therapy received during studytreatment must be recorded.

5.3 Supportive Care

Prophylactic aspirin or low molecular weight heparin are to be given to all patients receiving lenalidomide (as DVT prophylaxis) unless contraindicated. Prophylactic blood product support is discouraged however blood product transfusions are allowed for symptomatic patients who have clinically significant anemia or thrombocytopenia.

5.4 Removal from the study

Subjects may take the study drug indefinitely unless removed or withdrawn from the study.The following are criteria for removal from the study:

- Disease progression. Disease progression is defined as a bone marrow biopsy showing >5% myeloblasts.
- Intercurrent illness that prevents further administration of treatment,
- Occurrence of any adverse event (AE), intercurrent illness or abnormality in laboratory assessmentresults which, in the opinion of the investigator, warrants the subject’s permanentwithdrawal from the trial
- Treatment with a prohibited concomitant medication other than the use of appropriatemedications for the treatment of adverse events under direction of the

investigator (Protocol section 5.2)

- Participant becomes pregnant
- Participant is lost to follow-up
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

If a participant discontinues the trial prematurely, the reason given must be fully evaluated and recorded appropriately in source documents and the CRF. If the subject is being withdrawn because of an adverse event (AE), that AE should be indicated as the reason for withdrawal.

All participants have the right to withdraw at any time during treatment without prejudice.The investigator can discontinue a subject's participation in the trial at any time ifmedically necessary. The Principal Investigator should be notified promptly when a subject is withdrawn.

6 SCHEDULE OF EVENTS

Study procedures are same for subjects enrolled in Phase 1 and Phase 2(Table 4).

6.1. Screening Period (Post-transplant Day15 to Day100)

Subjects will be screened post autologous transplant after engraftment and up to day 100 of transplant which will qualify patients for study. Patients without adequate platelet count and/or WBC (see inclusion/exclusion criteria Sections 3.1 and 3.2) by day 100 will not be eligible for study.

A post-transplant bone marrow aspirate and biopsy will be required to document remission (defined here as <5% myeloblasts and CBC parameters as per Sections 3.1 and 3.2) before registration. Study procedures and information regarding the nature of the study will be reviewed with potential subjects and written informed consent will be obtained prior to any study related procedures. If deemed eligible, the followingdata will be obtained:

- Informed consent.

- Demographic information.
- Medical history, including previous treatment history (disease characteristics).
- A physical examination, including weight and height.
- Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Karnofsky performance status will be assessed. Data from Karnofsky status at initial diagnosis will also be obtained if available.
- Concomitant medications within four weeks prior to registrationwill be recorded.
- Adverse events will be assessed.
- Subjects will be instructed to avoid any restricted medications.
- 10ml of Bone marrow Aspirate will be sent to Amit Verma's laboratory (for shipping and handling instruction see Appendix C).
- Buccal Swab.
- Serum β-hCG for all female subjects.Serum β-hCG must be performed within 24 hours prior to prescribing lenalidomide. Subjects may not receive lenalidomide until the Investigator has verified that the results of these pregnancy tests are negative.

6.2. TreatmentPeriod

Cycle 1 - Day 1 (Week 1) visit:

All assessments must be completed prior to dosing with Lenalidomide. If Cycle 1 Day 1 date is no more than three days from screening laboratory assessments, then labs performed during screening may be used.

Assessments required for Cycle 1 Day 1:

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Start of Lenalidomide treatment

Cycle 1 - Week 2 &Week 3 visits:

All assessments must be completed on planned day of the week (± 1 day). Assessments required for Cycle 1 - Weeks 2 and 3:

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Lenalidomide treatment
- Review of Pill diary

Cycle 1 – Week 4 visit:

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Review of Pill diary

Cycle 2 - Week 1 Visit:

All assessments must be completed on planned day of the week (± 1 day). Assessments required for Cycle 2 - Weeks 1 visit:

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Lenalidomide treatment
- Review of pill diary

Cycle 2 - Week 3 Visit:

All assessments must be completed on planned day of the week (± 1 day). Assessments required for Cycle 2 - Weeks 3 visit:

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Lenalidomide treatment
- Review of pill diary

Cycle 3and onward - Week 1 Visits:

Beginning with cycle 3, study visits will be performed once every cycle, during the first week. Subjects will continue study treatment with Lenalidomide on *three-weeks-on, one-week-off* schedule. All assessments must be completed on planned day of the week (± 2 days). Assessments required for Cycle 3 - Weeks 1 visit:

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Lenalidomide treatment
- Review of pill diary

Bone marrow biopsies

Bone Marrow biopsy should be performed prior to or during Cycle 4 Week 1 visit, and should be repeated every four cycles during the first year, and every 6-8 cycles thereafterat the discretion of investigator, until disease progression.

6.3 End of Study Visit:

An End of study visit should be performed within two weeks after last dose or subject's removal decision. Criteria for removal from the study are outlined on Section 5.4.

- Physical Examination
- Karnofsky performance status

- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment
- Serum β-hCG for all female subjects.

6.4 Follow-up period

Follow-up Visits:

Any adverse eventthat occurred during the study treatment should be monitored by follow-up visits until it resolves to grade 1 or less, or until a new therapy is started. In case of an unresolved adverse event, a first follow-up visit must be scheduled no later than 30 days after end of study visit. Depending on the nature of the adverse events, follow-up visits should be scheduled regularly per investigator's discretion. If a subject is taken off the study before disease progression, bone marrow aspiration biopsies should be performed as described in Appendix C.Assessments required but not limited, during follow-up visits are;

- Physical Examination
- Karnofsky performance status
- Vital signs (blood pressure, heart rate and temperature)
- Complete Blood counts with differential and serum chemistries (see Appendix D for details).
- Concomitant medications assessment
- Adverse events assessment

Phone follow-ups:

After follow-up visits, subjects should be contacted to gather survival data every six months. Secondary malignancy information should also be obtained during phone follow-ups. Any secondary malignancy should be recorded and reported to coordinating center.

Table 4: Study Calendar

	Screening	Cycle 1 (Weeks)				Cycle 2 (Weeks)			Cycle 3	Cycle 4& onward	End of Study [5]	Follow up Visits [7]	Phone Follow-up [8]
		W1	W2	W3	W4	W1	W2	W3					
Informed Consent	X												
Demographic Information	X												
Medical History	X												
Physical Examination	X	X	X	X	X	X		X	X	X	X	X	
Performance Status (KPS)	X	X	X	X	X	X		X	X	X	X	X	
Vital Signs	X	X	X	X	X	X		X	X	X	X	X	
CBC with differential [1]	X	X[4]	X	X	X	X		X	X	X	X	X	
Serum chemistry [1]	X	X[4]	X	X	X	X		X	X	X	X	X	
Concomitant Medications [2]	X	<-	-	-	-	-	-	-	-	-	->	X	
Adverse Event Assessment [2]	X	<-	-	-	-	-	-	-	-	-	->	X	
Buccal Swab	X												
Bone Marrow Aspiration Biopsy [3]	X									X[3]			
Lenalidomide Treatment		X	X	X		X	X	X	X	X			
Review of pill diary			X	X	X	X		X	X	X	X		
Urine β-hCG [6]	X										X		
Instructions for restricted drugs [2]	X	<-	-	-	-	-	-	-	-	-	->	X	
Secondary malignancy reporting												X	X
Survival Information												X	X

See next page for footnotes.

Footnotes:

1. Lab requirements are outlined in Appendix D.
2. Continuous collection of data during the study.
3. Must be performed at baseline, prior to or during Cycle 4 Week 1 visit, and be repeated every four cycles during the first year, and every 6-8 cycles thereafter in the discretion of investigator until disease progression.
4. If Cycle 1 Day 1 date is no more than three days from screening laboratory assessments, then labs performed during screening may be used.
5. Must be performed within two weeks after last dose or subject's removal decision.
6. Required for all female subjects. Must be performed within 24 hours prior to prescribing lenalidomide.
7. Any adverse event occurred during the study treatment should be monitored by follow-up visits until resolves to grade 1 or less, or until a new therapy is started. In case of an unresolved adverse event, first follow-up visit must be scheduled no later than 30 days after end of study visit. Depending on the nature of the adverse events, follow-up visits should be scheduled regularly per investigator's discretion. . If a subject is taken off the study before disease progression, bone marrow aspiration biopsies should be performed as described in Appendix C of protocol.
8. Subject must be contacted every six months.

7 CORRELATIVE/SPECIAL STUDIES

Correlative studies will be performed on all subjects. Specifically, our aims will include:

Aim 1: To determine role of residual AML stem cells on efficacy of Lenalidomide maintenance after autotransplant.

Aim 2: To determine molecular markers of clinical response after Lenalidomide maintenance in AML

Myeloid malignancies can arise from a small population of quiescent cancer-initiating cells that are not eliminated by conventional cytotoxic therapies. Eliminating leukemia stem cells can reduce the rates of relapse and increase leukemia free survival. Thus it is important to evaluate the role of persistent leukemia stem cells in the efficacy of Lenalidomide maintenance regimens.

Aim 1: Analysis of AML stem cells pre and post treatment with lenalidomide and their correlation with response (Study co-investigator Dr. Ulrich Steidl is an expert in flow sorting of leukemia stem cells and their functional characterization, both in vitro and in vivo). Using high-speed multi-parameter fluorescence-activated cell sorting we will separate bone marrow progenitors into long-term and short-term hematopoietic stem cells as well as more committed myeloid, erythroid and megakaryocytic progenitors, as performed previously[16,17]. Sorted cells will have FISH studies done on them to estimate the number of stem cells with clonal cytogenetic alterations (Leukemic stem cells). Functional assays will also be done on these sorted cells to demonstrate clonogenic potential in vitro. A recently discovered marker of AML stem cells and putative predictor of overall survival, IL1RAP[15], will also be evaluated on AML stem cells and correlated with clinical response.

Our preliminary studies indicate that primitive stem cells (LT-HSCs and ST-HSCs) and certain progenitors (GMP) are expanded in AML and harbor initiating karyotypic abnormalities and striking alterations in DNA methylation with an accumulation of aberrantly hypermethylated loci. [14] Furthermore, we and others observe that karyotypically abnormal stem cells persist in the bone marrow even after morphological complete remission and thus may potentially lead to relapse of AML[18]. These preliminary data lead us to hypothesize that a pool of disease-initiating stem or early progenitor cells exists in AML that contains and progressively acquires genetic and epigenetic alterations and may persist after conventional treatments. The study of these AML cells-of-origin and disease-initiating cell populations will reveal fundamentally new information about the persistence and elimination with lenalidomide maintenance treatment,
Quantitative and cytogenetic assessment of AML stem and progenitor cells from marrow samples at the pretreatment and relapse stages will be performed. Karyotypic, transcriptomal, as well as methylomic analysis of these cells will be conducted to assess clonality and epigenetic profile . **Comparison of pretreatment and post treatment samples will demonstrate whether a decrease in clonal AML stem cells correlates with clinical response.** We will also determine whether persistence of clonal stem cells in responders correlates with a risk of future relapse.

Also, a recently discovered marker of AML stem cells, IL1RAP[2], will be evaluated on bone marrow aspirate cells, in combination with other known stem cell markers. IL1RAP expression on phenotypic stem cells and the frequency/kinetics of such cells upon treatment will be correlated with clinical outcomes.

Aim 2: Molecular markers of relapse and remission:

a. **Whole transcriptome and exome sequencing** of AML bone marrow stem and progenitors and correlation with response. Bone marrow aspirates will be used for these studies. Bone marrow stem cells will be sorted and RNA and DNA will be isolated. Sequencing libraries will be generated and subjected to massive parallel sequencing using an Illumina HiSeq2000 instrument.. The transcriptome patterns will be correlated with response. In addition to gene expression patterns, this study will also correlate mutations and single nucleotide polymorphisms (from exome sequencing) with response to lenalidomide. Somatic mutations will be identified by comparison of marrow and germline (toenail clippings or cheek swabs) samples.

b. **Methylome profiling and clinical response:** DNA methylation has been shown to have clinical relevance and can be used to define AML patients into prognostic subgroups.[19,20] DNA isolated from AML stem and progenitor cells will be used for global methylome analysis as done previously.[21,22] Methylation patterns will be correlated with response and overall survival.

A bone marrow (BM) aspirate will be performed within the framework of the routine staging as outlined in Section 6.2. An extra tube of 10cc will be sent within 24 hours to the Albert-Einstein College of Medicine Department of Molecular Biology Laboratory where they will be processed and centrally analyzed. A detailed description of specimen collection, handling and shipping can be found in **Appendix C.**

In addition to bone marrow aspiration buccal swabs with brushes will be done at baseline and stored in RNA later solution. RNA/DNA will be isolated from these samples and compared to bone marrow samples to determine somatic mutations in AML stem cells.

8 MEASUREMENT OF EFFECT

8.1 Response Assessment

For the purposes of this study, subjects' bone marrow aspirate and biopsies will be used at regular intervals to assess progression. Please see Section 6.2 for the schedule.

8.2 Survival End Points

Progression-Free Survival

Progression free survival (PFS) is defined as the duration of time from starting lenalidomide to time of progression.

Progressive disease[a] is defined by the following:

- 5% myeloblasts on bone marrow aspirate and biopsy.

Abbreviations: CR, complete response; DFS, disease-free survival.
[a]All relapse categories require two consecutive assessments made at anytime before classification as relapse or disease progression and/or theinstitution of any new therapy.

Event-free survival (EFS): The definition of an event is 1) disease progression or 2) death from any cause. EFS is the time from stem cell transplant until an event as defined above has occurred.

TTP (Time to Progression): This is the time from start of treatment to diseaseprogression with deaths owing to causes other than progressionnot counted, but censored. This is a helpful method toassess the durability of treatment benefit.

Overall survival (OS) is defined as the time from transplant until death of any cause.

9. DATA REPORTING

9.1 Definitions

Adverse Event: An Adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of lenalidomide whether or not considered related to lenalidomide.

During clinical trials, adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.)

AEs will be recorded in the case report form for the duration of the trial, regardless of whether or not the event(s) are considered related to trial medication. All AEs considered related to trial medication will be followed until resolution even if this occurs post-trial.

Serious adverse event (SAE)

An adverse event occurring at any dose that results in any of the following outcomes (CFR 312.32):

- death
- a life-threatening adverse drug experience
- inpatient hospitalization or prolongation of existing hospitalization excluding those for study drug administration, transfusional support, disease staging/re-staging procedures, bone marrow biopsy or placement of an indwelling catheter, unless associated with other serious events.
- persistent or significant disability or incapacity,
- congenital anomaly / birth defect.

The definition of serious adverse event (experience) also includes *important medical event*. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening orresult indeath or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. A new diagnosis of cancer during the course of treatment should be considered an important medical event.

The definition of "related" being that there is a reasonable possibility that the drug caused the adverse experience**.**

Unexpected Adverse Event

An adverse event that is not mentioned in the Investigator's Brochure or package insert or the specificity or severity of which is not consistent with the investigator's brochure or package insert.

Life-threatening

Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

Adverse events (AEs) will use the descriptions and grading scales found in the revised

Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

9.2 Determination of reporting requirements

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

Step 1:*Identify the type of event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0*. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov). Additionally, if assistance is needed, the NCI has an Index to the CTCAE that provides help for classifying and locating terms. All appropriate treatment locations should have access to a copy of the CTCAE.

Step 2:*Grade the event using the NCI CTCAE*.

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

Step 4: Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is consideredunexpected, for expedited reporting purposes only, when either the type of event is ***NOT***listed in section 2.5or in drug-insert.

Step 5: Review the ”Additional instructions, requirements, and exceptions for this protocolspecific requirements for expedited reporting of specific adverse events that require specialmonitoring.

Step 6: Determine if the protocol treatment given prior to the adverse event included investigational agent(s), a commercial agent(s), or a combination of investigational and commercial agents.

9.3 Reporting methods

Adverse event log form (using Montefiore-Einstein AE form) should be faxed or emailed to the Montefiore Medical Coordinating Center.

9.4 When to report an event in an expedited manner

This trial will be monitored by the Albert Einstein Cancer Center Data Safety Monitoring Committee (AECC DSMC). Data monitoring plan is provided in Section 11.1.

Follow guidelines in section 9.5 regarding criteria that required reporting of adverse events that required require 24-hour notification.

For any adverse events that occur more than 30 days after the last dose of treatment, only those that have an attribution of possibly, probably, or definitely must be reported on an expedited adverse event report form.

9.5 Expedited reporting

This section outlines adverse events that require expedited reporting.

The following be reported as a SAE:

- **Grade 2 (moderate) and Grade 3 (severe) Events** – Only events that are Unexpected and Possibly, Probably or Definitely Related/Associated with lenalidomide.

- **ALL Grade 4 (life threatening or disabling) Events** – Unless expected AND specifically listed in protocol as not requiring reporting (see section 9.8).

- **ALL Grade 5 (fatal) Events** – When participant is enrolled and actively participating in the trial OR when event occurs within 30 days of the last study intervention.

MEDWATCH 3500A form will be used to report SAEs to Montefiore Medical Coordinating Center and to Celgene Corporation for this study.

Expedited AE reporting timelines:

24 Hours; 5 calendar days – The investigator must initially report the AE within 24 hours of learning of the event followed by a completed AE report within 5 calendar days of the initial 24-hour report.

10 calendar days – A complete AE report on the AE must be submitted within ten calendar days of the investigator learning of the event. Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates **hospitalization (or prolongation of existing hospitalization)** must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol specific expedited adverse event reporting exclusions (Section 9.8)

Hospitalizations are defined as lasting 24 hours or longer and these events must be reported via an expedited manner.

Any event that results in persistent or significant disability/incapacity, congenitalanomaly, or birth defect must be reported via an expedited AE mechanism.

Additional instructions, requirements and exceptions for this protocol:

With respect to determining the specific day by which the event must be reported, the day the reporter learns of the adverse event constitutes “Day 0”.

9.6 Reporting Second Primary Malignancies (SPMs)

SPMs are considered events of interest and should be included as part of the assessment of adverse events throughout the course of the study. Investigators are to report any second primary malignancies as serious adverse events regardless of causal relationship to lenalidomide, occurring at any time for the duration of the study. For all subjects who develop second primary malignancies, sites will be required to submit all diagnostic reports (e.g. pathology, cytogenetics, flow cytometry results) from the indication diagnostic confirmation samples submitted at screening and all reports for the tumor samples from the SPM diagnosis. For SPMs diagnosed at another institution (outside the investigational site) sites are to make every effort to obtain these reports for the SPM confirmation.

9. 7 Reporting Pregnancies

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on lenalidomide, or within 30 days of the subject’s last dose of lenalidomide, are considered immediately reportable events. Lenalidomide is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Montefiore Medical Center Coordinating Center AND Celgene Drug Safety immediately by facsimile, or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form. Local IRB should be notified per institutional guidelines. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must notify Montefiore Medical Center Coordinating Center AND Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form, or approved equivalent form. If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Montefiore Medical Center Coordinating Center AND Celgene Drug Safety immediately by facsimile, or other

appropriate method, within 24 hours of the Investigator's knowledge of the event using MEDWATCH 3500A form.

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the in utero exposure to lenalidomide should also be reported to Montefiore Medical Center Coordinating Center AND Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using MEDWATCH 3500A form.

9.8 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require reporting in an expedited manner. The following AEs (Table 5) must be reported through the routine reporting mechanism (Section 9.1):

Table 5: Expedited Adverse Event Reporting Exclusions

CTCAE Category	Adverse Event	Grade	Hospitalization / Prolongation of Hospitalization	Attribution	Comments
Blood and lymphatic system	Anemia	3-4	n/a	Lenalidomide	lenalidomide produces expected myelosuppression.
Blood and lymphatic system	Neutropenia	3-4	n/a	Lenalidomide	As above; report only if longer than 15 days unless related to relapsed AML
Blood and lymphatic system	Thrombocytopenia	3-4	n/a	Lenalidomide	As above; report only if longer than 15 days unless related to relapsed AML

9.9 Responsibilities of Participating Sites

Adverse events will be reported to each participating institution according to protocolguidelines and local policies and procedures governing the local Institutional Review
Board.

A principal Investigator in a participating institution is required to notify Institutional Review Board (IRB) of a serious adverse event according to institutional policy.

A copy of all SAE's as defined in section 9.1 should be faxed to the Coordinating Center, Montefiore Medical Center within two working days by the participating site. All Events described in protocol sections 9.6 and 9.7 must be reported via an expedited AE mechanism.

Participating study sites should NOT report SAEs to the FDA. Rather, participating sites should report SAEs to the primary study site, Montefiore Medical Center and to Celgene. The primary site will be responsible for reporting to FDA.

9.10 Responsibilities of the Coordinating Center (Montefiore Medical Center) and CelgeneCorporation.

The Coordinating Center (Montefiore Medical Center) will maintain records andprovide reports to Celgene Corporation for the following Adverse Experiences:

1. All serious, unexpected, adverse events,
2. Any significant increase in the frequency of serious expected adverse events, and
3. An annual progress report including number of patients accrued, date on study, date off study, vital status (alive/dead and date alive/dead).

Celgene Corporation willsupply study drug, lenalidomide free of charge.

10. STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

The aim of this proposal is to provide an accurate estimate of the two-year relapse free survival rate for elderly AML patients treated with lenalidomide maintenance after autologous stem cell transplantation. With about 30 patients, the study will be able to estimate the two year relapse free survival rate with not more than 9% standard error(i.e., if the observed rate is 40%, the 95% CI will be (23%, 56%). In addition, our study will have over 80% power to conclude that the two year relapse rate is significantly reduced when patients are treated with lenalidomide, assuming 60% and 90% patients will relapse with and without lenalidomide, respectively. The observed relapse free survival rate will be calculated along with its 95% CI. A one sample test on proportion will be used to detect if the relapse free survival rate with lenalidomide is significantly higher than that without the treatment (relapse rate is expected to be >95%).Secondary analysis including Kaplan-Meier analysis on overall survival.

10.2 Sample Size/Accrual Rate

The primary and coordinating site will be the Montefiore-Einstein Cancer Center (MECC). The Bone Marrow Transplantation team at MECC sees on average 15 patients per year that would meet the eligibility criteria for the study.

We anticipate an annual accrual of 8 patients. The projected time for completion of the study is 45 months. Should the accrual be less than 8 patients per twelve month period from the start of activation of the protocol, we will be actively approaching other sites to participate in the study. Montefiore is a founding member of the Tri-State Transplant Consortium as well as a member of the Blood and Marrow Transplant Clinical Trials Network. Both of these cooperative groups may be utilized as sources of collaboration to boost accrual.

11 Data Management and Regulatory Requirements:

Data on toxicities and patient outcomes will be collected at the individual participating institution treating the patient on protocol. Every 4 weeks the collected data will be submitted to the Montefiore-Einstein Cancer Center Research Study Coordinator Lawrence Almanzar. Data submitted as Case Report Forms (CRF)will be submitted by participating institutions for central data management to the Montefiore-Einstein Cancer Center Research Study Coordination Center on a monthly basis, where they will be merged with the other data available for each study participant into one master data set.

11.1 Data Safety and Monitoring Plan

During the recruitment and follow up phases of the trial, the Albert Einstein Cancer Center Data and Safety Monitoring Committee (DSMC) will monitor data and oversee patient safety. The principal investigator will participate in the meetings as a non-voting member. The DSMC meets every monthand will review adverse events. DSMC will also reviewadherence to protocol and study progresson a quarterly basis. All adverse events will be reported, as per policy, to the CCI/IRB. The DSMC will make recommendations regarding study performance and continuation. Ira Braunschweig will provide requested data to the DSMC to assess progress toward resolving research questions.

In addition, Dr. Ira Braunschweig (PI) will review case report forms (CRFs) and any other toxicity data from all patients enrolled in this trial at regular frequent intervals. Biweekly conference calls will be conducted between the participating institutions to discuss trial patient enrolment, adverse events and protocol adherence.

Each participating investigator will have primary responsibility for the safety of individual participants under his/her care and will review the data and safety on an ongoing basis. In addition, the independent DSMC will have primary responsibility for monitoring the accumulating study data for sign of adverse trends in morbidity /

mortality and drug toxicity.

11.2 Recruitment and Consent

Patients who are identified by clinicians at the participating sites as being eligible for the study will be approached by the local PI or co-PI about their potential participation. An informed consent document in English and Spanish will be provided to the patient. The investigational nature of the study, its objectives, the procedures and the potential risks and benefits involved will be explained to the patient in simple and direct lay language avoiding medical terms. The patient and the person taking the consent will sign the consent form and a copy of it will be given to the patient. A copy of the consent and the protocol will be left in the patient's chart.

11.3 Patient Protection

This study will be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements. All research personnel will receive the required education and training needed for conducting clinical research related to the protection of human subjects and personal health information according to the Institutional Review board and the Health Insurance Portability and Accountability Act of 1996 Public Law 104-191. As mentioned above, each patient will be assigned a study identification number at the time of recruitment. Records will be kept confidential. As this research involves a drug, the U.S. Food and Drug Administration (FDA) may inspect the research records and medical records as may the Office for Human Research Protections (OHRP) and employees fromCelgene Pharmaceutical Co, Ltd. The research study doctors and research staff will review medical records and will keep the information private. No patient will be identified in any written or verbal report with the following exception: the local human research committees of the participating institutions may inspect the patient records. All hard copies of information will be stored in locked cabinets, to which only senior research personnel has access. The study data files will be stored on password protected computers.

11.4 Record Retention:

U.S. FDA regulations (21CFR312.62[c] require all records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consents forms, laboratory test results and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the investigation is discontinued and the FDA and the applicable local health authorities are notified.

11.5 Study Monitoring:

Site visits can be conducted by an authorized representative of the PI, Dr. Ira Braunschweig to inspect study data, subjects' medical records, and CRFs in accordance with ICH and GCP guidelines.

APPENDIX A

Performance Status Criteria

Karnofsky Performance Scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B – FORM 1 (REGISTRATION AND ELIGIBILITY)

SUBJECT Initials: _____ MR# _____ DOB: _____

CENTER:_____ Date: _____

Treating Physician: _____

"A Phase I/II study of lenalidomide maintenance after autologous stem cell transplant for elderly patients with Acute Myeloid Leukemia (AML)."

Inclusion Criteria: **The answers to the following questions must be YES in order for the patient to be eligible.**	**YES**	**NO**
3.1.1. Patient has a confirmed diagnosis of non-M3 AML. Antecedent MDS is acceptable.		
3.1.2. Post autologous Stem Cell Transplant Bone marrow biopsy core that is consistent with morphologic remission.		
3.1.3. Agebetween 60and 80years old.		
3.1.4. Patient received induction and consolidation chemotherapy, and autologous stem cell transplant for AML.		
3.1.5.Life expectancy of greater than 12 months.		
3.1.6. Karnofsky Performance Status 70 or greater (see Appendix A)		
3.1.7. Patients have normal organ and marrow function as defined below: - Leukocytes ≥2,000/mcL - Absolute neutrophil count≥ 1,000/mcL - Platelets≥ 75,000/mcL - Total bilirubin≤4 X institutional upper limit of normal unless 2nd to Gilbert's disease - AST(SGOT) and ALT(SGPT)≤ 4 X institutional upper limit of normal - Creatinine<1.5 X institutional upper limit of normal ORcreatinine clearance≥ 30 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.		
3.1.8 Able to take aspirin, or warfarin, or low molecular weight heparin as prophylactic anticoagulation.		
3.1.9.Ability to understand and the willingness to sign a written informed consent document.		
3.1.10 Patient will be registered into the mandatory RevAssist® program and be willing and able to comply with the requirement of RevAssist®.		

Exclusion Criteria: **The answers to the following questions must be NO in order for the patient to be eligible.**	**YES**	**NO**
3.2.1. Patient received chemotherapy or radiotherapy within 2 weeks prior to entering the study or has not recovered from adverse events due to agents administered more than 4 weeks earlier.		
3.2.2 Patient received another investigational agent after post autologous stem cell transplant.		
3.2.3 Patient will be receiving another investigational product during the study.		
3.2.4 Patient who is growth factor or transfusion dependent.		
3.2.5Patient has CNS leukemia.		
3.2.6Patient has a history of allergic reactions attributed to thalidomide orlenalidomide.		
3.2.7 Patient has a history of erythema nodosum, characterized by a desquamating rash while taking thalidomide or similar drugs.		
3.2.8History of metastatic malignancy.		
3.2.9Presence of uncontrolled illness including, but not limited to ongoing or active infection, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Patients must not have suffered recent (< 6 months) myocardial infarction, unstable angina, uncontrolled hypertension, or difficult to control cardiac arrhythmias.		
3.2.10 Evidence of uncontrolled Congestive Heart Failure (CHF).		
3.2.11Active Hepatitis B as defined by Hepatitis B surface antigen positivity, unless able to start dual anti-HepB therapy, or already on dual anti-HepB therapy		
3.2.12Patientis positive for Hepatitis B core antibody, but negative for the hepatitis B surface antigen, and is on lamivudine 100 mg daily until at least 3 months post-transplant		
3.2.13Patient is positive for HIV or HTLV-1.		
3.2.14 Patient is woman of childbearing potential (defined as a sexually mature woman who has not undergone a hysterectomy or who has had menses at any time in the preceding 24 consecutive months).		
3.2.15 Patient is a man who agreed not to father a child and agreed to use a latex condom during any sexual contact with women of childbearing potential while taking lenalidomide and for 4 weeks after therapy is stopped, even if they have undergone a successful vasectomy.		

Fax Registration Form to: Montefiore-Einstein Coordinating Center (see cover page)
To be completed by Coordinating Center and faxed back to Site:

Patient Eligible: **Yes** **No**_____

Patient ID :_____ **(this should be used as patient identifier)**

___ _____ _____
Coordinating Center Staff Signature **Date**

Page 2 of 2

APPENDIX C

HANDLING AND SHIPPING OF BONE MARROW SPECIMENS:

HANDLING AND SHIPPING OF BONE MARROW AND OTHER SPECIMENS FOR CORRELATIVE STUDIES:

All specimens for correlative studies must be labeled with the patient's code number, which will be assigned by the Data Management Office, and should not bare the patient's identity. The specimens must be kept at room temperature and sent by fedex to:

Amit Verma, MD
Albert Einstein College of Medicine,
1300 Morris Park Ave, Chanin 302B
Bronx, NY 10461
Tel : 718 430 8761
Fax: 718 430 8702
amit.verma@einstein.yu.edu

Bone Marrow Aspirate: 10cc of heparinized marrow aspirate will be required. We will perform flow cytometry and genome wide sequencing from these samples. RNA and DNA from patient bone marrow specimens at baseline will be sequenced to correlate expression patterns and mutational patterns with response. Three unstained slides containing bone marrow aspirate will be required for further studies

1. Prepare a conical tube for the bone marrow specimen by adding 0.5 ml of preservative-free sodium heparin (400 U/ml stock solution) to a 50 ml polypropylene conical tube.
2. Collect **10 cc, if possible** of bone marrow using a needle/syringe with preferably citrate or if citrate is not available, then with heparin, and transfer it to the tube containing heparin.
3. Add RPMI-1640, at 1:1 ratio (i.e.; 20 ml bone marrow plus 20 ml RPMI-1640).
4. Invert the tube to mix thoroughly and minimize clotting potential
5. Screw the cap on the tube, and tightly wrap the lower edge of the cap with parafilm.

Peripheral Blood: If the bone marrow aspirate cannot be obtained then we will require 3 green top tubes, with 8 cc of blood each. Mononuclear cells from patient's peripheral blood will be purified by Ficoll-Hypaque sedimentation and these cells used for flow cytometry and sorting. These samples will also be used for interphase FISH studies (if abnormal cytogenetics found at diagnosis). Cells will be frozen for future RNA extraction to perform gene expression studies.

Buccal swabs: Buccal swabs with brushes will be done at baseline and stored in RNA later solution. RNA/DNA will be isolated from these samples and compared to bone marrow samples to determine somatic mutations in AML stem cells.

Banking of Samples: Peripheral blood, bone marrow, cell lysates, and cell products obtained from the previously mentioned samples will be stored for subsequent research related to this project.

6. * Please page or call Dr. Amit Verma (phone: 646-468-1055) to notify him that a shipment has been sent and for any questions. Sodium heparin (400 U/ml stock solution) to a 50 ml polypropylene conical tube.
7. Collect **10 cc, if possible** of bone marrow using a needle/syringe with preferably citrate or if citrate is not available, then with heparin, and transfer it to the tube containing heparin.
8. Add RPMI-1640, at 1:1 ratio (i.e.; 20 ml bone marrow plus 20 ml RPMI-1640).
9. Invert the tube to mix thoroughly and minimize clotting potential
10. Screw the cap on the tube, and tightly wrap the lower edge of the cap with parafilm.

Appendix D –Required Laboratory Parameters

Complete Blood Count (CBC) with differential:

White Blood Cells (WBC) count
Red Blood Cells (RBC) count
Hemoglobin
Platelet count
Neutrophil (%)
Lymphocyte (%)
Monocyte (%)
Eosinophil (%)
Basophil (%)
Absolute Neutrophil count
Absolute Lymphocyte count

Biochemistries:

Sodium
Potassium
Chloride
$C0_2$
Glucose
Urea Nitrogen
Calcium
Creatinine
Albumin
Bilirubin (total)
Bilirubin (direct)
Alkaline Phosphatase
SGOT
SGPT
Total Protein

Clinical significant lab results must be graded and recorded in Adverse Event Page.

Appendix E – Cockcroft-Gault estimation of CrCl:

Cockcroft-Gault estimation of creatinine clearance (CrCl):
(Cockcroft, 1976; Luke 1990)

$$\text{CrCl (mL/min)} = \frac{(140 - \text{age}) \times (\text{weight, kg})}{72 \times (\text{serum creatinine, mg/dL})} \quad \textbf{(Males)}$$

$$\text{CrCl (mL/min)} = \frac{(140 - \text{age}) \times (\text{weight, kg})}{72 \times (\text{serum creatinine, mg/dL})} \times 0.85 \quad \textbf{(Females)}$$

Appendix F: Risks of Fetal Exposure, Pregnancy Testing Guidelines, Acceptable Birth Control Methods and Precautions.

Risks Associated with Pregnancy

The use of lenalidomide in pregnant females and nursing mothers has not been studied nor has the effect of the lenalidomide on human eggs and sperm. Lenalidomide is structurally related to thalidomide. Thalidomide is a known human teratogenic active substance that causes severe life-threatening birth defects. An embryo-fetal development study in animals indicates that lenalidomide produced malformations in the offspring of female monkeys who received the drug during pregnancy. The teratogenic effect of lenalidomide in humans cannot be ruled out. Therefore, a risk minimization plan to prevent pregnancy must be observed.

All study participants must be registered into the mandatory RevAssist® program, and be willing and able to comply with the requirements of RevAssist®.Criteria for females of childbearing potential (FCBP)This protocol defines a female of childbearing potential as a sexually mature female who:

1) has not undergone a hysterectomy or bilateral oophorectomy or
2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Contraception

Females of childbearing potential (FCBP) enrolled in this protocol must agree to use two reliable forms of contraception simultaneously or to practice complete abstinence from heterosexual intercourse during the following time periods related to this study:

1) for at least 28 days before starting lenalidomide;
2) throughout the entire duration of lenalidomide treatment;
3) during dose interruptions; and
4) for at least 28 days after lenalidomide discontinuation.

The two methods of reliable contraception must include one highly effective method and one additional effective (barrier) method. FCBP must be referred to a qualified provider of contraceptive methods if needed. The following are examples of highly effective and additional effective methods of contraception:

- Highly effective methods:
  - Intrauterine device (IUD)
  - Hormonal (birth control pills, injections, implants)

- Tubal ligation
- Partner's vasectomy

- Additional effective methods:
  - Male condom
  - Diaphragm
  - Cervical Cap

Implants and levonorgestrel-releasing intrauterine systems are associated with an increased risk of infection at the time of insertion and irregular vaginal bleeding. Prophylactic antibiotics should be considered particularly in patients with neutropenia.

Pregnancy testing

Medically supervised pregnancy tests with a minimum sensitivity of 50 mIU/mL must be performed for females of childbearing potential, including females of childbearing potential who commit to complete abstinence, as outlined below.

Before starting lenalidomide

Female Patients:

FCBP must have negative pregnancy test 24 hours prior to prescribing lenalidomide. The patient may not receive lenalidomide until the Investigator has verified that the results of these pregnancy tests are negative.

Male Patients:

Must agree to practice complete abstinence or agree to use a condom during sexual contact with pregnant females or females of childbearing potential throughout the entire duration of lenalidomide treatment, during dose interruptions and for at least 28 days following lenalidomide discontinuation, even if he has undergone a successful vasectomy.

The investigator must ensure that:

- Females of childbearing potential comply with the conditions for pregnancy risk minimization, including confirmation that she has an adequate level of understanding.
- Females NOT of childbearing potential acknowledge that she understands the hazards and necessary precautions associated with the use of lenalidomide.
- Male patients taking lenalidomide acknowledge that he understands that traces of

lenalidomide have been found in semen, that he understands the potential teratogenic risk if engaged in sexual activity with a female of childbearing potential or pregnant female, and that he understands the need for the use of a condom even if he has had a vasectomy, if engaged in sexual activity with a female of childbearing potential or pregnant female.

- Subject is continuing to use two reliable methods of birth control at each visit during the time that birth control is required.
- If pregnancy or a positive pregnancy test does occur in a study patient, lenalidomide must be immediately discontinued.
- Pregnancy testing and counseling must be performed if a patient misses her period or if her pregnancy test or her menstrual bleeding is abnormal. Lenalidomide treatment must be temporarily discontinued during this evaluation.
- Females must agree to abstain from breastfeeding during study participation and for at least 28 days after lenalidomide discontinuation.
- male subjects must practice complete abstinence or use a condom during sexual contact with pregnant females or females of childbearing potential throughout the entire duration of lenalidomide treatment, during dose interruptions and for at least 28 days following lenalidomide discontinuation, even if he has undergone a successful vasectomy.
- If pregnancy or a positive pregnancy test does occur in the partner of a male study patient during study participation, the investigator must be notified immediately.
- Male patients are instructed not to donate semen or sperm during therapy or for at least 28 days following discontinuation of lenalidomide.

Additional precautions

- Subjects should be instructed never to give lenalidomide to another person.
- Subjects should not donate blood during therapy and for at least 28 days following discontinuation of lenalidomide.
- Only enough lenalidomide for one cycle of therapy may be prescribed with each cycle of therapy.
- Any unused lenalidomide must be returned as instructed through RevAssist program.

Appendix G – Drug insert and Full Prescribing Information

Most recent drug insert; highlights and full prescribing information for lenalidomide are available online at:

http://www.revlimid.com/docs/Revlimid-Full-PI.pdf

Appendix H: FDA MEDWATCH 3500A Form:

FDA MedWatch 3500A Form is available online at:

http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/ucm103114.pdf

Appendix I : NCI CTCAE Version 4.0

Toxicity will be scored using NCI CTC Version 4.0 for toxicity and adverse event reporting. A copy of the NCI CTC Version 4.0 can be downloaded from the CTEP homepage: (http://ctep.info.nih.gov).

All appropriate treatment areas have access to a copy of the CTC Version 4.0

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