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TITLE: Phase 2 Study of Mitoxantrone, Etoposide, and Cytarabine (MEC) plus Lenalidomide for the Treatment of Adult Patients with Relapsed or Refractory Acute Myeloid Leukemia

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Other Agents:	Lenalidomide	Supplied by Celgene
	Mitoxantrone	Commercial Supply
	Etoposide	Commercial Supply
	Cytarabine	Commercial Supply

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SYNOPSIS AND SCHEMA

Title:

Phase 2 Study of Mitoxantrone, Etoposide, and Cytarabine (MEC) plus Lenalidomide for the treatment of Adult Patients with Relapsed or Refractory Acute Myeloid Leukemia (AML)

Primary Objective:

The primary objective of this study is to assess rates of complete remission (CR) and rates of complete remission with incomplete platelet recovery (CRp) after treatment with mitoxantrone, etoposide, cytarabine (MEC) and lenalidomide.

Secondary Objectives:

1. To evaluate the days to neutrophil recovery (the first of 3 days of ANC >500).
2. To evaluate the days to platelet recovery (platelet count >20K unsupported).
3. To evaluate the treatment-related mortality (the number of non-relapse related deaths in the first 50 days of starting treatment.)
4. To evaluate the transfusion support (the number of red blood cell and platelet transfusions needed in the first 50 days of treatment).
5. To determine if certain genetic profiles of the leukemia predict for better response (optional correlative study)
6. To evaluate relapse and survival following treatment with MEC and lenalidomide.

Study Design:

This is a single arm Phase 2 study which consists of determining the overall response rate of Lenalidomide + MEC in relapsed or refractory AML patients. A 45% complete remission rate will be considered clinically significant.

Simon's two stage optimal design is used to compute the sample size. A total number of 40 patients is needed in order to detect a 20% CR rate increase assuming the CR for null hypothesis is 25% with a 90% power and 9% type I error. The study will be reevaluated if 4 or fewer responses out of 18 patients are observed and the study will be declared inactive if fewer than 14 responses are seen in the total 40 patients. The probability of stopping at the first stage is 0.52.

Accrual Objective: 40 - 42 patients in order to enroll 40 evaluable patients.

Accrual Period: The estimated accrual period is 2.5 years

Eligibility Criteria:

The following criteria must be met prior to enrolling.

- Acute myelogenous leukemia diagnosed by WHO criteria with one of the following:
  - Primary refractory disease following $\geq$ 1 cycle of induction chemotherapy.

  - First relapse or higher. Patients with primary or secondary acute myelogenous leukemia are eligible. Patients with biphenotypic leukemia are eligible.
- 18-70 years
- LVEF $\geq$ 50%
- ECOG Performance status 0-2
- Creatinine <2.0 mg/dl and eGFR $\geq$ 60 mL/min.
- Total bilirubin < 1.5 x institutional ULN
- AST (SGOT) and ALT (SGPT) $\leq$ 3 x institutionalULN
- Able to adhere to study schedule and other protocol requirements
- Patients may receive hydroxyurea, steroids, or leukapheresis as necessary until Day 5 of treatment.

Treatment Description:

Patients will receive only one cycle of treatment, as below:
1. Lenalidomide Days 1-10
2. Mitoxantrone by IV infusion over approximately 6-10 minutes for 5 days (days 4-8)
3. Etoposide IV over approximately 1 hour daily for 5 days (days 4-8)
4. Cytarabine IV over approximately 1 hour daily for 5 days (days 4-8).

Table 1: Schema for reinduction chemotherapy treatment

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
Mitoxantrone (intravenous)				X	X	X	X	X		
Etoposide (intravenous)				X	X	X	X	X		
Cytarabine (intravenous)				X	X	X	X	X		
Lenalidomide (oral)	X	X	X	X	X	X	X	X	X	X

Study Duration:
Participants will be followed for at least 50 days after the start of protocol treatment.
Participants will be followed for relapse and survival at 3 months after treatment, and then every six months until 3 years from start of treatment. Patients removed from the study treatment will continue to be followed for relapse and survival for up to three years following initiation of study treatment

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SYNOPSIS AND SCHEMA

1. OBJECTIVES

1.1 Study Design

This is a Phase II study designed to test the efficacy of lenalidomide in combination with reinduction chemotherapy (mitoxantrone/etoposide/cytarabine) for patients with relapsed or refractory acute myeloid leukemia (AML).

1.2 Primary Objectives

The primary objective of this study is to assess rates of complete remission (CR) and rates of complete remission with incomplete platelet recovery (CRp) after treatment with mitoxantrone, etoposide, cytarabine (MEC) and lenalidomide.

1.3 Secondary Objectives

1. To evaluate the days to neutrophil recovery (the first of 3 days of ANC >500)
2. To evaluate the days to platelet recovery (platelet count >20K unsupported)
3. To evaluate the treatment-related mortality(the number of non-relapse related deaths in the first 50 days of starting treatment)
4. To evaluate the transfusion support (the number of red blood cell and platelet transfusions needed in the first 50 days of treatment)
5. To determine if certain genetic profiles of the leukemia predict for better response (optional correlate)

2. BACKGROUND

2.1 Acute Myeloid Leukemia

There are 12,000 new cases of acute myelogenous leukemia (AML) each year in the United States, with a median age of onset of 67. AML is characterized by an arrest in differentiation and uncontrolled proliferation of myeloid precursors in the bone marrow, leading to a decrease in the mature, infection fighting cells and severe infections. Unfortunately, successful treatment of AML remains a difficult challenge. Although complete remission can be achieved in approximately 60- 70% of patients using combination chemotherapy with an anthracycline and cytarabine, the majority of patients relapse. (1) Therefore, approximately 70% of patients with AML may be candidates for reinduction therapy. If a second remission can be achieved, the patient may be a candidate for a potentially curative allogeneic stem cell transplant. (2)

Reinduction attempts are less successful than primary induction therapy, and therefore, the 5 year disease free survival rate for all patients with AML is 25% and less than 10% for patients over the age of 60. (3, 4, 5) Since the majority of AML patients fail standard therapy, the development of more effective salvage reinduction chemotherapy is essential. The prognosis is particularly poor for patients who are older, who have disease derived from myelodysplastic syndromes (MDS) or myeloproliferative disorders (MPD), and those with secondary AML from prior chemotherapy. A complete remission is achieved in less than 40% of cases, with very poor survival rates of less than 10%. (6, 7).

The majority of relapses occur within the first year following primary induction. The high relapse rate has been attributed to the persistence of leukemic stem cells, which display resistance to cytotoxic chemotherapy. While several large randomized studies such as the United Kingdom Medical Research Council have compared primary induction regimens, there is much less published on reinduction treatment. (8)

2.2 Mitoxantrone, Etoposide, and Cytarabine

There are many regimens that have been employed for reinduction and no regimen is clearly the standard of care. One commonly employed regimen used to treat patients with relapsed and refractory AML involves mitoxantrone, etoposide, and cytarabine (MEC). (9) This regimen was introduced in 1991 for the treatment of relapsed or refractory AML and has been used alone and in combination with multi-drug resistance modulators and monoclonal antibodies. (10-12)

One hundred and ninety-one AML patients participated in a multicenter study of MEC chemotherapy given as mitoxantrone 8 mg/m2, etoposide 80 mg/m2, and cytarabine 1 gm/m2 daily for 6 days. (10) Patients were randomized to MEC alone or to receive MEC plus the CD33 antibody lintuzumab. Patients were either resistant to initial treatment or relapsed within 12 months of initial therapy. Forty-six percent of patients were over age 60. Induction related deaths were 13%. Side effects included nausea, vomiting, diarrhea, mucositis, elevated liver function tests, with mucositis reported in 21% of patients. Patients received one course of induction chemotherapy. Twenty-two percent of patients receiving MEC achieved a complete remission and 5% of patients achieved a CRp (complete remission without platelet recovery). Patients in CR received a second course of MEC with mitoxantrone 8 mg/m^2 daily x 2 days, etoposide 80 mg/m^2 daily x 4 days, and cytarabine 1 gm/ m^2 daily x 4 days. Median survival was 5 months. There was no difference in remission rates or survival between the MEC and MEC plus lintuzumab arms.

Tallman and colleagues reported on 38 patients, median age 47 years, with MEC chemotherapy with or without the multidrug resistance inhibitor cyclosporine. (11) The drugs were dosed as follows: mitoxantrone 10 mg/m^2 daily x 5 days, etoposide 100mg/ m^2 daily x 5 days, and cytarabine 1g/m^2 daily x 5 days. Patients were either in first recurrence after < 6 months of complete remission (11 patients), refractory to initial induction therapy or to one attempt at reinduction after recurrence (18 patients), in second recurrence (4 patients), or in recurrence after either allogeneic or autologous bone marrow transplantation (5 patients). There was one treatment related death. The remission rate was 23% and the median survival 104 days. Patients received a second cycle of MEC in identical doses if there was persistent disease on Day +14. There was no difference in remission rate or survival between the patients that did or did not receive cyclosporine.

A slightly modified version of the MEC regimen, randomized with and without the multidrug-resistance inhibitor PSC-833 (Valspodar), was studied in 129 patients, median age 58 years, with relapsed or refractory AML and high-risk MDS. (12) Eligible patients had relapsed less than 6 months after first complete remission, after allogeneic or autologous stem cell transplantation, or were in second or greater relapse, refractory to induction chemotherapy, or secondary AML, or MDS with greater than 10% blasts. The MEC patients received mitoxantrone 8 mg/m^2/d,

etoposide 100 mg/m^2/d, cytarabine 1 gm/m^2/d, all days 1 to 5. The treatment related death rate (non relapse) was 10%. Complete remission was achieved in 25% of the patients receiving MEC. The median time to CR was 40 days; 25% of patients required 2 courses of treatment to achieve CR. Patients with secondary AML and MDS (no prior induction chemotherapy) did slightly better with 44% CR rate, compared to 19% CR rate for the relapsed patients. The median survival was 5 months. There was no difference in the CR rates, median disease-free survival, or overall survival between patients who received MEC alone or MEC plus PSC-833.

More modern studies have shown similar remission rates. A retrospective analysis reported on 77 patients with relapsed or refractory AML treated with MEC at the following doses: mitoxantrone 8 mg/m^2/d, etoposide 100 mg/m^2/d, cytarabine 1 gm/m^2/d, all days 1 to 5. (13) Eighteen percent of patients achieved a complete remission and the overall survival was 6.8%. There was trend to improved response in patients with favorable risk cytogenetics.

There have been no prospective randomized trials comparing MEC chemotherapy to other salvage regimens. A retrospective study compared MEC to a clofarabine, cytarabine, and G-CSF regimen. The complete remission rate was 26% for reinduction after MEC, with no significant differences between the two regimens. (14) Single dose mitoxantrone has been compared to the conventional divided dose mitoxantrone; the single dose regimen was inferior, mainly due to an increased incidence of toxic deaths. (15)

Thus, patients receiving reinduction chemotherapy with MEC have a poor prognosis, with only 18-26% entering complete remission, and further investigation is warranted. There are several published versions with minor variations in the 5 day MEC regimen with no prospective comparisons. The current MEC version in use for relapsed AML at the Dana Farber/Harvard Cancer Center is mitoxantrone,8-10 mg/m^2/day, by IV infusion over 6-10 minutes for 5 days, etoposide, 100 mg/m^2/day, IV over 1 hour daily for 5 days, and cytarabine, 1000 mg/m^2/day, IV over 1 hour daily for 5 days. This regimen will serve as the basis chemotherapy regimen for this study.

2.3 Lenalidomide

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

Lenalidomide demonstrates pleiotropic activities in distinct cell types that result in direct antitumor effects on cancer cells, inhibition of stromal growth factor support, and enhancement of host anticancer immunity. (16-20) The clinical activity of lenalidomide has been most notable in B-cell malignancies including MM, MCL, DLBCL, FL, and B-cell CLL. (21-24)

The activity of lenalidomide may be further subdivided into the following categories: direct antiproliferative activity against hematopoietic tumor cells; immunomodulatory activity including upregulation of T cell and NK cell responses; and inhibition of monocyte responses, antiangiogenic activity, and pro-erythropoietic activity.

Lenalidomide's pleiotropic activities in a range of cell types including MM cells and immune effector cells suggest modulation of multiple molecular pathways. Studies conducted to identify

the molecular target(s) of lenalidomide have shown that it physically associates with the protein cereblon (encoded by the *CRBN* gene), a protein required for the teratogenic effects of thalidomide in zebrafish and chicken embryos (25). Cereblon is a substrate receptor for an ubiquitin E3-ligase complex containing deoxyribonucleic acid (DNA) damage-binding protein 1 (DDB1), cullin 4 (CUL4), and regulator of cullins 1 (Roc1) proteins ($CRL4^{cereblon}$) (25). Upon binding to Cereblon, lenalidomide induces the ubiquitination of substrate proteins, including Ikaros (encoded by the gene *IKZF1*) and Aiolos (encoded by the gene *IKZF3*), thus targeting them for proteasomal-dependent degradation (26-28). It was also demonstrated that the expression of Cereblon in MM cells is linked to the anti-proliferative effects of lenalidomide and to the acquired resistance in vitro (29-30). The downstream anti-proliferative and pro-apoptotic effects of Ikaros and Aiolos are linked to the subsequent downregulation of the MM growth-promoting factors c-Myc and IRF4.

Ikaros and Aiolos are zinc finger transcription factors initially discovered as regulators of the T cell receptor and are required for proper hematopoiesis particularly lymphocyte development and plasma cell maturation. Cereblon expression also mediates the T-cell response to lenalidomide through the targeted degradation of Ikaros and Aiolos (26,29). In activated T cells in which cereblon was transiently decreased, interleukin-2 (IL-2) and TNF-α induction by lenalidomide was markedly reduced. Since IL-2 and TNF-α are important for tumor surveillance by activated T cells, these results indicate that some of the immunomodulatory effects of lenalidomide are mediated via initial binding to cereblon. Specifically, upon engagement with the $CRL4^{cereblon}$, lenalidomide induced ubiquitination and proteasomal degradation of Ikaros and Aiolos in T cells in a time- and concentration-dependent manner (26-28). Because Ikaros and Aiolos are known repressors of the IL-2 promoter, their degradation in response to lenalidomide and other IMiDs® compounds, explains the enhanced T cell IL-2 production .

In summary, lenalidomide exhibits potent anti-tumor activity in B cell malignancies, which appears to be mediated through the protein target, cereblon. While specific cereblon interaction networks are still to be defined, these results are key for further understanding of the mechanism of lenalidomide effects.

2.3.1 Clinical experience in multiple myeloma with lenalidomide

In 2 phase I studies in multiple myeloma, a total of 41 patients have been treated with lenalidomide. In one study at the University of Arkansas, 15 patients who relapsed or were refractory to high dose melphalan therapy with stem cell transplant were treated for 4 weeks in an open-label safety study and were permitted to continue therapy in an extension phase of the trial. Patient cohorts were treated at the following daily doses: 5mg, 10mg, 25mg, and 50mg. (31) In a similar study at the Dana Farber Cancer Institute, 27 patients with rapidly advancing refractory multiple myeloma were enrolled. (32)

Anti-myeloma activity was observed in each of these 2 phase I studies. Decreases in neutrophil and platelet counts were the dose-limiting toxicities associated with lenalidomide. The maximum tolerated dose (MTD) was not reached within 28 days. Due to dose modifications associated with myelosuppression observed beyond Day 28 at the 25mg and 50mg daily dose levels, the dose schedule most widely used in future studies has been lenalidomide 25 mg on Days 1-21, repeated every 28 days.

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). 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. No plasma accumulation was observed with multiple daily dosing. Peak and overall plasma concentrations were dose proportional over the dosing range of 5mg to 50mg. (33)

A multicenter, randomized, phase II trial compared 2 syncopated dose schedules of lenalidomide used alone or in combination with dexamethasone in the treatment of relapsed or refractory multiple myeloma. All patients were treated on Days 1-21 of a 28-day cycle. Patients treated with 15mg BID experienced more myelosuppression and dose reductions compared with patients treated with 30mg daily. Anti-myeloma activity was observed with each dose and schedule of single agent lenalidomide. The addition of dexamethasone to lenalidomide yielded responses in some patients who had not responded to lenalidomide alone. (34).

A phase II trial utilizing lenalidomide plus dexamethasone for newly diagnosed multiple myeloma patients was reported by the Mayo Clinic. Lenalidomide was given orally 25 mg daily on days 1-21 of a 28-day cycle. Dexamethasone was given orally 40 mg daily on days 1-4, 9-12, 17-20 of each cycle. Objective response was defined as a decrease in serum monoclonal protein by 50% or greater and a decrease in urine M protein by at least 90% or to a level less than 200 mg/24 hours, confirmed by two consecutive determinations at least 4 weeks apart. Thirty-one of 34 patients achieved an objective response, including 2 (6%) achieving complete response (CR), and 11 (32%) meeting criteria for both very good partial response and near complete response, resulting in an overall objective response rate of 91%. Of the 3 remaining patients not achieving an objective response, two had minor response (MR) and one stable disease. Forty-seven percent of patients experienced grade 3 or higher non-hematologic toxicity, most commonly fatigue (15%), muscle weakness (6%), anxiety (6%), pneumonitis (6%) and rash (6%). Rev/Dex is a highly active regimen with manageable side effects in the treatment of newly diagnosed myeloma. (35)

Celgene Corporation sponsored 2 multicenter, randomized, double-blinded, placebo-controlled phase III trials [1 U.S. (MM-009) and 1 international (MM-010)] in patients with relapsed or refractory multiple myeloma. (36) More than 350 patients were enrolled into each of these studies. All patients had to be considered sensitive to dexamethasone and were treated with dexamethasone 40mg qd, Days 1-4, 9-12 and 17-20. In addition to receiving dexamethasone, patients were randomized to lenalidomide 25mg qd or placebo, Days 1-21. Cycles were repeated every 28 days. After 4 cycles, there was a predetermined reduction of the dexamethasone dose to 40mg qd, Days 1-4 repeated every 28 days. In both studies, a pre-specified interim analysis conducted by an Independent Data Monitoring Committee demonstrated that subjects receiving the combination of lenalidomide (Len) plus dexamethasone (Dex) had significantly longer times to progression and higher response rates than those treated with single-agent dexamethasone. These studies led to the FDA approval of lenalidomide in combination with dexamethasone for the treatment of multiple myeloma in patients that have received at least one prior therapy.

2.3.2 Clinical experience in myelodysplastic syndromes (MDS) with lenalidomide

An exploratory trial in 43 MDS patients with transfusion dependent or symptomatic anemia was conducted at the University of Arizona. (37) Patients received lenalidomide at doses of 25mg or 10mg per day, or of 10mg on Days 1-21, repeated every 28 days. All patients had had no response to erythropoietin or had a high endogenous erythropoietin level. Response rates were similar across the 3 dose schedules used. Responses were observed in 24 patients overall (56%) including 21 patients with a major response and 20 patients with sustained transfusion independence. Patients with a major response reached a median hemoglobin level of 13.2 grams per deciliter, with a corresponding 5.3 grams per deciliter median increase from baseline. After a median follow-up of 81 weeks, the median duration of major response had not been reached and was more than 48 weeks. Of 20 patients with karyotypic abnormalities, 10 (50%) patients had a complete cytogenetic remission. The response rate was highest in patients with a clonal interstitial deletion involving chromosome 5q31.1 (10 out of 12, 83%). Neutropenia and thrombocytopenia were the most common adverse events, and resulted in dose delays or reductions in 25 patients (58%).

Celgene Corporation sponsored a multicenter trial (MDS-003) of 148 MDS patients with a clonal interstitial deletion involving chromosome 5q31.1. Lenalidomide was given at a dose of 10mg on Days 1-21, repeated every 28 days, to 44 patients, and at a dose of 10mg daily to the other 104 patients. Transfusion independence was achieved in 93 patients (64%), with a median hemoglobin increase of 3.9g/dl. Cytogenetic response was achieved in 76% of transfusion independent patients with 55% achieving a cytogenetic complete response. Pathologic complete response was documented in 32 out of 110 (29%) evaluable patients. With a median follow-up of 9.3 months, the median response duration had not been reached. Neutropenia (39%) and thrombocytopenia (35%) were the most common adverse events requiring dose delays or reductions. (38)

Another Celgene sponsored trial (MDS-002) in patients with low to intermediate-1 risk MDS enrolled 215 patients, of whom, 166 were documented to have low to intermediate-1 risk MDS. Among the patients with documented low to intermediate-1 risk MDS, 84 patients (51%) responded to treatment. Transfusion independence was achieved in 54 patients (33%) and 30 patients (18%) achieved a minor response, defined as a 50% or greater decrease in blood transfusion requirement. The median duration of transfusion-independence was 41 weeks. The median baseline hemoglobin level was 8.0g/dl, which increased by 3.2g/dl in responding patients. Among 20 patients evaluable for cytogenetic response, 9 patients (45%) experienced a cytogenetic remission. (39)

Sixty-seven percent of patients with del (5q) MDS achieved transfusion independence after treatment with lenalidomide. (39) Treatment related cytopenia correlated with clinical response in patients with del (5q) myelodysplasia. (40) Treatment with lenalidomide also resulted in responses in patients with normal cytogenetics. (41) Two hundred and fourteen patients received lenalidomide 10 mg daily or 10mg days 1-21 of a 28 day cycle. Patients had low or intermediate-1 risk MDS non 5q- and were transfusion dependent. Transfusion independence was achieved in 26% of patients.

2.4 Rationale

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 and Revlimid® has recently been approved in the treatment of patients with mantle cell lymphoma whose disease has relapsed or progressed after two prior therapies, one of which included bortezomib.

2.4.1 Role of Lenalidomide in Acute Myeloid Leukemia

Recently, lenalidomide has been shown to have activity in acute myelogenous leukemia, given at a dose of 35-50 mg daily as a single agent. (42-44) Chandler and colleagues performed a dose escalation trial of lenalidomide. The 50 mg dose was the maximum tolerated dose with higher doses resulting in dose-limiting fatigue. (42) Blum et al reported on a Phase I study of 31 patients with relapsed or refractory AML who received lenalidomide in doses of 25-75 mg daily Days 1-21 of a 28 day cycle. (43) Fatigue was considerable at a dose of 75 mg. Five patients achieved a complete response.

Thirty-three patients over age 60 received lenalidomide 50 mg daily in 2 28 day cycles, followed by a low dose lenalidomide (10 mg) maintenance if they did not progress. Overall complete remission/complete remission with incomplete platelet recovery rate was 30%, with patients with low blast counts more likely to respond. (44) The CR rate was 53% in patients completing the high dose lenalidomide treatment. The median time to CR was 30 days and the duration of CR was 10 months. The most common toxicities were neutropenia, thrombocytopenia, and infection.

Two patients with AML with trisomy 13 cytogenetic abnormality responded to lenalidomide. (45) One patient received lenalidomide 50 mg daily for 14 days, followed by 30 days off therapy and 21 days of lenalidomide 50 mg daily, and the other patient received a dose of 35 mg daily Days 1-21 of repeated 28 day cycles. Both patients achieved a complete remission, and relapsed after 9 months of CR.

Sekeres and colleagues treated 37 older AML patients with del (5q) with lenalidomide 50 mg daily for induction, and 10 mg daily for maintenance. (46) The median age was 74 years. Fourteen patients completed induction, and five patients achieved a complete remission.

Combinations of lenalidomide with standard chemotherapy are currently under investigation.

Laboratory correlates of the AML patients treated with lenalidomide indicated suppression of the tumor clone, as assessed by fluorescence in situ hybridization for del (5q31). (47) Twenty-eight patients with AML or high risk MDS, all with chromosome 5 abnormalities, alone or in combination, were treated with lenalidomide 30 mg daily for 16 weeks. Nineteen percent of patients had a major cytogenetic response and eight percent had a minor cytogenetic response. Thirty-five percent of patients had a clinical response, including 9 of 10 who completed the full 16 weeks of treatment.

Data from Ohio State University and others has shown that microRNA (miR)-181 a and b is associated with improved outcomes in cytogenetically normal and cytogenetically abnormal AML. (48,49) More recent work suggests that lenalidomide increases miR-181a and b, which

may contribute to improved outcomes, and is consistent with pretreating patients with lenalidomide prior to induction chemotherapy. (50)

Lenalidomide has also been used to treat relapse after allogeneic stem cell transplant for AML; lenalidomide was given at a dose of 10 mg daily and CR was achieved. (51)

Recent data suggests lenalidomide may affect clonal architecture in patients with myelodysplasia. (52) Loss of treatment efficacy coincided with re-expansion of the dominant subclone.

A Phase I study (07-006) of lenalidomide and bortezomib for patients with MDS and AML has been completed at Massachusetts General Hospital and Dana Farber Cancer Institute. (53) Lenalidomide was given at a dose of 10 mg daily, Days 1-21 of 28 days cycles. The drug was tolerated well. 23 patients (14 men) were enrolled; one patient was inevaluable due to disease progression prior to starting protocol therapy. The median age was 73 years (range 54-87). There was 1 DLT observed, neutropenia, in 6 patients treated with 1.0 mg/m^2 bortezomib and no DLTs at 0.7 or 1.3 mg/m^2. The median number of cycles was 2 (range 2-9). Grade $\geq$ 3 toxicities possibly attributable to the treatment at any dose level were: anemia (2), thrombocytopenia (10), leukopenia (3), infection (1), rash (2), dyspnea (1), dizziness (1), hypotension (1), pneumonia (2) and neuropathy (1). Among the 14 patients with MDS, 1 patient with RARS experienced a CR and 2 with RAEB-2 experienced marrow CR (mCR). Among the 8 patients with AML, there was 1 CR.

Protocol 12-202, Phase I study of Lenalidomide plus mitoxantrone, etoposide, and cytarabine for patients with relapsed and refractory AML has just been completed at DF/HCC. Results were presented in abstract form at the American Society of Hematology, December 2015. (54)

The primary objective of this Phase I trial was to evaluate the safety of lenalidomide in combination with MEC for relapsed acute myelogenous leukemia and determine a safe Phase II dose and schedule. The secondary objectives included time to neutrophil (>500) and platelet recovery (> 20K), the complete response rate with and without platelet recovery, and the treatment-related mortality within the first 45 days. Patients received lenalidomide in escalating doses at dose levels: 5 mg, 10 mg, 25 mg and 50 mg. Lenalidomide was given initially Days 1-14, and then due to neutropenia was decreased to Days 1-10. The chemotherapy was given as follows: Mitoxantrone 8 mg/m^2/day Days 4-8, Etoposide 100 mg/m^2/day Days 4-8, and Cytarabine 1000 mg/m^2/day Days 4-8. Thirty-six patients were enrolled with a median age of 61 years. Forty-five percent of patients had received 2 or more prior chemotherapy regimens, and 22% had received a prior stem cell transplant**.** Thirteen patients entered complete remission and one patient had complete remission with incomplete count recovery for a response rate of 39%. The median time to ANC > 500 was 30 days and the median time to platelet recovery > 20,000 was 34 days. Two of 6 patients treated with lenalidomide for 14 days had no count recovery at Day 42. After the dose adjust to 10 days, only 1 of 13 patients at lenalidomide 50 mg daily x 10 days had prolonged count recovery. Thus, the recommended phase 2 dose is 50 mg daily for 10 days. There were 4 deaths on study, deemed unrelated to study drug. One patient died of sepsis, one of infection, and two of respiratory failure.

We propose to study the efficacy of lenalidomide and MEC chemotherapy as reinduction treatment in patients with relapsed and refractory AML. The survival is poor for patients with relapsed or refractory leukemia treated with standard chemotherapy. Lenalidomide has been demonstrated to have significant activity in AML. Our Phase I study demonstrated that lenalidomide in combination with MEC chemotherapy will be safely tolerated. Patients with relapsed and refractory AML will be eligible for this study. If this combination proves to be effective, a multicenter Phase III study would be considered.

2.5 Correlative Studies Background

Acute myeloid leukemia (AML) is a disease characterized by the combinatorial action of multiple somatic mutations that lead to an arrest in normal hematopoietic differentiation and uncontrolled growth of immature bone marrow myeloid blasts. (55, 56)

Advances in gene sequencing technology over the past decade have allowed for the identification of over 50 recurrent mutations in AML patients. Mutational testing of a limited number of genes is already considered an integral part of standard clinical practice. The presence of mutations in *FLT3* and *NPM1* help define prognosis and determine treatment algorithms. Recent clinical trial data have demonstrated the clinical efficacy of the tyrosine kinase inhibitor midostaurin in the presence of a *FLT3* mutation. AML patients with *IDH* mutations are now being enrolled in clinical trials utilizing small molecule inhibitors of this mutant enzyme (57). Some mutations may also predict response or resistance to certain therapeutic interventions. In myelodysplasia, a related myeloid disease, the finding of isolated deletion of 5q is already an approved indication for the use of lenalidomide (58). However, concomitant mutations in *TP53* have been demonstrated to predict a poor response to treatment with lenalidomide in these patients (59). Whether this observation holds true in AML remains an open question. Identifying genetic markers that predict response to specific agents so that we can triage patients appropriately is an important and unmet clinical need.

We now routinely perform DNA sequencing of recurrently mutated genes from clinical samples using next generation sequencing assays, including the Rapid Heme Panel or the Heme SNaPshot assay. These assays provide targeted next-generation sequencing of recurrently mutated genes as well as high-resolution genomic copy number data at these loci. Mutation and copy number status will be determined. With this genetic information we can classify patients into molecularly defined groups; for instance, *de novo* AML, secondary AML and *TP53* associated AML (60). We can also look for correlations between specific genetic lesions and response to therapy in this clinical trial. In doing this, we hope to identify specific factors associated with response and primary resistance to lenalidomide in AML.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study. All required tests must be performed within 21 days of the start of treatment.

3.1.1 Acute myelogenous leukemia diagnosed by WHO criteria with one of the following (patients with biphenotypic leukemia are eligible, provided that the treating physician determines an AML treatment regimen is appropriate)
- Primary refractory disease following $\geq$ 1 cycle of induction chemotherapy
- First relapse or higher. Patients with primary or secondary acute myelogenous leukemia are eligible.

3.1.2 Age 18-70 years old

3.1.3 LVEF $\geq$ 50 %

3.1.4 ECOG Performance status 0-2

3.1.5 Able to adhere to study schedule and other protocol requirements.

3.1.6 Participants must have normal organ function as defined below, unless felt due to underlying disease and approved by the overall PI. Patients with Gilbert's disease may have total bilirubin up to $\leq$ 3 x institutional ULN.
- Creatinine < 2.0mg/dl, and eGFR $\geq$ 60 mL/min
- Total bilirubin $\leq$ 1.5 x institutional ULN
- AST (SGOT) and ALT (SGPT) $\leq$ 3 x institutional ULN

3.1.7 Patients may receive hydroxyurea, steroids, or leukapheresis as necessary until Day 5 of treatment.

3.1.8 Patients must give voluntary written informed consent and HIPAA authorization before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

3.1.9 Patients may have had prior treatment for MDS or AML, including prior lenalidomide for MDS or AML or another condition.

3.1.10 Patient may have had prior autologous or allogeneic transplant (family member, unrelated donor, or cord blood) if there is at least 90 days between transplant and study entry.

3.1.11 Patients may also have had donor lymphocyte infusion if there is at least 60 days between donor lymphocyte infusion and study entry.

3.1.12 Patients on immunosuppression are also eligible.

3.1.13 Females of childbearing potential (FCBP)[†] must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL prior to receiving treatment with lenalidomide, and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 28 days before she starts taking lenalidomide. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy.

3.1.14 Ability to understand and the willingness to sign a written informed consent document.

3.1.15 All study participants must be registered into the mandatory Revlimid REMS ® program, and be willing and able to comply with the requirements of the REMs ® program. Females of reproductive potential must adhere to the scheduled pregnancy testing as required in the Revlimid REMS® program.

[†] A female of childbearing potential is 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)

3.2 Exclusion Criteria

3.2.1 Known hypersensitivity to thalidomide or lenalidomide (if applicable).
3.2.2 The development of erythema nodosum if characterized by a desquamating rash while taking thalidomide or similar drugs.
3.2.3 Known seropositive for human immunodeficiency virus (HIV). HIV testing is not required. Hepatitis testing is not required.
3.2.4 Patients who have had a myocardial infarction within 6 months of enrollment or has New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities.
3.2.5 Any serious medical condition laboratory abnormality or psychiatric illness that would prevent the subject from signing the consent form.
3.2.6 Any condition, including laboratory abnormalities, that in the opinion of the investigator places the subject at unacceptable risk if he/she were to participate in the study or confounds the ability to interpret data from the study.
3.2.7 Patients with major surgery within 28 days prior to treatment.
3.2.8 Patients with any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol.
3.2.9 Patient has received an investigational agent or cytotoxic chemotherapy (excluding hydroxyurea) within 7 days of study entry. If an LP is performed for clinical care, intrathecal chemotherapy (although not systemic chemotherapy) may be given as prophylaxis per institutional standard of care, but should be completed >24 hours prior to administration of protocol therapy.
3.2.10 Patients with acute promyelocytic leukemia.
3.2.11 Females who are pregnant

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. Women and minorities are included in the eligibility criteria. We do not anticipate that women or minorities will have increased toxicity with this treatment approach. Gender, race and ethnicity will be recorded in our database, per standard procedures.

3.4 Consent

Patients who are referred to the participating institutions for consideration of treatment of acute myelogenous leukemia will be considered for participation. Patients with AML who have relapsed or refractory disease are eligible for this study. Treatment recommendations are discussed thoroughly with patient and family. The alternative forms of therapy, as far as they exist, are presented as objectively as possible. The risks and hazards of the procedure are explained to the patient and family. It will be pointed out specifically that some aspects of this treatment are considered experimental. Consent is obtained using forms approved by participating site's Institutional Review Board.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at Massachusetts General Hospital by the Coordinating Center. All sites should contact the Coordinating Center to verify treatment availability.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator/Sponsor. If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. The Coordinating Center should be notified of cancellations as soon as possible.

4.4 Registration Process for Other Investigative Sites

To register a subject, the following documents should be completed by the participating institution and forwarded to the Coordinating Center:

- Copy of source documentation for inclusion/exclusion criteria and screening procedures, including but not limited to
    - Pathology report
    - Medical history and physical exam
    - Laboratory reports
    - Radiology results/reports
    - Echo or MUGA report
    - Concomitant medication list

- Demographics information
- Signed study consent form
- Study Entry Note
- HIPAA authorization form, if applicable
- Eligibility checklist

The Coordinating Center will review the above documentation to verify eligibility and consent. To complete the registration process, the Coordinating Center will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. Once registered, a confirmation email with the participant study number, and if applicable the dose treatment level, will be sent to the participating site.

NOTE: Registrations can only be conducted by the Coordinating Center during the business hours of 8:30 AM and 5:00 PM Eastern Standard Time (or Eastern Daylight Time when applicable), Monday through Friday. A complete registration packet, including all documents listed above, must be received at least 24 hours *prior to* the anticipated registration to ensure adequate review. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Lead Institution.

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

5. TREATMENT PLAN

5.1 Treatment Regimen

Table 1: Schema for reinduction chemotherapy treatment

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
Mitoxantrone (intravenous)				X	X	X	X	X		
Etoposide (intravenous)				X	X	X	X	X		
Cytarabine (intravenous)				X	X	X	X	X		
Lenalidomide (oral)	X	X	X	X	X	X	X	X	X	X

Patients will receive all treatment on an inpatient basis. All chemotherapy doses will be based on actual weight obtained on Day 1 of the treatment cycle. The total administered doses of the MEC chemotherapy will be calculated using actual weight and may be rounded up or down within a range of 5% of the actual calculated dose. Any dose modifications in the mitoxantrone, etoposide, and cytarabine chemotherapy are per physician discretion or institutional practice (please refer to section 6.3, table 5), with approval from the overall PI.

Administer chemotherapeutic agents according to institutional policies and procedures. Mitoxantrone will be administered first, followed by etoposide, followed by cytarabine. Patients will receive only one cycle of treatment as outlined in tables 1 and 2. There is no retreatment as a part of this protocol. See section 6 (Dosing delays/Dose modifications) for further information on dose modifications and interruptions. Premedications will be given per institutional standards.

Table 2: Dosing information

Agent	Dose	Route	Schedule
Mitoxantrone	8 mg/m^2/day	IV infusion over approx 6-10 minutes daily or per institutional standards for 5 days	Days 4 – 8
Etoposide	100 mg/m^2/day	IV over approx 1 hour daily or per institutional standards for 5 days	Days 4 – 8
Cytarabine	1000 mg/m^2/day	IV over approx 1 hour daily or per institutional standards for 5 days	Days 4 – 8
Lenalidomide	50 mg	Orally	Days 1– 10

Following reinduction therapy, patients may be treated further according to their treating physician. Patients who achieve a CR or CRi, as well as those who are otherwise deemed eligible, are recommended to proceed with allogeneic hematopoietic stem cell transplantation if possible. Otherwise patients are recommended to have further consolidation according to their treating physicians.

5.2 Pre-Treatment Criteria

Patients must meet eligibility in order to be enrolled on the study. Eligibility criteria does not need to be reconfirmed on Day 1 of treatment. There are no additional pretreatment criteria on Day 4 of treatment.

5.3 Agent Administration

Treatment will be administered on an inpatient basis. Expected toxicities and potential risks for mitoxantrone, etoposide, cytarabine, and lenalidomide are outlined in Toxicities and Dosing Delays/Dose Modification (section 6).

Administer chemotherapeutic agents according to institutional policies and procedures. Lenalidomide should be administered prior to MEC each day. During the administration of MEC, mitoxantrone will be administered first, followed by etoposide, followed by cytarabine.

General dosing guidelines for lenalidomide are provided below. Please refer to the dosage and

administration schedule specified in the prescribing information.

5.3.1 Lenalidomide Dosing Guidelines

Lenalidomide should be taken orally at about the same time each day. The capsules should not be opened, broken, or chewed. Lenalidomide capsules should be swallowed whole, preferably with water, either with or without food. If a dose of lenalidomide is missed, it should be taken as soon as possible on the same day (until midnight). If it is missed for the entire day, it should not be made up. Vomited doses will not be made up. Do not take 2 doses at the same time.

5.3.1.1 Patients Receiving Concomitant Digoxin

Periodic monitoring of digoxin levels in patients receiving this medication concomitantly with lenalidomide is recommended in accordance with clinical judgment and based on standard clinical practice.

5.3.1.2 Patients Receiving Concomitant Warfarin

Monitoring of warfarin concentration in accordance with standard practice is advised during treatment with lenalidomide.

5.3.1.3 Elderly Patients

No dedicated clinical studies have been conducted to evaluate the PK of lenalidomide in the elderly. However, results of population PK analyses (subject ages ranging from 39 to 85 years old) suggest that age does not influence the disposition of lenalidomide. No lenalidomide dose adjustments are needed.

5.3.1.4 Patients with Impaired Renal Function

Lenalidomide is primarily excreted unchanged by the kidney; for other approved indications, starting dose adjustment is recommended in patients with CKD. Based on a PK study in subjects with CKD due to nonmalignant conditions, lenalidomide starting dose adjustments are recommended for patients with CrCl < 60 mL/min; therefore, patients must have a CrCl ≥ 60 mL/min to be eligible for this study.

Because lenalidomide is primarily excreted unchanged by the kidney, monitoring of renal function is advised. No dose adjustments are required for patients with CrCl ≥ 60 mL/min. Patients must have eGFR of ≥ 60 mL/min and Cr < 2 mg/dL to enroll on the study. Refer to Section 6.2, Table 4 for lenalidomide dose adjustment recommendations if patients develop worsened renal function while on treatment.

5.3.1.5 Prohibited Medications

Any investigational agent or medication intended to treat the malignancy.

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 Encouraged Supportive Care and Allowed Concomitant Medications

Infection Prophylaxis may be per institutional guidelines. The following practices are suggested prophylactic antibiotics (such as Levaquin or other appropriate agent) against gram negative organisms while patient is neutropenic; antiviral therapy against herpes viruses with acyclovir or an appropriate substitution; and antifungal prophylaxis with fluconazole (or another appropriate agent). Patients should have excellent oral care per institutional standards. Patients with fever and neutropenia should be treated with broad spectrum antibiotics per institutional guidelines.

Recombinant Granulocyte Colony Stimulating Factor (G-CSF) may be administered intravenously or subcutaneously at the discretion of the investigator and per institutional guidelines. G-CSF is recommended for patients older than age 60 after the completion of chemotherapy and for all patients with serious infections.

Prophylaxis against tumor lysis syndrome is recommended and can be administered per institutional guidelines.

Transfusion support per Dana-Farber/Harvard Cancer Center guidelines will be provided, and patients may be managed per local standards.

Central venous access is suggested prior to the start of chemotherapy.

Antiemetics will be used per standard practices.

Hyperalimentation (TPN) will be provided at the treating physician's discretion.

Decadron eye drops are recommended and can be dosed per institutional guidelines. Suggested dosing is as follows:

- Two drops of dexamethasone suspension 0.1% to both eyes every 6 hours starting with first dose of cytarabine on day 4 and continued for 72 hours after the last dose of cytarabine on day 8.

Neurologic assessments for cerebellar function are recommended while patient is receiving cytarabine.

After hospitalization, subjects will be followed closely to monitor for complications related to chemotherapy. Patients will receive standard post-chemotherapy discharge teaching and guidelines to prevent infection.

Prophylactic anticoagulation is not recommended considering the low platelet count after induction chemotherapy.

5.4.2 Anticoagulation consideration

Lenalidomide increases the risk of thrombotic events in patients who are at high risk or with a history of thrombosis, in particular when combined with other drugs known to cause thrombosis. Due to the thrombocytopenia with this chemotherapy regimen, anticoagulation and antiplatelet agents are not recommended.

When lenalidomide is combined with other agents such as steroids (e.g. dexamethasone, prednisone), anthracyclines (Doxil, Adriamycin) and erythropoietin, the risk of thrombosis is increased.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s) or those listed in table 3 as requiring treatment discontinuation
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- The subject needs a medication that is not part of this study (e.g additional chemotherapy for relapsed or progressive disease)
- The investigator feels this study protocol would not be in the subject's best interest
- Patient completed all treatment as outlined in the protocol

Participants will be removed from the protocol therapy when any of these criteria apply. Alternative care options will be discussed with the participant.

The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). The DF/HCC research team will update the relevant Off Treatment/Off Study information in OnCore. The Coordinating Center will update this information for external site participants.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Andrew Brunner, MD at [redacted].

5.6 Duration of Follow Up

Participants will be followed for at least 50 days after the start of protocol treatment. Participants will be followed for relapse and survival at 3 months after treatment, and then every

six months until 3 years from start of treatment, including those patients who are removed from the study treatment prior to completing the 50 day toxicity period.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Taking a Participant Off Study

Study subjects may withdraw their consent to participate in this trial at any time. Once withdrawn, the study subject will receive usual care considered standard and routine for their individual case.

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death
- Completed all follow-up per protocol

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). The DF/HCC research team will update the relevant Off Treatment/Off Study information in OnCore. The Coordinating Center will update this information for external site participants.

6. DOSING DELAYS/DOSE MODIFICATIONS

Any dose modifications or delays in the reinduction chemotherapy regimen of mitoxantrone, etoposide, and cytarabine should be per the treating physician and institutional practice, with approval from the overall PI (also please refer to section 6.3, table 5).

There may be two dose reductions in lenalidomide per patient. Reduction of lenalidomide dose may be modified as follows:

Table 3: Lenalidomide Dose Reductions

Dose Level	Lenalidomide dose
1 (starting dose)	50 mg
-1	25mg
-2	10 mg

No Lenalidomide will be given after Day 10, even if there are missed doses.

Dose delays and modifications will be made as indicated in Table 4. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Toxicity Management

Significant toxicity is expected in this leukemia reinduction protocol. Patients will receive supportive care and medical management of toxicity.

Antiemetics are recommended for management of nausea and vomiting.

Stool cultures for C. Diff are recommended for patients with diarrhea. If negative, antidiarrheals should be used as clinically indicated

For management of neutropenia/fever myeloid growth factors can be considered. Platelet transfusions should be considered for management of patients with a platelet count of <10K or bleeding.

Local steroid creams are recommended as clinically indicated for the management of rashes.

6.2 Dose Modifications for Lenalidomide

Table 4: Dose modifications for Lenalidomide

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	no change in dose	no change in dose	no change in dose	no change in dose
Vomiting	no change in dose	no change in dose	no change in dose	no change in dose
Diarrhea	no change in dose	no change in dose	no change in dose	no change in dose
Neutropenia	no change in dose	no change in dose	no change in dose	Continue lenalidomide dose. Follow CBC. Consider G-CSF.
Thrombocytopenia	no change in dose	no change in dose	Continue lenalidomide dose. Follow CBC. Consider platelet transfusion	Continue lenalidomide dose. Follow CBC. Consider platelet transfusion
Neutropenia associated with fever (temperature ≥ 38.5º C)	N/A	N/A	Continue lenalidomide dose. Follow CBC. Consider G-CSF.	Continue lenalidomide dose. Follow CBC. Consider G-CSF.

Table 4: Dose modifications for Lenalidomide				
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Non-blistering rash thought related to Lenalidomide	no change in dose	no change in dose	Hold lenalidomide. If resolves to ≤ grade 1 restart lenalidomide at next lower dose level and continue through the remainder of the scheduled treatment. Omitted doses are not made up.	Discontinue lenalidomide. Remove patient from study.
Desquamating (blistering) rash thought related to lenalidomide	Discontinue lenalidomide. Remove patient from study	Discontinue lenalidomide. Remove patient from study	Discontinue lenalidomide. Remove patient from study	Discontinue lenalidomide. Remove patient from study
Neuropathy	no change in dose	no change in dose	Hold (interrupt) lenalidomide. If resolves to ≤ grade 1, restart lenalidomide at next lower dose level and continue through the remainder of the scheduled treatment. Omitted doses are not made up.	Discontinue lenalidomide. Remove patient from study.
Venous thrombosis or embolism	No change in dose	No change in dose	Hold (interrupt) lenalidomide and start therapeutic anticoagulation, if appropriate. Restart lenalidomide at investigator's discretion (maintain dose level).	Hold (interrupt) lenalidomide and start therapeutic anticoagulation, if appropriate. Restart lenalidomide at investigator's discretion (maintain dose level).

Table 4: Dose modifications for Lenalidomide				
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Acute kidney injury	No change in dose	Decrease lenalidomide dose by one level according to Table 3.	Hold lenalidomide. If resolves to ≤ grade 2, restart lenalidomide at next lower dose level and continue through the remainder of the scheduled treatment. Omitted doses are not made up.	Hold lenalidomide. If resolves to ≤ grade 2, restart lenalidomide at next lower dose level and continue through the remainder of the scheduled treatment. Omitted doses are not made up.
Other clinically significant, non-hematologic toxicity thought to be related to lenalidomide	No change in dose	No change in dose	Hold lenalidomide. If resolves to ≤ grade 2, restart lenalidomide at next lower dose level and continue through the remainder of the scheduled treatment. Omitted doses are not made up.	Hold lenalidomide. If resolves to ≤ grade 2, restart lenalidomide at next lower dose level and continue through the remainder of the scheduled treatment. Omitted doses are not made up.

6.3 Dose Modifications for Mitoxantrone, Etoposide, Cytarabine

Table 5: Dose modifications for mitoxantrone, etoposide, cytarabine				
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Nausea	no change in dose	no change in dose	no change in dose	no change in dose
Vomiting	no change in dose	no change in dose	no change in dose	no change in dose
Diarrhea	no change in dose	no change in dose	no change in dose	no change in dose
Neutropenia	no change in dose	no change in dose	no change in dose	no change in dose
Thrombocytopenia	no change in dose	no change in dose	no change in dose	no change in dose
Non-blistering	no change in	no change in	no change in dose	no change in dose

rash	dose	dose		

Table 5: Dose modifications for mitoxantrone, etoposide, cytarabine

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Desquamating (blistering) rash	no change in dose	no change in dose	no change in dose	no change in dose
Neuropathy	no change in dose	no change in dose	no change in dose	no change in dose
Other clinically significant non-hematologic toxicity	no change in dose	no change in dose	Per physician discretion or institutional practice	Per physician discretion or institutional practice

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values, regardless of etiology. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the CRF rather than the individual signs or symptoms of the diagnosis or syndrome. An overdose, accidental or intentional, whether or not it is associated with an AE, or abuse, withdrawal, sensitivity or toxicity to an investigational product should be reported as an AE. If an overdose is associated with an AE, the overdose and adverse event should be reported as separate terms. All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject's clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, or findings from other tests and/or procedures.

All AEs will be recorded by the Investigator from the first dose of study treatment until 28 days after the last dose of lenalidomide and those SAEs made known to the investigator at any time thereafter that are suspected of being related to lenalidomide. AEs and serious adverse events (SAEs) will be recorded on the AE page of the CRF and in the subject's source documents. All SAEs must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

Toxicity will be scored using CTCAE Version 4.03 for toxicity and adverse event reporting. A copy of the CTCAE Version 4.03 can be downloaded from the CTEP homepage (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.03. All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to MEC, relationship to lenalidomide, and for its seriousness. The investigator must appraise all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test drug and about the patient's outcome.

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until 28 days after the last dose of study treatment. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

7.1 Expected Toxicities

7.1.1 Adverse Event List for Mitoxantrone

The most common adverse events include myelosuppression, fever and chills, alopecia, mucositis, and nausea and vomiting. Other commonly reported events include GI bleeding, malaise, fatigue, cardiomyopathy, anorexia and weakness. Other adverse events include cardiac arrhythmias, bleeding, abdominal pain, hepatitis, cough, shortness of breath, infertility, neuropathy and headache. Rare side effects include urticaria, rash, anaphylaxis, extravasation at the IV site, chest pain, pneumonitis, second cancers, and tachycardia.

7.1.2 Adverse Event List for Etoposide

The most common side effects are myelosuppression, mucositis, nausea, vomiting, and alopecia. Hypotension can occur during administration. Allergic reactions can also occur. Other adverse events include facial and tongue swelling, cough, back pain, abdominal pain, fatigue, fever, pulmonary fibrosis, rash, and hepatic toxicity. Rare toxicities include second cancers, rash, urticaria, itching, constipation, transient cortical blindness, optic neuritis, anaphylaxis, congestive heart failure, myocardial infarction, somnolence, and thrombophlebitis.

7.1.3 Adverse Event List for Cytarabine

The most common side effects include myelosuppression, nausea, vomiting, diarrhea, skin rash, mucositis, alopecia, fever, and reduced kidney function. Conjunctivitis may also occur. Other adverse events include diarrhea, urinary retention, confusion, jaundice, dizziness, and abdominal pain. Rare toxicities include pancreatitis, cough, and anaphylaxis.

7.1.4 Adverse Event List for Lenalidomide

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: leukopenia, neutropenia, febrile neutropenia, granulocytopenia, lymphopenia, pancytopenia, anemia, thrombocytopenia, blurred vision, diarrhea, abdominal pain, toothache, constipation, dyspepsia, nausea, vomiting, asthenia, fatigue; edema, pyrexia, chills, pneumonia, bronchitis, upper respiratory tract infection, urinary tract infection, erysipelas, gastroenteritis, Herpes simplex, Herpes zoster, Influenza, lower respiratory tract infection, sinusitis, sepsis, bacteremia, nasopharyngitis, pharyngitis, rhinitis, weight loss; decreased appetite, hyperglycemia, hypokalemia, hypocalcemia, hypophosphatemia, hypomagnesemia, hyponatremia, pain in extremity, pain in limb, arthralgia, back pain, bone pain, muscle spasms, musculoskeletal pain, muscle cramp, chest pain, myalgia, dizziness, dysgeusia, headache; cataracts, hypoaesthesia; neuropathy, peripheral neuropathy, peripheral sensory neuropathy, tremor, cough, dyspnea, epistaxis, pulmonary embolism, deep vein thrombosis, dry skin, pruritus, rash, hypersensitivity (in uncommon category), depression, insomnia and recently vertigo.

Complete and updated adverse events are available in the Investigational Drug Brochure

7.2 Adverse Event Characteristics

7.2.1 CTCAE term (AE description) and grade

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.03 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- Grade 1 = Mild – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required
- Grade 2 = Moderate – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required
- Grade 3 = Severe – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible
- Grade 4 = Life-threatening – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable
- Grade 5 = Death - the event results in death

7.2.2 Serious Adverse Events

A serious adverse event (SAE) is any AE occurring at any dose that:

- Results in death;

- Is life-threatening (i.e., in the opinion of the Investigator, the subject is at immediate risk of death from the AE);
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay);
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions);
- Is a congenital anomaly/birth defect;
- Constitutes an important medical event

Important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

7.2.3 Attribution

The Investigator must determine the causal relationship between the administration of lenalidomide, the administration of MEC, and the occurrence of an AE/SAE as Not Related or Related as defined below:

Not Related: Means a causal relationship of the adverse event to IP administration is unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event.

Related: Means there is a reasonable possibility that the administration of IP caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the IP and the adverse event.

Causality should be assessed as per the categories below and provided for every AE/SAE based on currently available information. Causality is to be reassessed and provided as additional information becomes available.

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

If an event is assessed as possibly, probably or definitely related to a comparator, ancillary or additional IP that has not been manufactured or provided by Celgene, please provide the name of the manufacturer when reporting the event.

7.2.4 Duration, Action Taken and Outcome

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event

The Investigator will report the action taken with IP as a result of an AE or SAE, as applicable (e.g., discontinuation, interruption, or dose reduction of IP, as appropriate) and report if concomitant and/or additional treatments were given for the event.

The Investigator will report the outcome of the event for both AEs and SAEs. All SAEs that have not resolved upon discontinuation of the subject's participation in the study must be followed until recovered (returned to baseline), recovered with sequelae, not recovered or death (due to the SAE).

7.3 Reporting Requirements for Specific Adverse Events

7.3.1 Abnormal Laboratory Values

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study, and
- is judged to be of significant clinical importance

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event. If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE. If possible, the laboratory abnormality should be recorded as a medical term and not simply as an abnormal laboratory result (e.g., record thrombocytopenia rather than decreased platelets).

7.3.2 Second Primary Malignancies

Second primary malignancies will be monitored as events of interest and must be reported as serious adverse events the subject is in. This includes any second primary malignancy, regardless of causal relationship to study drugs occurring at any time for the duration of the study, from the time of signing the ICD for at least 3 years from the date the last subject is enrolled into the study. Events of second primary malignancy are to be reported using the SAE report form and must be considered an "Important Medical Event" if no other serious criteria apply; these events must also be documented in the appropriate page(s) of the CRF (i.e., AE and SPM CRF) and subject's source documents. Documentation on the diagnosis of the second primary malignancy must be provided at the time of reporting as a serious adverse event (e.g., any confirmatory histology or cytology results, X-rays, CT scans, etc.).

7.3.3 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 study treatment, or within 28 days from last study treatment , are considered immediately reportable events. Study treatment is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Coordinating Center for submission to Celgene Drug Safety

immediately by facsimile, or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form. The female subject may be referred to an obstetrician-gynecologist (not necessarily one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The Investigator will follow the female subject until completion of the pregnancy, and the Coordinating Center and Celgene notified 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 Celgene immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

If a female partner of a male subject taking investigational product becomes pregnant, the male subject taking lenalidomide should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

7.3.4 Overdose

Overdose, as defined for this protocol, refers to chemotherapy drug dosing only.

On a per dose basis, an overdose is defined as the following amount over the protocol-specified dose of lenalidomide, mitoxantrone, etoposide, or cytarabine assigned to a given patient, regardless of any associated adverse event or sequelae:

- PO-any amount over the protocol-specified dose
- IV-10% over the protocol-specified dose

On a schedule or frequency basis, an overdose is defined as anything more frequent than the protocol required schedule or frequency.

On an infusion rate basis, an overdose is defined as any rate faster than the protocol-specified rate. Complete data about drug administration including any overdose, regardless of whether the overdose was accidental or intentional, should be reported in the case report forms.

The Coordinating Center will submit reports of all suspected or confirmed pregnancies, pregnancy outcomes, neonatal deaths or overdoses to Celgene Drug Safety immediately by

facsimile, or other appropriate methods on behalf of the participating site.

Coordinating Center

[redacted]

Celgene Drug Safety Contact Information:
Celgene Corporation
Global Drug Safety and Risk Management

[redacted]

7.3.5 On Study Death

A death on study requires both expedited reporting to the Coordinating Center and routine reporting in the toxicity case report forms regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

7.4 Expedited Adverse Event Reporting

All adverse event reports must include the patient number, age, sex, weight, severity/grade of reaction (e.g. mild, moderate, severe), relationship to drug attribution (e.g. probably related, unknown relationship, definitely not related), date and time of administration of study medications and concomitant medications, and medical treatment provided. The investigator is responsible for evaluating all adverse events to determine whether criteria for "serious" as defined above are present. The investigator is responsible for reporting adverse events to the Coordinating Center as described below.

Investigators **must** report to the Overall PI and Coordinating Center any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the MedWatch 3500A Mandatory Reporting Form.

7.4.1 Expedited Reporting to Celgene

For the purpose of regulatory reporting, Celgene Drug Safety will determine the expectedness of events of being related to lenalidomide based on the Investigator Brochure. In the United States, all determined unexpected serious adverse reactions (SUSARs) will be reported in an expedited manner in accordance with 21 CFR 312.32.

Serious adverse events (SAE) are defined above. The investigator must inform the Coordinating Center in writing using a Celgene SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. **A final report to document resolution of the SAE is required.** The Celgene tracking number (RV-CL-AML-PI006779) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the patient records.

The Coordinating Center will submit all SAE reports to Celgene Drug Safety immediately by facsimile, or other appropriate methods on behalf of the participating site.

7.4.2 Reporting to the IRB

For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution must abide by the reporting requirements set by the DF/HCC. In addition to SAEs that meet criteria set forth in Section 7.2.2, the DF/HCC requires reporting of any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report AEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the Coordinating Center within the timeframes detailed in the table below. The Coordinating Center will submit AE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days[#]	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days[#]	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

Participating investigators must report each adverse event to the Coordinating Center in accordance with these timeframes. In the event that the participating investigator does not become aware of the adverse event immediately (e.g., participant sought treatment elsewhere) or within the reporting timeframes listed in the table above, the participating investigator is to report the event within 1 business day after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by email or facsimile to:

[redacted]

7.4.3 Expedited Reporting to the FDA

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Coordinating Center, on behalf of the Sponsor, will report to the FDA any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Serious adverse events (SAEs) that are **unlisted/unexpected, and at least possibly associated to the drug,** and that have not previously been reported in the Investigators brochure, or reference safety information document should be reported promptly to the Coordinating Center using Form FDA 3500A (Mandatory Reporting Form for investigational agents).

Fatal or life threatening SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 7 calendar days after awareness of the event. All other SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 15 calendar days after awareness of the event. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

7.4.4 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form).

- Hematologic toxicity of any grade does not need to be reported.
- Routine chemotherapy toxicity of any grade including nausea, diarrhea, constipation, vomiting, alopecia, mucositis, fatigue, grade 3-4 lab abnormalities that resolve to grade 0-2 with appropriate follow-up or intervention within 48 hours do not need to be reported.

Events not considered to be SAEs are hospitalizations for:

- A standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.
- Routine treatment or monitoring of the studied indication not associated with any deterioration in condition

- The administration of blood or platelet transfusion as routine treatment of studied Indication. However, hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.
- A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
- A procedure that is planned (i.e., planned prior to starting of treatment on study); must be documented in the source document and the CRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.
- An elective treatment of or an elective procedure for a pre-existing condition unrelated to the studied indication.
- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, both the AE CRF and the SAE Report Form must be completed. For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to study drugs, action taken regarding study drugs, and outcome.

7.4.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

Celgene shall notify the Investigator via an IND Safety Report of the following information:

- Any AE suspected of being related to the use of drug in this study or in other studies that is both serious and unexpected.
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

The Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

The Investigator must keep copies of all pertinent safety information on file, including correspondence with Celgene and the IRB/EC.

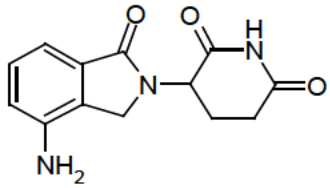
8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational and other agents administered in this study can be found in Section 7.1.

8.1 Lenalidomide

8.1.1 Description

REVLIMID® (lenalidomide), a thalidomide analogue, is an antineoplastic and immunomodulatory agent. The chemical name is 3-(4-amino-1-oxo 1,3-dihydro-2*H*-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-2*H*-isoindol-2-yl) piperidine-2,6-dione]

The empirical formula for lenalidomide is $C_{13}H_{13}N_3O_3$, and the gram molecular weight is 259.3.

Lenalidomide is an off-white to pale-yellow solid powder. It is soluble in organic solvent/water mixtures, 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.

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.

8.1.2 Clinical Pharmacology

8.1.2.1 Mechanism of Action

Lenalidomide is an analogue of thalidomide with immunomodulatory, antiangiogenic, and antineoplastic properties. Lenalidomide inhibits proliferation and induces apoptosis of certain hematopoietic tumor cells including multiple myeloma, mantle cell lymphoma, and del (5q)

myelodysplastic syndromes *in vitro*. Lenalidomide causes a delay in tumor growth in some *in vivo* nonclinical hematopoietic tumor models including multiple myeloma. 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.

Immunomodulatory properties of lenalidomide include activation of T cells and natural killer (NK) cells, increased numbers of NKT cells, and inhibition of pro-inflammatory cytokines (e.g., TNF-α and IL-6) by monocytes. Lenalidomide inhibited the secretion of pro-inflammatory cytokines and increased the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. Lenalidomide inhibited the expression of cyclooxygenase-2 (COX-2) but not COX-1 in vitro. In multiple myeloma cells, the combination of lenalidomide and dexamethasone synergizes the inhibition of cell proliferation and the induction of apoptosis.

8.1.2.2 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.. However, in the pivotal MM and MDS registration trials where the efficacy and safety were established for lenalidomide, the drug was administered without regard to food intake. Thus, lenalidomide can be administered with or without food.

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. (20) Exposure (AUC) in multiple myeloma patients is 57% higher than in healthy male volunteers.

8.1.2.3 Distribution

In vitro (^{14}C)-lenalidomide binding to plasma proteins is approximately 29% in healthy subjects and 23% in MM patients.

8.1.2.4 Metabolism and Excretion

The metabolic profile of lenalidomide in humans has not been studied. In healthy subjects and patients with MM or MDS, approximately 65% to 85% of the administered dose 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.

8.1.3 Form

Celgene Corporation will supply Revlimid® (lenalidomide) to study participants at no charge through the REMS® program. Dosage form Lenalidomide will be supplied as capsules for oral

administration. Lenalidomide is an off-white to pale-yellow solid.

8.1.4 Storage and Stability

Lenalidomide will be shipped directly to the participating site. Bottles will contain a sufficient number of capsules for one cycle of dosing (10 days). Lenalidomide should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

8.1.5 Compatibility

Lenalidomide is compatible with the chemotherapy drugs and supportive care drugs as outlined in the protocol. Additional questions not contained within this protocol may also be investigated by referencing the investigator's brochure.

8.1.6 Handling

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

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.7 Availability

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 REMS® program of Celgene Corporation.

8.1.8 Preparation

Lenalidomide will be supplied in oral formulation. No on site preparation is needed. Lenalidomide will be shipped directly to the participating site. Bottles will contain a sufficient number of capsules for one cycle of dosing**.**

8.1.9 Administration

Dosing should be taken at approximately the same time each day and is recommended to be given in the morning. If a dose of lenalidomide is missed, it should be taken as soon as possible on the same day (until midnight). If it is missed for the entire day, it should not be made up. Vomited doses will not be made up. Patient may take lenalidomide with or without food. Lenalidomide should be administered prior to other chemotherapy on a given day.

Patients who take more than the prescribed dose of lenalidomide should be instructed to seek emergency medical care if needed and contact study staff immediately.

8.1.10 Ordering

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. Celgene Corporation will supply Revlimid® (lenalidomide) to study participants at no charge through Celgene's Revlimid Risk Evaluation and Mitigation Strategy® (REMS) (formerly known as RevAssist® Program). Lenalidomide will be provided in accordance with Celgene Corporation's Revlimid REMS® program. Per standard Revlimid REMS® program 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 Revlimid REMS®program.

Further information about the Revlimid REMS® program is available at www.celgeneriskmanagement.com.

Drug will be shipped on a per patient basis by the contract pharmacy to the participating site. The treatment will be shipped directly to the research pharmacy for distribution to the patient. Only enough lenalidomide for one cycle of therapy will be supplied to the patient each cycle.

8.1.11 Accountability

The Investigator or designee is responsible for taking an inventory of each shipment of study drug received, and comparing it with the accompanying study drug accountability form. The Investigator will verify the accuracy of the information on the form, sign and date it, retain a copy in the study file, and return a copy to Celgene or its representative. The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.12 Destruction and Return

Celgene will instruct the Investigator on the return or destruction of unused study drug. If any study drug is lost or damaged, its disposition should be documented in the source documents. Study drug supplies will be retained at the clinical site pending instructions for disposition by Celgene. Patients will be instructed to return empty bottles or unused capsules to the clinic site

8.2 Mitoxantrone

8.2.1 Description

Mitoxantrone is a synthetic antineoplastic anthracenedione for intravenous use. It is a DNA-reactive agent that intercalates into deoxyribonucleic acid (DNA) through hydrogen bonding, causing cross links and strand breaks. Mitoxantrone also interferes with ribonucleic acid (RNA) and is a potent inhibitor of topoisomerase II, an enzyme responsible for uncoiling and repairing damaged DNA. It has a cytocidal effect on both proliferating and nonproliferating cultured human

cells. Mitoxantrone has been shown in vitro to inhibit B cell, T cell, and macrophage proliferation and to impair antigen presentation.

8.2.2 Form

Mitoxantrone Injection, USP (concentrate) is a sterile aqueous solution containing Mitoxantrone hydrochloride at a concentration equivalent to 2 mg Mitoxantrone free base per ml supplied in vials for multiuse dose.

8.2.3 Storage and Stability

Store between 20 to 25 degrees C. Do not freeze. Opened vials may be stored at room temperature for 7 days or under refrigeration for up to 14 days. Solutions diluted for administration are stable for up to 7 days at room temperature or under refrigeration.

8.2.4 Compatibility

Mitoxantrone should not be mixed in the same infusion as heparin since a precipitate may form. Because specific compatibility data is not available, mitoxantrone should not be mixed in the same infusion with other drugs.

8.2.5 Handling

Skin accidentally exposed to Mitoxantrone should be rinsed copiously with warm water and if the eyes are involved, standard irrigation techniques should be used immediately. Procedure for proper handling and disposal of anticancer drugs should be followed.

8.2.6 Availability

Mitoxantrone is commercially available. It is supplied by APP Pharmaceuticals. It will not be provided for this study.

8.2.7 Preparation

Mitoxantrone is supplied as concentrate that must be diluted prior to injection. The dose of mitoxantrone should be diluted to at least 50 ml with either 0.9% Sodium Chloride Injection (USP) or 5% Dextrose Injection (USP). Mitoxantrone may be further diluted into Dextrose 5% in Water, Normal Saline, or Dextrose 5% with Normal Saline and used immediately. Product should be visually inspected for particulate matter and discoloration whenever solution and container permit.

8.2.8 Administration

Mitoxantrone should be given by intravenous infusion over approximately 6-10 minutes. Severe local tissue damage may occur if there is extravasation. The tubing should be attached to a butterfly needle or other suitable device and inserted preferably into a large vein. If any signs or symptoms of extravasation have occurred, including burning, pain, pruritus, erythema, swelling,

blue discoloration¸ or ulceration, the injection or infusion should be immediately terminated and started in another vein.

8.2.9 Ordering

Mitoxantrone is commercially available. It will be ordered by the oncology attending and supplied via the inpatient oncology pharmacy per institutional guidelines.

8.2.10 Accountability

Mitoxantrone accountability will be handled via institutional chemotherapy policies.

8.2.11 Destruction and Return

N/A or handled via institutional chemotherapy policies

8.3 Etoposide

8.3.1 Description

Etoposide is used in the treatment of certain neoplastic diseases. It is a 4'-demethylepipodophyllotoxin 9-(4, 6-0-(R)-ethylidene-B-D-glucopyranoside). It is very soluble in methanol and chloroform, slightly soluble in ethanol and sparingly soluble in water and ether. Its main effect appears to be at the G2 portion of the cell cycle in mammalian cells. The predominant macromolecular effect of etoposide appears to be the induction of DNA strand breaks by an interaction with DNA topoisomerase II on the formation of free radicals.

8.3.2 Form

Etoposide Injection USP is available in 100 mg(5ml) and 250 mg(12.5ml) sterile, multiple dose vials. The pH of the clear, colorless to pale yellow solution is 3 to 4. Each ml contains 20 mg Etoposide, 2 mg anhydrous citric acid, 30 mg benzyl alcohol, 80 mg polysorbate, 650 mg polyethylene glycol, and 30.5 percent (v/v) dehydrated alcohol.

8.3.3 Storage and Stability

Unopened vials of Etoposide Injection USP are stable for 24 months at room temperature. Vials diluted as recommended to a concentration of 0.2 or 0.4 mg/ml are stable for 96 and 24 hours, respectively, at room temperature under normal room fluorescent light in both glass and plastic containers.

8.3.4 Compatibility

Because specific compatibility data is not available, etoposide should not be mixed in the same infusion with other drugs.

8.3.5 Handling

Procedures for proper handling and disposal should follow institutional guidelines. If Etoposide solution contacts the skin or mucosa, immediately and thoroughly wash the skin with soap and water and flush the mucosa with water.

8.3.6 Availability

Etoposide is commercially available and will not be provided for this study. It is supplied at 100mg/5ml and 250 mg/12.5 multiple dose vials.

8.3.7 Preparation

Etoposide Injection USP must be diluted prior to use with either 5% Dextrose Injection or 0.9% Sodium Chloride Injection, to give a final concentration of 0.2 to 0.4 mg/ml or per institutional standard. If solutions are prepared at concentrations above 0.4 mg/ml, precipitation may occur.

8.3.8 Administration

Hypotension following rapid intravenous administration has been reported; hence, it is recommended that the Etoposide solution be administered over a 30-60 minute period. A longer duration of administration may be used if the volume of fluid to be infused is a concern. Etoposide should not be given by rapid intravenous injection. The bag of etoposide should be inspected prior to administration and held if the solution is cloudy or precipitated drug is observed.

8.3.9 Ordering

Etoposide is commercially available. It will be ordered and supplied per institutional guidelines.

8.3.10 Accountability

Etoposide accountability will be handled via institutional chemotherapy policies.

8.3.11 Destruction and Return

N/A or handled via institutional chemotherapy policies.

8.4 Cytarabine

8.4.1 Description

Cytarabine (1-beta-D-arabinofuranosylcytosine) is a synthetic antineoplastic agent. The exact mechanism of action for cytarabine is unknown. It is thought that the drug is an antimetabolite, interfering with the synthesis of deoxyribonucleic acid (DNA). The inhibition of the conversion of cytidine to deoxycytidine is the presumed primary site of action. Cytarabine may be incorporated into DNA and RNA as in vitro chromosome breaks have been associated with the

drug, and the clinical effects are limited to tissues with a high rate of cellular proliferation. Alternatively, cytarabine may have a differentiating role rather than an antimitotic effect as the mechanism of action. Cytarabine reportedly also has immunosuppressive activity. Two important properties of cytarabine include:

- A relatively unique synergistic effect with other classes of drugs including alkylating agents, thiopurines, and anthracycline antibiotics.

- Clinical effectiveness that is significantly affected by the schedule of administration. Cytarabine is effective for refractory leukemia and the addition of anthracyclines may enhance this effect.

8.4.2 Form

Cytarabine (Cytosar-U, Cytosine Arabinoside, Ara-C) for Injection, USP, is a sterile lyophilized material for reconstitution. It is available in multi-dose vials containing 100 mg, 500 mg, 1 g or 2 g sterile cytarabine. Cytarabine is commercially available and will not be provided for this study.

8.4.3 Storage and Stability

Cytarabine lyophilized powder should be stored at 150 to 300 C. Solutions reconstituted with Bacteriostatic Water for Injection with Benzyl Alcohol to 0.945% weight/volume may be stored at 15^0 to 30^0 C for up to 48 hours. The pH of the reconstituted solution is about 5. Solutions with a slight haze should be discarded. Solutions reconstituted with a preservative should be used immediately. Undiluted vials should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature.

8.4.4 Compatibility

For intravenous cytarabine administration, cytarabine lyophilized powder should be reconstituted with Bacteriostatic Water for Injection with Benzyl Alcohol 0.945% weight/volume.

8.4.5 Handling

Cytarabine is manufactured by the Upjohn Company in Kalamazoo, MI, USA. Cytarabine is commercially available. Procedures for proper handling and disposal should follow institutional guidelines.

8.4.6 Availability

Cytarabine is commercially available and will not be provided for this study.

8.4.7 Preparation

Cytarabine should be reconstituted according to institutional standard; recommendations for reconstitution in Bacteriostatic Water for Injection with Benzyl Alcohol 0.945% weight/volume

as a diluent as follows are provided below:

Vial lyophilized dose (mg)	Bacteriostatic Water for Injection with Benzyl Alcohol 0.945% (ml)	Final Cytarabine Concentration (mg/ml)
100	5	50
500	10	50
1000	10	100
2000	20	100

8.4.8 Administration

Cytarabine is administered intravenously, over approximately one hour. Cytarabine injection may induce hyperuricemia secondary to rapid lysis of leukemia cells. Periodic checks of bone marrow, liver and kidney functions should be performed in patients receiving cytarabine.

8.4.9 Ordering

Cytarabine is commercially available. It will be ordered and supplied per institutional guidelines.

8.4.10 Accountability

Cytarabine accountability will be handled via institutional chemotherapy policies.

8.4.11 Destruction and Return

N/A or handled via institutional chemotherapy policies.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Correlative studies are optional. Patients who consent will have a purple top tube sent as standard of care for NGS molecular testing according to site SOC (e.g., Rapid Heme Panel, Heme SNaPshot assay) testing at the time of study entry, and subsequent bone marrows as clinically indicated. Missed samples due to weekends, holidays, insufficient volume or patient refusal will not be considered violations. Additional DNA will be stored for future testing, in the event of new genetic mutations.

9.1 Laboratory Correlative Studies

CORRELATIVE STUDY 1: Understanding the leukemia genetics of relapsed and refractory AML.

Acute myeloid leukemia (AML) is a disease characterized by the combinatorial action of multiple somatic mutations that lead to an arrest in normal hematopoietic differentiation and uncontrolled growth of immature bone marrow myeloid blasts (55,56). Advances in gene

sequencing technology over the past decade have allowed for the identification of over 50 recurrent mutations in AML patients. Mutational testing of a limited number of genes is already considered an integral part of standard clinical practice. In myelodysplasia, a related myeloid disease, the finding of isolated deletion of 5q is an approved indication for the use of lenalidomide (57). Individual mutations can be associated with sensitivity to specific therapies and the development of resistance. For instance, mutations in *TP53* along with loss of chromosome 5q have been demonstrated to predict a poor response to treatment with lenalidomide (58). Recent work has also demonstrated that MDS clonal structure is dynamic during treatment with lenalidomide as some initially dominant clones disappear and founder clones tend to become dominant over time (59,60). Understanding these changes and how they relate to treatment response is an important next step in finding predictive markers to identify appropriate patients for selected therapies. In order to address these issues in this study of salvage chemotherapy combined with lenalidomide we plan to sequence recurrently mutated genes in myeloid malignancies using standard sequencing panels available at the institutions involved in the trial, such as the Heme SNaPshot assay available at Massachusetts General Hospital, and the Rapid Heme Panel assay at the Brigham and Women's Hospital Center for Advanced Molecular Diagnostics (CAMD). Sequencing using one of these assays or a similar assay is recommended at clinical trial enrollment, and then on subsequent bone marrows as clinically indicated. This assay can be performed from a single purple top tube collected from a bone marrow aspirate or per local SOP.

9.1.1.1 Collection of Specimen(s)

Collection of these samples is optional but recommended. A purple top tube of bone marrow aspirate (or other appropriate tube based on the assay) will be collected from each consenting patient: at study enrollment; the day + 35 (+/-3 days) bone marrow biopsy if indicated or at count recovery if sooner;); as well as at relapse, if this occurs. For this correlative, the baseline mutation testing may be performed at the time of relapse and may occur up to 2 months prior to enrollment as part of clinical care, provided that no intervening therapy has been given except hydroxyurea or 6MP. If it cannot be obtained off the bone marrow, a sample using leukemic blood will be adequate.

9.1.1.2 Handling and Shipping of Specimens

- Sequencing and any sample shipping will be performed locally according to standard of care at each local institution.

CORRELATIVE STUDY 2: Assessing lenalidomide function in AML.

In myelodysplasia, a related myeloid disease, the finding of isolated deletion of 5q is an approved indication for the use of lenalidomide. This sensitivity to lenalidomide is mediated through selective proteosomal degradation CSNK1A1, a haploinsufficient gene found in the commonly deleted region of 5q. Lenalidomide induces degradation by binding to the E3 ubiquitin ligase cereblon thus altering its affinity for some targets such as CSNK1A1 and leading to its ubiquitination and eventual degradation. In multiple myeloma, another disease in which lenalidomide is approved, the proteins targeted for degradation are IKZF1 and IKZF3, which are crucial B-cell transcription factors. In both diseases, the genes targeted by lenalidomide show

decreased protein levels but no alteration in the RNA level. Therefore, the only way to assess this function of lenalidomide is through direct measurement of protein levels. In multiple myeloma, sensitivity to lenalidomide has been associated with the protein levels of cereblon and its associated complex.

Understanding whether these previously identified targets are also degraded in AML and whether cereblon levels might predict response to lenalidomide is important for understanding its mechanism of action in AML. We have previously used mass spectrometry to measure the levels of cereblon and lenalidomide targets in cell lysates. We propose to collect samples before and after treatment with lenalidomide to measure the levels of target proteins.
Peripheral blood and bone marrow will be collected at the following time points:

- Study enrollment, prior to initiation of treatment: 5ml peripheral blood and 2ml bone marrow aspirate
- On day 4, prior to initiation of MEC chemotherapy, a 5ml peripheral blood sample will be collected. This may be obtained before or after the dose of lenalidomide that day.
- Day +35+/- 3 days (or at count recovery if sooner): 5ml peripheral blood and 2ml bone marrow aspirate (at the time of marrow assessment)

This correlative study is recommended but optional. Study samples will be collected and processed according to local tissue banking protocols.

MGH: patient samples will be collected and stored using the local tissue bank prior to analysis.

BWH/DFCI: patient samples will be collected and stored using the local tissue bank prior to analysis.

BIDMC: patient samples will be collected and stored using the local tissue bank prior to analysis.

UVA: patient samples will be collected and stored using the local tissue bank prior to analysis.

CORRELATIVE STUDY 3: Assessing the impact of therapy on tumor-reactive T-cells

In the phase I study on Lenalidomide and MEC, we found that exposure to this chemotherapy combination results in the expansion of IFN-ɣ expressing T-cells when exposed to leukemia cell lysate from the time of relapse. Using leukemia cells collected at baseline, when T-cells from pre-treatment and then at the end of therapy are exposed to the leukemia cell lysate, we saw a significant increase in the number of reactive T-cells following MEC and Lenalidomide. At the peak following MEC and lenalidomide therapy, there was a mean 3.8-fold and 7.5-fold increase in the level of IFN-γ expressing CD4+ and CD8+ T cells in the peripheral blood, respectively, consistent with the expansion of tumor-reactive T-cells. We therefore propose an optional correlative study to collect bone marrow and peripheral blood samples at diagnosis and at response to further evaluate the development of a leukemia-specific T-cell response after exposure to leukemia cells.

Blood and bone marrow specimens should be collected in heparinized tubes (large green top

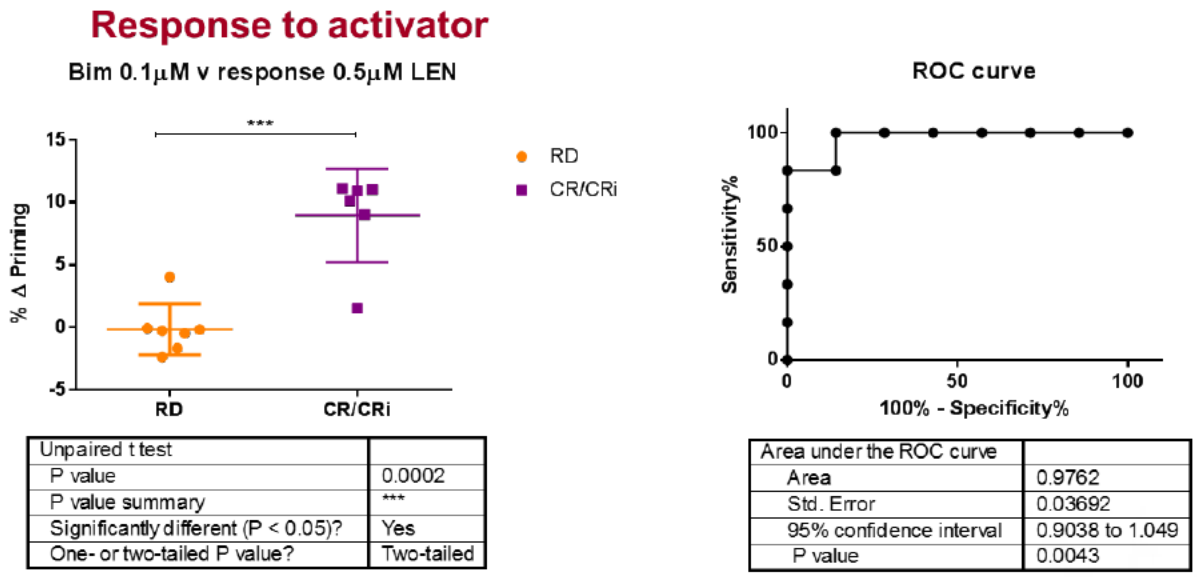
tubes). We will plan to collect approximately 25ml (3 large green top tubes) of peripheral blood and 4 ml (one small green top tube) of BM aspirates. The green top tubes should be sent to the Avigan lab and delivered at room temperature within a couple of hours from collection using standard local shipping methods. If samples are not delivered immediately after collection, samples should be placed on cold packs and shipped. Samples may be shipped Monday-Thursday.

- Tubes will be collected at the following time points: Study enrollment, prior to treatment initiation: 3 large heparinized green top tubes (~25ml) of peripheral blood and 1 small green top tube (4ml) of bone marrow aspirate
- Count recovery or day 35 marrow: 3 large heparinized green top tubes (~25ml) of peripheral blood and 1 small green top tube (4ml) of bone marrow aspirate

Samples should be sent to:
Attn: Dina Stroopinsky,
Beth Israel Deaconess Medical Center, Center for Life Sciences,
[redacted]

CORRELATIVE STUDY 4: Dynamic BH3 profiling to predict clinical response to lenalidomide and MEC chemotherapy.

Background: One mechanism by which lenalidomide combined with MEC chemotherapy may impact responses to chemotherapy is by activating pro-apoptotic signaling. Apoptosis is regulated by the BCL-2 family of proteins, which encompass a number of pro-apoptotic and anti-apoptotic proteins. This family of proteins is classified by structure and BCL-2 homology (BH) domains; a subset of the BCL-2 family, containing BH3-only proteins, have pro-apoptotic function. The magnitude of apoptotic response of a tumor after being exposed to proapoptotic peptides is a measure of how "primed" a cell is to respond to BH3, and is associated with response to treatment with standard cytotoxic chemotherapy (Vo T-T et al. Cell. 2012(151): 344). More recently, the Letai lab has developed a functional assay to profile BH3 response after cancer cells have been incubated with a specific chemotherapy, and have shown that this correlates with the response to these agents ([redacted]). In a previous study, lenalidomide was given prior to MEC chemotherapy for patients with AML, and baseline samples underwent dynamic BH3 profiling after exposure to lenalidomide (Figure below).



Unpaired t test	
P value	0.0002
P value summary	***
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed

Area under the ROC curve	
Area	0.9762
Std. Error	0.03692
95% confidence interval	0.9038 to 1.049
P value	0.0043

BH3 profiling performed on pre-treatment leukemia samples after exposure to lenalidomide showed that there was a significant increase in priming in the samples from patients who subsequently achieved a complete remission compared to those who did not respond to therapy (p=0.0002), suggesting that dynamic BH3 profiling may be a way to predict which patients will most likely respond to this combination, and which patients may be better served by alternative therapies. We therefore propose an optional correlative study to collect bone marrow or leukemic blood samples at diagnosis to perform BH3 profiling and correlate with responses.

Blood and bone marrow samples will be collected during treatment and sent to the Letai lab for analysis. We will plan to collect samples at baseline (prior to starting chemotherapy, 5mL bone marrow aspirate and 10mL peripheral blood) and at day 3 of treatment (peripheral blood only, 10mL sample). Baseline samples should be collected Monday-Friday; if collected Friday, they should be collected in the morning to allow same day shipping. Samples collected on day 3 should be sent in a similar fashion; if day 3 lands on a Saturday samples should be sent on day 2 (Friday), while if day 3 is a Sunday, samples may be collected on the morning of day 4 prior to MEC administration. Samples may be collected and shipped directly to the Letai lab, or may be stored and sent in batches, per the local site preferences/capability.

1. If samples are to be shipped one at a time directly from the center treating the patient, a heparinized aspirate tube may be used, shipped on blue ice to maintain a 4 degree F temperature (not wet ice or dry ice) after wrapping the tube with a dry pad. Samples should reach the Letai lab within 24 hours, and it would help a lot if we had notice so we were prepared to get it.
2. Preferred: samples that are stored and shipped as a batch should be viable frozen (e.g., in 10% DMSO, 90% serum).

Samples should be sent to:
Dana-Farmer Cancer Institute
Attn: Shruti Bhatt
[redacted]

Boston-02215

If possible, as samples are being prepared, contact Shruti Bhatt at [redacted]

10. STUDY CALENDAR

10.1 Pre-Treatment Evaluations

All required tests must be performed within 21 days of the start of treatment.

Clinical Evaluations:

1. A complete history with full details of the patient's previous treatment and response will be obtained.
2. A complete physical examination.
3. Marrow aspiration and biopsy with cytogenetics, flow cytometry, and molecular genetic NGS sequencing panel (e.g. "heme snapshot" or "rapid heme" panel, per local site). Patients are eligible who have a dry aspirate.
4. Echocardiogram or MUGA to assess ejection fraction.
5. Chest X-ray
6. Performance Status
7. All study participants must be registered into the mandatory REMS program, and be willing and able to comply with the requirements of REMS.

Laboratory Evaluations:

1. CBC with differential
2. Comprehensive chemistry profile, including measures of hepatic and renal function, such as electrolytes, BUN, creatinine, alkaline phosphate, bilirubin, and transaminases, as per institutional guidelines.
3. Pregnancy Test (serum or urine) in child bearing female. Refer to inclusion criteria 3.1.13 for details.

10.2 Bone Marrow Assessments

All patients will have bone marrow examined at the time of count recovery or, if counts have not yet recovered, at Day +35 +/- 3 days. Patients with persistent leukemia will be followed for overall survival as noted in section 10.4.

Patients who have count recovery within one week of the Day 35 marrow do not need a repeat bone marrow. Patients who have not experienced count recovery by Day 45 will have a marrow evaluation performed at Day 45+/-3 days regardless of counts. Patients may have additional bone marrows performed as clinically indicated.

10.3 Post Treatment Evaluations

Standard post-treatment testing, including complete blood counts and, serum chemistries will be

performed per institutional guidelines and consistent with clinical good practice. Suggested practice would be to perform three times weekly.

10.4 Long Term Follow Up

Participants will be followed for at least 50 days after the start of protocol treatment to assess for response to treatment and adverse events. At the completion of the study treatment, participants will be followed for relapse and overall survival outcomes for a total of 3 years from the start of treatment. These outcomes should be assessed at approximately 3 months after treatment, and then approximately every six months until the 3 year follow-up has been reached.

Patients removed from the study treatment early, for instance patients with refractory disease who go onto another therapy within 45 days of the study therapy, and will continue to be followed for relapse and survival for up to three years following initiation of study treatment.

Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. They will then also be followed at the above approximate intervals for relapse and survival outcomes.

Baseline evaluations are to be conducted within 21 days prior to the start of protocol therapy.

Assessments must be performed prior to administration of any study agent. Study assessments should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted.

Table 6. Required Study Data.

	Pre-treatment (within 21 days)	Weekly (+/-3 days)	Count recovery or Day +35 (+/- 3 days)	Day +38	Day +45 +/- 3days
Medical History and Physical Exam	X				
CBC with diff	X	X (Diff optional)			X
Comprehensive chemistry with renal and liver functions[1]	X	X			X
ABO/Rh	X				X
Bone marrow biopsy and aspirate[2]	X		X		X
Informed Consent	X				
History and physical	X	X			X
Performance Status	X				
EKG	X				
MUGA or ECHO	X				

Chest x-ray	X				
Toxicity assessment[4]	X	ongoing throughout			
Enroll in REMS	X				
Pregnancy Test Child Bearing Female	X			X	
Optional Research Studies	X[5]	b	X		

[1] Chemistry testing to include creatinine, eGFR, BUN, potassium, sodium, chloride, HCO3, glucose, magnesium, phosphorus, calcium. Liver function testing to include ALT, AST, total bilirubin, alkaline phosphatase, albumin.
[2] All patients will have bone marrow examined at count recovery or at Day +35 +/- 3 days. Patient will be off treatment if persistent leukemia or need for further treatment. Patients who have count recovery within one week of the Day 35 marrow do not need a repeat bone marrow. Patients who have not experienced count recovery by Day 45 will have marrow at Day 45+/-3 days regardless of counts. Patients may have additional bone marrows performed as clinically indicated.
[3]The day 4 peripheral blood research sample should be obtained prior to the initiation of MEC chemotherapy (may be obtained before or after lenalidomide on that day).
[4] Toxicity will be assessed from the time of first dose of study treatment through 30 days past the last dose of study treatment.
[5]Molecular testing (correlative 1) may be performed at the time of disease relapse and within 2 months of study enrollment, provided no intervening systemic chemotherapy has been administered (excluding hydroxyurea or 6MP).
[b]Samples for optional correlative 4 may be collected at baseline prior to chemotherapy, and on day 3 of treatment (or day 2 or 4 if on a weekend, but prior to MEC administration).

11. MEASUREMENT OF EFFECT

11.1 Count Recovery

11.1.1 Time to Neutrophil Recovery

The time to neutrophil engraftment is defined as the first 3 consecutive days of absolute neutrophil count >500.

11.1.2 Time to Platelet Recovery

Platelet recovery is defined as a platelet count ≥ 20,000/μL for three consecutive measurements over three or more days. The first of the three days will be designated the day of platelet engraftment. Subjects must not have had platelet transfusions during the preceding 3 days or in the following 7 days after the day of engraftment, unless the platelet transfusion is being given specifically to achieve a platelet threshold to allow an elective invasive procedure, such as a central catheter removal. The time to a platelet count ≥ 100,000/μL will be collected as well.

11.2 Treatment-related Mortality

All deaths in the absence of relapse of the primary malignancy will be considered treatment

related mortality. The cumulative incidence of treatment related mortality at 50 days will be measured.

11.3 Relapse-Free and Overall Survival

Both overall survival and relapse-free survival will be assessed at 45 days after the start of study treatment, as well as over the 3 year planned follow-up period. Overall survival is defined as time from diagnosis of disease until date of death or censored on the last known date alive if patients are still alive. Testing for recurrent malignancy in the blood, marrow or other sites will be used to assess relapse after treatment. Relapse is defined by morphological evidence of the original malignancy consistent with pre-treatment features. Minimal residual disease is defined by the sole evidence of malignant cells by flow cytometry, or fluorescent in situ hybridization (FISH), or polymerase chain reaction (PCR), or other techniques, in absence of morphological or cytogenetic evidence of disease in blood or marrow. Since the frequency of testing for minimal residual disease is highly variable, and the sensitivity is highly variable among laboratory techniques, evidence of minimal residual disease will not be sufficient to meet the definition of relapse in the context of this study. Relapse free survival is defined from time of diagnosis to disease relapse or death whichever occurs first or censored on the last known date of free from disease relapse.

11.4 Second Malignancies

Patients will be followed for the development of second cancers, including lymphoproliferative disorder and myelodysplasia/myeloproliferative disorder. See Section 7.3.2

11.5 Evaluation of Response

All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete remission, 2) complete remission without neutrophil or platelet recovery, 3) persistent disease, 4) early death from leukemia 5) early death from toxicity, or 6) unknown (not assessable, insufficient data). World Health Organization (WHO) criteria will be used to assess response. Patients will be assessed for complete remission or complete remission without platelet recovery per WHO criteria. (43) Patients who experience toxicity but die with persistent leukemia will be classified as 3) death of persistent disease.

Morphologic Complete Remission (CR):
Defined as morphologic leukemia-free state, including <5% blasts in Bone Marrow aspirate with marrow spicules, no persistent extramedullary disease, ANC >1000/mm3 and platelet count >100,000/mm3.

Morphologic Complete Remission without neutrophil (CRi) or platelet recovery (CRp):
Defined as CR with the exception of neutrophil count < 1,000/mm3 (CRi) or platelet count < 100,000/mm3 (CRp).

Persistent Disease:
Greater than 5% blasts in the marrow.

Recurrence/morphologic relapse:
Defined as reappearance of >5% blasts in the bone marrow, not attributable to any other cause, *after* the documentation of complete remission (CR)

Early death from leukemia:
Defined as death within 50 days of treatment attributed to persistent leukemia.

Early death from toxicity:
Defined as death within 50 days of start of treatment attributed to therapy in the absence of leukemia.

11.6 Transfusion Requirements

Patients will receive transfusion support per institutional guidelines. The number of red blood cell and platelet transfusions needed in the first 50 days of treatment will be assessed.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Participating sites within are responsible for submitting data and/or data forms to the ODQ according to the schedule set by ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; any

response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

This protocol will adhere to the policies and requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Overall PI, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix B.

- The Overall PI/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the study agent(s) directly from supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

12.4 Collaborative Agreements Language

N/A

13. STATISTICAL CONSIDERATIONS

Primary objective
This is a single arm study to evaluate the efficacy of lenalidomide in combination with reinduction chemotherapy (mitoxantrone/etoposide/cytarabine) for patients with relapsed or refractory AML.

Sample size
The primary endpoint is complete remission (CR) and CRp after MEC+lenalidomide. A 45% complete remission rate will be considered clinically significant. The response evaluation will be assessed after the first (and only) cycle of treatment. There is no retreatment as a part of this protocol. We will plan to evaluate patients who receive the study treatment; we will plan to replace up to 2 patients who are not able to complete the ten days of therapy.

A two-stage design is considered here. A total number of 40 patients is needed in order to detect a 20% response rate increase, assuming the null hypothesis is 25%, with a 90% power and 9% one-sided type I error rate. The study will be reevaluated if 4 or fewer responses out of 18 patients are observed and the treatment will be declared inactive if fewer than 14 responses are seen in the total 40 patients. Factors during treatment, such as the rate of treatment completion, may be considered in evaluating any responses. The probability of stopping at the first stage is 0.52, if the true response rate is 25%.

We chose this study design over other possible phase II study designs, including randomized phase II designs, because it is powered toward identifying a clinically significant improvement in response (20% increase compared to historical rates); a smaller randomized study may be underpowered to identify the true response rate.

Primary analysis
The rate of CR+CRp will be calculated along with the corresponding 90% two-stage exact binomial CI.

The analyses for secondary objectives will be descriptive in nature. Proportion of patients with ANC recovery/platelet recovery (as defined in Section 11.1) will be reported, along with median days to counts recovery. Total transfusion requirements will be reported as the median and range of each blood product. Cumulative incidence of treatment related mortality (defined in Section 11.2) will be estimated using relapse and early death from relapse as competing risks. Overall survival and Relapse-free survival (defined in Section 11.3) will be estimated using the Kaplan-Meier method.

Participants who never start protocol therapy, or who do not complete the 10 days of protocol based chemotherapy will be excluded from the analyses and will be replaced. The estimated accrual period is 2.5 years.

13.1.1 Evaluation of toxicity

All participants will be evaluable for toxicity from the time of their first treatment up until day 50 or the initiation of other therapy. In the phase I study of 35 patients, 3 dose limiting toxicities were noted due to prolonged count recovery. In the current study, we will monitor for toxicities and assess them as a part of the two stage design described above. If, within the first 10 patients on study, 3 or more experience prolonged neutrophil or platelet recovery beyond day 45 in the absence of disease, or other unacceptable toxicities felt related to the study regimen, we will put the study on hold to investigate further enrollment. The probability of putting the study on hold is 7%, if the true probability of delayed count recovery is 10%.

13.1.2 Evaluation of the Primary Efficacy Endpoint

Analyses will be performed on an intent-to-treat basis. Specifically, all eligible participants, except those who are removed and replaced, who are then included in the study, will be assessed for response/outcome to therapy, even if there are major protocol therapy deviations.

14. PUBLICATION PLAN

The results should be made public within 18 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	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.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

DFCI IRB Protocol #: 16-574

APPENDIX B

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

TABLE OF CONTENTS

1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP serves as a reference for any sites external to DF/HCC that are participating in a DF.HCC clinical trial.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-Center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Boston Children's Hospital (BCH), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (CTEP, Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies. The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Overall Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol

document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Office of Data Quality (ODQ): A group within DF/HCC responsible ensuring high-quality standards are used for data collection and the ongoing management of clinical trials, auditing, and data and safety monitoring. ODQ also coordinates quality assurance efforts related to multi-center clinical research.

DF/HCC Clinical Trials Research Informatics Office (CTRIO): A group within DF/HCC responsible for providing a comprehensive data management platform for managing clinical trial data.

2. GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Andrew Brunner, M.D., will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. CTEP, FDA, OBA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with the FDA (investigator-held IND trials) as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.

- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to Participating Institutions as needed.
- Review and approve Participating Site informed consent forms
- Conduct and document initial and ongoing protocol training
- Oversee the data collection process from Participating Institutions.
- Maintain documentation and cumulative reports of Serious Adverse Event (SAE) reports and Deviations/Violations across all sites and provide to the DF/HCC Sponsor for timely review and submission to the DFCI IRB, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all Participating Institutions.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out approved protocol monitoring plan either by on-site or remote monitoring.
- Maintain essential regulatory documents of all Participating Institutions which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites, and protocol training documentation
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc) and maintain documentation all relevant communications.

2.3 Participating Institution

Each Participating Institution is expected to comply with all applicable federal regulations and DF/HCC requirements, the protocol and HIPAA requirements.

The general responsibilities for each Participating Institution may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.

- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Adverse Event (SAE) reports to local IRB per institutional requirements and to the Coordinating Center, in accordance with DF/HCC or other sponsor requirements.
- Submit protocol deviations and violations to local IRB per institutional requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution upon request.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that for all interventional drug, biologic, or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution’s IRB.
- Participating Institution’s IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPPA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an authorization statement. This authorization statement may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB and if applicable NCI/CTEP, will provide a consent template, with information regarding authorization for the disclosure of protected health information.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected. DF/HCC has chosen to use authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned DF/HCC QACT case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.7 DF/HCC Multi-Center Protocol Registration Policy

All participants must be registered with DF/HCC prior to conducting any research-related procedures

3.7.1 Participant Registration and Randomization

Please refer to protocol **Section 4.0: Registration Procedures**

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the Participating Institution receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.7.3 Eligibility Exceptions

CTEP specifically prohibits registration of a participant on any NCI Sponsored protocol that does not fully and completely meet all eligibility requirements. No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All Participating Institutions are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.8 DF/HCC Protocol Case Number

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. Participating Institutions should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

3.8.1 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms “violation”, “deviation” and “exception” to describe departures from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.8.2 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is prospectively approved prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol departure that was not prospectively approved by the IRB prior to its initiation or implementation.

3.8.3 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions,

deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission. The deviation may not be implemented without all required approvals

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines. DF/HCC will forward all violation reports to CTEP via an internal DF/HCC process, as applicable.

3.9 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated therapy will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center. Protocols using CTEP supplied agents must report these toxicities via the Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS). The DF/HCC Sponsor will be notified of these events via CTEP-AERS.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.9.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol **Section 7: Adverse Events**.

Participating Institutions must report the SAEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB Adverse Event Reporting Policy.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Institutions will review and submit to their IRB according to their institutional policies and procedures

3.9.2 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. Participating Institutions will review /submit to their IRB according to their institutional policies and procedures.

3.10 Data Management

DF/HCC CTRIO develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC CTRIO provides a web based training for all eCRF users.

3.10.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

If study forms are not submitted on schedule, the Participating Institution will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

4. REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol **Section 8: Pharmaceutical Information**.

Participating Institutions should order their own agent regardless of the supplier. (i.e., NCI or a pharmaceutical company.)

If the agent is commercially available, check with the local Director of Pharmacy and/or the Research Pharmacy to ensure that the agent is in stock. If the agent is not stocked, ensure that the agent can be ordered once the protocol is approved by the local IRB.

If the agent is investigational, ensure that the pharmacy will be able to receive and store the agent according to state and federal requirements. The local IRB should be kept informed of who will supply the agent (i.e., NCI or a pharmaceutical company) so that any regulatory responsibilities can be met in a timely fashion.

5. MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

Participating Institutions will be required to submit participant source documents to the Coordinating Center for eligibility confirmation as well as for ongoing, remote monitoring. Participating Institution are also be subject to on-site monitoring conducted by the Coordinating Center.

Participating Institutions will undergo on-site monitoring by the Coordinating Center within 3 months of enrollment of the first patient; Combination on-site and remote monitoring will occur every 4-6 months thereafter while patients are on treatment or in active follow-up. Remote monitoring may be done in lieu of on-site monitoring if no active patients are on trial at that site. Once all site participants are off treatment and in long-term follow-up, remote monitoring will be conducted annually for confirmation of long term follow-up data and regulatory compliance.

For remote monitoring visits, Participating Institutions will be asked to provide remote electronic medical record access to the monitor or will be required to forward redacted copies of participants' medical record and source documents to the Coordinating Center to aid in source data verification. The participants and CRFs to be reviewed at the visit will be communicated at least 2 weeks in advance of the scheduled monitoring visit. Source documentation can be provided to the Coordinating Center via an encrypted memory stick

or via a secure file transfer system. During remote monitoring visits, the Site Specific File will be reviewed in lieu of the site regulatory binder.

On-Site Monitoring will be scheduled several weeks in advance and will be conducted over a 2-3 day period. During an on-site monitoring visit, 2-4 participants will be monitored as well as the complete regulatory binder. Source documentation verification (SDV) will be conducted by having access to participants' complete medical record and source documents. Participating Institutions will be expected to coordinate the necessary resources for the monitor, including a desk, access to all participant medical and research records (electronic and hard copy), the regulatory binders and access to a photocopier. The Participating Institution will also be asked to assist in scheduling a pharmacy visit and a brief exit interview on the final day of the visit with the Study Coordinator and the Site investigator.

All Participating Institutions will be required to participate in monthly Coordinating Center initiated teleconferences. Once all participants have completed treatment, teleconferences will be scheduled as needed.

5.2 Monitoring Reports

Following each monitoring visit, a monitoring follow-up report will be provided to the Participating Site (i.e. Site PI and Coordinator). The monitoring report will summarize any issued queries or data clarification requests, identify any reportable events or required follow-up on prior events and will specify details of any non-compliance. Participating Sites are requested to respond to all queries and data clarifications requests within 10 business days.

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

The **minimum** accrual per participating site is 3-5 patients annually in consideration of the regulatory and monitoring cost and effort of overseeing each site.

6. AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance and involves the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and

analyzed, and accurately reported per the protocol, applicable Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 Audit Plan: NCI Sponsored Trials

N/A

6.2 DF/HCC Internal Audits

All Participating Institutions are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.3 Audit Notifications

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal or NCI) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.4 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.5 Participating Institution Performance

The DF/HCC Sponsor, DFCI IRB and the NCI for CTEP trials, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

Participating Institutions that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation. A DF/HCC Sponsor and/or the DFCI IRB may terminate a site's participation if it is determined that a site is not fulfilling its responsibilities as described above.