Amendment

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Protocol Title: Lenalidomide Maintenance Therapy in Multiple Myeloma: A Phase II Clinical and Biomarker Study

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* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: Expedited

DEC Clearance Date: 01/18/2017

Protocol Version Date: 01/06/2017

Abbreviated Title: Revlimid Maintenance for MM
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Lenalidomide Maintenance Therapy in Multiple Myeloma: A Phase II Clinical and Biomarker Study

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E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes
G. Some/all research activities performed outside NIH

Commercial Agents:
Lenalidomide

Identifying Words: Revlimid, immunomodulatory drugs, T cell activity, gene expression

Précis

Background:

- Multiple myeloma (MM) remains largely an incurable disease with an estimated median survival of 6-7 years in standard risk myeloma and 2-3 years in high risk disease despite treatment with autologous stem cell transplantation (ASCT).
- Maintenance therapy to achieve sustained suppression of residual disease following chemotherapy or ASCT has long been viewed as a desirable approach for extending survival in MM.
- Giving the immunomodulatory drug lenalidomide after induction or re-induction treatment may stimulate the immune system in various ways to stop or slow the return of cancer.
- It is not yet known how immune stimulatory effects of extended dosing with lenalidomide in the maintenance setting correlate with clinical benefits.

Objective:

- Assess T cell (CD4, CD8), NKT and NK cell numbers in peripheral blood during the course of lenalidomide maintenance therapy in treated MM patients.

Eligibility:

- Patients with multiple myeloma who have achieved stable disease or better reponse, assessed at ≥ 4 weeks after completing induction or re-induction treatment
- Age ≥ 18 years
- ECOG PS of 0-2
- Adequate hematological parameters defined by: absolute neutrophil count ≥ 1.0 K/uL, hemoglobin ≥ 8 g/dL, and platelet count ≥ 75 K/uL
- Adequate hepatic function, with bilirubin < 1.5 x the ULN, and AST and ALT < 3 x ULN
- Adequate renal function with creatinine clearance (CrCl) of greater than or equal to 40 mL/min. CrCl will be calculated using the Cockcroft-Gault method. If the calculated CrCl based on Cockcroft-Gault method is <40 mL/min, patient will have a 24 hr urine collection to measure CrCl. The measured CrCl must also be ≥ 40 ml/min

Design:

- Single arm, single stage, phase II trial of lenalidomide maintenance for treated MM patients who have stable or responsive disease.
- After screening, eligibility determination, and enrollment; subjects will receive lenalidomide 10 mg by mouth daily on days 1-21 of repeated 28-day cycles. When necessary, lenalidomide will be held and restarted in accordance with accepted clinical dose modification guidelines.
- Subjects may continue lenalidomide until disease progression, unacceptable toxicity or completion of two years of lenalidomide therapy and the 30 day safety follow-up visit.
- Blood will be obtained to assess changes in T cell (CD4, CD8), NKT and NK cell numbers by flow-cytometric analysis at pre-specified time points during lenalidomide maintenance.
- Blood samples and/or bone marrow samples where possible, will be used for additional research studies, which may include functional analyses of immune-cell subsets, analyses for cytokines, chemokines, antibodies, tumor cell antigen targets, and/or other markers.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective:

- Assess T cell (CD4, CD8), NKT and NK cell numbers in peripheral blood of MM patients during the course of lenalidomide maintenance therapy.

1.1.2 Secondary Objectives:

- Determine progression free survival
- Determine duration of response
- Assess NK cell function and activity
- Assess changes in B cell subsets, myeloid derived suppressor cells and T regulatory cells by phenotypic analysis during the course of therapy
- Assess expression of CRBN, and how it relates to NK cell number and activity

1.2 BACKGROUND AND RATIONALE

Multiple myeloma overview

Multiple myeloma (MM) accounts for 1% of all cancers and approximately 10% of all hematologic malignancies [1]. It is defined as a clonal proliferation of malignant plasma cells in the bone marrow, with the presence of a monoclonal protein in serum or urine and with features of end-organ damage including hypercalcemia, renal insufficiency, anemia, and bone lytic lesions [2]. Almost all patients evolve from an asymptomatic premalignant state termed monoclonal gammopathy of undetermined significance (MGUS) [3]. MM is incurable with an estimated median survival of 6-7 years in standard risk myeloma and 2-3 years in high risk disease despite treatment with autologous stem cell transplantation (ASCT) [4].

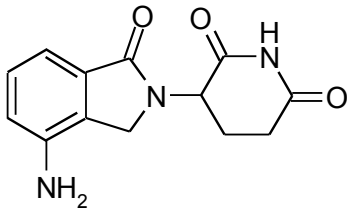
Maintenance therapy in MM

Maintenance therapy to achieve sustained suppression of residual disease following chemotherapy induction or ASCT has long been viewed as a desirable approach for extending survival in MM. Even the most intensive therapy followed by ASCT is usually unable to extend progression-free survival (PFS) to beyond 36 months, with the majority of patients eventually experiencing relapse. Efforts to establish long-term maintenance regimens using alkylating agents, interferons, and corticosteroids have been hindered by unacceptable toxicity and have largely failed to improve survival [5]. One study in which patients were randomized to receive either prednisone 50mg or 10mg per day showed an overall survival (OS) benefit in the 50mg arm of 37 v. 26 months, P=0.05 [6]. However, these results have not been confirmed in a subsequent controlled clinical trial, and patients treated with corticosteroid maintenance are less responsive to these agents at relapse [7]. Increased survival in MM has been achieved with the introduction of novel therapeutic agents including the immunomodulatory drug lenalidomide (Revlimid; Celgene) [8].

Investigational drug lenalidomide

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

Chemical Structure of Lenalidomide



Lenalidomide possesses diverse tumoricidal and immunomodulatory properties and a favorable toxicity profile. Lenalidomide overcomes many of the issues associated with long-term thalidomide therapy, especially neuropathy. Lenalidomide in combination with dexamethasone received FDA approval after two parallel studies demonstrated response rates of 61% for lenalidomide plus dexamethasone versus 22% for dexamethasone alone in patients with relapsed/refractory multiple myeloma [9]. The drug has also been studied in newly diagnosed patients in combination with low-dose dexamethasone, as well as with bortezomib and dexamethasone.

Lenalidomide role as maintenance therapy

A pilot phase 2 study showing the feasibility and efficacy of lenalidomide consolidation and maintenance therapy laid the basis for further clinical testing of lenalidomide. Recently, three large phase III trials comparing lenalidomide maintenance to placebo found significant progression-free survival (PFS) benefit for both ASCT-eligible [10, 11] and ineligible [12] patients. Additionally, one study [11] reported a significant difference in 3 year OS between the lenalidomide and placebo arms, 88% v. 80%, p=0.03. In all three studies [10-12], the progression-free survival benefit of lenalidomide was independent of the response to induction therapy at randomization, age, disease stage, β2-microglobulin level, use or nonuse of lenalidomide or thalidomide induction therapy, and presence or absence of cytogenetic abnormalities (though cytogenetic data were available only in the study by Attal et al. [10] and only in a few patients).

These findings are summarized in table 1.

Table1: Lenalidomide maintenance studies after ASCT and conventional chemotherapy

Study	Induction	No.	Maintenance	PFS/TTP (median)	OS	Discontinue due to AE
IFM 2005-02 [10]	VAD/other→ Single or double ASCT → Len. consolidation x 2 cycles	a. 307	a. Len. 10 mg /d with dose adaptations (5-15 mg/d)	a. 41 mo	a. 73% (4 y OS)	a. 27%
		b. 307	b. Placebo	b. 21 mo p <0.001 HR 0.50	b. 75% p = 0.70 HR 1.06	b.15%
CALGB 100104 [11]	Any → ASCT	a. 231	a. Len. 10-15 mg/d	a. 46 mo (TTP)	a.88%) (3 y OS)	a. 12%
		b. 229	b. Placebo	b. 27mo p <0.001	b. 80% p= 0.03	b. 2%
MM 015 [12]	a. Mel. + Pred. + Len.	a. 152	a. Len. 10 mg/d, days 1-21	a. 31 mo	a. 70% (3 y OS)	a. 20%
	b. Mel. + Pred. + Len.	b. 153	b. Placebo	b. 14 mo	b. 62%	b. 16%
	c. Mel. + Pred.	c. 154	c. Placebo	c. 13 mo p <0.001	c. 66% p =0.254	c. 2.9%

PFS- progression free survival; TTP- time to progression; OS- overall survival; SPM- second primary malignancies; VAD- vincristine, adrimaycin, dexamethasone; ASCT- autologous stem cell transplant; Mel.-melphalan; Pred.-prednisone; Len.- lenalidomide; AE - adverse events

Lenalidomide maintenance therapy was well tolerated with expected and manageable side effects including mild hematotoxicity and infections. There was no increase in neurotoxicity or thromboembolic complications. Most patients were able to continue maintenance treatment without the need to discontinue therapy because of adverse events. Of note, there was a 7 to 8% rate of second primary cancers in the lenalidomide groups as compared with 3 to 4% in the placebo groups. This increase in second cancers led to discontinuation of lenalidomide in the trial reported by Attal et al [10]. Post hoc analysis with second cancers scored as events showed a median event-free survival of 40 to 43 months versus 23 to 27 months in both transplantation trials [10, 11] and 29 months versus 13 months in the trial that compared MPR-R with placebo [12], providing support for a magnitude of benefit associated with lenalidomide that seems to outweigh the risk of second cancers. The development of second primary cancers may also be due in part to other factors, some of which include prior use of various MM drugs with known leukemogenic potential, MM-related disease factors, host-related factors, as well as environmental and behavioral factors [13]. Further studies are needed to evaluate the true risk of this complication, to identify risk factors for its development, and hopefully, to develop strategies for the prevention of second primary cancers. Before more information is available, physicians and patients must weigh the benefits of lenalidomide maintenance therapy against the low but relevant risk of second cancers.

Lenalidomide mechanism of action

The mechanism of action of lenalidomide remains to be fully characterized. Although the exact mechanisms of lenalidomide's action are unknown, a number of mechanisms are postulated to be responsible for lenalidomide's ability to provide disease control. The anti-MM effect of lenalidomide is thought to be mediated by the combined effects of activation of immune effector cells (both T and NK cells), tumor down regulation of critical pro-survival cytokines (including

IL-6, TNF-α, PDGF and VEGF), direct inhibition of plasma cell proliferation, and suppression of angiogenesis [14, 15, 16].

Lenalidomide effect on NK, NKT, and T cells

Data suggest that part of the therapeutic efficacy of lenalidomide may be via the promotion of NK cell–mediated responses against MM cells. A number of studies and our unpublished preclinical work from (table 2) has demonstrated that as the disease progresses to advanced states from MGUS to MM, there is an associated decline in the NK cell number, and associated anti-MM activity[17, 18, 19].

Table 2: Data from natural history study 10-c-0096 comparing NK cell numbers in subjects with monoclonal gammopathy of uncertain significance and smoldering multiple myeloma

Disease	N	Variable	Mean (k/μL)	p-value
MGUS	33	WBCs	6.41	0.03
SMM	37		5.32	
MGUS	33	NK cells	0.89	0.07
SMM	37		0.69	
Mayo Clinic SMM Risk Score 1	17	NK cells	0.85	0.004
Mayo Clinic SMM Risk Score 2	18		0.53	

MGUS- monoclonal gammopathy of undetermined significance; SMM- smoldering multiple myeloma

Lenalidomide can augment NK cell number, cytotoxicity and NK cell–driven antibody-dependent cell-mediated cytotoxicity (ADCC) in several ways [14]. In vitro studies have shown that peripheral blood mononuclear cells (PBMCs) are required for lenalidomide-induced NK cell function, and this is achieved indirectly through activation of nuclear factor of activated T cell 2 (NFAT2) in T cells [20, 21]. In this way, lenalidomide activates T cells to produce IL-2, which in turn stimulates NK cell activation and function against MM [20]. Lenalidomide causes enhancement of antigen-specific CD8+ T-cell cytolysis, upregulation of Fc-gamma receptor signaling leading to increased NK cell activity, and activation of NKT cells [14]. Lenalidomide has been shown to increase CD16 expression on NK cells and facilitate ADCC against MM [22]. Lenalidomide can also down-modulate suppressor of cytokine signaling (SOCS) 1 expression in NK cells, resulting in a higher sensitivity to activating stimuli in the microenvironment [23]. Finally, lenalidomide also appears to favorably modulate the balance of NK cell activating and inhibitory ligand expression on MM targets. This action of lenalidomide may be of particular interest as initiation of a cytotoxic response by an NK cell is mediated through balance of signals obtained through inhibitory receptors such as killer immunoglobulin-like receptors (KIRs) and CD94–NKG2A [24], and activating receptors including NKG2D, DNAM-1, and Nkp46 [24]. Lenalidomide up regulates expression of ULBP-1 on MM cells and decreases expression of PD-L1, which both result in improved NK cell surveillance, recognition and lysis of MM tumor targets [26, 27]. Changes in NK cell receptor ligand expression on MM cells and, hence, their resistance to NK cell–mediated killing may underlie the development of more proliferative and refractory plasma cell clones as MM progresses [28, 29] and treatment with lenalidomide in maintenance setting may provide a means to promote NK cell–mediated control over MM .

Furthermore, prior smaller investigations in relapsed multiple myeloma have reported lenalidomide to increase the numbers of T cells (CD4, CD8), NKT and NK cells patients [30, 31]. Similar findings have been reported in studies based on myeloma cell lines [32, 33]. In a recent study lenalidomide was found to be particularly active in patients with higher cereblon (CRBN) expression, suggesting CRBN plays a role in lenalidomide-mediated immune activation [34]; however, the relationship between these observations and a clinical benefit is unclear.

Lenalidomide in combination with dexamethasone has been FDA-approved to treat relapsed and refractory MM. Interestingly, however, in the setting of a clinical trial of lenalidomide and dexamethasone, correlative studies demonstrated that while NK cell number was increased from a mean of 2.20 ± 0.05 × 10^5/mL (baseline) to a mean of 3.90 ± 0.03 × 10^5/mL (cycle 6; P = .05), cytotoxicity was irreversibly reduced from 48.9% ± 6.8% to 27.6% ± 5.1% (P = .0028), attributed mainly to blunting of lenalidomide's immunostimulatory effects on patient NK cells by concurrent dexamethasone administration [35]. Hence, single-agent lenalidomide seems to be the logical choice for maintenance treatment when tumor load has already been reduced significantly and control of the residual tumor cells by active immune surveillance is the clinically relevant priority. Given the presumed broad immune stimulatory effects of lenalidomide, it may be of clinical and scientific importance to investigate changes in immune cells in the setting of extended dosing (i.e. maintenance therapy) with this agent. Currently, there is no information available on this topic.

Lenalidomide effect on B cell subsets, MDSC and T regulatory cells

Regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) have been shown to inhibit the activation and function of T cells that participate in antigen-specific immune responses. Tregs are potent immunosuppressive cells of the immune system that promote progression of cancer through their ability to limit antitumor immunity and promote angiogenesis. Higher proportions of Tregs have been reported in the PBMCs of patients with several types of tumors [36, 37, 38] including MM [39, 40]. Lenalidomide has been shown to inhibit the proliferation of FOXP3+ CTLA-4+CD4+CD25high Tregs in vitro [41]. In humans, MDSCs are defined as CD14-CD11b+ or cells that express the myeloid marker CD33 and lack markers for mature myeloid and lymphoid cells in combination with low expression of HLA-DR [42]. Recently, new populations of CD14+HLA-DR−/low MDSCs have been identified in patients with hepatocellular carcinoma [43] and MM [44]. MDSCs accumulate as a consequence of factors associated with inflammation, such as increased secretion of vascular endothelial growth factor (VEGF), GM-CSF, IL-1β, IL-6 and PGE2, factors that are also elevated in patients with MM [45] These changes in Treg and MDSC populations have not been characterized in the setting of lenalidomide maintenance in patients with MM. Cytokines and growth factors directly regulate proliferation, differentiation and death of normal B-lymphocytes, and abnormal cytokine networks clearly exist within the MM microenvironment. A recent in vitro study showed that lenalidomide significantly suppresses immunoglobulin synthesis by B cells [45], but we have limited knowledge of lenalidomide's effect on B cell populations in patients receiving maintenance therapy.

To conduct a comprehensive evaluation of a range of immune cells in relation to lenalidomide maintenance therapy, we will include analyses of B-, T-, NK and NKT cells and important signaling cytokines.

Proposed Study Investigation with Biomarker Studies

The primary objective of this study is a longitudinal assessment of T cell (CD4, CD8), NKT and NK cell populations during the course of lenalidomide maintenance therapy in patients with MM. Peripheral blood samples from all enrolled patients will be collected and batched for biomarker analysis at baseline, after cycles 1, 3, and 6, and then after every 6 cycles while receiving lenalidomide therapy, until completion of the study. The assays will be run on the batched samples for first ten patients at baseline, after cycles 1, 3, and 6 to determine the appropriate time points of assays for the subsequent patients. Samples will also be collected from all patients at the time of disease progression. Blood samples and bone marrow samples will be used for additional research studies, which may include functional analyses of immune-cell subsets, analyses for cytokines, chemokines, antibodies, tumor cell antigen targets, and/or other markers. Effective with Amendment D, bone marrow aspirate and biopsy will be obtained for research studies at the discretion of the PI. Patients' tumor cells, where possible, will be characterized by gene expression profiling to enhance our understanding of lenalidomide efficacy and safety in a genetically diverse tumor population.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients with multiple myeloma treated with induction therapy or re-induction therapy, who at the time of study enrollment have documented evidence of stable disease response or better according to International Myeloma Workshop Consensus Panel as provided in Section **6.2**. The response assessment must occur at least 4 weeks after completion of their last treatment.

2.1.1.2 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of lenalidomide in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials.

2.1.1.3 ECOG performance status ≤ 2 (see Appendix A).

2.1.1.4 Patient must have adequate hematologic, renal, hepatic, and cardiac function as defined by:

- Absolute neutrophil count ≥ 1.0K /μL independent of growth factor support
- Platelets ≥ 75K/μL
- Hemoglobin ≥ 8 g/dL (transfusions are permissible)
- Calculated creatinine (CrCl) clearance of greater than or equal to 40 mL/min. using the Cockcroft-Gault method (see Appendix E). If the calculated CrCl based on Cockcroft-Gault method is <40 mL/min, patient will have a 24 hr urine collection to measure CrCl. The measured CrCl must also be ≥ 40 ml/min.
- Total bilirubin ≤ 1.5 mg/dL, AST (SGOT) and ALT (SGPT) ≤ 3 x ULN

2.1.1.5 Females of childbearing potential (FCBP) must agree to use two effective forms of contraception simultaneously or to practice complete abstinence from heterosexual intercourse during the following time periods related to this study: 1) for at least 28 days before starting study drug; 2) while participating in the study; and 3) for at least

28 days after discontinuation from the study. The two methods of effective contraception must include one highly effective method (i.e. intrauterine device (IUD), hormonal [birth control pills, injections, or implants], tubal ligation, partner's vasectomy) and one additional effective (barrier) method (i.e. latex condom, diaphragm, cervical cap). FCBP must be referred to a qualified provider of contraceptive methods if needed.

2.1.1.6 A FCBP is defined as a sexually mature woman 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).

2.1.1.7 A FCBP must have two negative serum or urine pregnancy tests prior to starting study drug. The first pregnancy test must be performed within 10-14 days prior to the start of study drug and the second pregnancy test must be performed within 24 hours prior to prescribing the study drug. The prescriptions of study drug must be filled within 7 days.

2.1.1.8 Male patients must agree to use a latex condom during sexual contact with FCBP while participating in the study and for at least 28 days following discontinuation from the study even if he has undergone a successful vasectomy.

2.1.1.9 Patient must be able to take aspirin (81 or 325 mg) daily as prophylactic anticoagulation. Patients intolerant to ASA may use warfarin or low molecular weight heparin.

2.1.1.10 Patient must understand and voluntarily sign an informed consent form, with the understanding that the patient may withdraw consent at any time without prejudice to future medical care.

2.1.2 Exclusion Criteria

2.1.2.1 Patients with progressive or refractory MM, as defined by International Myeloma Workshop Consensus Panel criteria provided in section **6.2**

2.1.2.2 Refractory to lenalidomide in the most recent line of therapy, as defined by the International Myeloma Consensus Panel criteria [47]- as failure to achieve minimal response or development of progressive disease while on lenalidomide or within 30 days of lenalidomide therapy

2.1.2.3 Patients who are receiving any other investigational agents with the intent to treat myeloma. Permitted concurrent therapies include:

- Bisphosphonates
- Radiotherapy to single stable disease site

2.1.2.4 Plasma cell leukemia

2.1.2.5 Pregnant or lactating females. Because there is a potential risk for adverse events to nursing infants secondary to treatment of the mother with lenalidomide, lactating females must agree not to breast feed while taking lenalidomide.

2.1.2.6 Uncontrolled hypertension or diabetes

2.1.2.7 Active hepatitis B or C infection

2.1.2.8 Diagnosed or treated for another malignancy within 3 years prior to study enrollment, with the exception of complete resection of non-melanoma skin cancer, or an in situ malignancy.

2.1.2.9 Previous diagnosis of another malignancy with any evidence of residual disease.

2.1.2.10 Patients seropositive for the human immunodeficiency virus (HIV), and/or those who are taking anti-retroviral treatment for HIV/AIDS

2.1.2.11 Prior organ transplant requiring immunosuppressive therapy

2.1.2.12 Prior allogeneic stem cell transplant

2.1.2.13 Patients requiring continuous, systemic immunosuppressive therapy

2.1.2.14 Patients with myocardial infarction within 6 months prior to enrollment, New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, severe uncontrolled cardiac arrhythmias, or electrocardiographic evidence of acute ischemia

2.1.2.15 Patients with conditions that would prevent absorption of the study drug

2.1.2.16 Uncontrolled intercurrent illness including but not limited to uncontrolled infection or psychiatric illness/social situations that would compromise compliance with study requirements

2.1.2.17 Significant neuropathy ≥Grade 3 at baseline

2.1.2.18 Contraindication to concomitant anticoagulation prophylaxis

2.1.2.19 Major surgery within 1 month prior to enrollment

2.1.2.20 Patients who were previously exposed and who developed severe adverse events, hypersensitivity or desquamating rash to either thalidomide or lenalidomide

2.1.3 Recruitment Strategies

1) Patients with newly diagnosed MM who have completed induction treatment with CRd on trial 11-C-0221.
2) Patients with relapsed multiple myeloma after completing treatment on expanded access CRd trial 12-C-0043 or other appropriate NCI trials.
3) Other participant sources will be from outside physician referrals.
4) Our ongoing multiple myeloma treatment study and outside physician referral network has a high representation of minorities.

2.2 SCREENING EVALUATION

2.2.1 Clinical evaluation must be completed within 4 weeks prior to study enrollment:

A complete history and physical examination with documentation of type of response (sCR, CR, VGPR, PR, MR or SD according to criteria described in section **6.2**) to induction or re-induction treatment, the time interval from last treatment to documentation of response, and assessment of performance status using the ECOG scale must be performed prior to study entry. Patients will be evaluated for baseline neuropathy.

2.2.2 The following studies and laboratory tests will be completed within 4 weeks prior to study entry:

2.2.2.1 CBC with differential

2.2.2.2 Reticulocyte count

2.2.2.3 Acute care panel, calculated creatinine clearance using Cockgroft-Gault formula (within 2 weeks prior to study entry)

2.2.2.4 Mineral panel, Hepatic panel, Uric acid, LDH, and Beta-2 Microglobulin

2.2.2.5 PT, PTT

2.2.2.6 Serum protein electrophoresis (SPEP) and immunofixation to assess for presence and quantity of monoclonal protein (M-protein)

2.2.2.7 Random urine sample for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria). Collect a 24 hour urine sample if necessary for confirmation of multiple myeloma response to prior (re)induction treatment, or if calculated creatinine clearance is <40 ml/min.

2.2.2.8 Serum free light chain studies

2.2.2.9 Quantitative immunoglobulins

2.2.2.10 Viral serologies:

- Hepatitis B surface antigen
- Anti Hepatitis C (HCV) antibody. If positive, will follow with HCV RNA PCR
- HIV ELISA screening test. If positive, will follow with confirmatory Western Blot test

2.2.2.11 Review of bone marrow core biopsy or aspirate

2.2.2.12 Serum or urine pregnancy test in women of child-bearing potential (complete within 10-14 days and again within 24 hours prior to prescribing lenalidomide for cycle 1)

2.2.2.13 12-lead EKG

2.2.2.14 Skeletal survey of the axial and appendicular skeleton. Exception may be made if skeletal survey has been performed within the past 3 months and was found to be positive. In this case, films will be forwarded to the Clinical Center for confirmatory interpretation by the Department of Radiology.

2.3 REGISTRATION PROCEDURES

2.3.1 Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 BASELINE EVALUATION

2.4.1 Research and clinical laboratory tests to be performed within 4 weeks of study entry and prior to starting therapy. Refer to section 5 for details on biospecimen collection and biomarker studies.

2.4.2 Peripheral Blood/Urine (see section 5)

2.4.2.1 For storage and establishing a biobank (AppendixC in section **15**)

2.4.2.2 Flow cytometry and other assays (see section 5):

- Phenotypic analysis of NK, NKT, and T cells
- Phenotypic analysis of B cell subsets
- Proliferation assays for human T cells (CD4 and CD8)
- Functional NK cell analysis
- Regulatory T cell and myeloid derived suppressor cell (MDSC) phenotype
- Chemokine and cytokine assay
- Selected tumor cell antigen targets

2.4.3 Bone Marrow (see section **5**). Bone marrow aspirate and biopsy will be used for histopathological evaluation, to determine the immunophenotyping of aberrant clonal plasma cells by multiparametric flowcytometry, CD 138+ and CD 138- fractions cell sorting with subsequent correlatives on both fractions, and the expression of CRBN by gene expression profiling and quantitative PCR.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

- Patients with newly diagnosed or relapsed MM with stable disease or better clinical response after induction or re-induction treatment will be enrolled on this phase II study and treated with a maintenance dose of lenalidomide 10 mg daily on days 1 to 21 of repeated 28-day cycles.
- Patients who complete at least 3 cycles will be evaluable for primary endpoints. Patients who do not complete three cycles of therapy will not be included in the analysis of primary or non-primary endpoints; and will be replaced by new patients.
- Using flow cytometry on peripheral blood, the four primary outcomes of T cell (CD4, CD8), NKT and NK cell numbers will be measured at pre-specified treatment time points to determine the difference in the counts of these parameters for each patient.

3.2 DRUG ADMINISTRATION

- Lenalidomide10 mg once daily, given by mouth on days 1-21, of repeated 28-day cycles, to continue until disease progression, unacceptable toxicity or completion of two years of lenalidomide therapy and the 30 day safety follow-up visit.
- Lenalidomide will be prescribed monthly by Clinical Center physicians. Upon completion of phone counseling per the REMS® program, the NIH Clinical Center physician will prescribe a 28 day supply of lenalidomide to be sent to the patient by express delivery beginning with Cycle 4.

- Females of childbearing potential (FCBP) patients will be required to have pregnancy testing as outlined in AppendixAppendix B: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods; and may have the pregnancy test performed by local treating physician. In these cases, the local physician will submit the results to NIH Clinical Center for review. Upon receipt of negative pregnancy test result and completion of phone counseling per the REMS® program, NIH Clinical physician will prescribe a 28 day supply of lenalidomide to be sent to the patient by express delivery.
- Lenalidomide capsules should be swallowed whole, and should not be broken, chewed or opened.
- If vomiting occurs within 15 minutes and the whole capsule is present in the emesis then the dose can be repeated.
- If a dose of lenalidomide is missed, it should be taken as soon as possible on the same day. If it is missed for the entire day, it should not be made up.
- 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.
- 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. Per standard REMS® 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 REMS® program. Prescriptions must be filled within 7 days for females of childbearing potential and 14 days for all other risk categories. Only enough lenalidomide for one cycle of therapy will be supplied to the patient each cycle.
- Patients should be instructed never to give lenalidomide to another person and to return any unused capsules to their research coordinator at the end of treatment.
- Patients should not donate blood during therapy and for at least 28 days following discontinuation of lenalidomide.
- Patients will be required to take oral Aspirin 81 mg or 325 mg or alternative anticoagulation therapy every day for the duration of their participation in the study.

3.3 DOSE MODIFICATIONS

Before each cycle, patients will be evaluated for possible toxicities that may have occurred after the previous dose(s). Toxicities will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) (http://ctep.cancer.gov/reporting/ctc.html). Each adverse event (AE) attributed to lenalidomide will be carefully recorded, so that the dose modifications can be made accordingly. If multiple toxicities are noted, the dose adjustments and/or delays will be made according to guidelines that address the most severe toxicity.

3.3.1 Dose reduction steps:

Dose	Lenalidomide
Baseline dose	10 mg
One level dose reduction	5 mg
Two level dose reductions	5 mg every other day

3.3.2 Dose adjustments for Thrombocytopenia:

Thrombocytopenia	Recommended Course
First Episode- Platelet count $< 25 \times 10^9$/L	Hold lenalidomide. Hold prophylactic anti-coagulation. Monitor CBC weekly. Platelet count returns to $\geq 25 \times 10^9$/L. Resume lenalidomide at 10 mg. Resume prophylactic anti-coagulation, if no bleeding.
Subsequent Episodes- Platelet count $< 25 \times 10^9$/L	Hold lenalidomide. Hold prophylactic anti-coagulation. Monitor CBC weekly. Platelet count returns to $\geq 25 \times 10^9$/L. Resume lenalidomide at next lower dose level. Resume prophylactic anti-coagulation, if no bleeding. Do not decrease dose below 5 mg every other day.

*In case of thrombocytopenia, the decision to modify the dose of lenalidomide, administer platelet transfusions, and resume anti-coagulation may be based on the clinical judgement of the PI.

3.3.3 Dose adjustments for Neutropenia:

Neutropenia	Recommended Course
First episode- (Neutropenia is the only hematologic toxicity) ANC $< 0.5 \times 10^9$/L (grade 4) ANC $< 1.0 \times 10^9$/L (grade 3) with fever/sepsis	Hold lenalidomide. Consider growth factors. Monitor CBC weekly. ANC returns to $\geq 0.5 \times 10^9$/L, and fever/sepsis have resolved. Resume lenalidomide at same dose level, and consider stopping growth factors.
First episode- (Hematologic toxicities other than neutropenia are observed) ANC $< 0.5 \times 10^9$/L (grade 4) ANC $< 1.0 \times 10^9$/L (grade 3) with fever/ sepsis	Hold lenalidomide. Consider growth factors. Monitor CBC weekly. ANC returns to $\geq 0.5 \times 10^9$/L, other toxicities and fever/sepsis have resolved. Resume lenalidomide at next lower dose level, and consider stopping growth factors.

Subsequent Episodes- ANC < 0.5 x 10^9/L (grade 4) ANC < 1.0 x 10^9/L (grade 3) with fever/sepsis	Hold lenalidomide. Add growth factors. Monitor CBC weekly. ANC returns to ≥ 0.5 x 10^9/L, and fever/sepsis have resolved. Resume lenalidomide at next lower dose level and consider stopping growth factors. Do not decrease dose below 5 mg every other day.

*Fever defined as single temperature of 38.3°C or sustained temperature of 38°C for > 1 hour. In case of neutropenia, the decision to start/continue growth factors (such as filgrastim, pegfilgrastim), and/or reduce the dose of lenalidomide may be based on the clinical judgment of the PI.

3.3.4 Dose adjustments for Non hematologic toxicity

Lenalidomide Common Non-hematologic Toxicities	**Dose Modifications**
Non Blistering Rash	If Grade 3, hold lenalidomide, and restart at next lower dose level once rash improves to ≤ Grade 2.
Blistering, exfoliative or bullous rash (any grade), or suspected Stevens Johnson Syndrome (SJS) or Toxic Epidermal Necrolysis (TEN)	Discontinue lenalidomide and remove patient from therapy.
Venous or arterial thrombosis/embolism	Hold lenalidomide and start therapeutic anticoagulation. Restart lenalidomide at investigator's discretion at current or reduced dose level. Patient should continue anticoagulation therapy during the course of lenalidomide treatment. Additional work up to determine the cause of thromboembolic events may be done as clinically indicated at the discretion of investigator.
Renal Dysfunction CrCl based on Cockcroft-Gault formula: CrCl = (140 – Age) x Mass (in kilograms) x [0.85 if Female] 72 x Serum Creatinine (in mg/dL)	• CrCl 31-60 ml/min – no dose modifications • CrCl ≤30 mL/min (not requiring dialysis) – Dose reduce Lenalidomide to 10 mg every 48 hours • CrCl ≤30 mL/min (requiring dialysis) – Decrease Lenalidomide to 5 mg daily and on dialysis days, dose lenalidomide after dialysis.
Other non-hematologic grade 3/4 toxicities judged to be related to lenalidomide. Readily reversible electrolyte and metabolic abnormalities or infections controlled by appropriate therapy are exempt.	Hold treatment and restart at next lower dose level when toxicity has resolved to ≤Grade 2

3.3.5 Monitoring (see study calendar for more specific details) Please Note: Day 1 may be delayed for up to one week for Cycle 2 and beyond.

3.3.5.1 Routine labs (CBC with diff, retic count, acute care panel, mineral panel, hepatic panel, LDH, uric acid) will be performed on Day 1 (within 3 days prior) and Day 15

(within 3 days prior) of first cycle, on Day 1 (within 3 days prior) of cycles 2, 4 and 7 and on Day 1 (within 3 days prior) of every third cycle starting at cycle 10 at scheduled clinic visits, at the time of documented CR, and at disease progression (see study calendar).

3.3.5.2 Random urine sample for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria) will be performed at baseline, on Day 1 (within 3 days prior) of cycles 2, 4 and 7 and on Day 1 (within 3 days prior) of every third cycle starting at cycle 10 at scheduled clinic visits, at the time of documented CR, and at disease progression (see study calendar).

3.3.5.3 Myeloma tests including serum protein electrophoresis, serum immunofixation, serum free light chains, quantitative immunoglobulins, beta-2 microglobulin will be performed at baseline, on Day 1 (within 3 days prior) of cycles 2, 4 and 7 and on Day 1 (within 3 days prior) of every third cycle starting at cycle 10 at scheduled clinic visits, at the time of documented CR, and at disease progression (see study calendar).

3.3.5.4 Patients will have clinic visits with H&P or standard progress notes assessing for toxicity/side effects on Day 1(within 3 days prior), and 15 (within 3 days prior) of cycle 1 and Day 1 (within 3 days prior) of cycles 2, 4 and 7 and on Day 1 (within 3 days prior) of every third cycle starting at cycle 10 at scheduled clinic visits (see study calendar).

3.3.5.5 Skeletal survey to assess disease status will be performed at baseline. The skeletal survey at the time of documented CR or disease progression is optional.

3.3.5.6 Evaluation of bone marrow for disease status will be performed at baseline, and then at the time of documented CR or disease progression. Additional bone marrow samples to evaluate disease status, treatment effect, or biomarker studies will be obtained at the discretion of PI, if patients are willing.

3.3.5.7 Additional laboratory studies and clinic visits may be performed as clinically indicated.

3.3.5.8 Patients removed from protocol therapy for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Further follow-up can be assessed either by phone consultation or clinic appointment at the discretion of the PI.

3.4 STUDY CALENDAR

Study	Pre-treatment (Screening/ Baseline)	Maintenance Treatment until progression, unacceptable toxicity or completion of two years of lenalidomide therapy and the 30 day safety follow-up visit					Progressive disease	Follow up- off protocol therapy[l, m]	
		Cycle 1		Cycles 2, 4 and 7	Cycle 10 and beyond-every 3 cycles	CR achieved	Any cycle	Determine at PI's discretion	
		Day[k] 1	Day[k] 15	Day[m] 1	Day[m] 1			Any day	
Medical Record Review	x								x
H&P/clinic visit	x	x	x	x	x	x	x	x	
ECOG	x	x	x	x	x	x	x	x	
Informed Consent	x								
Routine Labs[a]	x	x	x	x	x	x	x	x	
Viral Studies[b]	x								
Register for REMS	x								
Pregnancy Test[c]	x[c]	x[d]	x[e]	x[e]	x[e]	x[e]			
Myeloma tests[f]	x			x	x	x	x	x	
Urine for UPEP and IFE [g]	x			x	x	x	x	x	
Bone marrow/aspirate[h]	x					x	x	x	
Blood for - biomarkers		x		x	x	x	x	x	
12 lead EKG	x								
Skeletal survey	x						x[j]	x[j]	
Adverse Events/Toxicity		x	→	→	→	→	→	→	
Storage blood/urine		x		x	x	x	x	x	

a. Routine tests include CBC, reticulocyte count, acute care panel, mineral panel and hepatic panel, uric acid, LDH and calculated CrCl. PT and PTT will only be performed at baseline.

b. Viral studies include: HIV, Hep B surface antigen and Hep C antibody. If Hep C antibody positive, Hep C RNA PCR will be performed.

c. Pregnancy tests (urine or serum) for females of childbearing potential. A female of childbearing potential (FCBP) 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)

d. Pregnancy tests (urine or serum) must occur within 10 – 14 days and again within 24 hours prior to prescribing lenalidomide for Cycle 1 (prescriptions must be filled within 7 days).

e. FCBP with regular or no menstruation must have a pregnancy test (serum or urine) weekly for the first 28 days and then every 28 days while on therapy (including breaks in therapy); at discontinuation of lenalidomide and at Day 28 post the last dose of lenalidomide. Females with irregular menstruation must have a pregnancy test (serum or urine) weekly for the first 28 days and then every 14 days while on therapy (including breaks in therapy), at discontinuation of lenalidomide and at Day 14 and Day 28 post the last dose of lenalidomide (see Appendix A: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods).

f. Myeloma tests include serum protein electrophoresis, serum immunofixation, , serum free light chains, quantitative immunoglobulins, and beta-2 microglobulin will be performed at baseline, on Day 1 (within 3 days prior) of Cycle 2, Cycle 4, and Cycle 7, and thereafter every 3 cycles, at the time of documented CR, and at disease progression.

g. Random urine sample for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria) will be performed at baseline, on Day 1 (within 3 days prior) of Cycle 2, Cycle 4, and Cycle 7, and thereafter every 3 cycles, at the time of documented CR, and at disease progression.

h. Bone marrow aspirate and biopsy can be performed +/- 21 days of intended cycle day. Bone marrow aspirate and biopsy will be sent to Hematology Service of the Department of Laboratory Medicine and Flow cytometry for immunophenotyping of plasma cells. CD 138 sorting/GEP/quantitative PCR/ storage will be done in Molecular pathology or Lymphoid Malignancies Branch lab... For questions regarding these samples, please contact Mark Roschewski at 301-451-9021. Effective with Amendment D, bone marrow aspirate and biopsy will be obtained for research studies at the discretion of the PI.

i. Blood for biomarker analysis will be collected on D1 (within 3 days prior) of Cycle 1, Cycle 2, Cycle 4, Cycle 7 and every 6 cycles thereafter.

j. Skeletal survey to assess disease status will be performed at baseline. The skeletal survey at the time of documented CR or disease progression is optional.

k. Variations of +/- 3 days of scheduled visits are permitted.

l. Patients removed from protocol therapy for unacceptable toxicities will be followed until resolution or stabilization of the adverse event. Further follow-up for patients taken off protocol therapy can be assessed either by phone consultation or clinic appointment at the discretion of the PI.

m. Day 1 may be delayed for up to one week for Cycle 2 and beyond.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.5.1 Criteria for removal from protocol therapy

3.5.1.1 Patients with medically concerning grade 3 or 4 adverse events related to study drug may be taken off therapy at the discretion of the principal investigator.

3.5.1.2 Toxicity has not resolved after 8 weeks of withholding treatment

3.5.1.3 Grade 4 non-blistering rash, or blistering rash of any grade

3.5.1.4 Grade 4 neuropathy

3.5.1.5 Grade 4 hypersensitivity reaction

3.5.1.6 Progression of disease

3.5.1.7 Patient chooses to go off therapy

3.5.1.8 The principal investigator may remove patient from protocol therapy if deemed necessary due to medical conditions, compliance, etc.

3.5.1.9 Diagnosis of new malignancy

3.5.1.10 Patient becomes pregnant.

3.5.2 Criteria for removal from study

3.5.2.1 Patient requests to be withdrawn from study

3.5.2.2 Death

3.5.2.3 Physician's determination that withdrawal is in the patient's best interest

3.5.2.4 Effective with amendment D, version date February 12, 2015, patients, will be removed from the study when they have completed two years of lenalidomide therapy and the 30 day safety follow-up visit. Patients who have on-going toxicity(ies) will be followed as described in Section **7.1.1**.

3.5.2.5 Study closure

3.5.3 Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and faxed to 301-480-0757.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 TUMOR LYSIS SYNDROME

4.1.1 Allopurinol is optional and will be prescribed at the Investigator's discretion. Allopurinol dose should be adjusted according to the package insert. Subjects who do not tolerate allopurinol should be discussed with the Lead Principal Investigator.

4.2 BONE DISEASE/EXTRAMEDULLARY DISEASE

4.2.1 Subjects may receive radiation if clinically indicated for treatment of uncontrolled pain, cord compression, vertebral instability/impending fracture, etc, in the face of non progressive disease

4.2.2 Kyphoplasty/Vertebroplasty: Subjects may receive kyphoplasty/vertebroplasty for symptomatic vertebral compression fractures in the face of non progressive disease.

4.2.3 Bisphophonate therapy: Approved bisphophonate therapy (zoledronic acid or pamidronate) is allowed. Patients will be monitored for renal function and osteonecrosis of the jaw. Patients may require prior evaluation from dental specialist before instituting bisphosphonates.

4.3 USE OF STEROIDS

4.3.1 Use of corticosteroids during maintenance therapy with the intent to treat myeloma is not permitted. However, corticosteroids may be used as medically indicated for non-myeloma related acute indications e.g. to control symptoms of asthma flare. Corticosteroids should be discontinued as soon as possible.

4.4 HYPERCALCEMIA

4.4.1 Patients may receive treatment for hypercalcemia including hydration, bisphophonates, furosemide, steroids, calcitonin, etc.

4.5 TRANSFUSIONS/GROWTH FACTORS

4.5.1 Subjects may receive RBC or platelet transfusions if clinically indicated.

4.5.2 Subjects may receive supportive care with erythropoietin or darbopoetin.

4.5.3 Prophylactic use of growth factors (i.e. filgrastim, pegfilgrastim) is not permitted. However, therapeutic use of filgrastim or sargramostim in patients with serious neutropenic conditions, such as sepsis, febrile neutropenia may be considered at the investigator's discretion. Colony-stimulating factors may be used if grade 4 neutropenia occurs but should not be given prophylactically.

4.6 ANTI-COAGULATION

4.6.1 Oral Aspirin 81 mg or 325 mg or suitable alternative anti-coagulation for thrombotic prophylaxis every day for the duration of their participation in the study.

4.7 ANTI-EMETICS

4.7.1 Anti-emetics should not be given prophylactically. If a patient demonstrates the need for anti-emetics, their use will be at the treating physician's discretion.

4.8 PCP PROPHYLAXIS

4.8.1 The use of medication (e.g. Bactrim) for PCP prophylaxis is at the investigator's discretion.

5 BIOSPECIMEN COLLECTION

5.1 BIOMARKER STUDIES FOR RESEARCH

1) Blood/Urine

Sampling Time Points for biomarker studies/ biospecimen banking

	Baseline	C 2 D1 (within 3 days prior)	C 4 D1 (within 3 days prior)	C 7 D1 (within 3 days prior)	Every 3 cycles starting at cycle 7 D1 (within 3 days prior)	Every 6 cycles starting at cycle 7 D1 (within 3 days prior)	Disease progression	At CR
Blood for biomarker analysis	x	x	x	x		x	x	x
Bone marrow*	x						x	x
Blood/Urine Biopspecimen storage bank	x	x	x	x	x		x	x

a. Blood samples for biomarker studieswill be collected from all patients at baseline, after cycles 1, 3, and 6, and then after every 6 cycles while receiving lenalidomide therapy, until completion of the study. The samples will be batched for later analysis.

b. The assays will be run on the batched samples for first ten patients at baseline, and after cycles 1, 3, and 6 to determine the appropriate time points of assays for the subsequent patients.

c. Blood and urine specimens will be collected from all patients who agree to participate in optional studies and stored for establishing biospecimen storage bank. These specimens will be collected at baseline, after cycles 1, 3, and 6 and after every three cycles while receiving lenalidomide. Specimens will also be collected and stored at the time of documented CR and disease progression. IRB approval will be obtained before using any samples to conduct studies that are not described within this protocol.

d. Collection and storage of peripheral blood and urine for biospecimen storage bank is outlined in appendix C.

e. Processing of peripheral blood for biomarker studies is outlined in appendix D.

f. At any given time, up to 100cc of peripheral blood will be collected. The amount of blood collected will be dictated by the number of experiments to be performed, and by the patient's peripheral blood count.

g. The standard number of peripheral blood tubes drawn for storage at each of the above time points may include but are not limited to the following: one 7-8 mL serum tube, one 7-8 mL plasma heparinized tube, and one 7-8 mL EDTA tube.

h. The standard number of peripheral blood tubes drawn for biomarker analysis at each of the above time points may include but are not limited to the following: two 7-8 mL plasma heparinized tubes.

i. At any given time, approximately 45 mL of urine will be collected into a standard urine collection cup and sent for analysis and storage at each of the above timepoints.

j. Sample Requirements and Handling: The date and exact time of each blood draw should be recorded on the sample tube. Serum samples should be kept at room temperature for 30-60min prior to being refrigerated. Please page Dr. Figg's lab at 102-11964 for immediate pick-up.

2) Bone Marrow

Effective with Amendment D, bone marrow aspirate and biopsy will be obtained for research studies at the discretion of the PI.

a. Bone marrow specimens (aspirate and biopsy) will be collected for at baseline, and then at the time of documented CR or disease progression. Bone marrow samples will be used to determine the molecular characteristics of the tumor cells and the expression of CRBN by gene expression profiling and quantitative PCR.

b. Additional bone marrow samples for other biomarker studies will be obtained at the discretion of PI, if patients are willing.

c. Collection of bone marrow, sorting of bone marrow and storage of bone marrow samples is outlined in appendix F.

d. Biomarker studies associated with bone marrow specimen will be performed and related to clinical outcome if the results of the study indicate a clinical or translational rationale for analyzing the samples. Such studies may include but are not limited to the following:

i. Pathology/Immunohistochemistry: Bone marrow biopsy and aspirate will be sent to Clinical Center Department of Laboratory Medicine, Hematology Service for morphological evaluation by Irina Maric, MD and

Katherine Calvo, MD, PhD. Immunohistochemical staining will be performed under the direction of Irina Maric, MD. Plasma cell burden will be assessed using immunohistochemistry markers such as CD 138, light chains, CD56 etc.

ii. Flow cytometry: Immunophenotyping of aberrant plasma cells by flow cytometry currently involves, but is not limited to, the use of the following reagents: CD138, CD19, CD45, CD38, and CD56. Characteristic changes in immunophenotypically abnormal plasma cells (CD138 positive) include but are not limited to absent CD19 and CD45, decreased CD38, and increased CD56. These studies will be performed under the direction of Maryalice Stetler-Stevenson of the flow cytometry unit in the NCI Laboratory of Pathology.

iii. Cell Sorting, molecular characterization - Marrow aspirate will be sent to the Lymphoid Malignancies Branch lab and sorted into CD 138 + and CD 138 – fractions. For questions regarding these samples, please contact Mark Roschewski at 301-451-9021.

- GEP profiling: CD138+ plasma cells will be purified from bone marrow aspirates harvested at each indicated time point. GEP will be performed in the Lymphoid Malignancies Branch lab using the Affymetrix U133 Plus 2.0 microarray platform. Gene expression profiles will be analyzed to identify potential markers of response to lenalidomide such as CRBN and markers for progression. Changes in selected genes will be confirmed by quantitative PCR if suggested to be related to response or progression.
- Both CD 138+ and CD 138- fractions will be collected, batched, and entered into a biobank. See section **5.3.1** and Appendix F in section **18** for storage of bone marrow biobank.

5.2 BIOMARKER STUDIES: METHODS

While it has been shown that lenalidomide-induced NK cell activation is IL-2 dependent, the specific subset of cells responsible for mediating this effect has not been clearly elucidated. To conduct a comprehensive evaluation of a range of immune cells in relation to lenalidomide maintenance therapy, we will include analyses of B-, T-, NK and NKT cells and important signaling cytokines.

- **Phenotypic analysis of PBMCs:** Patients' PBMCs will be FACS-sorted into NK cells ($CD3^{-}CD16^{+}CD56^{+}$), CD4 ($CD3^{+}CD4^{+}$), and CD8 ($CD3^{+}CD8^{+}$) T-cell subsets. The percentage of T cells (CD4, CD8), NKT and NK cells will be determined by phenotypic analysis of peripheral blood mononuclear cells (PBMCs) stained for CD56, CD3, CD4, CD8, and CD16. To obtain total numbers per milliliter of peripheral blood, the percentage of CD4 T or NK cells will be multiplied by the total lymphocyte count.
- **Phenotypic analysis of B cell subsets:** B cell subsets will be analyzed by phenotypic analysis of PBMCs stained for CD19, CD38 CD45, CD27 and CD138.

- **Proliferation assay for human T cells (CD4 and CD8):** 100 µl of PBMC (1 x 10^6cells/ml) will be added to each well of a in a 96-well round-bottom plate. The plate is coated with 100 µl anti-CD3 (OKT3; eBioscience) at a concentration of 10 µg/ml. Incubate plate in a humidified 37o C, 5% CO2 incubator. All cells are cultured in medium RPMI 1640 (Mediatech, Inc., Herndon, VA) supplemented with 10% heat inactivated human AB serum (Gemini Bio-Products, Woodland, CA); 100 units/ml penicillin and 100µg/ml streptomycin (Mediatech, Inc.); 2 mM L-glutamine (Mediatech, Inc.). Cells are incubated for 3 days. Proliferation will be assessed by [3H]thymidine (1 µCi [0.037 MBq] per well) (Perkin-Elmer, Boston, MA) incorporation pulsed on day 4 and quantified 18 hours later using a liquid scintillation counter (Wallac, Gaithersburg, MD). All experiments will be performed in triplicate.
- **Functional NK analysis:** A 4-h 111In-release assay will be used to measure NK activity in patients. Cell line K562 will be used as target. Target cells will be labeled with 50 µCi of 111In-oxyquinoline in a volume of 0.25 ml of RPMI-1640 for 15 min at 37º C according to a modification of the procedure described by Wiltrout et al [48]. Target cells (1 x 104) in 50 µl will be added to each well of assay plates containing 96-U-bottom wells. Effector cells (sorted NK cells) to target cell (E/T) ratios of 50:1, 25:1, 12.5:1 and 6.25:1 will be assayed. The plates will be incubated for 4-h at 37o C in a humidified atmosphere containing 5% CO2. Supernatants will be harvested for gamma counting using Skatron Harvesting Frames. Experiments will be carried out in triplicate. Specific lysis will be calculated using the formula:

$$\text{Lysis (\%)} = \frac{\text{Observed release (cpm)-spontaneous release (cpm)}}{\text{Total release (cpm)-spontaneous release (cpm)}} \times 100$$

- Regulatory T cell (Treg) and MDSC phenotype: The number and phenotype of Tregs in PBMCs from patients in this study will be determined by 9-color flow cytometry analysis. Cells will be resuspended in staining buffer (phosphate-buffered saline containing 3% fetal bovine serum) and stained for CD4, CD25, CD127, FoxP3, CTLA-4, CD45RA, PDL-1, DR and CD8. The ratios of Treg to effector cells will also be analyzed. MDSCs will be analyzed by staining for CD34, CD33, CD11b, CD15, CD13 CD14 and DR.
- **Chemokine and Cytokine Assay:** The presence of IL-4, IL-10, IL-8, IL-13, TNF-alpha, IFN-gamma, IL-12p70, GM-CSF, IL-5, and IL-6 in serum obtained from patients pre- and post-treatment will be determined using a multi-plex assay (Meso Scale Discovery, Gaitherburg, MD). Assays will also be performed for cytokines such as TGF-beta, IL-17, VEGF and IL-23.
- **Other Assays:** Using flow cytometry and immunohistochemistry, we will determine selected tumor cell antigen targets including, but not limited to MUC1, Brachyury, PD1 ligands, MAGE-A, and NYESO. Patient serum and bone marrow samples will be evaluated for previously studied prognostic markers including M-spike, percent bone marrow plasma cells, serum free light-chain ratio, total immunoglobulin levels,

immunophenotype by flow cytometry, albumin, and ß2-microglobulin. Patients' tumor cells will be characterized by gene expression profiling to enhance our understanding of lenalidomide efficacy and safety in a genetically diverse tumor population.

- **Future Assays:** The information obtained from this study regarding immune effector cells in MM will provide the framework for the development of immune-based therapeutic options like novel vaccines or monoclonal antibodies, and the development of therapies designed specifically to increase NK cell cytotoxicity against MM. It will also allow for analysis of myeloma cell-immune cell interactions.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.3.1 Procedures for Collecting, Processing, and Storage of Bone Marrow biopsies

- See Appendix F
- Orders for bone marrow biopsies should be placed in the Clinical Research Information System (Clinical Research Center, NIH, Bethesda, MD). Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.
- Bone marrow biopsies will be submitted in native condition to the NCI Laboratory of Pathology and handled according to routine procedures for diagnosis. Bone marrow core biopsies will be fixed and paraffin embedded for histological and immunohistochemical analysis and long-term storage. Bone marrow aspirates will be prepared according to routine procedures. Five to ten air-dried aspirate smears will be stored long-term.
- Materials for research studies will be documented on form NIH 2803-1.
- Initial processing of bone marrow aspirates for research will depend on the size of the aspirate. CD138 positive plasma cells will be isolated from a subset of these samples.
- For the purposes of storage, all research samples except stored serum and urine will be assigned a unique number and cataloged. Viably frozen cells will be stored in a temperature controlled, alarm secured nitrogen tank in the NCI Department of Hematopathology. Frozen bone marrow biopsies and processed biologic material (such as RNA and protein) will be stored at -80°C in a temperature-controlled, alarm-secured freezer. All research samples except stored serum will be stored in the Lymphoid Malignancies Branch lab. For questions regarding these samples, please contact Mark Roschewski at 301-451-9021.
- Frozen specimens will be wrapped in aluminum foil labeled with the patient's name and accession number, put into a resealable polyethylene freezer bag, and stored in a liquid nitrogen freezer. The liquid nitrogen freezers are monitored daily for temperature variations. A FileMaker Pro database called HP Patient Information and Specimen Inventory is used for tracking the samples.

5.3.2 Procedures for stored serum, peripheral blood, and urine specimens:

- See Appendix C for processing and storage procedures for Dr. Figg's lab.

5.3.3 Procedures for Collecting, Processing, and Storage of Blood samples for biomarker studies

- See Appendix D for processing and storage procedures for Dr. Figg's lab
- Batched blood samples for research purposes will be further analyzed for biomarker studies in Dr. Schlom's lab as per the methods detailed in section **5.1**

5.3.4 Protocol Completion & Sample Destruction

- The principal investigator will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. a broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples, or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the Center for Cancer Research of the NCI.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

- Data will be prospectively collected and entered into the NCI C3D clinical trials database. All adverse events that are Grade 3 or higher related to lenalidomide and/or research procedures will be collected into the NCI C3D database. All data will be kept secure. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.
- When patients complete protocol treatment but remain on-study, the following data will be collected:
  - adverse events related to lenalidomide
  - survival status which will be collected by phone or clinic visit
  - additional cancer therapy received
- Record Keeping:

  Complete records must be maintained on each patient; these records will consist of the hospital chart as well as any other information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on a computer database from which formal analyses are done. The primary source documentation will include patient eligibility data, patient history, flow sheets (including specialty forms for pathology, radiology, or surgery), an off-study summary sheet, and a final assessment by the treating physician.
- Forwarding of Patient Data from Other Institutions:

  Either due to extenuating medical circumstances or for convenience, some patients may elect to have certain routine laboratory studies or protein marker analyses performed at an outside institution between scheduled interval visits to the CRC for this protocol. These results will be forwarded to the Myeloma Research Nurse who

will enter the data into the study database. Additional blood or tissue samples drawn on patients enrolled in this protocol between scheduled visits may be forwarded and entered into the database as well.

6.2 RESPONSE CRITERIA

6.2.1 Response assessments will be performed on Day 1 (+/- 3 days) of cycles 2, 4 and 7; and on Day 1 (+/-3 days) of every third cycle starting at cycle 10 at scheduled clinic visits, at the time of documented CR, and at disease progression Disease Parameters

- Patients who have a measurable serum or urine M-protein. A "measurable" serum M-protein is ≥ 1 g/dL and a "measurable" urine M-spike is ≥ 200 mg/24 hours. If patient does not have a "measurable" serum or urine M-protein, but has either a serum kappa or lambda FREE light chain of 10 mg/dL along with an abnormal kappa to lambda free light chain ratio, patient is considered to have "measurable" disease.
- The serum free light chain (FLC) assay is of particular use in monitoring response to therapy in patients who have oligo-secretory disease. When using this assay, it is important to note that the FLC levels vary considerably with changes in renal function and do not solely represent monoclonal elevations. Thus both the level of the involved and the uninvolved FLC isotype (i.e., the involved/uninvolved ratio or involved-uninvolved difference) should be considered in assessing response. The serum FLC assay should be used in assessing response only if the baseline serum and/or urine M proteins are not “measurable” by traditional criteria (serum M protein ≥ 1gm/dL and/or urine M protein ≥ 200 mg/24), and the baseline level of the involved FLC is 10mg/dL and clonal (abnormal ratio). Patients included on the study on the basis of FLC alone (i.e., no measurable serum/urine) should be the only ones who are evaluated using FLC response criteria. The others should follow usual criteria and ignore FLC results.
- In order to be classified as a hematologic response, confirmation of serum monoclonal protein, serum immunoglobulin free light chain (when primary determinant of response) and urine monoclonal protein (when primary determinant of response) results must be made by verification on two consecutive determinations.
- Caution must be exercised to avoid rating progression or relapse on the basis of variation of radiological technique alone. Compression fracture does not exclude continued response and may not indicate progression. When progression is based on skeletal disease alone, it should be discussed with the PI before removing the patient from the study.

6.2.2 Response Criteria from International Myeloma Workshop Consensus Panel Criteria[47]:

6.2.2.1 Evaluation of Response Criteria

- **Stringent Complete Response (sCR)**
  Complete Response as defined below plus:

Normal FLC ratio and absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence (presence/ absence of clonal cells is based on the kappa/ lambda ratio).

- **Complete Response (CR)**
  Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and ≤5% plasma cells in bone marrow. Patients in whom the only measurable disease is by serum FLC levels: CR in such patients indicates a normal FLC ratio of 0.26 to 1.65 in addition to CR criteria listed above.
- **Very Good Partial Response (VGPR)**
  Serum and urine M-protein detectable by immunofixation but not on electrophoresis or 90% or greater reduction in serum M-protein plus urine M-protein level <100mg per 24h. If the serum and urine M-protein are unmeasureable, a ≥90% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria.
- **Partial Response (PR)**
  ≥50% reduction of serum M-protein and reduction in 24-h urinary M-protein by ≥90% or to <200mg per 24h. If the serum and urine M-protein are unmeasureable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria.
- **Minimal Response (MR)**
  ≥ 25% but ≤ 49% reduction of serum M protein and reduction in 24-hour urine M-protein by 50%-89% In addition to the above criteria, if present at baseline, 25%-49% reduction in the size of soft tissue plasmacytomas is also required . If the serum and urine M-protein are unmeasureable, a 25- 49% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. No increase in size or number of lytic bone lesions is required (development of compression fracture does not exclude response)
- **Stable Disease (SD)**
  Not meeting criteria for CR, VGPR, PR, MR or progressive disease. All categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.
- **Progressive disease (PD)**
  Requires any one or more of the following:
  - Increase of ≥25% from baseline or lowest reponse value in Serum M component, Urine M component, FLC or bone marrow plasma cell percentage. Lowest response value does not need not to be a confirmed value.
  - Serum M-component absolute increase must be ≥0.5g/dl. The serum M-component increases of ≥1 gm/dl are sufficient to define relapse if starting M-component is ≥5g/dl.
  - Urine M-component absolute increase must be ≥200mg/24h
  - Only in patients without measurable serum and urine M-protein levels: the absolute increase in difference between involved and uninvolved FLC levels must be >10mg/dl.

- Only in patients without measurable serum and urine M protein levels and without measurable disease by FLC levels, bone marrow plasma cell absolute percentage must be ≥10%
- Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in size of existing bone lesions or soft tissue plasmacytomas
- Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL) that can be attributed solely to the plasma cell proliferative disorder

- **Refractory Myeloma**
  Refractory myeloma is defined as disease that is nonresponsive while on primary or salvage therapy, or progresses within 30 days of last therapy. Nonresponsive disease is defined as either failure to achieve minimal response or development of progressive disease (PD) while on therapy.
- **Relapse from CR**
  Any one or more of the following:
  - CR patients will need to progress to the same level as VGPR and PR patients to be considered PD. A positive immunofixation alone is therefore not sufficient.
  - Development of ≥ 5% plasma cells in the bone marrow
  - Appearance of any other sign of progression (ie, new plasmacytoma, lytic bone lesion, hypercalcemia

6.2.3 Duration of Best Response

The duration of overall response is measured from the time measurement criteria are met for best response until the first date that recurrent or progressive disease is objectively documented.

6.2.4 Progression-Free Survival

PFS is defined as time of start of treatment to time of progression or death, whichever occurs first.

6.2.5 Overall Survival

Overall survival is defined as the time of start of treatment to death from any cause.

6.2.6 Overall response rates

Overall response rates (ORR) is PR+VGPR+CR after n cycles of therapy.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

Attribution of the AE:

- 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.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs that are Grade 3 or higher must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per section **7.2.**

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 Suspected adverse reaction

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

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or,

if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Per Celgene: Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.
- Pregnancy

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

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

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.11 Pregnancies

7.1.11.1 Reporting Pregnancy to Celgene

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on lenalidomide, or within 28 days of the subject's last dose of lenalidomide, are considered immediately reportable events. Lenalidomide is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Drug Safety immediately by facsimile or email using the Pregnancy Initial Report Form. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report 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 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.

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 investigational product (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.

Male Subjects

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 her healthcare provider immediately.

Celgene Drug Safety Contact Information:

Celgene Corporation
Global Drug Safety and Risk Management
Connell Corporate Park
300 Connell Dr. Suite 6000
Berkeley Heights, NJ 07922
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

7.2 NCI-IRB REPORTING

7.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All serious non-compliance

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All Serious Events regardless of attribution.

NOTE: Grade 1 and Grade 2 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.2.4 SAE Reporting to Celgene

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

Serious adverse events (SAE) are defined above. The investigator must inform Celgene in writing using a Celgene SAE form or MEDWATCH 3500A form of any SAE as soon as possible or at least within 24 hours of being aware of the event. The date of awareness should be noted on the report. The written report must be completed and supplied to Celgene by facsimile within 24 hours/1 business day at the latest on the following working day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. 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-MM-NCI-0718) 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.

Celgene Corporation
Drug Safety
86 Morris Avenue
Summit, N.J. 07901
Toll Free: (800)-640-7854
Phone: (908) 673-9667
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

7.3 DATA AND SAFETY MONITORING PLAN

The following plans will be used for this study.

7.3.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient in detail. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator in a timely manner. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

8.1 STUDY DESIGN/PRIMARY ENDPOINTS

This is a single arm, single stage, phase II trial to evaluate maintenance therapy with the immunomodulatory agent lenalidomide in treated MM patients who have stable or responsive disease. After screening, eligibility determination, and enrollment; subjects will receive lenalidomide 10 mg by mouth daily on days 1-21 of a 28-day cycle. Subjects may continue lenalidomide until disease progression, diagnosis of new malignancy, unacceptable toxicity, investigator discretion, or voluntary withdrawal. When necessary, lenalidomide will be held and restarted in accordance with accepted clinical dose modification strategies.

The primary objective of this study is a longitudinal assessment of T cell (CD4, CD8), NKT and NK cell populations during the course of lenalidomide maintenance therapy in patients with MM. Using flow cytometry on peripheral blood obtained at baselines and after 3 cycles of therapy, we will determine the primary measures at both time points. Comparisons at other time points will be considered exploratory..

With 28 patients enrolled, we will have 80% power to detect an effect equal to 0.67 SD of the difference in counts (0.67 SD effect size) of T cells (CD4, CD8), NKT and NK cells with a two-tailed paired t-test and a significance level of 0.013 for each test. Thus, using a strict Bonferroni adjustment, we would have an overall significance level of 0.05 for the four tests. In practice, a less overly conservative Hochberg adjustment may be used instead of a Bonferroni adjustment. In addition, if the difference in the numbers of any type of cells is not normally distributed ($p<0.05$ by a Shapiro- Wilks test), then a Wilcoxon signed rank test will be performed instead.

Results will be presented in a descriptive format.

8.2 SAMPLE SIZE/ACCRUAL RATE

It is anticipated that 2-3 patients per month may enroll on this trial; thus, approximately 14 months may be required to enroll the 28 patients.

8.3 STATISTICAL ANALYSIS OF SECONDARY ENDPOINTS

Secondary endpoints include duration of response and progression-free survival. Duration of response is defined as time from response to disease progression or death. Progression-free survival is defined as the time from study entry until progression or death. Duration of response and progression-free survival will be estimated using the Kaplan-Meier method. Where possible, blood samples and bone marrow samples will be used for additional research studies, which may include functional analyses of immune-cell subsets, analyses for cytokines, chemokines, antibodies, tumor cell antigen targets, and/or other markers. Due to the sample size, the results from those efforts will be exploratory, and the main goal is to generate data for future studies.

9 COLLABORATIVE AGREEMENTS

9.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

There is an approved collaborative agreement, CRADA, with Celgene Corporation for lenalidomide.

9.2 Material Transfer Agreement (MTA)

An MTA will be executed before the samples described in Section 5 are shipped to Ola Landgren, MD, PhD at Memorial Sloan Kettering Cancer Center.

The samples and accompanying personally identifiable information, or code to personally identifiable information, will be used for analyses of the causation, diagnostics and prognostics, and natural history of multiple myeloma and its precursor conditions. Additionally, the samples may be used for analyses of related hematologic malignancies and their precursors states (including chronic lymphocytic leukemia and monoclonal B-cell lymphocytosis (MBL); Waldenstrom's macroglobulinemia and IgM MGUS), as well as myeloproliferative neoplasms. The samples will also be used for clinical, molecular and imaging based characterization of the above disorders.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

MM is an almost always incurable plasma cell neoplasm that comprises approximately 10% of all hematologic malignancies. MGUS, SMM, and MM increase in incidence with age. According to SEER statistics, from 2003-2007, the median age at death for myeloma is 75 years of age. Incidence rates of myeloma are higher among Blacks compared to Caucasians, affecting 14.3 black males per 100, 000 males and 10.0 black females per 100,000 females compared to 6.7 white males per 100, 000 males and 4.1 white females per 100, 000 women. Despite this, MM affects all genders and races. As such, we expect that the majority of patients enrolled in this trial will be older adults of either gender or race. MM patients enrolled on this study will consist of patients referred to and screened at the NIH Clinical Center. There will be no subject selection bias with regard to gender, ethnicity, or race. This protocol excludes lactating and pregnant women from receiving this investigational drug to avoid any possible risks to the fetus or newborn.

10.2 PARTICIPATION OF CHILDREN

Pediatric patients with MM are extremely rare. Patients under the age of 18 are excluded from this study because inclusion of a rare younger patient will not provide adequate generalizable information to justify their inclusion in this study.

10.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Currently, MM is an incurable malignancy with frequent complications of skeletal fractures, anemia, renal failure and hypercalcemia. Maintaining or improving upon the clinical responses achieved following induction therapy is viewed as an important strategy to prolong survival in MM. Lenalidomide is an attractive drug with immunomodulatory activity for maintenance

therapy with the advantage of oral administration. Two trials of lenalidomide maintenance after ASCT and one trial after conventional chemotherapy induction therapy showed a significant risk reduction for PFS, with one of the transplant trials also showing a prolongation of OS. Lenalidomide maintenance therapy was well tolerated with almost negligible hematotoxicity, no neurotoxicity, and no increase in thromboembolic complications or infections. The observation of an increased occurrence of second primary malignancies (SPM), however, is notable. There was a 7 to 8% rate of second primary cancers in the lenalidomide groups as compared with 3 to 4% in the placebo groups. Post hoc analysis supported for a magnitude of benefit associated with lenalidomide that seems to outweigh the risk of second cancers. The development of second primary cancers is not just attributed to lenalidomide and may also be due in part to other factors, some of which include prior use of various MM drugs with known leukemogenic potential, MM-related disease factors, host-related factors, as well as environmental and behavioral factors.

10.4 RISKS/BENEFITS ANALYSIS

On a clinical note, for most patients, multiple myeloma still remains an incurable malignancy and, on average, the general risk of dying is substantially higher than the risk of developing a second cancer. Lenalidomide maintenance has significantly improved progression free survival in three clinical trials.

Further studies are needed to evaluate the true risk of developing second primary cancers, to identify risk factors for their development, and hopefully, to develop strategies for the prevention of second primary cancers. Before more information is available, physicians and patients must weigh the benefits of lenalidomide maintenance therapy against the low but relevant risk of second cancers.

The procedures required to obtain samples/data for experimental purposes (venipuncture, urine collection, PET/CT scan and bone marrow biopsy) are of limited risk to the patient. Although patients will suffer some additional pain or discomfort from bone marrow biopsies, clinical experience has shown that the medical risk is limited.

10.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

Informed consent will be obtained from all patients on this trial by one of the investigators. There will be no minors enrolled < 18 years of age; therefore, assent is unnecessary. The informed consent procedure contains all elements required for consent. In addition, the Principal Investigator or an associate investigator or member of the research team will discuss the protocol in detail with the patient and will be available to answer all patient questions to allow the patient to give informed consent.

11 PHARMACEUTICAL INFORMATION

11.1 LENALIDOMIDE

11.1.1 Source

REVLIMID® (lenalidomide) is provided to investigator by Celgene Inc. under Cooperative Research and Development Agreement (CRADA).

NOTE:
Before lenalidomide is dispensed, patients must 1) have a negative pregnancy test (if applicable) and 2) be counseled by a trained counselor. Pharmacists may be trained counselors (see Lenalidomide Counselor Program Site Counselor Identification Form in the protocol). The counseling requirements for investigational-use lenalidomide are separate from the REMS program. Only a 28-day supply may be dispensed to a patient at one time.

Mode of Action: Lenalidomide, a thalidomide analog, is an immunomodulatory agent with a spectrum of activity that is still under investigation. Some of its effects include inhibition of inflammation, inhibition of angiogenesis, inhibition of hematopoetic tumor cell proliferation, modulation of stem cell differentiation and upregulation of T cell and NK cell responses.

11.1.2 Toxicity

Fetal Risk

Do not use REVLIMID during pregnancy. Lenalidomide, a thalidomide analogue, caused limb abnormalities in a developmental monkey study. Thalidomide is a known human teratogen that causes severe life-threatening human birth defects. If lenalidomide is used during pregnancy, it may cause birth defects or death to a developing baby. In women of childbearing potential, obtain 2 negative pregnancy tests before starting REVLIMID® treatment. Women of childbearing potential must use 2 forms of contraception or continuously abstain from heterosexual sex during and for 4 weeks after REVLIMID treatment

Hematologic Toxicity

REVLIMID can cause significant neutropenia and thrombocytopenia.

In the pooled MM studies Grade 3 and 4 hematologic toxicities were more frequent in patients treated with the combination of REVLIMID and dexamethasone than in patients treated with dexamethasone alone

Deep Vein Thrombosis and Pulmonary Embolism

Venous thromboembolic events (predominantly deep venous thrombosis and pulmonary embolism) have occurred in patients with MM treated with lenalidomide combination therapy. A significantly increased risk of DVT and PE was observed in patients with MM who were treated with REVLIMID and dexamethasone therapy in a clinical trial.

Allergic Reactions

Angioedema and serious dermatologic reactions including Stevens-Johnson syndrome

(SJS) and toxic epidermal necrolysis (TEN) have been reported. These events can be fatal. Patients with a prior history of Grade 4 rash associated with thalidomide treatment should not receive REVLIMID. REVLIMID interruption or discontinuation should be considered for Grade 2-3 skin rash. REVLIMID must be discontinued for angioedema, Grade 4 rash, exfoliative or bullous rash, or if SJS or TEN is suspected and should not be resumed following discontinuation for these reactions.

Tumor Lysis Syndrome

Fatal instances of tumor lysis syndrome have been reported during treatment with lenalidomide. The patients at risk of tumor lysis syndrome are those with high tumor burden prior to treatment. These patients should be monitored closely and appropriate precautions taken.

Most common adverse reactions (≥20%)

Fatigue, neutropenia, constipation, diarrhea, muscle cramp, anemia, pyrexia, peripheral edema, nausea, back pain, upper respiratory tract infection, dyspnea, dizziness, thrombocytopenia, tremor and rash

11.1.3 Formulation and preparation

Lenalidomide will be supplied as capsules for oral administration. Celgene Inc. will provide lenalidomide 5, 10, mg capsules while patients are being treated on this protocol.

11.1.4 Stability and Storage

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

11.1.5 Administration procedures

Celgene Corporation will supply Revlimid® (lenalidomide) to the Clinical Center Pharmacy to be dispensed to study participants at no charge through the REMS® program. Lenalidomide will be shipped directly to patients or picked up directly from the Clinical Center pharmacy. Bottles will contain a sufficient number of capsules for one cycle of dosing**.**

11.1.6 Incompatibilities

Results from human in vitro metabolism studies and nonclinical studies show that REVLIMID is neither metabolized by nor inhibits or induces the cytochrome P450 pathway suggesting that lenalidomide is not likely to cause or be subject to P450- based metabolic drug interactions in man.

Digoxin

When digoxin was co-administered with lenalidomide, the digoxin AUC was not significantly

different; however, the digoxin Cmax was increased by 14%. Periodic monitoring of digoxin plasma levels, in accordance with clinical judgment and based on standard clinical practice in patients receiving this medication, is recommended during administration of lenalidomide.

Warfarin

Co-administration of multiple doses of 10 mg of lenalidomide had no effect on the single dose pharmacokinetics of R- and S-warfarin. Co-administration of single 25-mg dose warfarin had no effect on the pharmacokinetics of total lenalidomide. Expected changes in laboratory assessments of PT and INR were observed after warfarin administration, but these changes were not affected by concomitant lenalidomide administration.

Concomitant Therapies That May Increase the Risk of Thrombosis

Erythropoietic agents, or other agents that may increase the risk of thrombosis, such as estrogen containing therapies, should be used with caution in MM patients receiving lenalidomide with dexamethasone.

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13 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.

14 APPENDIX B: RISKS OF FETAL EXPOSURE, PREGNANCY TESTING GUIDELINES AND ACCEPTABLE BIRTH CONTROL METHODS

Risks Associated with Pregnancy

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

All study participants must be registered into the mandatory REMS® program, and be willing and able to comply with the requirements of REMS®.

Criteria for females of childbearing potential (FCBP)

This protocol defines a female of childbearing potential as a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

The investigator must ensure that:

- Females of childbearing potential comply with the conditions for pregnancy risk minimization, including confirmation that she has an adequate level of understanding
- Females NOT of childbearing potential acknowledge that she understands the hazards and necessary precautions associated with the use of lenalidomide
- Male patients taking lenalidomide acknowledge that he understands that traces of lenalidomide have been found in semen, that he understands the potential teratogenic risk if engaged in sexual activity with a female of childbearing potential or pregnant female, and that he understands the need for the use of a condom even if he has had a vasectomy, if engaged in sexual activity with a female of childbearing potential.

Contraception

Females of childbearing potential (FCBP) enrolled in this protocol must agree to use two reliable forms of contraception simultaneously or to practice complete abstinence from heterosexual intercourse during the following time periods related to this study: 1) for at least 28 days before starting lenalidomide; 2) throughout the entire duration of lenalidomide treatment; 3) during dose interruptions; and 4) for at least 28 days after lenalidomide discontinuation.

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

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

    - Tubal ligation
    - Partner's vasectomy
  - Additional effective methods:
    - Male condom
    - Diaphragm
    - Cervical Cap

Because of the increased risk of venous thromboembolism in patients with multiple myeloma taking lenalidomide and dexamethasone, combined oral contraceptive pills are not recommended. If a patient is currently using combined oral contraception the patient should switch to one of the effective methods listed above. The risk of venous thromboembolism continues for 4−6 weeks after discontinuing combined oral contraception. The efficacy of contraceptive steroids may be reduced during co-treatment with dexamethasone.

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

Pregnancy testing

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

Before starting lenalidomide

Female Patients:

FCBP must have two negative pregnancy tests (sensitivity of at least 50 mIU/mL) prior to prescribing lenalidomide. The first pregnancy test must be performed within 10-14 days prior to prescribing lenalidomide and the second pregnancy test must be performed within 24 hours prior to prescribing lenalidomide. The patient may not receive lenalidomide until the Investigator has verified that the results of these pregnancy tests are negative.

Male Patients:

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

During study participation and for 28 days following lenalidomide discontinuation

Female Patients:

- FCBP with regular or no menstrual cycles must agree to have pregnancy tests weekly for the first 28 days of lenalidomide treatment, including dose interruptions and then every 28 days throughout the remaining duration of lenalidomide treatment, including dose interruptions, at lenalidomide discontinuation, and at Day 28 following lenalidomide discontinuation. If menstrual cycles are irregular, the pregnancy testing must occur weekly for the first 28 days of lenalidomide treatment, including dose interruptions, and then every 14 days throughout the remaining duration of lenalidomide treatment,

including dose interruptions, at lenalidomide discontinuation, and at Day 14 and Day 28 following lenalidomide discontinuation.

- At each visit, the Investigator must confirm with the FCBP that she is continuing to use two reliable methods of birth control at each visit during the time that birth control is required.
- If pregnancy or a positive pregnancy test does occur in a study patient, lenalidomide must be immediately discontinued.
- Pregnancy testing and counseling must be performed if a patient misses her period or if her pregnancy test or her menstrual bleeding is abnormal. Lenalidomide treatment must be temporarily discontinued during this evaluation.
- Females must agree to abstain from breastfeeding during study participation and for at least 28 days after lenalidomide discontinuation.

Male Patients:

- Must practice complete abstinence or use a condom during sexual contact with pregnant females or females of childbearing potential throughout the entire duration of lenalidomide treatment, during dose interruptions and for at least 28 days following lenalidomide discontinuation, even if he has undergone a successful vasectomy.
- If pregnancy or a positive pregnancy test does occur in the partner of a male study patient during study participation, the investigator must be notified immediately.
- Male patients should not donate semen or sperm during therapy or for at least 28 days following discontinuation of lenalidomide.

Additional precautions

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

Requirements for REMS

- Patients will be asked to take part in a mandatory confidential survey prior to initiation of lenalidomide. To take the survey, they will be instructed to call the Celgene Customer Care Center at 1-888-423-5436. Male patients will be asked to take the survey monthly. Female patients will be asked to take survey periodically (monthly if females of childbearing potential and every 6 months if females of not childbearing potential).
- All patients will be required to sign the REVLIMID, Patient-Physician Agreement Form.

Females not of childbearing potential must sign the the REVLIMID, Patient-Physician Agreement Form that says you are presently not pregnant and do not have the ability to have children.

15 APPENDIX C: Peripheral Blood and Urine Collection And Storage

Venipuncture

- Up to 100 mL of peripheral blood will be collected into heparinized tubes or EDTA tubes. The amount of blood collected will be dictated by the number of experiments to be performed, and by the patient's peripheral blood count.
- Serum
    - Collect 7-8 mL blood in a serum separator tube (SST or red top).
    - Allow the blood to clot by standing at room temperature for 30 minutes. o Separate serum from cells by centrifuging at 4 degrees C for 5 minutes at 1200xg.
    - Pipette 3 aliquots of 1mL each into three 2mL cryovials.
    - Freeze immediately at -20C or lower.o Maintain in -80C freezer for storage until shipment.
- Plasma
    - Collect 7-8 mL blood in a sodium heparin tube (green top).
    - Place immediately on wet ice and refrigerate until time of processing. o Separate plasma from cells by centrifuging at 4 degrees C for 5 minutes at 1200xg.
    - Pipette 3 aliquots of 1mL each into three 2mL cryovials. o Freeze and store in -80C freezer.
- Complete blood count
    - A venous blood sample for a CBC will be collected in a 10ml EDTA lavender top (BD EDTA 366643) tube. Keep at room temperature until processing begins.
- Mononuclear Cells
    - A venous blood sample for harvesting mononuclear cells will be collected in a 10ml EDTA lavender top (BD EDTA 366643) tube. Keep at room temperature until processing begins.

Urine Sample Collection

- Approximately 45 mL of urine will be collected into a standard urine collection cup for further analysis. The amount of urine collected will be dictated by the number of experiments to be performed.
- Transfer two even aliquots to two screw-cap 50 mL conical tubes.
- Freeze immediately at -20C or lower.
- Maintain in -20C freezer for storage until shipment.

Labeling of Samples

- All specimens are to be labeled per the local site's standard procedures. The following information, if not provided on the specimen label, must be linked to the specimen label and provided on the inventory sheet:
    - patient study ID #
    - sample type
    - date/time of draw (DD/MMM/YY 24:00)
    - timepoint (ex. C1D1 pre, C1D1 24hr post)
    - any collection issues (short draw, delayed processing, etc.)

  - protocol title/number
  - institute name
  - contact information
- Do not include the patient name, medical record number, or initials.

Sample Data Collection:

- All samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in the Patient Sample Data Management System (PSDMS) utilized by the CPP. This is a secure program, with access to the PSDM System limited to defined CPP personnel, who are issued individual user accounts. Installation of PSDMS is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All CPP personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.
- PSDMS creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without PSDMS access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Sample Storage:

- Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80□ according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the PSDM System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.
- Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.
- If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the

PSDMS. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these

16 APPENDIX D: ISOLATION AND CRYOPRESERVATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

A. Isolation of mononuclear cells by Lymphocyte Separation Medium (LSM) (Ficoll-Hypaque solution)

1. Place **fresh heparinized blood (not longer than 1 hour after drawing)** (approx. 15 ml) into a 50-ml conical centrifuge tube. Using a sterile pipet, add an equal volume of room-temperature PBS. Mix well.
2. Slowly layer the Lymphocyte Separation Medium (LSM) underneath the blood/PBS mixture by placing the tip of the pipet containing LSM at the bottom of the sample tube. Use 3 ml of LSM per 10 ml blood/PBS mixture.
3. Centrifuge 30 min. at 2000 rpm (900 xg), 18°C to20°C, with no brake.
4. Using a sterile pipet, remove the upper layer that contains the plasma and most of the platelets. Using another pipet, transfer the mononuclear cell layer to another tube. Wash cells by adding excess Hanks balanced salt solution (HBSS) with 3X the volume of the removed supernatant. Resuspend cells in HBSS, and repeat the wash once to remove most of the platelets.
5. Resuspend mononuclear cells in RPMI-1640 medium containing 10% FCS. Count cells and determine viability by Trypan Blue exclusion.

B. Cryopreservation of PBMC

1. Prepare and label cryovials with patient #, cell concentration and date. (**It is best to have all vials labeled prior to pelleting of the cells**).
2. Aspirate supernatant.
3. Resuspend cells in a volume of formulate freezing medium to give desired concentration (approx 5-10 x 10^6 cells/vial). **Please use Sarstedt Cryovial=0.5 and 2 ml screw cap Micro Tube (1-800-257-5101; catalogue # 72.694.006).**

Preparation of Freezing media:

(a) Measure out 45ml of **heat inactivated human AB serum** into a 50 ml centrifuge tube.
(b) Add 5 ml DMSO to the tube.
(c) Cap and invert tube for several times to mix.
(d) If not all freezing media is to be used within 2 hours, aliquot extra portion into 15 ml centrifuge tubes and transfer to -20°C freezer for storage. Freezing media will expire one year after preparation.

4. Immediately dispense cells into the labeled cryovials ensuring that the cell concentration in each vial is labeled correctly.
5. Place full cryovials in a programmed freezer cooled to 4°C and begin the freezing at **-1°C/min.**
6. After freezing has been completed, transfer all vials to liquid nitrogen storage.

17 APPENDIX E: COCKCROFT-GAULT EQUATION

(140-age [years])*(Weight [kg])*(0.85 if female) / 72 * serum creatinine [mg/dL]

Source: Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1):31-41.

18 APPENDIX F: BONE MARROW ASPIRATE COLLECTION, SORTING AND STORAGE

Collection of Bone Marrow

- Refer to section **5.1** for biomarker study bone marrow timepoints and study calendar section **3.4**
- Orders for bone marrow biopsies should be placed in the Clinical Research Information System (Clinical Research Center, NIH, Bethesda, MD).
- Notify the CCR Hematology lab that flow immunophenotyping is being performed

  (301-496-4473). The hematology BM collection tech will bring a 10 mL tube sodium heparin Vacutainer tube to the specimen collection site and prepare an extra smear for the Flow Cytometry Laboratory.

- Get sterile heparin suitable for injection from the nurse's station. Rinse syringe and needle with sterile heparin, leaving no less than 0.5 mL in syringe.

- Bone marrow samples will be collected as bone marrow core biopsies and aspirates for analyses. Aspirate first 2 cc of marrow for morphology first and give specimen to CCR Hematology lab technician to be given to CCR Pathology department (1 mL will go to Hematopathology and 1 mL will be delivered to Irina Maric, MD for research assessing proteasomes). Reposition needle and, for cellular specimens, slowly aspirate 5-8 mL of bone marrow for flow cytometry and cell sorting.

- Bone marrow core biopsies and one fraction of marrow aspirates will be fixed and paraffin-embedded for histological/immunohistochemical analysis and long term storage. One fraction of marrow aspirates will be stored as air-dried aspirate smears and the rest will be frozen.

- For aspirate specified for flow cytometry under the direction of Maryalice Stetler-Stevenson, immediately discharge 1 mL of aspirated marrow syringe into a 10mL sodium heparin Vacutainer, cap tube tightly and mix by gentle inversion 5-6 times. Label tube with patient name, unique identifier number and date. Deliver immediately to the Flow Cytometry Laboratory B1B58 (specimens containing hematopoietic neoplasms have a tendency to clot and must be processed immediately). Call for STAT Escort pickup and delivery if you cannot deliver the specimen yourself (301-496-9295).

- For aspirate designated for sorting, GEP profiling, microenvironment studies, send remaining aspirate sample to the Lymphoid Malignancies Branch lab. For questions regarding these samples, please contact Mark Roschewski at 301-451-9021. Place aspirate sample in EDTA syringe immediately on ice. Transfer within 30 minutes of sampling to the lab for processing.