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Carfilzomib, Lenalidomide, and Dexamethasone in Newly Diagnosed Multiple Myeloma: Clinical and Correlative Phase II Study

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Investigational Agents:

Drug Name:	Carfilzomib
IND Number:	112587
Sponsor:	Center for Cancer Research
Manufacturer	Amgen, Inc.

Commercial Agents: Dexamethasone, Lenalidomide

PRÉCIS

Background:

- Multiple myeloma (MM) is an incurable plasma cell neoplasm with a median survival of 3-4 years.
- Novel agent combinations with proteasome inhibitors demonstrate improved response rates while increasing survival in MM patients.
- A common debilitating side effect of the proteasome inhibitor bortezomib is neuropathy.
- Carfilzomib is a new proteasome inhibitor with potent anti-MM effects and decreased peripheral neuropathy

Objective:

- To evaluate toxicity, including peripheral neuropathy, of carfilzomib, lenalidomide, and dexamethasone (CRd) in untreated MM patients

Eligibility:

- Newly diagnosed patients with histologically confirmed multiple myeloma
- Age ≥ 18 years
- Creatinine Clearance (CrCl) ≥ 60 ml/min. CrCl will be calculated using the Cockcroft-Gault method. If the calculated CrCl based on Cockcroft-Gault method is <60 mL/min, patient will have a 24 hr urine collection to measure CrCl. The measured CrCl must also be ≥ 60 ml/min.
- Without serious co-morbidity that would interfere with receipt of CRd
- Absolute neutrophil count (ANC) ≥ 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 < 3.0 x ULN

Design:

- Single arm, single stage phase II trial of combination therapy (carfilzomib, lenalidomide, and dexamethasone) for untreated multiple myeloma patients with an early stopping rule for toxicity
- Patients will receive 8 cycles of induction combination therapy of CRd
- Each cycle consists of 28-days
- After 4 cycles of therapy, transplant eligible patients will undergo stem cell collection
- Patients achieving stable disease or better after 8 cycles of CRd will receive lenalidomide extended dosing (phase I) for 12 cycles. After 12 cycles, patients will have the option to continue extended dosing (phase II) for one additional year.
- Patients will have routine blood work with SPEP and free light chains monthly during the induction phase. Laboratory evaluations may be spread out to every 3 months during the

maintenance and follow-up phases.

- Pre- and post-treatment in addition to follow-up bone marrow biopsies will be obtained for confirmation of diagnosis, response and durability, and correlative studies.
- Patients will also undergo evaluation for minimal residual disease at regular interval time points, using multi-parametric flow cytometry and FDG PET-CT.
- A single stage phase II design will be employed, with an early stopping rule. Unless 4 or more patients in the first 20 have Grade 3 or higher neurologic toxicity in the first 2 completed cycles, a total of 45 evaluable patients will be enrolled in this study.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

To evaluate toxicity, including peripheral neuropathy, of carfilzomib, lenalidomide, and dexamethasone (CRd) in untreated MM patients

1.1.2 Secondary Objectives

- To determine best response rates according to International Myeloma Working Group criteria after 8 cycles of CRd combination therapy
- To determine progression free survival (PFS)
- To determine duration of response (DOR)
- To determine overall survival (OS)
- To evaluate biological activity of carfilzomib and correlate to clinical outcomes, including gene expression profiling on pre- and post-carfilzomib exposure bone marrow samples
- To assess the impact of minimal residual disease by flow cytometry and PET/CT in relation to outcome

1.1.3 Exploratory Objectives

- To assess MRD detection by next generation VDJ sequencing, exome sequencing, and PD-1/PD-L1 expression in the bone marrow compartment
- To evaluate radiographic changes (signal intensity) in bone marrow heterogeneity and focal lesions by Diffusion Weighted MRI (DW-MRI) over time.
- To evaluate radiographic changes (FDG avidity) in bone marrow heterogeneity and focal infiltration by PET/CT overtime.

1.2 BACKGROUND AND RATIONALE

1.2.1 Introduction

Multiple myeloma (MM) is characterized by clonal proliferation of malignant plasma cells in the bone marrow, affecting an estimated 20,000 people annually(1). Disease hallmarks include presence of monoclonal protein in serum or urine and features of end organ damage, including hypercalcemia, renal insufficiency, anemia, and bone lytic lesions(2). MM remains incurable with an estimated median survival of 3-4 years with conventional therapies and longer with newer agents(3, 4).

1.2.2 Proteasomes and Bortezomib

Proteasomes play a critical role in protein turnover and degradation, thereby affecting essential cell functions of cell cycle control, signal transduction, apoptosis, and stress responses. The 26S proteasome complex consists of the 20S barrel–like core and 19S regulating component. The 20S proteasome has three main catalytic domains that contribute to protein breakdown: chymotryptic-like activity site, tryptic-like activity site, and caspase-like activity site(5). Inhibiting proteasomes in malignant cells leads to buildup of ubiquinated proteins, resulting in eventual cell death. Such inhibitor effects likely extend beyond just a simple overaccumulation of cell waste. Rather, proteasome inhibitors also exert direct effects on the myeloma microenvironment and enable neoplastic cells to “re-direct” cell proliferation/apoptotic signaling while overcoming drug resistance mechanisms.

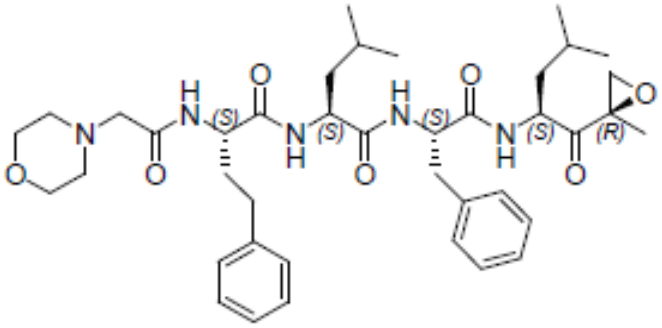
Bortezomib is a dipeptide boronate reversible inhibitor of the chymotryptic domain of the 26S proteasome. In combination with other agents, bortezomib demonstrates a potent anti-myeloma effect in initial treatment of transplant and non-transplant candidates, as well as in relapsed/refractory disease settings(6, 7). A recent phase I/II study shows promising efficacy of the novel combination: bortezomib, lenalidomide, and dexamethasone in newly diagnosed MM patients, with 74% of subjects achieving very good partial response (VGPR) or better(8).

Despite undisputable benefits of novel agents, bortezomib and others pose certain clinical challenges. Bortezomib drug toxicity is common with side effects including neuropathy, GI distress (diarrhea), myelosuppression, thrombocytopenia and herpes zoster re-activation. Bortezomib associated peripheral neuropathy can be experienced in up to 33% (Grade 1 and 2) and up to 18% (Grade 3 and 4) of newly diagnosed MM patients(9). In the aforementioned phase I/II study, with combination bortezomib, lenalidomide, and dexamethasone, 80% of treated patients experienced sensory neuropathy all grades(8). In addition, as increasing numbers of patients are treated with novel agents, drug resistance and refractoriness seem to present a significant dilemma. Myeloma patients refractory or resistant to bortezomib and at least one IMID (lenalidomide or thalidomide) demonstrate median overall survival and event free survival of 6 months and 1 month, respectively(10).

1.2.3 Investigational Drug Carfilzomib

Carfilzomib is a tetrapeptide ketoepoxide-based irreversible inhibitor that forms a covalent bond with N-terminal threonine residue of the chymotrypsin domain. Compared to bortezomib, carfilzomib demonstrates equal potency but greater selectivity for the chymotrypsin activity site over the tryptic and caspase domains.

Chemical Structure of Carfilzomib



Carfilzomib is less reactive to non-proteasome proteases compared to bortezomib, likely contributing to lower levels of neuropathy and myelosuppression(11-13). In vitro models suggest carfilzomib has activity against bortezomib resistant myeloma cell lines(12). Carfilzomib can also work synergistically with dexamethasone to enhance tumor cell death(12). A number of phase I and phase II studies are currently investigating carfilzomib toxicity and efficacy in MM.

As of July 2019, carfilzomib has been examined in approximately 11,000 people in a research setting. It has been found that carfilzomib can cause a reactivation of hepatitis B virus. Additionally, there have been 4 cases of Progressive Multifocal Leukoencephalopathy (PML) possibly linked to Carfilzomib.

1.2.3.1 Phase I Carfilzomib Monotherapy

A Phase 1 clinical trial, PX-171-002, testing carfilzomib in subjects with relapsed/refractory hematologic malignancies, is being completed(14). During the dose escalation portion of the trial, 36 subjects received carfilzomib on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle. 20 subjects with MM were enrolled on the study.

No dose limiting toxicities (DLTs) were observed in the initial seven cohorts (doses ranged from 1.2 to 15 mg/m^2) of three subjects each. At the 20 mg/m^2 dose level, one of eight patients had a Grade 3 renal failure at Cycle 1, Day 2 which was considered possibly related to study drug and lasted for six days. The patient continued on study for the remainder of Cycle 1 before having disease progression. At the 27 mg/m^2 dose level, one of six subjects experienced a DLT during Cycle 1, consisting of severe hypoxia with pulmonary infiltrates following Day 2 of dosing. In subjects where the 27 mg/m^2 dose was efficacious, a "first dose effect" was seen that included a constellation of findings that appeared to be the clinical sequelae of rapid tumor lysis syndrome (TLS) and/or cytokine release. This effect was notable for fever, chills, and/or rigors occurring during the evening following the first day of infusion. On the second day, three of five subjects with MM experienced an increase in creatinine to Grade 2 including the subject with the DLT. This elevation was rapidly reversible and all three subjects were rechallenged with carfilzomib without recurrence of the events. Interestingly, all three subjects had a rapid decline in serum and/or urine M-protein levels. There were no consistent changes in potassium, calcium, phosphorous, or uric acid levels although some increases in LDH and other markers of tumor lysis were noted. Because of the possible TLS and reversible creatinine elevations, hydration and very-low dose dexamethasone prophylaxis were instituted in subsequent studies and have essentially eliminated clinically significant TLS/creatinine elevations and the other "first-dose" effects.

Four of the twenty MM patients achieved a partial response (PR), one of two at the 15 mg/m^2 dose, one of six at the 20 mg/m^2 dose, and two of five at the 27 mg/m^2 dose.

1.2.3.2 Phase II Carfilzomib Monotherapy

Two Phase 2 clinical studies are ongoing with carfilzomib in MM patients, PX-171-003-A0 (N=46) in relapsed and refractory MM and PX-171-004 (N=39) in relapsed MM.(15, 16) In both studies, patients were dosed with 20 mg/m^2 on Days 1, 2, 8, 9, 15, and 16 on a 28 day schedule. In these studies there were four cases of suspected or documented TLS prior to institution of the prophylaxis guidelines. Since these guidelines were implemented, no further cases of TLS have been reported including in >350 additional patients with relapsed or refractory MM treated in ongoing Phase II studies.

A "stepped up" dosing schedule, referred to as 20/27 mg/m^2, has subsequently been incorporated into the PX-171-003 study (referred to as PX-171-003-A1) in order to maximize the clinical benefit of carfilzomib. Patients receive 20 mg/m^2 for the first cycle and 27 mg/m^2 thereafter. The study completed enrolment of 266 patients by the end of 2009 and formed the basis for NDA filing in beginning of 2011.

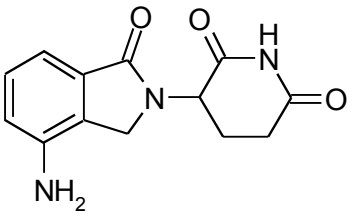
No cases of TLS were observed and rates of BUN and creatinine elevation dropped sharply, with Grade 3/4 renal impairment dropping to 2.2% in A1(from 15% in A0), most likely due to hydration and very low dose dexamethasone. The other most common adverse events were similar to the A0 portion of the study. Treatment-emergent peripheral neuropathy remains low on this portion of the study with 15% Grade 1/2 and one (0.7%) Grade 3/4 event reported to date

on PX-171-003-A1(15). In addition, anemia rates in the PX-171-003-A1 (higher dose) were lower than those reported in the PX-171-003–A0 portion of the study, possibly indicating that the higher dose of carfilzomib is achieving better clearing of neoplastic cells in the bone marrow allowing superior normal marrow reconstitution. Rates of thrombocytopenia and neutropenia were similar in the two cohorts, with Grade 3 neutropenia in ~5% without any Grade 4 neutropenia to date.(15)

1.2.4 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-2*H*-isoindol-2-yl) piperidine-2,6-dione and it has the following chemical structure:

Chemical Structure of Lenalidomide



The mechanism of action of lenalidomide remains to be fully characterized. Although the exact antitumor mechanism of action of lenalidomide is unknown, a number of mechanisms are postulated to be responsible for lenalidomide's activity against MM. Lenalidomide has been shown to increase T cell proliferation, which leads to an increase in IL-2 and IFN-gamma secretion. The increased level of these circulating cytokines augment natural killer cell number and function, and enhance natural killer cell activity to yield an increase in MM cell lysis. In addition, lenalidomide has direct activity against MM and induces apoptosis or G1 growth arrest in MM cell lines and in MM cells of patients resistant to melphalan, doxorubicin and dexamethasone. Revlimid® is approved in combination with dexamethasone for the treatment of patients with MM that have received at least one prior therapy.(17, 18) The drug has also been studied in newly diagnosed patients in combination with low-dose dexamethasone, as well as with bortezomib and dexamethasone as mentioned earlier.(8, 19)

1.2.5 Phase I Combination Therapy Carfilzomib, Lenalidomide, and Dexamethasone in Refractory/Relapsed MM

PX-171-006 is an ongoing Phase 1b study in patients with relapsed MM in which carfilzomib is administered in combination with lenalidomide (Revlimid®) and dexamethasone.(20) "Low-dose" dexamethasone 40 mg/day is given on Days 1, 8, 15, and 22 in all cases. Carfilzomib is administered IV on Days 1, 2, 7, 8, 15, and 16; lenalidomide is administered PO on Days 1 through 21. Enrollment has closed in this study, and no MTD was reached. The maximum per protocol doses of carfilzomib (20/27mg/m2) with lenalidomide 25mg and low dose dexamethasone are being used. After 8 patients tolerated these doses well, an additional 44 patients were enrolled in an "expansion" cohort at this level. To date, 40 patients were treated in cohorts 1-6 and 44 in the cohort 6 expansion. 27/32 patients in cohorts 1–5 are evaluable for safety and 29/32 for response. No dose-limiting toxicities or deaths attributed to study treatment have been observed. No treatment emergent fatigue, neuropathy, or thrombotic events ≥ Grade (G) 3 were observed. Hematological AEs ≥G3 (thrombocytopenia [n=6], anemia [n=4], and neutropenia [n=6]) were reversible. 4 patients had drug-related SAEs as follows: transient G3

sinus bradycardia, G3 upper respiratory tract infection, febrile neutropenia, and G3 diarrhea + G3 urinary infection. ORR and CBR for the 29 evaluable patients are 59% and 72%, respectively. Response data is shown in the table below.

	CRd: Cohorts 1–5 (CFZ: 15 to 20 mg/m2; LEN: 10 to 25 mg)		
Response	Relapsed (n=16)	Refractory (n=13)	Overall (n=29)
≥ CR/nCR	5 (31)	1 (8)	6 (21)
≥ VGPR	7 (44)	4 (31)	11 (38)
≥ PR	9 (56)	8 (62)	17 (59)
≥ MR	11 (67)	10 (77)	21 (72)

1.2.6 Phase I Combination Therapy Carfilzomib, Lenalidomide, and Dexamethasone in Newly Diagnosed MM

Results of a phase I/II study in newly diagnosed MM patients was recently published evaluating carfilzomib, lenalidomide, and dexamethasone.(21) Carfilzomib was the only escalating agent, ranging from 20 mg/m2 (dose level 1), 27 mg/m2 (dose level 2), to a recently amended dose of 36 mg/m2 (dose level 3) based on recent toxicity assessments. Lenalidomide was administered at 25 mg PO (days 1-21) while dexamethasone was given at 40/20 (cycles 1-4/5-8) mg PO weekly.

The study enrolled 53 patients, 4 patients at 20 mg/m2, 13 at 27 mg/m2, 18 at 36 mg/m2 and additional 18 in the expanded phase 2 cohort at 36 mg/m2. One patient had a DLT at dose level 27 mg/m2 with an episode of non-febrile neutropenia. Two patients experienced DLT at 36 mg/m2 dose level with episodes of dyspnea and pulmonary edema. Data indicated a dose dependent trend of increasing DLTs, however, the DLT probability was estimated to be less than 20% set for the MTD using the TITE-CRM algorithm. The MPD of 36 mg/m2 was selected as the expansion dose for phase II. Hematological AEs ≥G3 (were thrombocytopenia 17%, anemia 21%, and neutropenia 17%). Non-hematologic AEs ≥G3 include hypophosphatemia 25%, hyperglycemia 23%, DVT/PE 9%, rash 8%, and elevated liver function test 8%. Peripheral neuropathy was experienced by 23% and limited in severity to Grade 1(17%) and 2 (6%). There was no Grade 3 or 4 peripheral neuropathy reported. After a median of 12 cycles of treatment, 62% achieved at least near CR and 42% achieved a stringent CR.

1.2.7 Minimal Residual Disease (MRD) in MM

Current response criteria, which are defined by M-spike, plasma cell burden, and abnormal free light chains, are often limited in predicting clinical response and duration of response. MRD in MM is still under current investigation. Immunophenotyping of abnormal plasma cells using multi-parametric flow cytometry has prognostic value in the post-autologous stem cell transplantation setting.(22) At day 100 post auto-transplant, patients achieving negative MRD flow status compared to those with residual positive MRD flow status had longer OS (median not reached vs. 89 months, p = 0.002). Other techniques, such as, polymerase chain reaction (PCR) of Ig heavy chain (IGH) rearrangements and light chain genes have also been employed in

evaluating for MRD and found to have prognostic significance in showing prolonged PFS after intensive therapy.(23)

FDG-PET CT has been valuable at localizing intramedullary and extramedullary disease in multiple myeloma. FDG uptake is able to distinguish between active lesions and chronic disease, scar tissue, necrotic tissue, radiation changes, and other benign disease. The sensitivity of FDG-PET in detecting myelomatous involvement is approximately 85%, and its specificity is approximately 90%.(24)

Incorporating such techniques into prospective clinical trials may have a role in evaluating response to therapy.

1.2.8 Proposed Study Investigation with Correlative Studies

Given carfilzomib's potent anti-myeloma activity and lack of peripheral neuropathy, we propose a phase II investigation of combination therapy (Cycles 1-8 carfilzomib, lenalidomide, dexamethasone) in newly diagnosed MM patients followed by extended dosing with lenalidomide (days 1-21 of 28 day cycle) for 12 cycles. Patients will be enrolled on study to determine if the CRd 3 drug-combination demonstrates a rate of grade 3 or worse neurologic toxicity lower than 10% in the first two completed cycles. After 4 cycles, transplant eligible patients will undergo autologous stem cell harvesting. After 8 cycles, patients achieving SD or better will proceed to extended dosing with lenalidomide. Patients not achieving SD will be off treatment. Response criteria is outlined in Section **5.3**. Proposed correlative studies include gene expression profiling on purified CD 138 + pre- and post- carfilzomib plasma cells, identification of potential biomarkers (blood, urine, bone marrow aspirates), proteasome quantification and activity, ubiquination assays, and effects on downstream signaling targets. Patients will also undergo evaluation for minimal residual disease at regular interval time points, using multi-parametric flow cytometry, FDG PET-CT, and detection of clonality using heavy and/or light chain immunoglobulin rearrangement. Effective with Amendment L (version date 07/15/2015), bone marrow aspirate and biopsy will be obtained for research studies at the discretion of the PI.

FDG-PET CT has been valuable at localizing intramedullary and extramedullary disease in MM. FDG uptake is able to distinguish between active lesions and chronic disease, scar tissue, necrotic tissue, radiation changes, and other benign disease. The sensitivity of FDG-PET in detecting myelomatous involvement is approximately 85%, and its specificity is approximately 90%. Along the lines of more advanced imaging techniques, DW-MRI has recently been found as a potential complementary imaging modality that focusses on the actual myeloma plasmacytoma disease rather than subsequent bone destruction. The MY-RADS group state that because skeletal survey and CT predominantly help to detect the destructive effects of myeloma on trabecular and cortical bone rather than disease within the bone marrow space, sensitivity and capability as a restaging tool are inherently limited. Myeloma infiltrates within bone marrow can be observed on CT if they lie within the marrow spaces adjacent to fatty bone marrow. However, in trabecular bone spaces (i.e. vertebral bodies), myeloma infiltrates are difficult to evaluate given factors including the trabeculae, degenerative changes, benign lesions, and osteoporosis. In contrast, MRI allows direct imaging of the bone marrow given its superb sensitivity, soft-tissue contrast, and early detection of focal myeloma lesions. Although PET/CT can also detect myeloma lesions, MRI is more sensitive especially with newer techniques, i.e. DW-MRI, which have shown a sensitivity of 77% compared to 47% for PET/CT. Current whole-body MRI protocols can incorporate DW-MRI sequences that are sensitive to cellular density and viability and are important for disease detection and monitoring. Another benefit of DW-MRI sequences is that they are quick to perform (~45 minutes) and interpret. Finally,

the relationship of apparent diffusion coefficient values with cell density has the potential to assess response to treatment and response heterogeneity prior to changes in lesion size. The recent MY-RADS recommendations were published in an attempt to promote standardization and decrease variations in the acquisition, interpretation, and reporting of whole-body MRI and allow better response assessments across cancer centers. MY-RADS recommendations require validation within clinical trials, including assessments of reproducibility and therefore we will be guided by the group's recommendations especially in regard to MRI data acquisition and analysis protocols.

1.2.9 Continuation of Lenalidomide

Since the initiation of the protocol new data has been published regarding the efficacy and safety of extended dosing with lenalidomide in multiple myeloma patients. Three double blind randomized control trials were published in the same May 2012 issue of NEJM showing that patients receiving extended dosing with lenalidomide (10-15 mg) after induction type regimens have a significant progression free survival benefit in the lenalidomide therapy arms (24-26). Two studies (McCarthy et al. and Attal et al.) were conducted after autologous HSCT and one study (Palumbo et al.) assessed transplant ineligible patients after induction therapy with melphalan, lenalidomide and prednisone. Importantly, McCarthy and colleagues showed an overall survival benefit in patients receiving lenalidomide compared to placebo (15% vs. 23%, p = 0.03)(26). A small increased risk of secondary primary malignancies was noted in the lenalidomide groups compared to the placebo groups in all three trials. However, it should be noted that such a risk has been well known and established in multiple myeloma patients receiving alkylator therapy, such as melphalan(27, 28). All three of the aforementioned randomized trials used lenalidomide after melphalan exposure, a significant and notable difference between our trial (11-C-0221) and theirs. Despite this increased risk of secondary malignancy, post ad hoc analysis for all three trials with second malignancies being scored as "events" still showed a significant event free survival benefit for lenalidomide arms compared to placebo(24-26, 29).

In accord with these recent publications and lack of alkylator exposure in our protocol using CRd, we increased lenalidomide 10 mg extended dosing from the initially proposed 1 year to 2 years in patients that maintain their current clinical response and do not show signs of progression as outlined in Section **6.3** or reasons for removal from protocol therapy listed in Section **3.5**.

1.2.10 Updated Interim Analysis- ASH 2012

In an interim analysis presented at ASH 2012, we updated results on the first 20/45 patients. Among the first 20 patients, none developed ≥ grade 3 neuropathy. Best responses with a median of 7 cycles showed 15/20(75%) nCR/sCR, 2/20(10%) VGPR, 2/20 (10%) PR, and 1/20 (5%) SD. Patients demonstrating ORR (PR or higher) was 19/20 (95%). Patients with ≥ grade 3 non-hematologic toxicities include: LFT elevation (20%), fatigue (15%), rash/pruritus (15%), dyspnea (10%), CHF (10%), general disorders and administration site conditions (5%), mood alterations (5%), and electrolyte disturbances (5%). Patients with ≥ grade 3 hematologic toxicities include: lymphopenia (60%), and anemia (5%).

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Newly diagnosed patients with histologically confirmed Multiple Myeloma (MM based on the following criteria:

a) Clonal plasma cells in the bone marrow

b) Measurable disease within the past 4 weeks defined by any one of the following:

- Serum monoclonal protein ≥ 1.0 g/dL
- Urine monoclonal protein >200 mg/24 hour
- Serum immunoglobulin free light chain > 10 mg/dL AND abnormal kappa/lambda ratio

c) Evidence of underlying end organ damage attributed to underlying plasma cell proliferative disorder meeting at least one of the following:

- Hypercalcemia: serum calcium ≥ 2.65 mmol/L
- Renal Insufficiency: serum creatinine > 2.0 mg/dL
- Anemia: hemoglobin value <10 g/dL or 2 g/dL < normal reference
- Bone disease: lytic lesions, severe osteopenia or pathological fractures

2.1.1.2 Creatinine Clearance ≥ 60 ml/min. CrCl will be calculated by Cockcroft-Gault method. CrCl (calculated) = (140 – Age) x Mass (in kilograms) x [0.85 if Female] 72 x Serum Creatinine (in mg/dL). If calculated CrCl based on Cockcroft-Gault method is <60 mL/min, patient will have a 24 hr urine collection to measure CrCl. The measured CrCl must also be ≥ 60 ml/min.

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

2.1.1.4 Eastern Cooperative Oncology Group (ECOG) performance status 0-2

- Absolute neutrophil count (ANC) ≥ 1.0 K/uL, hemoglobin ≥ 8 g/dL (transfusions are permissible), and platelet count ≥ 75 K/uL
- Adequate hepatic function, with bilirubin < 1.5 x the ULN, and AST and ALT < 3.0 x ULN.

2.1.1.5 All study participants must be able to tolerate one of the following thromboprophylactic strategies: aspirin, low molecular weight heparin or warfarin (coumadin).

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

2.1.1.7 Females of childbearing potential (FCBP)[†] must have a negative serum or urine

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

pregnancy test within 10 – 14 days and again within 24 hours prior to prescribing lenalidomide for Cycle 1 (prescriptions must be filled within 7 days) and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 28 days before she starts taking lenalidomide. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy.

2.1.1.8 Subjects must be able to give informed consent

2.1.2 Exclusion Criteria

2.1.2.1 Prior or concurrent systemic treatment for MM.

- Treatment of hypercalcemia or spinal cord compression or aggressively progressing myeloma with corticosteroids is permitted.
- Bisphosphonates are permitted.
- Treatment with corticosteroids for indications other than MM is permitted.
- Radiotherapy is permitted.
- Treatment for smoldering myeloma is permitted.

2.1.2.2 Plasma cell leukemia

2.1.2.3 Pregnant or lactating females. Because there is a potential risk for adverse events in nursing infants secondary to treatment of the mother with carfilzomib in combination with lenalidomide, breastfeeding should be discontinued if the mother is treated with carfilzomib and lenalidomide. These potential risks may also apply to other agents used in this study.

2.1.2.4 Uncontrolled hypertension or diabetes

2.1.2.5 Active hepatitis B or C infection

2.1.2.6 Has significant cardiovascular disease with NYHA Class III or IV symptoms, or hypertrophic cardiomyopathy, or restrictive cardiomyopathy, or myocardial infarction within 3 months prior to enrollment, or unstable angina, or unstable arrhythmia as determined by history and physical examination. Echocardiogram will be performed if clinically warranted.

2.1.2.7 Has refractory GI disease with refractory nausea/vomiting, inflammatory bowel disease, or bowel resection that would prevent absorption

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

2.1.2.9 Significant neuropathy ≥Grade 3 at baseline

2.1.2.10 Contraindication to any concomitant medication, including antivirals, anticoagulation prophylaxis, tumor lysis prophylaxis, or hydration given prior to therapy

2.1.2.11 Major surgery within 1 month prior to enrollment

2.1.3 Recruitment Strategies

Participant sources will be from outside physician referrals and from standard websites such as clinicaltrials.gov and the NCI CCR website.

2.2 SCREENING EVALUATION

2.2.1 Clinical Evaluation

2.2.1.1 A complete history and physical examination with documentation of measurable disease and assessment of performance status using the ECOG scale must be performed prior to study entry.

a) Patients will be evaluated for baseline neuropathy. Patients with grade 3 or higher will be excluded.

2.2.1.2 The following laboratory tests will be completed 4 weeks prior to study entry:

a) CBC with differential and reticulocyte count
b) Acute Care (sodium, potassium, chloride, CO2, glucose, BUN, creatinine), Mineral (serum calcium, phosphate, magnesium and albumin) and Hepatic (alkaline phosphatase, ALT, AST, total and direct bilirubin) Panels, and eGFR determination
c) Uric acid, LDH, and Beta-2 Microglobulin
d) PT, PTT
e) Serum protein electrophoresis (SPEP) and immunofixation to assess for presence and quantity of monoclonal protein (M-protein)
f) 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 diagnosis.
g) Serum free light-chain studies, determined using the FreeliteTM assay system
h) Quantitative immunoglobulins
i) Viral serologies
    1) Hepatitis B surface antigen
    2) Anti-Hepatitis C (HCV) antibody. If positive, will follow with HCV RNA PCR
j) Review of bone marrow core biopsy and aspirate.
k) Serum or urine pregnancy test in women of child-bearing potential.
l) 12-lead EKG

2.2.1.3 A skeletal survey of the axial and appendicular skeleton will be performed. 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 an additional reading by the Department of Radiology.

2.3 REGISTRATION PROCEDURES

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 sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. 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.

Note: As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

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 correlative studies.

2.4.1.1 Bone Marrow (see Section **5**)

a) Immunophenotyping of aberrant clonal plasma cells by multiparametric flow cytometry
b) Interphase FISH/cytogenetics
c) CD 138+ fractions and CD 138- fractions cell sorting with subsequent correlatives on both fractions
d) Proteasome activity and quantification
e) Markers for autophagy of myeloma plasma cells on pre and post exposure of carfilzomib
f) Histopathological evaluation on bone marrow aspirate and biopsy
   1) Immunoglobulin heavy and/or light chain rearrangement

2.4.1.2 Peripheral Blood/Urine

a) Peripheral blood and urine samples for storage and establishing a biobank. (**Appendix C**)
b) Samples for pharmacokinetics
c) Subunit profiling and activity of circulating proteasomes by enzyme-like immunosorbent assay
d) Apoptosis assays to identify necrotic or late stage apoptotic cells
e) Immunolocalization studies.
f) Flow cytometry
   1) Peripheral blood for circulating plasma cells
   2) Immune cells – including, but not limited to T cells (CD4 and CD8), LGL, and NK cells
   3) Autophagy

2.4.2 Novel imaging studies (FDG PET/CT scan) within 4 weeks of study entry and prior to starting therapy

Prior to ^{18}F-FDG PET/CT imaging, the subject will be fasted and have not received any sugar containing substance (i.e. glucose, sucrose, dextrose) for 4-6 hours. Subjects will be encouraged to drink water during this period to reduce radiation dose to the kidneys and will be asked to void prior to ^{18}F-FDG injection. Women of childbearing potential will have a documented report of negative pregnancy test from the CC or another accredited lab performed on the day of the scan or the day before the scan.

^{18}F-FDG, [18F]-fludoeoxyglucose is an FDA approved radiopharmaceutical.

Immediately prior to injection, the subject's blood glucose level will be evaluated via fingerstick. Non-diabetic subjects with fasting blood glucose levels above 150 mg/dl may be rescheduled at the discretion of the PI. Subjects will be asked to refrain from excessive physical exertion for the 24 hours prior to injection. Patients will report to the NCI Molecular Imaging Clinic (MIC) on the day of their F-18 PET/CT scan and peripheral venous access will be obtained (most commonly via IV in the antecubital fossa). The ^{18}F-FDG injection procedure will be injected and be followed by a ~20 ml saline (sodium chloride IV infusion 0.9% w/v) flush over a period of ~20 seconds. The injection site will be evaluated pre- and post administration for any reaction (e.g. bleeding, hematoma, redness, or infection).

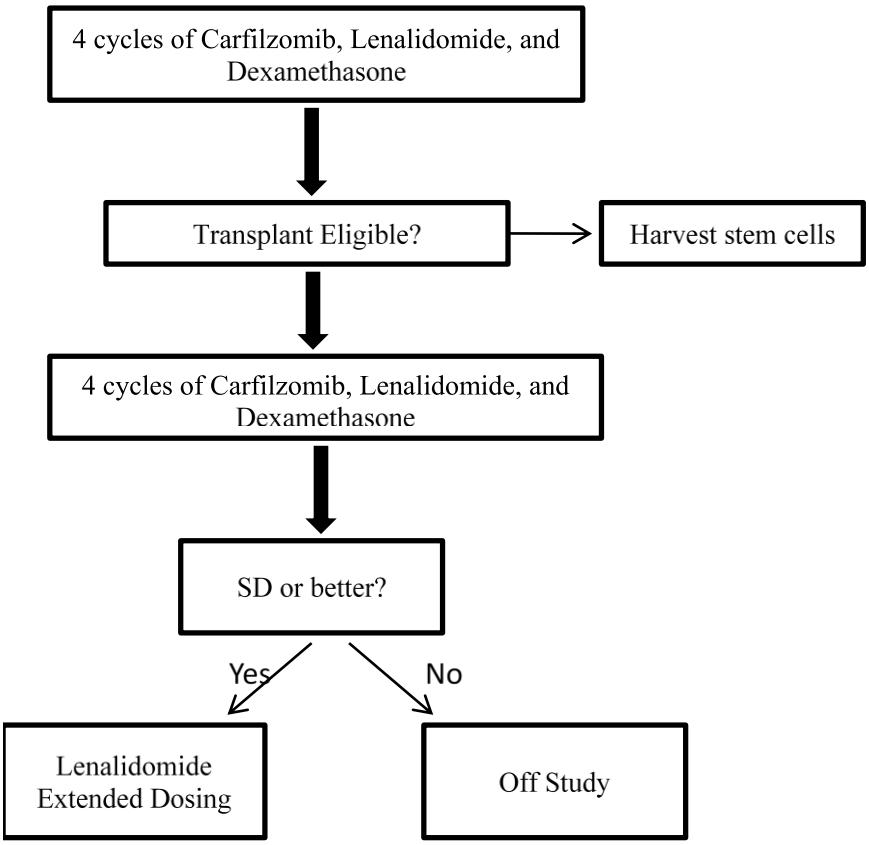
Whole body (vertex to toes) static PET/CT imaging will be performed beginning at 1-hour, and again at 2-hours post injection. PET/CT standard operating procedures. The patient will be instructed to maintain good hydration in order to reduce the radiation dose.

The radiation dose from the procedure will be a maximum of 2.1 rem per year; this is within the RSC guidelines of 5.0 rem per year for adults.

3 STUDY IMPLEMENTATION

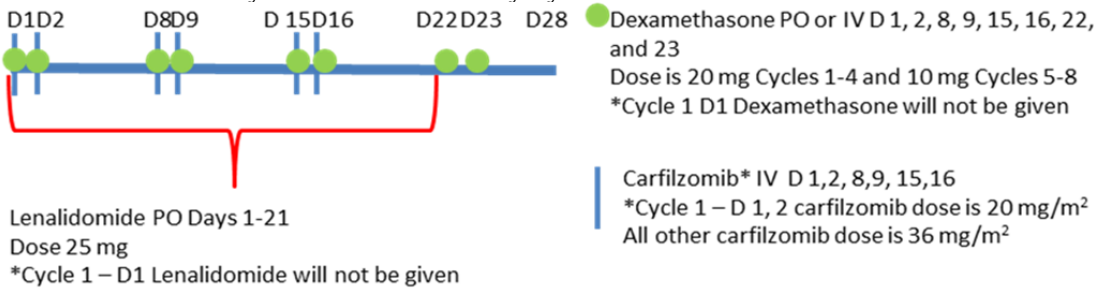
3.1 STUDY DESIGN

- Patients with newly diagnosed MM will be enrolled on the Phase II study and treated with 3 drug combination (Cycles 1-8 carfilzomib, lenalidomide, dexamethasone) followed by extended lenalidomide dosing.
- After receiving first 4 cycles, patients who are considered to be eligible for subsequent high dose therapy/autologous stem cell transplant (ASCT) will undergo autologous stem cell harvesting for potential use in the future. In accord with current clinical standards, the decision whether a given patient is eligible for subsequent high dose therapy/ASCT, or not, will be based on a clinical assessment conducted by the transplant center that evaluates the patient.
- For patients who choose to undergo stem cell harvest, next cycle may be delayed for up to 5 weeks.
- After 8 cycles, patients achieving SD or better will proceed to extended dosing phase I of lenalidomide (Days 1-21 of 28 day cycle) for 12 cycles. After 12 cycles of extended dosing of lenalidomide, patients who maintain their current clinical response and do not meet criteria of PD as defined in Section **6.3** will have the option to continue lenalidomide as clinically indicated for an additional year on extended dosing phase II.
- Patients not achieving SD will be off treatment.
- Patients will be enrolled on study to determine if the CRd 3 drug-combination demonstrates a rate of grade 3 or worse neurologic toxicity of 10% or lower in the first two completed cycles. A rate greater than 10% will be considered excessive.



3.2 DRUG ADMINISTRATION

3.2.1 Induction Phase: Cycles 1-8 with 28 day cycles



3.2.2 Agents

3.2.2.1 Carfilzomib

- Cycle 1: 20 mg/m² IV infusion over 30 minutes on days 1 and 2, then 36 mg/m² IV on days 8, 9, 15, and 16
- Cycle 2-8: 36mg/ m² IV infusion over 30 minutes on days 1, 2, 8, 9, 15, and 16

3.2.2.2 Lenalidomide

- Cycle 1: 25 mg oral days 2-21 of 28-day cycle
- Cycle 2-8: 25 mg oral days 1-21 of 28-day cycle
- Lenalidomide capsules should be swallowed whole, and should not be broken, chewed or opened.

- 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. Only enough lenalidomide for one cycle of therapy will be supplied to the patient each cycle**.**

3.2.2.3 Dexamethasone

- Cycle 1: 20 mg oral or IV on days 2, 8, 9, 15, 16, 22, and 23
- Cycle 2-4: 20 mg oral or IV on days 1, 2, 8, 9, 15, 16, 22, and 23
- Cycle 5-8: 10 mg oral or IV on days 1, 2, 8, 9, 15, 16, 22, and 23

3.2.3 In Cycle 1, the following adjustments to the dosing schema will be implemented:

- Cycle 1 Day 1, carfilzomib will be adminstered alone.
- Patients will be admitted and observed as an inpatient while receiving Cycle 1 Day 1 and 2 therapy.
- Carfilzomib will be given at a lower dose of 20 mg/m^2 on Days 1 and 2 of Cycle 1.
- Lenalidomide and Dexamethasone will not be given on day 1
- Bone marrow biopsy with aspirtation will be performed at baseline and Cycle 1 Day 2
- Hydration will be administered prior and subsequent to carfilzomib dosing.
  1) Oral hydration: All subjects must be well hydrated (i.e., volume replete). Begin oral hydration equal to approximately 30 mL/kg/day (~6–8 cups of liquid per day), starting 48 hours prior to the planned first dose of carfilzomib. Compliance must be reviewed with the subject and documented by the site personnel prior to initiating treatment with carfilzomib; treatment is to be delayed or withheld if oral hydration is not deemed to be satisfactory.
  2) IV hydration: 500 mL (250 mL before & 250 mL after carfilzomib) OR 1000 mL (500 mL before & after carfilzomib) of normal saline or other appropriate IV fluid formulation must be given before *and* after each carfilzomib dose during Cycle 1 D1 and D2. Total volume will be determined at the discretion of clinician and volume status of patient. If lactate dehydrogenase (LDH) or uric acid is elevated at Cycle 2, Day 1, then the recommended IV hydration should be repeated for Cycle 2. The goal of the hydration program is to maintain robust urine output, (e.g., ≥ 2 L/day). Subjects should be monitored periodically during this period for evidence of fluid overload.
  3) In subjects considered to be still at risk for TLS at completion of Cycle 1, hydration should be continued into subsequent cycles if clinically indicated

3.2.4 Harvesting Stem Cells

- After 4 cycles of induction combination therapy, patients who are clinically considered to be transplant eligible and who do not opt out from that procedure will be sent for evaluation of harvesting autologous stem cells at a transplant center.
- Transplant eligible patients will be defined as age <75 years of age with no significant disease co-morbidities and ECOG ≤2. Individual transplant centers will evaluate patient eligibility based on their own criteria.
- For patients who choose to undergo stem cell harvest, next cycle may be delayed for up to 5 weeks.

3.2.5 Extended Dosing Phase I: Cycles 9-20

- Patients that achieve a SD or better after 8 cycles of therapy will go on to receive extended dosing with oral lenalidomide 10 mg, given daily for 21 days of a 28 day cycle, for 12 cycles.
- Lenalidomide ordered in the extended dosing phase of the study will be prescribed monthly by NIH 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.
- Females of childbearing potential (FCBP) patients will be required to have pregnancy testing as outlined in **Appendix A: Requirements for REMS®**; 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.

3.2.6 Extended Dosing Phase II (optional): Cycles 21through 32 (effective with Amendment H version date: 05/17/2013):

- After 12 cycles of extended dosing of lenalidomide (phase I), patients who maintain their current clinical response and do not meet criteria of PD as defined in Section **6.3** will have the option to continue lenalidomide as clinically indicated for one additional year. For those patients who are ready to begin extended dosing phase II, treatment may be delayed until amendment H is approved by the IRB.
- Lenalidomide ordered in extended dosing phase II will be prescribed monthly by NIH 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.
- FCBP patients will be required to have pregnancy testing as outlined in **Appendix A**; 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.

3.3 DOSE MODIFICATIONS

3.3.1 Dose Reductions: Induction Phase

	Lenalidomide	Carfilzomib	Dexamethasone Cycles 1-4	Dexamethasone Cycles 5-8
Baseline dose	25 mg daily on Days 1-21 of 28 day cycle	36 mg/m^2	20 mg	10 mg
One level dose reduction	20 mg daily on Days 1-21 of 28 day cycle	27 mg/m^2	10 mg	4 mg
Two level dose reduction	15 mg daily on Days 1-21 of 28 day cycle	20 mg/m^2	4 mg	0 mg
Three level dose reduction	10 mg daily on Days 1-21 of 28 day cycle		0 mg	
Four level dose reduction	5 mg daily on Days 1-21 of 28 day cycle			

3.3.1.1 If more than 2 dose reductions are required with Carfilzomib, study treatment will be discontinued and the patient will go off therapy.

3.3.1.2 If there is no resolution of toxicity after 2 weeks of withholding treatment or up to 3 weeks for infection related treatment, the subject will go off therapy

3.3.2 Hematologic Toxicity: Induction Phase

3.3.2.1 Due to the nature of the disease originating in the bone marrow, events occurring within the first two cycles of treatment and deemed to be due to disease burden will be exempt from dose reductions at the discretion of the investigator.

3.3.2.2 On day 1 of each new cycle (or day 1 of every third cycle during extension phase), patients must meet the following criteria or dose modify based on dose reductions in table 3.3.1:

- ANC $\geq 1.0 \times 10^9$/L
- Platelet count $\geq 75 \times 10^9$/L during cycles 1-8; Platelet count $>50 \times 10^9$/L during cycles 9 and beyond

3.3.2.3 If these conditions are not met on Day 1 of a new cycle, a new cycle of treatment will not be initiated until the toxicity has resolved. If there is no resolution after 2 weeks of withholding treatment or up to 3 weeks for infection related treatment, the subject will go off therapy.

3.3.2.4 If the investigator chooses to dose reduce according to table 3.3.1, the investigator will determine which drug will be modified based on side effect profile and clinical judgment.

3.3.2.5 If a patient develops thrombocytopenia or neutropenia during the cycle, then the following actions would take place (see Thrombocytopenia/Neutropenia table below).

Thrombocytopenia	**Lenalidomide**	**Carfilzomib**
Fall to $< 25 \times 10^9$/L	Hold both Lenalidomide and Carfilzomib, follow CBC weekly. Hold prophylactic anti-coagulation.	

Thrombocytopenia	**Lenalidomide**	**Carfilzomib**
Return to ≥ 25 x 10^9/L	Resume lenalidomide at next dose reduction	Resume carfilzomib at full dose.*
Subsequent fall to < 25 x 10^9/L	Hold both Lenalidomide and Carfilzomib, follow CBC weekly. Hold prophylactic anti-coagulation.	
Return to ≥ 25 x 10^9/L	Resume lenalidomide at next dose level reduction	Resume carfilzomib at full dose.*

*Carfilzomib may be dose reduced at the clinical discretion of investigator

Neutropenia (Absolute Neutrophil Count)	**Lenalidomide**	**Carfilzomib**
Falls to < 0.5 x 10^9/L or to < 1.0 x 10^9/L with fever	Hold Lenalidomide and Carfilzomib. Add filgrastim if Grade 3 with fever (single temperature of 38.3° or sustained temperature of 38° for > 1 hour) or Grade 4. Follow CBC weekly	
Returns to ≥ 1.0 x 10^9/L	Resume Lenalidomide at next dose reduction.	Resume Carfilzomib at full dose.*
Subsequent drop to < 0.5 x 10^9/L or to < 1.0 x 10^9/L with fever	Hold Lenalidomide and Carfilzomib. Add filgrastim if Grade 3 with fever or Grade 4. Follow CBC weekly	
Returns to ≥ 1.0 x 10^9/L	Resume Lenalidomide at next dose reduction.	Resume Carfilzomib at full dose.*

*Carfilzomib may be dose reduced at the clinical discretion of investigator

3.3.3 Non-Hematologic Toxicity: Induction Phase

3.3.3.1 Toxicity ≥ grade 3 will require appropriate study drug to be held until resolved to ≤ Grade 1 unless specified below. Investigator will determine which drug will be held based on side effect profile and clinical judgment.

3.3.3.2 Once toxicity has resolved ≤ grade 1, subsequent doses will be reduced at next dose level (according to table in Section **3.3.1**) if the adverse event was deemed to be treatment related by the PI. If the adverse event was deemed to be unrelated to treatment, the patient may continue the full dose.

3.3.3.3 Readily reversible electrolyte and metabolic abnormalities or infections controlled by appropriate therapy are exempt.

Common Lenalidomide Toxicities	**Dosing Modifications**
Non Blistering Rash	• If Grade 3, hold lenalidomide dose and restart at next dose reduction once rash improves to ≤ Grade 1 • If Grade 4, discontinue lenalidomide and remove patient from therapy
Blistering Rash (Any Grade)	Discontinue lenalidomide and remove patient from therapy

Common Lenalidomide Toxicities	**Dosing Modifications**
Venous thrombosis/embolism	Hold lenalidomide and start therapeutic anticoagulation. Restart lenalidomide at investigator's discretion at current dose level.
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 – Dose reduce lenalidomide to 10 mg daily from Days 1-21 • CrCl ≤30 mL/min (not requiring dialysis) – Dose reduce Lenalidomide to 15 mg every 48 hours and see carfilzomib dosing below. • CrCl ≤30 mL/min (requiring dialysis) – Decrease Lenalidomide to 5 mg daily and on dialysis days dose lenalidomide after dialysis.

Common Carfilzomib Toxicities	**Dosing Modifications**
Allergic Reaction/Hypersensitivity	• Grade 2-3. Hold carfilzomib until ≤ Grade 1 and resume at full carfilzomib dose • Grade 4. Discontinue protocol therapy
Tumor Lysis Syndrome (≥ 3 of the following: ≥50% increase in creatinine, uric acid, or phosphate; ≥30% increase in potassium; ≥20% decrease in calcium; or 2-fold increase in LDH	Hold carfilzomib until all abnormalities in serum chemistries have resolved. Resume at full dose
Herpes zoster or simplex of any grade	Hold carfilzomib until lesions are dry. Resume at full dose
Neuropathy	• Grade 2 treatment emergent neuropathy with pain or Grade 3 neuropathy. Continue to dose. If neuropathy persists for more than two weeks hold carfilzomib until resolved to ≤ Gr 2 without pain. Then restart at next dose level reduction. • Grade 4 neuropathy. Discontinue protocol therapy
Congestive Heart Failure	Any subject with symptoms of congestive heart failure, whether or not drug related, must have the dose held until resolution or return to baseline. After which, treatment may continue at reduced dose or patient may be withdrawn from therapy.

3.3.4 Dose Reductions: Extended Dosing Phases I and II

	Lenalidomide
Baseline dose	10 mg daily on Days 1-21 of 28 day cycle
One level dose reduction	5 mg daily on Days 1-21 of 28 day cycle

3.3.5 Hematologic Toxicity: Extended Dosing Phases I and II

3.3.5.1 On day 1 (or within 7 days prior to day 1) of every third cycle during extension phase, patients must meet the following criteria or dose modify based on dose reductions in

table 3.3.4:

- ANC ≥ 1.0 x 10^9/L
- Platelet count >50 x 10^9/L during cycles 9 and beyond

If these conditions are not met on Day 1 of every third cycle, treatment will not be initiated until the toxicity has resolved. If there is no resolution after 2 weeks of withholding treatment or up to 3 weeks for infection related treatment, the subject will go off therapy.

3.3.5.2 If a patient develops thrombocytopenia or neutropenia during the cycle, then the following actions would take place (see Thrombocytopenia/Neutropenia table below).

Thrombocytopenia	**Lenalidomide**
Fall to < 25 x 10^9/L	Hold Lenalidomide, follow CBC weekly. Hold prophylactic anti-coagulation.
Return to ≥ 25 x 10^9/L	Resume lenalidomide at next dose reduction if deemed drug related
Subsequent fall to < 25 x 10^9/L	Hold Lenalidomide. Hold prophylactic anti-coagulation
Return to ≥ 25 x 10^9/L	Patient will go off therapy if deemed drug related

Neutropenia (Absolute Neutrophil Count)	**Lenalidomide**
Falls to < 0.5 x 10^9/L or to < 1.0 x 109/L with fever	Hold Lenalidomide. Add filgrastim if Grade 3 with fever (single temperature of 38.3o or sustained temperature of 38o for > 1 hour) or Grade 4. Follow CBC weekly
Returns to ≥ 1.0 x 10^9/L	Resume Lenalidomide at next dose reduction.
Subsequent drop to < 0.5 x 10^9/L or to < 1.0 x 109/L with fever	Hold Lenalidomide. Add filgrastim if Grade 3 with fever or Grade 4. Follow CBC weekly
Returns to ≥ 1.0 x 10^9/L	Patient will go off therapy if deemed drug related

3.3.6 Non-Hematologic Toxicity- Extended Dosing Phase

3.3.6.1 Toxicity ≥ grade 3 will require study drug to be held until resolved to ≤ Grade 1 or baseline unless specified below.

3.3.6.2 Once toxicity has resolved ≤ grade 1 or baseline, subsequent doses will be reduced at next dose level (according to table in Section **3.3.4**) if the adverse event was deemed to be treatment related by the PI. If the adverse event was deemed to be unrelated to treatment, the patient may continue the full dose. In the case of renally adjusted doses, dosing modifications are made according to table below.

3.3.6.3 Readily reversible electrolyte and metabolic abnormalities or infections controlled by appropriate therapy are exempt.

Common Lenalidomide Toxicities	**Dosing Modifications**
Non Blistering Rash	• If Grade 3, hold lenalidomide dose and restart at next dose reduction once rash improves to ≤ Grade 1 • If Grade 4, discontinue lenalidomide and remove patient from therapy
Blistering Rash (Any Grade)	Discontinue lenalidomide and remove patient from therapy
Venous thrombosis/embolism	Hold lenalidomide and start therapeutic anticoagulation. Restart lenalidomide at investigator's discretion at current dose level.
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 – Dose reduce lenalidomide to 10 mg daily from Days 1-21 • CrCl ≤30 mL/min (not requiring dialysis) – Dose reduce Lenalidomide to 15 mg every 48 hours and see carfilzomib dosing below. • CrCl ≤30 mL/min (requiring dialysis) – Decrease Lenalidomide to 5 mg daily and on dialysis days dose lenalidomide after dialysis.

3.3.7 Monitoring

3.3.7.1 Patients will be observed in the hospital for administration of cycle 1 Days 1 and 2 of therapy.

3.3.7.2 Routine labs (cbc, chemistry panel 14, LDH, magnesium, uric acid, phosphate) will be performed on Day 1, 2, 8, 15, and 22 of cycle 1 and Day 1 (or within 2 days prior to day 1) of each cycle during cycles 1-8 and on Day 1 (or within 7 days prior to day 1) of every third cycle during extension phases I and II.

3.3.7.3 Myeloma tests include serum protein electrophoresis, serum immunofixation, serum free light chains, quantitative immunoglobulins, beta-2 microglobulin and will be performed at baseline and Day 1 (or within 2 days prior to day 1) of each cycle in the induction phase and on Day 1 (or within 7 days prior to day 1) of every third cycle during extension phase I and II. Subsequent serum immunofixation will only be performed on those patients clinically indicated.

3.3.7.4 Random urine sample for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria) to be performed at baseline, day 1 (or within 2 days prior to day 1) of each cycle during cycles 1-8, and day 1 (or within 7 days prior to day 1) of every third cycle during extension phase I and II. If patient obtains CR, then a 24 hour UPEP will be performed to confirm CR status. In patients whose measurable disease is best determined by measuring Bence-Jones protein quantification, a 24-hr UPEP will be performed at baseline, day 1 of each cycle 1-8, and day 1 of every third cycle during extension phase I and II. Otherwise, 24-hr UPEP Bence-Jones quantification will be estimated from random UPEP specimens performed at baseline, day 1 of each cycle 1-8, and day 1 of every third cycle during extension phase.

3.3.7.5 Patients will have clinic visits with H&P or standard progress notes assessing for toxicity/side effects on Day 1, 2, 8, 15, and 22 of cycle 1 and Day 1 of each cycle during cycles 1-8 and on Day 1 of every third cycle during extension phases I and II.

(This can be delayed up to one week per PI discretion for clinical and/or logistical reasons).

3.3.7.6 Additional laboratory studies and clinic visits will be performed if clinically indicated.

3.3.7.7 FDG-PET scan will be performed on patients in the Molecular Imaging Department at baseline, during cycles 1-8 if patient obtains CR or at the end of cycle 8 if no CR is achieved, during cycles 9-20 if patient obtains CR or at the end of cycle 20 if no CR is achieved, and during cycle 21 and beyond if patient obtains CR or at treatment termination During extension phase lenalidomide, FDG-PET are optional for those patients that are MRD negative at the end of cycle 8 regardless of response. At PI discretion, patient may be asked to have an additional PET-CT scan and/or DW-MRI at progression. The radiation dose from the procedure will be a maximum of 2.1 rem per year; this is within the RSC guidelines of 5.0 rem per year for adults.

3.3.7.8 With amendment P, patients who remain on study will be asked to undergo DW-MRI on an annual basis, however, these will not be mandatory.

3.3.7.9 During lenalidomide extension phases I and II, FCBP patients will still be required to have pregnancy testing as outlined in **Appendix A**. Local treating physicians will be required to submit results to NIH clinical center for review.

3.4 STUDY CALENDAR

Study	Pre-treatment	Induction Treatment							Extended Treatment				End of Treatment and Follow-Up	Disease Progression at any Time Point[l, p]
		Cycle 1					Cycle 2-8		Extended Dosing Phase (Phases I and II)					
		Day 1	Day 2	Day 8	Day 15	Day 22	Day 1[p]	CR Achieved/ End of Cycle 8[m,n,o,p]	Day 1 of Cycles 9, 12, 15, 18, 21, 24, 27, 30[c,e,r,s]	Day 1 Cycles 9, 12, 15, 18[p,r,s]	Day 1 Cycles 9-32[r,s]	Phase I-CR Achieved (Cycle 9-20)/ End of Cycle 20 and Phase II-CR achieved (Cycle 21-32)/ Treatment termination [m,n,o,p,r,s]	Every 3-6 months ([j,p,r,s,t]) or annual ([u])	
Medical Record Review	x												x	
H&P	x	x	x	x	x	x	x		x					
ECOG	x						x		x					
Informed Consent	x													
Routine Labs[a]	x	x	x	x	x	x	x		x				x	x
Urine for UPEP and IFE [m]	x	x[m]					x[m]	x[m]	x[m]			x[m]	x	x
Viral Studies[b]	x													
Register for REMS®	x													
Pregnancy Test[c]	x[c]	x[d]	x[d]	x[e]	x[e]	x[e]	x[c]		x[c,e]		x[c.e]	x[e]	x[e]	
Myeloma Tests[f]	x						x		x			x[q]	x	x
Pharmacokinetic Studies		x[k]					x			x				
Research Blood/Urine	x		x	x	x		x	x	x			x[r]	x	x
Bone Marrow/Aspirate	x[g]		x[h]					x[i, o]				x[i, o]	x[i]	x
Skeletal Survey	x													
FDG PET-CT	x							x[n]				x[n]	x[n]	x
DW-MRI													x[u]	

Adverse Events/Toxicity		x										→	

a. Routine tests include CBC, reticulocyte count, Acute Care (sodium, potassium, chloride, CO2, glucose, BUN, creatinine), Mineral (serum calcium, phosphate, magnesium and albumin) and Hepatic (alkaline phosphatase, ALT, AST, total and direct bilirubin) Panels, uric acid, eGFR determination and LDH. PT and PTT will only be performed at baseline
b. Viral studies include 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). During extension phase, FCBP patients will have pregnancy tests per footnote "e". Local treating physicians will be required to submit results to NIH clinical center for review.
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, urine electrophoresis, urine immunofixation, serum free light chains, quantitative immunoglobulins, beta-2 microglobulin and will be performed at baseline. Subsequent serum immunofixation and urine immunofixation will only be performed on those patients clinically indicated.
g. Baseline bone marrow aspiration and biopsy will be sent to Dept of Pathology, flow cytometry, FISH/cytogenetics, CD 138 sorting/GEP/storage and heavy/light chain immunoglobulin rearrangement and/or NRAS/KRAS mutations in Molecular Pathology
h. Cycle 1 Day 2 bone marrow require aspirate alone sent for CD138 sorting (GEP studies and microenvironment studies), flow cytometry for proteasomes and (optional) autophagy markers.
i. Bone marrow aspirate and biopsy can be performed +/- 21 days of intended cycle day. Bone marrow aspirate and biopsy will be sent to Hematology Section, DLM, flow cytometry (bone marrow immunophenotyping of plasma cells, autophagy of plasma cells, and +/- optional assessment of proteasomes), CD 138 sorting/GEP/storage, and +/- heavy/light chain immunoglobulin rearrangement and/or NRAS/KRAS mutations in Molecular Pathology (optional). During the follow up phase, BM biopsy and aspirate studies along with peripheral blood flow cytometry will be done on a yearly basis at the discretion of the PI.
j. At minimum, follow-up will be every 3-6 months plus or minus 6 months until progression of disease, institution of alternative therapy (if patients decide to receive lenalidomide to continue maintenance therapy from an outside institution, they are allowed to stay on study), or death. Patients may be followed at more frequent time intervals if clinically indicated, i.e. following post-therapy toxicity.
    i. Patients who finish all 32 cycles of treatment and who do not continue therapy outside of this study and who have not progressed will be followed at least every 6 months by clinic visit with laboratory evaluation and by bone marrow biopsy yearly at the discretion of the investigator. Of note, patients who do not progress, but decide to continue or resume therapy at an outside facility, may be followed by clinical and laboratory examination every 3-6 months. Alternatively, this data may be captured by investigator-patient or investigator-physician phone or email communication if the required examinations have been performed at the outside facility.
    ii. Patients who have progressive disease while on study will be followed with restaging scans and laboratory tests as clinically indicated. Patients who are taken off treatment or who have subsequently progressed may continue to be followed for survival by phone or clinic visit at the discretion of

the PI. In certain circumstances (at the discretion of the patient and PI), patients who are being followed by an outside oncologist and institution may continue to stay on study without a formal NIH visit, but by investigator-patient or investigator-physician phone or email communication to capture clinical and laboratory data. During the follow up phase, research blood/urine collection is optional and at the discretion of the PI.

k. Pharmacokinetic studies Cycle 1 Day 1 will be collected at the following time points: pre-dose, end of infusion, 5, 15, and 30 minutes and 1, 1.5, 2, 4, 6, and 24 hours post administration for carfilzomib.
l. At disease progression, marrow and FDG-PET/CT are optional.
m. Urine for protein electrophoresis (UPEP) and immunofixation to assess for monoclonal protein in the urine (Bence-Jones proteinuria) at baseline, day 1 of each cycle during cycles 1-8, day 1 of every third cycle during cycles 9-32, and at treatment termination. If patient achieves CR, a 24 hour UPEP will be performed to confirm CR status. In patients whose measurable disease is best determined by measuring Bence-Jones protein quantification, a 24 hour UPEP will be performed at baseline, day 1 of each cycle 1-8, and day 1 of every third cycle during extension phase I and II. Otherwise, 24-hr UPEP Bence-Jones quantification will be estimated from random UPEP specimens performed at baseline, day 1 of each cycle 1-8, and day 1 of every third cycle during extension phase.
n. FDG-PET scan will be performed on patients in the Molecular Imaging Program at baseline, during cycles 1-8 if patient achieves CR and/or at the end of cycle 8 if no CR is achieved, during cycles 9-20 (extended dosing phase I) if patient achieves CR and/or at the end of cycle 20 if no CR is achieved, and during cycles 21-32 (extended dosing phase II) if CR is achieved or at treatment termination. FDG-PET scan can be performed +/- 21 days of intended cycle day. During extension phase lenalidomide, FDG-PET scans are optional for those patients that are MRD negative at the end of cycle 8 regardless of response. During the follow up phase, PET scans will be done on a yearly basis at the discretion of the PI.
o. Bone marrow biopsy and aspirate will be performed on patients at baseline, during cycles 1-8 if patient achieves CR or at the end of cycle 8 if no CR is achieved during cycles 9-20 (extended dosing phase I) if patient achieves CR or at the end of cycle 20 if no CR is achieved, and during cycles 21-32 (extended dosing phase II) if CR is not achieved or at treatment termination. Bone marrow aspirate and biopsy can be performed +/- 21 days of intended cycle day. For patients who are on extended dosing with lenalidomide and are found to be negative by immunohistochemistry and flow cytometry of the bone marrow (MRD negative), clearance of abnormal protein in blood and/or urine does not mandate a repeated bone marrow and the results will be interpreted as complete response. However, a bone marrow will be repeated at the discretion of investigator.
p. Variations of +/- 3-14 days of scheduled visits are permitted. For patients who choose to undergo stem cell harvest, next cycle may be delayed for up to 5 weeks. In the follow up phase, patients may be allowed to "skip" appointment visits at the discretion of the PI.
q. Repeat myeloma tests at treatment termination.
r. Within 7 days prior to D1 of each cycle
s. May be delayed up to 7 days per PI discretion for clinical or logistical reasons
t. Every attempt should be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy. If subjects cannot return to clinic, this may be done by phone.
u. With amendment P, patients will be offered DW-MRI on an annual basis; these are optional. Diffusion weighted MRI (DW-MRI) will also be performed at the discretion of the PI. DW-MRI will be performed by standard techniques in the NCI Molecular Imaging Branch.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.5.1 Criteria for removal from protocol therapy

- Patients with medically concerning grade 3 or 4 adverse events related to drug therapy may be taken off therapy at the discretion of the principal investigator.
- Patients require more than 2 dose reductions of carfilzomib
- Toxicity has not resolved after 2 weeks of withholding treatment or up to 3 weeks for infection related treatment
- Grade 4 non-blistering rash or blistering rash of any grade
- Grade 4 neuropathy
- Grade 4 hypersensitivity reaction
- Patient completes the protocol as outlined in Section **3.2**
- Progression of disease
- Patient chooses to go off therapy
- The principal investigator may remove patient from protocol therapy if deemed necessary due to medical conditions, compliance, etc.
- Patient becomes pregnant.

3.5.2 Off-Study Criteria

- Patient requests to be withdrawn from study
- Death
- Physician's determination that withdrawal is in the patient's best interest.
- Non-protocol therapy for myeloma; however, patients who have not progressed but choose to continue or resume lenalidomide maintenance beyond 32 cycles at an outside facility may stay on study at the discretion of the investigator.

3.5.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 TUMOR LYSIS SYNDROME

4.1.1 Hydration and Fluid Monitoring:

- Oral hydration: All subjects must be well hydrated (i.e., volume replete). Begin oral hydration equal to approximately 30 mL/kg/day (~6–8 cups of liquid per day), starting 48 hours prior to the planned first dose of carfilzomib. Compliance must be reviewed with the subject and documented by the site personnel prior to initiating treatment with carfilzomib; treatment is to be delayed or withheld if oral hydration is not deemed to be satisfactory.

- IV hydration: 500 mL (250 mL before & 250 mL after carfilzomib) OR 1000 mL (500 mL before & 500 mL after carfilzomib) of normal saline or other appropriate IV fluid formulation must be given before *and* after each carfilzomib dose during Cycle 1 D1 and D2. Total volume will be determined at the discretion of clinician and volume status of patient. If lactate dehydrogenase (LDH) or uric acid is elevated at Cycle 2, Day 1, then the recommended IV hydration should be repeated for Cycle 2. The goal of the hydration program is to maintain robust urine output, (e.g., ≥ 2 L/day). Subjects should be monitored periodically during this period for evidence of fluid overload.
- In subjects considered to be still at risk for TLS at completion of Cycle 1, hydration should be continued into subsequent cycles if clinically indicated.

4.1.2 Laboratory Monitoring:

- Appropriate chemistries, including creatinine, and complete blood counts (CBC) with platelet count should be obtained and reviewed prior to carfilzomib dosing. Results of laboratory studies must be reviewed and deemed acceptable prior to administering the carfilzomib dose.
- Subjects with laboratory abnormalities consistent with lysis of tumor cells (e.g., serum creatinine ≥ 50% increase, LDH ≥ 2-fold increase, uric acid ≥ 50% increase, phosphate ≥ 50% increase, potassium ≥ 30% increase, calcium ≥ 20% decrease) prior to dosing should not receive the scheduled dose

4.1.3 Clinical Monitoring:

- Signs and symptoms indicative of TLS, such as fevers, chills/rigors, dyspnea, nausea, vomiting, muscle tetany, weakness, or cramping, seizures, and decreased urine output.
- Patients will be admitted to the inpatient hospital and observed while receiving Cycle 1 Days 1 and 2 of therapy.

4.1.4 Management:

- If TLS occurs, cardiac rhythm, fluid, and serial laboratory monitoring should be instituted. Correct electrolyte abnormalities, monitor renal function and fluid balance, and administer therapeutic and supportive care, including dialysis, as clinically indicated.
- All cases of TLS must be reported to Amgen as a Serious Adverse Event (SAE) through the normal process within 24 hours of the clinical site becoming aware of the event.

4.1.5 Optional medication for high risk TLS patients:

- Allopurinol is optional and will be prescribed at the Investigator's discretion. These subjects may receive allopurinol 300 mg PO BID (Cycle 1 Day -2, Day -1), continuing for 2 days after Cycle 1 Day 1 (total of 4 days), then reduce dose to 300 mg PO QD, continuing through Day 17 of Cycle 1. 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 Radiation Therapy

Subjects may receive radiation for treatment of uncontrolled pain, cord compression, vertebral instability/impending fracture, etc.

4.2.2 Kyphoplasty/Vertebroplasty

Subjects may receive kyphoplasty/vertebroplasty for symptomatic vertebral compression fractures.

4.2.3 Bisphosphonate Therapy

Approved bisphosphonate 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 HYPERCALCEMIA

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

4.4 TRANSFUSIONS/GROWTH FACTORS

- Subjects may receive RBC or platelet transfusions if clinically indicated.
- Subjects may receive supportive care with erythropoietin or darbopoetin.
- Colony-stimulating factors may be used if neutropenia occurs but should not be given prophylactically.

4.5 ANTI-COAGULATION

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.

4.6 HSV, VSV PROPHYLAXIS

Oral Valacyclovir of 500 mg daily or oral Acyclovir of 800 mg BID throughout all cycles in which carfilzomib is given.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE SAMPLES FOR RESEARCH/PHARMACOKINETIC STUDIES

NOTE: Effective with Amendment L (version date 07/15/2015), bone marrow aspirate and biopsy will be obtained for research studies at the discretion of the PI. Effective with Amendment N (version date 07/25/2017), samples for next generation sequencing (NGS) were added.

Correlate	Baseline	Day 2 Post 24 Hour Infusion	During Cycles 2-8 If CR achieved or End of Cycle 8	During Extended Dosing Phases (Phase I and II) if CR achieved during cycles 9-20/ End of cycle 20 and if CR achieved during cycles 21-32/End of Cycle 32/treatment termination	Progression of disease
Pathology/IHC	X		X	X	X
Multiparametric Flow Cytometry	X		X	X	X
FISH/Cytogenetics (optional)	X				X
Molecular pathology for light or heavy chain immunoglobulin rearrangement and/or KRas and NRas mutation analysis	X		X (optional)	X (optional)	X
CD138+ Sorting/GEP Profiling/Microenvironment	X	X	X	X	X
Flow cytometry for proteasomes	X	X	X (optional)	X (optional)	X
Flow cytometry for autophagy	X	X	X	X	X
Other research labs as below including Next Generation Sequencing (NGS)	(Added with Amendment N; subjects beyond this cycle)			X	X
Storage	X	X	X	X	X

5.1.1 Bone Marrow and Peripheral Blood

a. During extension phase lenalidomide, bone marrow biopsy and aspirates are optional for those patients that are MRD negative at the end of cycle 8 regardless of response.
b. Patients may be asked to undergo bone marrow at progression of disease at the discretion of the PI.
c. Collection of bone marrow, sorting of bone marrow and storage of bone marrow samples is

outlined in **Appendix B** and Section **5.2.1**.

d. Correlative 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 Section 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. Plasma cells and microenvironment interactions will also be assessed using various immunohistochemistry markers for osteoblasts, osteoclasts, stromal cells and proteasomes.
   ii. Minimal Residual Disease: 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. FISH and cytogenetics: Interphase FISH/cytogenetics will be performed on patients enrolled in this protocol under the direction of Diane Arthur of the Molecular Diagnostics Core Laboratory of the NCI Branch. (**NOTE:** D. Arthur no longer at NIH/NCI, her involvement ended and these studies no longer being done as of Amendment M.)
   iv. Myelomatous plasma cells will be studied at the single cell level by multiparametric flow cytometry for markers of autophagy, pre- and post-therapy, including but not limited to the technique described by S.T. Rosen *et al. J. Biol. Chem.* 284:26816-26830, 2009 under the direction of Jane Trepel.
   v. Flow cytometry for proteasomes under the direction of Irina Maric, MD.
   vi. Cell Sorting, GEP profiling, whole exome sequencing, microRNA analysis and microenvironment interactions - Marrow aspirate will be sent to the lab of Dr. Katherine Calvo (Building 10 Room 2C418A) in the Department of Laboratory Medicine and sorted into CD 138 + and CD 138 – fractions. Dr. Calvo and Dr. Wang will also perform the below assays for unsorted bone marrow aspirate for VDJ sequencing and assessment of MRD negativity.
      - GEP profiling targeted exome sequencing, and VDJ sequencing for MRD assessment: Bone marrow aspirates will be collected and sent to the Calvo Lab after 8 cycles of induction or CR, after 20 and 32 cycles of treatment and at annual follow-up milestones where BM aspirates are collected (if the samples were collected from the patients.) At baseline: CD138+ plasma cells will be purified from bone marrow aspirates harvested. Baseline CD138+ cells will be sent for VDJ sequencing and to Memorial Sloan Kettering (Dr. Ola Landgren) for targeted/whole exome sequencing and molecular expression analyses (depending on resources) in a coded, linked manner. At subsequent time points, once patient is in CR, plasma cells will not be purified and aspirates will be viably frozen. Samples will be sent out in batches. Of note, some samples

may be sent to FDA/OHOP for next generation sequencing, see below.

- Bone marrow, blood and urine samples, and associated clinical lab data will be sent to Adaptive Biotechnologies Corp. for deep sequencing of the VDJ sequence. Samples and data will be submitted to Adaptive Biotechnologies Corp. in a coded, linked manner.
- CD 138- fractions will be analyzed for microenvironment interactions such as cytokine profiling, miRNAs, etc. in the Calvo Lab. A fraction of CD138- cells will also be cultured to isolate bone marrow stromal cells for further analyses. At the same time points, one 6 ml EDTA tube of peripheral blood will be collected for parallel analysis.
- Both fractions will be collected, batched, and entered into a biobank. See Section **5.2.1** for storage of bone marrow biobank. As of Amendment N, aspirate samples will be stored in DLM under Dr. Calvo.
- Aspirate samples may also undergo identification of downstream signaling targets, proteasome activity, and ubiquitination pathways on cell lysate or marrow aspirate.

vii. Additional but optional molecular profiling including DNA-, RNA- and protein-based assays in bone marrow, blood, and urine samples will be performed. The purpose of these assays will be to further characterize underlying biology and correlate with clinical outcomes in an exploratory manner. These are not limited to, but include the following:

- Next Generation Sequencing (NGS) collaboration with Office of Hematology and Oncology Products (OHOP) FDA

This study will be included in the multi-protocol CCR collaborations with OHOP/FDA in terms of the translational and correlative aspects utilizing OHOP's "wet lab" to perform NGS assays on various human samples enrolled on a variety of CCR clinical trials. The transfer of samples will be performed under a CCR "umbrella" MTA. The samples will be locally biobanked in the BPC (Figg Lab) and batched at the NIH until time for shipment, at which time, the samples will be sent to:

ATTN: Elliot Rosen
DBRR III/OBP/OPQ/CDER
Food and Drug Administration
10903 New Hampshire Ave,
Bldg. 52/72, Room 2248,
Silver Spring, MD 20993
Tel: 240-402-7353
Email: Elliot.Rosen@fda.hhs.gov

The correlative NGS assays to be performed will be dependent on final agreement in investigating mutually important questions of interest with our collaborators. These include but are not limited to all or some of the following but are all optional:

1. T-cell receptor repertoire (immunoseq): More recently research in the literature has shown that patients most likely to benefit from immunotherapies are those who are found to have anti-tumor T cell clonality. Lenalidomide is an immunomodulatory and therefore it is important to analyze this along with immune subsets below in

SMM. NGS DNAseq will be used to analyze the VDJ sequence of T-cells to determine clonality. Input source may be PBMC or whole blood and approximately 2 ug of input gDNA will be needed which will amount to a whole blood collection of two 10 ml EDTA tubes. Collection time points include the following milestones, baseline, C8 or CR, 1 and 2 years, and during follow up phase.

2. Germline single nucleotide polymorphisms (SNP) and copy number variations (CNV) DNAseq: Whole exome or genome sequencing will be performed on peripheral blood to determine baseline germline SNPs and CNVs. Whole blood (one 10 mL EDTA tube) sample will be collected at baseline for germline DNA extraction. This DNA will be used to analyze and compare germline vs. somatic/tumor genetic alterations based on sequence data. Furthermore, germline normal polymorphic variation will be analyzed as genome-wide association study (GWAS) to determine whether certain normal variations predispose patients with treated SMM to progress to biochemical or overt symptomatic disease. Additionally, these germline variations may be analyzed for association with treatment related adverse events.
3. ctDNA: Targeted DNAseq will be performed on ctDNA for assessment of minimal residual disease (MRD). In addition to the VDJ targeted commercial assay to determine presence of the malignant clone, the library will also include other known recurrent genetic aberations. Approximately 45% of myeloma patients have hyperdiploidy of one of the odd numbered chromosomes, the other 45% have specific translocations/deletions, well characterized, including translocations of 6;14, 11;14, 4;14, 14;16, 16;18, 17p del, and cMYC. Therefore, the assay will focus on both VDJ as with other hematologic malignancies and recurrent genetic alterations similar to the approach used in solid malignancies. Blood collection will involve two 10 mL EDTA tubes.
4. Gene expression profiling: RNAseq will be performed on CD138+ myeloma cells and correlated with DNAseq results. Additionally, peripheral blood/PBMC will analyzed for immune related signatures. One 10 mL EDTA tube will be used for this purpose.

5.1.2 Research Blood/Serum and Urine

Blood:

a. 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.

   Typical time points include: baseline, Day 2, Day 8 and Day 15 of Cycle 1, Day 1 of every cycle during cycles 2-8, Day 1 of every third cycle during cycles 9 and beyond during extended dosing phases (I and II), during Cycles 1-8 if CR is achieved or at the end of cycle 8, during cycles 9-20 if CR is achieved or at the end of cycle 20, during cycles 21-32 if CR is achieved or at treatment termination, and at any time point if the patient has progression of disease.

   Samples may be collected within 2 days prior to each of the above time points during the induction phase; and within 7 days prior to each of the above time points during the extension phase I and II phase. This does not include the blood draws for

pharmacokinetic analysis as outlined below. Samples may also be collected every 3-6 months during the post-maintenance follow-up period.

b. The standard number of peripheral blood research tubes drawn for collection and storage at each of the above timepoints may include but are not limited to the following: one 8 mL serum separator tube (SST), one 10 mL sodium heparin tube (GGT), and one 10 mL EDTA lavender top tube for storage in the Blood Processing Core (BPC) in 10/5A09 (Figg Lab) for biobanking. Additionally, up to six 10 ml EDTA tubes may be collected for various experiments to be performed with FDA collaborators, see below and one 6 mL EDTA lavender top tube for analysis and storage in the Calvo Lab.

Urine:

a. 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 time points.

   Typical time points include: baseline, Day 2, Day 8 and Day 15 of Cycle 1, Day 1 of every cycle during cycles 2-8, Day 1 of every third cycle during cycles 9 and beyond during extended dosing phases (I and II), during Cycles 1-8 if CR is achieved or at the end of cycle 8, during cycles 9-20 if CR is achieved or at the end of cycle 20, during cycles 21-32 if CR is achieved or at treatment termination, and at any time point if the patient has progression of disease.

   Samples may be collected within 2 days prior to each of the above time points during the induction phase; and within 7 days prior to each of the above time points during the extension phase I and II phase. The amount of urine collected will be dictated by the number of experiments to be performed.

All Samples:

a. Collection and storage of peripheral blood and urine outlined in **Appendix C**. 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 e-mail Julie Barnes at Julie.barnes@nih.gov and Paula Carter pcartera@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

   For sample pickup, page 102-11964.

   For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

   For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.

b. Peripheral blood and/or urine samples from patients will be analyzed for potential serum or urine biomarkers as well as drug concentrations, and correlated to clinical outcomes if the results of the study indicate a clinical or translational rationale for analyzing the samples. Such biomarkers may include but are not limited to:
   i. Peripheral blood flow cytometry assessing for circulating plasma cells under the direction of Maryalice Stetler-Stevenson, MD.
   ii. Subunit profiling and activity of circulating proteasomes by enzyme-like

immunosorbent assay

iii. Apoptosis assays pre and post-carfilzomib to identify necrotic or late stage apoptotic cells
iv. Immunolocalization studies
v. Markers of bone turnover and disease activity
vi. Bone marrow, blood and urine samples, and associated clinical lab data will be sent to Adaptive Biotechnologies Corp for deep sequencing of the VDJ sequence.
vii. Peripheral blood will be assessed for immune cell populations including, but not limited to T cells (CD4 and CD8), LGL, and NK cells using flow cytometry under the direction of Irina Maric, MD

5.1.3 Pharmacokinetic Samples

a. PK Sampling Schedule: Samples will be collected at the following time points on C1D1: pre-dose, end of infusion, 5, 15, and 30 minutes and 1, 1.5, 2, 4, 6, and 24 hr post administration for carfilzomib. The plasma levels of lenalidomide will be collected on Cycles 2-8 D1 during induction phase and every 3 cycles during extended dosing with lenalidomide to determine steady-state lenalidomide levels.
b. Sample Requirements and Handling: A venous blood sample will be collected in a 6-ml sodium heparin (green top) tube (BD Sodium Heparin 367878 or 367879) at each time point. Samples should immediately be placed on wet ice and refrigerated. The date and **exact** time of each blood draw should be recorded on the sample tube and the PK sheet. Questions regarding the collection of these samples can be directed to Dr. Mark Roschewski. A 6.0 ml aliquot of whole blood will be spun to obtain the plasma, which will be kept frozen at -70°C until analysis. For sample pickup, page 102-11964.

   For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

   For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.

c. Sample Analysis: The determination of lenalidomide and carfilzomib concentrations in all plasma samples, as well as the pharmacokinetic data analysis, will be performed by the Blood Processing Core (BPC) (For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044). Samples will be analyzed using a validated LC-MS/MS method developed at the end of the trial.
d. Pharmacokinetic Analysis: Non-compartmental and compartmental pharmacokinetic and pharmacodynamic modeling of carfilzomib in plasma will be performed using WinNonlin 5.0 (Pharsight, Inc., Mountain View, CA). PK parameters derived from plasma concentration-time data may include C_{MAX}, T_{MAX}, AUC, CL, Vd, and $t_{1/2}$.

5.1.4 Imaging

5.1.4.1 FDG-PET/CT

5.1.4.1.1 Schedule

FDG-PET scan will be performed on patients at baseline, during cycles 1-8 if patient obtains CR or at the end of cycle 8 if no CR obtained, during cycles 9-20 if patient obtains CR or at the end

of cycle 20 if no CR obtained, during cycles 21-32 if patient obtains CR or at treatment termination. During extension phase lenalidomide, FDG-PET scans are optional for those patients that are MRD negative at the end of cycle 8 regardless of response. Patients who remain on study will have PET scans performed on an approximate annual basis during the post-maintenance follow-up phase. At PI discretion, patient may be asked to have an additional PET-CT at progression.

5.1.4.1.2 Procedures

Prior to 18F-FDG PET/CT imaging, the subject will have fasted and not received any sugar containing substance (i.e., glucose, sucrose, dextrose) for 4-6 hours. Subjects will be encouraged to drink water during this period to reduce radiation dose to the kidneys and will be asked to void prior to 18F-FDG injection.

Women of childbearing potential will have a documented report of negative pregnancy test from the CC or another accredited lab performed on the day of the scan or the day before the scan.

18F-FDG, [18F]-fluorodeoxyglucose is an FDA approved radiopharmaceutical. Immediately prior to injection, the subject's blood glucose level will be evaluated via finger stick. Non-diabetic subjects with fasting blood glucose levels above 150 mg/dl may be rescheduled at the discretion of the PI. Subjects will be asked to refrain from excessive physical exertion for the 24 hours prior to injection.

Patients will report to the NCI clinic on the day of the F-18 PET/CT scan and peripheral venous access will be obtained (most commonly via IV in the antecubital fossa). The 18F-FDG injection procedure will be injected and be followed by a ~20 ml saline (sodium chloride IV infusion 0.9% w/v) flush over a period of ~20 seconds. The injection site will be evaluated pre- and post-administration for any reaction (e.g., bleeding, hematoma, redness, or infection).

Whole body (vertex to toes) static PET/CT imaging will be performed beginning at approximately 1-hour post injection per PET/CT standard operating procedures. The patient will be instructed to maintain good hydration in order to reduce the radiation dose.

5.1.4.1.3 Research Radiation Exposure

The radiation dose from the procedure will be a maximum of 2.1 rem per year; this is within the RSC guidelines of 5.0 rem per year for adults.

5.1.4.2 DW-MRI

5.1.4.2.1 Schedule

We plan to obtain DW-MRI scans on patients on an annual basis through completion of follow up for comparison with PET/CT findings. We plan to obtain DW-MRI scans on current patients, for whom we may not have a baseline DW-MRI scan, at the same timepoints, as applicable.

5.1.4.2.2 Procedures

DW-MRI exploits differences in the diffusion of water in various tissues to internal physiology. The image contrast in reflects the difference in rate of diffusion between tissues. All attempts will be made to perform the DW-MRI scans on the same day as PET/CTs but this not mandatory. For DW-MRI, no external contrast will be used and fasting is not required. Standard clinical operating procedures will be used for image collection. DW-MRI evaluations will be considered purely exploratory.

5.1.4.2.3 Results

Patients will be informed of the results of the DW-MRI scans. But given the exploratory and research nature of the scans, these results will not be used for clinical decision-making purposes.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

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

- See **Appendix B**.
- Bone marrow biopsies will be submitted in native condition to the NIH Clinical Center Department of Laboratory Medicine 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 and stored in Calvo Lab/DLM).
- 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 NIH Clinical Center 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. Prior to Amendment N, all research samples were stored in the laboratory of the Lymphoid Malignancies branch. As of Amendment N, these samples will be stored in a temperature controlled, alarm secured nitrogen tank/deep freezer in the NIH Department of Laboratory Medicine. For information regarding these samples, please contact Dickran Kazandjian, M.D. at 240-383-6311 or Katherine Calvo, MD, PhD at 301-594-9578.
- 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.

5.2.2 Procedures for stored serum, peripheral blood, and urine specimens in the Blood Processing Core:

See **Appendix C** for processing of samples.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

- All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC. This is a secure program, with access to LABrador limited to defined BPC personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen. All BPC lab personnel with access to patient information complete the NIH online Protection of Human Subjects course.
- LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador 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.).
- Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services 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 LABrador. 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 BPC. 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). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.
- 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 LABrador. 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 variables.

5.2.3 Samples processing in the Department of Laboratory Medicine and NCI Laboratory of Pathology

- Biopsy, bone marrow or peripheral blood samples will be obtained from the Hematology Section (HS) of the NCI Laboratory of Pathology (LP) or the Hematology Section (HS)

of the DLM, CC.

- Samples are coded by the LP HS, DLM HS or Calvo Lab. Tissue sections, or peripheral blood samples will be obtained and stored in the Calvo lab without any personal identifiers and will be labeled with the anonymous LP HS or DLM HS accession ID number.
- The relationship between the accession ID number and the patient clinical information will be stored in a secure database that is maintained and regularly backed up by Dr. Katherine Calvo.
- The Calvo laboratory may prepare tissue lysates, RNA and DNA from each sample. Samples, lysates and derived biologic molecules will be stored in Eppendorf tubes marked with the sample accession ID number in locked -80° C freezers in the Calvo laboratory.

5.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.3.1 Description of the scope of genetic/genomic analysis

Genomic testing will be done as described in Section **5.1.1**.

5.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Confidentiality for genetic samples will be maintained as described in Section **5.2**. In addition, a Certificate of Confidentiality has been obtained for this study.

5.3.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet:

https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists).

Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory within the NIH or as a send-out test to another CLIA certified lab.

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.3.4 Genetic counseling

If the research findings are verified in the CLIA certified lab, the subject will be referred to a genetic healthcare provider within the NIH for the disclosure of the results. These activities will be funded by the Center for Cancer Research.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist

with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed from Study Day 1 through 30 days after the last dose of study drug or until return to baseline or stabilization of event, whichever is later, or until the subject comes off-study. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

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.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per the requirements in section **7.2**.

6.1.1 Long-term follow-up

When patients enter the long-term follow-up period, the following data will be collected:

- adverse events related to Carfilzomib
- survival status which will be collected by phone or clinic visit
- additional cancer therapy received

6.1.2 Record Keeping

Complete records must be maintained on each patient; these records will consist of the hospital chart as well as any other outside information obtained from outside laboratories, radiology reports, or physician's records. 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.

6.1.3 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, data will be entered into the study database and filed in the medical record. Additional blood or tissue samples/results drawn on patients enrolled in

this protocol between scheduled visits may be forwarded and entered into the database as indicated.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository. _X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_X_ Identified or coded, linked or identified data with approved outside collaborators under appropriate agreements. **How and where will the data be shared?**

Data will be shared through:

_X_ An NIH-funded or approved public repository. Insert name or names: ClinicalTrials.gov, dbGAP

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements.

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

6.3.1 Timing

Response assessments will be performed Day 1 of every cycle during Cycles 1-8 and every third cycle during Cycles 9-20: Cycles 9, 12, 15 and 18 and every third cycle during Cycles 21-32: Cycles 21, 24, 27, 30 and end of 32.

6.3.2 Disease Parameters

- Measurable is defined as any of the following: serum M-protein is ≥ 1 g/dL or "measurable" urine M-spike is ≥ 200 mg/24 hours or serum kappa or lambda FREE light chain of 10 mg/dL along with an abnormal kappa to lambda free light chain ratio.
- 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.
- Appearance of monoclonal or oligoclonal bands that are different from original isotype may not be defined as "relapse from CR". Oftentimes, such bands may indicate fluctuations in immunological parameters that are not reflective of MM disease. In these situations, immunofixation and electrophoresis will be interpreted by the clinician before being labeled as "relapse"(30).

6.3.3 Response Criteria from International Myeloma Working Group Criteria(31) and addition of nCR category(6, 21):

6.3.3.1 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
- **Near Complete Response (nCR)**
  Defined as absence of myeloma protein on electrophoresis, independent of immunofixation status(6, 21)
- **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 unmeasurable, 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 unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria
- **Stable Disease (SD)**
  Not meeting criteria for CR, VGPR, PR 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 lowest response value in the following on 2 consecutive measurements:
  - Serum M-component and/or (the 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 and/or (the absolute increase must be ≥200mg/24h
  - Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels. The absolute increase must be >10mg/dl.
  - Bone marrow plasma cell percentage: the absolute % 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 that can be attributed solely to the plasma cell proliferative disorder

- **Relapse from CR**
  Any one or more of the following:
  - Reappearance of serum or urine M-protein by immunofixation or electrophoresis. (Appearance of monoclonal or oligoclonal bands that are different from original isotype may not be defined as "relapse from CR". Oftentimes, such bands may indicate fluctuations in immunological parameters that are not reflective of MM disease. In these situations, immunofixation and electrophoresis will be interpreted by the clinician before being labeled as "relapse".(32, 33))
  - Development of ≥ 5% plasma cells in the bone marrow
  - Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, hypercalcemia

6.3.4 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.

6.3.5 Progression-Free Survival

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

6.3.6 Overall Survival

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

6.3.7 Overall response rates after 8 cycles

Overall response rates (ORR) is PR+VGPR+CR after 8 cycles of therapy.

6.4 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).

6.4.1 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 *is 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 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet once a week 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. Events meeting requirements for expedited reporting as described in section **7.2** will be submitted within the appropriate timelines. .

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.

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8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

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 event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- 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.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of

either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.

- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

As death due to disease progression is part of the study objectives, and captured as an endpoint in this study, it will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section **8.3**.

8.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

All events listed below must be reported in the defined timelines to CCRsafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

8.5.1 SAE Reporting to Amgen

All SAEs, except for TLS, occurring after the subject has signed the informed consent form (ICF) until 30 days after the last dose of any study treatment must be fully documented and reported to Amgen Global Safety as soon as possible but no later than 7 calendar days of initial receipt of the information. **All cases of TLS must be reported to Amgen as a Serious Adverse Event (SAE) through the normal process within 24 hours of the clinical site becoming aware of the event**

The SAE report forms and the SAE Supplemental Form or MedWatch Form 3500a should be faxed/emailed together to Amgen Global Safety.

FAX: 888-814-8653 (toll-free, US only)
Toll-free Number: 888-814-8653
Email: svc-ags-in-us@amgen.com

8.5.2 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-PI-0663) 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

8.5.3 E-mail: drugsafety@celgene.com Reporting Pregnancy to Amgen

If a subject or spouse or partner of a subject becomes pregnant while enrolled in this clinical trial or up to three months following administration of carfilzomib, Amgen Drug Safety must be notified within 24 hours of the Investigator, designee, or site personnel learning of the pregnancy (See Amgen Drug Safety and Pharmacovigilance Contact information above). If the subject is pregnant, carfilzomib must be withheld.

Subjects, spouses, or partners will be followed through the outcome of the pregnancy. The Investigator will be required to report the results to Amgen Drug Safety.

If the outcome of the pregnancy meets a criterion for immediate classification as an SAE—spontaneous abortion (any congenital anomaly detected in an aborted fetus is to be documented), stillbirth, neonatal death, or congenital anomaly—the Investigator should repeat the procedures for expedited reporting of SAEs as outlined above.

8.5.4 Reporting Pregnancy to Celgene

Pregnancy of a female subject or the female partner of a male subject occurring while the subject is on lenalidomide or within 4 weeks after the subject's last dose of lenalidomide are considered expedited reportable events. If the subject is on lenalidomide, it is to be discontinued immediately and the subject is to be instructed to return any unused portion of lenalidomide to the Investigator. The pregnancy must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the pregnancy by phone and facsimile using the SAE Form.

The Investigator will follow the pregnant female until completion of the pregnancy, and must notify Celgene Drug Safety of the outcome as specified below. The Investigator will provide this information as a follow-up to the initial SAE.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for Expedited Reporting of SAEs to Celgene (i.e., report the event to Celgene Drug Safety by facsimile within 24 hours of the Investigator's knowledge of the event).

Any suspected fetal exposure to lenalidomide must be reported to Celgene within 24 hours of

being made aware of the event. The pregnant female should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling.

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the *in utero* exposure to lenalidomide should also be reported.

In the case of a live "normal" birth, Celgene Drug Safety should be advised as soon as the information is available

8.6 REPORTING PREGNANCY TO CCR

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 28 days after the last dose of study therapy.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 28 days after the last dose should, if possible, be followed up and documented.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL NON-ADHERENCE REPORTING

Protocol non-adherence is defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the

study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol non-adherence identified by the Staff or the site Monitor on the OSRO Site Protocol Non-Adherence Log. The protocol-specific, cumulative non-adherence log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month over the duration of the study**. In addition, any non-adherence to the protocol should be documented in the participant's source records and reported to the local IRB per their guidelines. OSRO required protocol non-adherence reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 STUDY DESIGN/PRIMARY ENDPOINTS

This is a single arm phase II study designed to evaluate efficacy of combinational therapy of carfilzomib, dexamethasone, and lenalidomide in newly diagnosed untreated MM patients. The primary objectives of this study are to determine if the combination of carfilzomib, lenalidomide and dexamethasone has acceptable toxicity in patients with newly diagnosed MM.

10.2 SAMPLE SIZE DETERMINATION

The sample size on which the trial will be based will be that necessary to demonstrate if the rate of grade 3 or worse neurologic toxicity is lower than 10%, or if it could be consistent with a rate which is greater than 10%, in which case it would be considered excessive.

We will plan to enroll a total of 45 evaluable patients in a single cohort and score each patient for the development of grade 3 or worse neurologic toxicity in the first two completed cycles of treatment. If we enroll 45 patients and if 5 or more have grade 3 or worse neurologic toxicity,

then the probability that the true rate of grade 3+ neurologic toxicity is 5% is 7.3% while the probability that the true rate of grade 3+ neurologic toxicity is 15% is 82.5%. Thus, the appearance of 5 or more patients with grade 3+ neurologic toxicity in 45 patients will provide evidence that the true rate of toxicity is consistent with 15%, and will be considered excessive.

10.3 STATISTICAL ANALYSES

10.3.1 Analysis of the Primary Endpoints
The 90% and 95% confidence intervals will be constructed around the observed fraction of patients with grade 3+ neurologic toxicity, and will be reported along with the observed incidence. If 5/45 are noted to have grade 3+ neurologic toxicity, the exact two-sided 90% CI bound extends from 4.5% to 22.0%, thus confirming that this rate of toxicity is likely to exceed 5%, and is consistent with as high as 22%.

As an early stopping rule for toxicity for the first 20 patients: if 4 patients are found to have grade 3+ neurologic toxicity in the first two completed cycles of treatment, no further patients will be enrolled. As soon as this can be determined, since the lower 80% one-sided confidence bound around 4/20 is 11.7%, which, being greater than 10%, would be considered demonstration of excessive toxicity. In fact, the lower 80% bounds range from 67% for observing 4 patients with grade 3+ neurologic toxicity in the first 4 patients to 12% for observing 4 patients with grade 3+ neurologic toxicity in 20 patients.

The rates of VGPR+CR and CR alone will also be estimated and reported. These rates are expected to be fairly high. Two-sided 95% confidence interval bounds will be formed around each of these measures.

In order to allow for a small number of inevaluable patients, the accrual ceiling will be set at 50.

It is anticipated that 2-3 patients per month may enroll on this trial; thus, 2 years is anticipated as the accrual period for this study.

10.3.2 Analysis of the Secondary Endpoints
Secondary endpoints are to estimate overall response rates (ORR) according to International Myeloma Working Group criteria after 8 cycles(31), progression free survival (PFS), duration of response and overall survival. Progression free survival, duration of response and overall survival will be estimated using the Kaplan-Meier method and reported along with 95% confidence intervals at appropriate time points.

A number of correlative studies will be performed in order to assess carfilzomib in vitro biological activity and investigate minimal residual disease in MM. Minimal residual disease will be further explored using multi-parametric flow cytometry and PET-CT imaging.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

11.1.1 Cooperative Research and Development Agreement (CRADA)
The following CRADAs are associated with this protocol: Amgen, Inc. (formerly directly with Onyx Pharmaceuticals), Inc. (#02695) for Carfilzomib; Celgene Corporation (#02696) for Lenalidomide.

11.1.2 Material Transfer Agreements (MTA)

11.1.2.1 Memorial Sloan Kettering Cancer Center (MSKCC)
An MTA was executed before any of the samples described in Section **5** were shipped to Ola Landgren, MD, PhD at Memorial Sloan Kettering Cancer Center (MSKCC #37998). Dr. Landgren will use samples 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.

Dr. Landgren is a former NIH staff member and PI/AI on this study whose ongoing role was originally intended to be covered by an NIH FWA Coverage Agreement to continue work on this study. In reviewing the samples sent to Dr. Landgren, it has been determined that samples received by him were coded in such a manner that it would not be possible for him to identify the subjects from which the samples were obtained. Dr. Landgren also does not have any access to the code key to identify the subjects. Therefore, with Amendment P, Dr. Landgren's involvement on study has been updated to a Non-NIH Collaborator as he does not have access to patient identifiers.

11.1.2.2 Food and Drug Administration (FDA)
An MTA will be executed to allow the samples described in Section **5** to be shipped to the Office of Biotechnology at the FDA as part of the collaborative effort. The MTA will be a global CCR MTA (#43656) to include multiple protocols; however, Dr. Kazandjian will be the responsible PI regarding the multiple myeloma protocols both at the NIH and FDA site.

12 HUMAN SUBJECTS PROTECTIONS

12.1 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.(1) Incidence rates of myeloma is 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.(1) 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.

12.2 PARTICIPATION OF CHILDREN

Pediatric patients with SMM 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

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.4**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 RISKS/BENEFITS ANALYSIS

Currently, MM is an incurable malignancy with frequent complications of skeletal fractures, anemia, renal failure and hypercalcemia. Combination use of novel agents, such as proteasome inhibitors, have significantly prolonged survival in this disease population. However, toxicity from bortezomib has impacted patients with frequent episodes of debilitating neuropathy. Carfilzomib, a new-generation proteasome inhibitor, has been tested in phase 1 trials in combination with lenalidomide and dexamethasone.

Such combinations seem to be well-tolerated with minimal toxicity and significant benefit. The most recent phase 1 trial in newly diagnosed MM patients has shown 100% PR or better in 19 patients with a median of 4 months administered. More importantly, this treatment protocol takes a unique approach to investigating minimal residual disease in MM by applying novel techniques such as multiparametric flow cytometry and FDG PET CT as it relates to traditional IMWG response criteria.

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

Adults who become unable to consent are allowed to remain on study because the protocol offers a prospect of direct benefit. The ongoing risks and benefits of participation for adults who become unable to consent are no different than those described for less vulnerable patients.

12.5 CONSENT PROCESS AND DOCUMENTATION

Informed consent will be obtained from all patients on this trial. There will be no minors enrolled < 18 years of age; therefore, assent is unnecessary. The informed consent 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.

12.5.1 Telephone Re-consent
Telephone consent applies to instances of re-consent only. Telephone re-consent will be obtained and documented per OHSRP/IRBO and CCR policies and procedures.

13 PHARMACEUTICAL INFORMATION

13.1 CARFILZOMIB (IND # 112587)

13.1.1 Source
Carfilzomib was provided to investigator by Onyx Pharmaceuticals, Inc. under a CRADA. Onyx provided carfilzomib during the active recruitment and treatment of this study. However, ongoing communication and sharing of information, including updated risks for carfilzomib, is through an agreement with Amgen.

13.1.2 Toxicity
A comprehensive listing of all toxicities (i.e., very common, common, etc.) are listed in the informed consent document. The approved USPI may also be referenced for current information related to this agent.

Amendment P, new information from Amgen, Inc: As of July 2019, carfilzomib has been examined in approximately 11,000 people in a research setting. It has been found that carfilzomib can cause a reactivation of hepatitis B virus. Additionally, there have been 4 cases of Progressive Multifocal Leukoencephalopathy (PML) possibly linked to Carfilzomib

13.1.3 Formulation and Preparation
Carfilzomib for Injection will be provided as a lyophilized powder which, when reconstituted, contains 2 mg/mL isotonic solution of carfilzomib Free Base in 10 mM sodium citrate buffer (pH 3.5) containing 10% (w/v) sulfobutylether-β-cyclodextrin (SBE-β-CD, Captisol®). Lyophilized Carfilzomib for Injection is stored in a refrigerator at 2°C–8°C. Water for injection is the only acceptable solution for reconstitution. After addition of the appropriate amount of water for injection and vigorous mixing, the solution is administered as an IV infusion. Vials are for single use. A volume of carfilzomib appropriate for a patient's dose will be added to 5% Dextrose Injection (D5W) in a sufficient amount to yield a volume to administer 100 mL (qs. 100 mL) in a polyvinyl chloride or polyolefin container.
For clinical use, Carfilzomib products will contain excess drug-containing fluid to compensate for product container and administration set priming volumes.
Before dispensing Carfilzomib products from the Pharmacy, an administration set suitable for a portable pump (e.g., Gemstar set 13758-28) will be attached, the administration set tubing will be primed with drug-containing fluid, air will be purged from the tubing (but not the product container), and the administration set will be capped with a Luer locking cap.

Lyophilized Drug Product: Lyophilized Carfilzomib for Injection is an investigational therapeutic agent provided in a single-dose vial as a sterile, lyophilized powder in the following dosages:

- 25 mg Single-Use Glass Vial / 8 pk Carton
  Each single-dose vial contains 25 mg of carfilzomib in a 20 mL labeled glass vial with an elastomeric stopper and Red flip-off lid. The product is supplied to the site in labeled

carton(s) containing eight (8) single-use vials/carton and is shipped and stored between 2°C - 8°C (36°F - 46°F). Remove the Red flip-off lid on the vial and aseptically add 12 mL of Water for Injection, USP to the lyophilized drug. Gently invert the vial multiple times and let stand to yield a clear solution containing 2 mg/mL carfilzomib. After reconstitution as instructed, a maximum total of 12.5 mL deliverable volume containing 25 mg of carfilzomib can be withdrawn from the vial.

- 45 mg Single-Use Glass Vial / 6 pk Carton
  Each single-dose vial provides 45 mg of carfilzomib in a 30 mL labeled glass vial with an elastomeric stopper and Yellow flip-off lid. The product is supplied to the site in labeled carton(s) containing six (6) single-use vials/carton and is shipped and stored between 2°C - 8°C (36°F - 46°F). Remove the Yellow flip-off lid on the vial and aseptically add 22 mL of Water for Injection, USP to the lyophilized drug. Gently invert the vial multiple times and let stand to yield a clear solution containing 2 mg/mL carfilzomib. After reconstitution as instructed, a maximum total of 22.5 mL deliverable volume containing 45 mg of carfilzomib can be withdrawn from the vial.
- 60 mg Single-Use Glass Vial / 4 pk Carton
  Each single-dose vial provides 60 mg of carfilzomib in a 50 mL labeled glass vial with an elastomeric stopper and Blue flip-off lid. The product is supplied in labeled carton(s) containing four (4) single-use vials/carton and is shipped and stored between 2°C - 8°C (36°F - 46°F). Remove the Blue flip-off lid on the vial and aseptically add 29 mL of Water for Injection, USP to the lyophilized drug. Gently invert the vial multiple times and let stand to yield a clear solution containing 2 mg/mL carfilzomib. After reconstitution as instructed, a maximum total of 30 mL deliverable volume containing 60 mg of carfilzomib can be withdrawn from the vial.

Inspection: The reconstituted drug solution in the vial should be a clear liquid. Inspect all vials for the presence of any suspended particles, particulate matter, discoloration or hazy solution prior to administration.

If the solution is not clear or particles exist in inspected vials, record the observation in the appropriate Drug Accountability Log and notify Amgen immediately.

- DO NOT USE THE DRUG.
- Place the vial(s) into a plastic bag labeled as "Quarantined" with the date.
- Store labeled quarantined drug in a temperature-monitored refrigerator and ensure they are physically segregated from the drug that is available for use.
- Amgen will instruct the clinical site on how to proceed with quarantined vial(s).
  Calculation of Dose: Each dose will consist of Carfilzomib for Injection administered on a mg/m2 basis, and should be based on the patient's actual calculated body surface area (BSA).

The BSA should be calculated based upon the institution's practice and method of calculation should remain consistent throughout a subject's participation in the trial.

Subjects with a BSA > 2.2 m2 will receive a dose based upon a 2.2 m2 BSA.

Dose adjustments do not need to be made for weight gains/losses of ≤ 20%.

13.1.4 Stability and Storage

Lyophilized Drug Product: Lyophilized Carfilzomib for Injection must be kept in the labeled

drug cartons and stored at 2°C - 8°C (36°F - 46°F) in a refrigerator.

If procedures permit, the refrigerator should be continuously monitored and temperature records retained for review.

The refrigerator should also be on a backup generator and alarmed for temperature deviations if available. Lyophilized Carfilzomib for Injection exposed at any time to temperatures exceeding 30°C / 86°F must be discarded

Reconstituted Drug Product: Once a drug vial is reconstituted and inspected, the clear solution can be stored in a refrigerator (recommended) controlled from 2°C - 8°C (36°F - 46°F) or at room temperature from 15°C - 30°C (59°F - 86°F) until use. Once reconstituted, Carfilzomib for Injection must be used on the day of reconstitution or else it must be destroyed. Prior to administration, all reconstituted drug should be equilibrated to room temperature. DO NOT FREEZE LYOPHILIZED OR RECONSTITUTED DRUG.

Diluted Drug Product: After dilution with D5W for clinical use, Carfilzomib should be stored under refrigeration.

13.1.5 Administration Procedures
Carfilzomib will be administered by intravenous infusion over 30-minutes through a peripheral or central venous access device via portable (ambulatory) pump. Care should be taken in placing and maintaining the product container at a level physically higher than the pump to avoid advancing air into the administration set tubing.

If the patient has a dedicated line for carfilzomib administration, the line must be flushed with a minimum of 20 mL of normal saline prior to and after drug administration.

13.1.6 Incompatibilities
In an in vitro study using human liver microsomes, carfilzomib showed modest direct and time-dependent inhibitory effect on human cytochrome CYP3A4/5. Given that the clearance of carfilzomib likely occurs extrahepatically via the activity of epoxide hydrolase and peptidase activities, the clinical relevance of these in vitro results is not clear. No clinically significant drug interactions have been noted to date in patients receiving a variety of agents metabolized by CYP3A4. Moreover, no dose adjustments have been required for any concomitant medication in patients receiving carfilzomib. However, caution should be exercised in administration of concomitant medications which are substrates of human CYP3A4.

13.2 LENALIDOMIDE

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

13.2.2 Toxicity
A comprehensive listing of all toxicities (i.e., very common, common, etc.) are listed in the informed consent document. The approved USPI may also be referenced for current information related to this agent. See below information regarding most significant toxicities:

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

13.2.3 Formulation and Preparation

Lenalidomide will be supplied as capsules for oral administration. Celgene Inc. will provide lenalidomide 5, 10, 15 and 25 mg capsules for the Induction Phase of the protocol and for the extended dosing phase(s).

13.2.4 Stability and Storage

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

13.2.5 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; no more than a one-month supply of lenalidomide may be dispensed at one time.

13.2.6 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

13.3 DEXAMETHASONE

13.3.1 Source

Dexamethasone will be provided from commercial sources by the NIH Clinical Center Pharmacy Department.

13.3.2 Toxicity

Common:

- Cardiovascular: Hypertension
- Dermatologic: Atrophic condition of skin, Finding of skin healing, Impaired
- Endocrine metabolic: Cushing's syndrome, Decreased body growth
- Gastrointestinal: Disorders of gastrointestinal tract
- Immunologic: At risk for infection
- Musculoskeletal: Osteoporosis
- Ophthalmic: Cataract (5%), Raised intraocular pressure (25%)
- Psychiatric: Depression, Euphoria
- Respiratory: Pulmonary tuberculosis

Serious:

- Endocrine metabolic: Hyperglycemia, Primary adrenocortical insufficiency
- Ophthalmic: Conjunctival hemorrhage (22%), Glaucoma, Vitreous detachment (2%)

13.3.3 Formulation and Preparation

- Oral Tablet (Scored): 4 mg
- Injection, solution, as sodium phosphate: 4 mg/mL (1 mL, 5 mL, 30 mL)

13.3.4 Administration Procedures

- Oral: Administer with meals to decrease GI upset.
- I.V.: Administer intravenously over 10 minutes.

13.3.5 Incompatibilities

- Contraindicated: Praziquantel (theoretical), Rotavirus Vaccine, Live (established)
- Major: Aldesleukin (theoretical), Bupropion (theoretical), Darunavir (theoretical), Dasatinib (theoretical), Etravirine (theoretical), Fosamprenavir (theoretical), Imatinib (theoretical), Ixabepilone (theoretical), Lapatinib (theoretical), Nilotinib (theoretical), Quetiapine (probable), Romidepsin (theoretical), Sunitinib (theoretical), Temsirolimus (theoretical), Thalidomide (probable)

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15 APPENDICES

15.1 APPENDIX A: REQUIREMENTS FOR REMS®

Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods

Requirements for REMS®

- Patients should be instructed never to give lenalidomide to another person.
- 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).
- Female patients should not donate blood during therapy and for at least 28 days following discontinuation of lenalidomide.
- Male patients should not donate blood, semen or sperm during therapy or 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. Monthly phone counseling is required per the REMS® program in order to prescribe a one-month supply of lenalidomide.
- All patients will be required to sign the REVLIMID, Patient-Physician Agreement Form.
- Males 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. See below for further details
- Females of childbearing potential 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. See below for further details.

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

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, 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 method 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.

15.2 APPENDIX B: BONE MARROW ASPIRATE COLLECTION, SORTING AND STORAGE

NOTE: Refer to Section **5.1** for correlative study bone marrow timepoints and study calendar Section **3.4**

Collection of Bone Marrow:

- 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 Hematology lab technician to be given to Hematology Section, Department of Lab Medicine (1 mL will go to Hematopathology and 1 mL (optionally) 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.
- After processing in the pathology department, clot sections will be sent to the Molecular Diagnostics Core Laboratory, LP, NCI under the direction of Mark Raffeld, MD for determination immunoglobulin heavy and/or light chain rearrangement, and KRAS/NRAS mutations.
- 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). Aspirate from marrows at baseline, end of cycle 8/or CR reached between cycles 1-8, end of cycle 20/or CR reached between cycles 9-20 and during treatment termination/or CR reached between cycles 21 and beyond will be sent for plasma cell flow cytometry immunophenotyping at Maryalice Stetler-Stevenson's lab.
- For aspirate specified for flow cytometry under the direction of Jane Trepel, immediately discharge 1 mL of aspirated marrow syringe into heparin syringe tube.
- For aspirate designated for CD138 sorting, VDJ sequencing, exome sequencing, and microenvironment studies, send remaining aspirate sample Calvo Laboratory in the Department of Laboratory Medicine Clinical Center (Attn: Dr. Weixin Wang, Building 10 Room 2C418A). Place aspirate sample in EDTA syringe immediately on ice. Transfer within 30 minutes of sampling to the lab for processing. CD138+ plasma cells will be viably frozen and batched to be sent to Adaptive Technologies for VDJ

sequencing and to MSKCC for exome sequencing. CD138- fractions will be studied by Dr.'s Wang and Calvo. At subsequent time points, CD138+ sorting will not be done if the patient is in remission.

- For aspirate specified for cytogenetics/FISH, aspirate will be sent to Diane Arthur. (**NOTE:** D. Arthur no longer at NIH/NCI, her involvement ended and these studies no longer being done as of Amendment M.)

15.3 APPENDIX C: RESEARCH PERIPHERAL BLOOD AND URINE COLLECTION AND STORAGE

NOTE: Refer to Sections **3.4** Study Calendar and **5.1** for research blood and urine collection time points for samples to the Blood Processing Core (BPC).

Venipuncture:

a. Up to 100 mL of peripheral blood will be collected into serum separator tubes (SST), sodium heparin tubes (GGT), 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.
b. Serum
    - Collect 7-10 mL blood in a serum separator tube (SST).
    - Allow the blood to clot by standing at room temperature for 30 minutes.
    - Separate serum from cells by centrifuging at 4 degrees C for 5 minutes at 1200xg.
    - Pipette 2 aliquots of 1.5mLs each into two 2mL cryovials.
    - Freeze immediately at -20 or lower
    - Maintain in -80 freezer for storage until shipment
c. Plasma
    - Collect 7 mL blood in a sodium heparin tube (green top).
    - Place immediately on wet ice and refrigerate until time of processing.
    - Separate plasma from cells by centrifuging at 4 degrees C for 5 minutes at 1200xg.
    - Pipette 2 aliquots of 1.5mLs each into two 2mL cryovials.
    - Freeze and store in -80C freezer.
d. 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.

Urine Sample Collection:

a. 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.
b. Transfer to a screw-cap conical tube
c. Freeze immediately at -20 or lower
d. Maintain in -80 freezer for storage until shipment

Labeling of Samples:

a. 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

b. Do not include the patient name, medical record number, or initials.