Protocol Title: A phase I/II trial of very low to low-doses of continuous azacitidine in combination with standard doses of lenalidomide and low-dose dexamethasone in patients with relapsed or refractory multiple myeloma

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PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator:		
	Signature of Investigator	Date
	Frederic J. Reu, M.D.	
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, instructions from Celgene representatives, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

STUDY PERSONNEL

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1 Protocol Synopsis

PROTOCOL TITLE: A phase I/II trial of very low to low-doses of continuous azacitidine in combination with standard doses of lenalidomide and low-dose dexamethasone in patients with relapsed or refractory multiple myeloma	
DATE PROTOCOL FINAL:	
INDICATION:	Relapsed or refractory multiple myeloma
STUDY PHASE:	Phase I/II
BACKGROUND AND RATIONALE: The best currently available treatment for patients with relapsed or refractory multiple myeloma uses regimens that include novel agents like lenalidomide, thalidomide, and bortezomib but still yield unsatisfactory long term tumor control with median remission duration not more than one year and median survivals ranging between 1 and 3 years in most studies (1). As myeloma progresses, clones emerge that have more tumor suppressor genes silenced by DNA methylation which may lead to resistance towards used therapies, escape from immune attack, and independence from the bone marrow microenvironment (2-6). In contrast to mutations such epigenetic changes can be corrected with aza nucleoside analogues like azacitidine, which inhibits the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT) (7-9) and has been shown to improve survival in MDS and WHO AML compared to conventional therapy (10). One of the most active current regimens for relapsed/refractory myeloma uses lenalidomide in combination with dexamethasone yielding a response duration of over 11 months and an overall survival of about 30 months (11). In the upfront therapy setting, low dose dexamethasone in combination with lenalidomide appears to yield at least equivalent survival rates but cause less toxicity than conventional dose dexamethasone (12). This regimen (lenalidomide 25mg day 1-21, every 28 days with dexamethasone 40mg weekly) has rapidly been adapted as one of the standard approaches to newly diagnosed and relapsed myeloma patients. By combining this regimen with azacitidine, resistant myeloma clones, which continue to synthesize DNA despite therapy, may become sensitive through reactivation of silenced tumor suppressor genes leading to a deeper and longer response. In vitro, non-toxic single low doses of azacitidine (1μM) can reactivate tumor suppressor genes in myeloma cell lines for 3-7 days (unpublished observations). Doses higher than 2-5μM, depending on the cell type, can exert immediate toxicity likely related to nucleoside analogue properties of the drug. Pharmacokinetic data in humans suggest that a single dose of 50mg/m^2 subcutaneously reaches concentrations that should be sufficient for DNA demethylation but could cause classical nucleoside analogue toxicity in some patients (2.89μM +/- 1.35 μM SD), higher doses like 75mg/m2 reach 4.88 μM +/- 1.4μM SD and thus almost certainly exert classic nucleoside analogue toxic effects (13). In this study we aim to avoid pronounced nucleoside analogue side effects while maximally exploiting DNA demethylating effects of azacitidine. We will therefore assess the safety of combination therapy with azacitidine given at low but increasing doses up to 50mg/m^2	

twice a week, together with standard dose lenalidomide and low dose dexamethasone in relapsed or refractory myeloma patients in a phase I/II study with 10 additional patients enrolled at the highest tolerated low azacitidine dose to obtain an initial impression of efficacy and to evaluate whether this dose exerts the expected pharmacodynamic effects in myeloma cells before a larger phase II trial may be conducted. In light of new data supporting use of standard-dose azacitidine in myeloid neoplasia patients with kidney dysfunction(14) and GFR-adjusted lenalidomide for myeloma patients(15), amendment 7 extends the safety and preliminary efficacy evaluations to myeloma patients with GFR 30-59 ml/min (Cockroft-Gault). Lenalidomide will be used at a fixed dose of 10mg daily from day 1-21 every 28 days which yielded equal AUC concentrations in myeloma patients with GFR 30-49m/min as 25mg daily from day 1-21 every 28 days in patients with GFR $\geq$ 80 ml/min in a recent study(15). Only two dose levels of azacitidine will be tested. Dose level "CKD 1" will use azacitidine at 40 mg/m^2 s.c. twice a week which has been found tolerable in patients with GFR $\geq$ 60 ml/min; one of six patients developed a dose limiting toxicity, culture negative neutropenic fever at an absolute neutrophil count (ANC) nadir of 0.48K/mm^3,.with resolution the next day at an ANC of 0.89K/mm^3 without G-CSF use. At the time of amendment 7, two patients with normal kidney function have completed the first cycle at dose level 5 (azacitidine 50mg/m^2 s.c. twice a week) without dose limiting toxicities. Six patients with GFR 30-59 ml/min will be treated at CKD 1 if dose limiting toxicities (DLTs) do not occur in more than one patient. If 2 of 3 or 2 of up to six patients develop DLTs at CKD1, subsequent patients will be treated at "CKD -1" (30mg/m^2 s.c. twice a week). Otherwise (no more than one patient with DLTs at CKD1), subsequent patients will receive azacitidine at 50mg/m^2 s.c. twice a week (= dose level CKD2). Lower or higher dose levels will not be tested. This modified dose escalation scheme increases safety by enrolling at least 6 patients per dose level and guarantees that the tolerability of twice a week azacitidine in patients with GFR 30-59 ml/min will be defined by enrolling maximally 12 additional patients. Omission of weekly azacitidine is based on the observation that clinical benefit response rates were more than twice as high with twice a week than with weekly azacitidine dosing (40% versus less than 15%) in patients with GFR > 60ml/min, an encouraging result taking into consideration that patients had received a median of 4.5 prior regimens, with myeloma refractory to previous lenalidomide in 88%, to previous protesome inhibitors in 81%, and to both lenalidomide and proteasome inhibitors in 75%. Once the highest tolerated low dose for patients with reduced GFR (HTLD-CKD) has been defined, enrollment at this dose level may contribute to the 10 patient phase II extension. Before amendment 7 eighteen patients have started treatment, including two at dose level 5. Assuming up to 6 patients will be enrolled per dose level; the maximal number of enrolled patients will therefore increase from previously estimated 40 to 44 after amendment 7.

STUDY OBJECTIVES:

Primary:

- Define the highest tolerated low doses (HTLD and HTLD-CKD for GFR > 60 ml/min and 30-59ml/min, respectively) and safety of azacitidine given at low but increasing doses up to 50mg/m^2 twice a week concurrently with GFR-adjusted lenalidomide doses and low dose dexamethasone in patients with relapsed or refractory multiple myeloma.

Secondary:

- Response according to international response criteria (≥PR) and clinical benefit response (≥minor response according to adapted EBMT criteria), see under 7
- Correlate response with plasma activity of the azacitidine inactivating enzyme cytidine deaminase (CDA)
- Progression-free survival and overall survival
- Peripheral blood hematopoietic progenitor (CD34+) yield and time to neutrophil and platelet recovery in patients undergoing autologous stem cell transplantation
- Promoter demethylation and gene reactivation in myeloma cells and hematopoietic progenitors treated at the HTLD / HTLD-CKD level after one cycle of therapy
- Changes in global gene expression in myeloma cells treated at the HTLD / HTLD-CKD level after one cycle of therapy

STUDY DESIGN:

Two institutions phase I/II study, classical 3x3 design for patients with GFR ≥ 60ml/min, modified 3x3 design for patients with GFR 30-59ml/min, with 10 additional patients treated at the highest tolerated low dose (HTLD / HTLD-CKD).

Patient population: Multiple myeloma, with relapsed or refractory disease (see under 5.3).

The Cockroft-Gault formula will be used for all GFR estimations throughout this study (Appendix IV).

STUDY ENDPOINTS

Primary:

- The highest tolerated low dose (HTLD and HTLD-CKD for GFR > 60 ml/min and 30-59ml/min, respectively) of azacitidine in combination with GFR-adjusted lenalidomide will be defined as the dose level below which dose limiting toxicity (DLT) occurs during the first 28-day cycle in 2 out of three or out of up to 6 patients. If one out of the first 3 patients with GFR ≥ 60ml/min develops DLT, up to three additional patients are treated at the same dose level. In patients with GFR 30-59ml/min each dose level will enroll 6 patients unless more than 1 patient develops DLT. The dose will not be escalated above 50mg/m^2 twice a week for reasons outlined under background and rationale of this synopsis. Any dose level evaluated as the possible HTLD / HTLD-CKD must include 6 patients assessed for DLT. DLT is defined in Section 5.1. Toxicity according to CTCAE v.4.0 will be assessed with weekly CBC w. Diff and CMP during the first 6 cycles, subsequently before each cycle, weekly history and physical during the first 28-day cycle, then before each cycle until cycle 12.

Secondary:

- Response will be determined monthly during the first 12 cycles and every two months thereafter with serum and 24hr urine protein electrophoresis, and as appropriate, supplemented by immunofixation, serum free light chain assay, and

bone marrow examination. Response before high dose melphalan and autologous stem cell transplant will also be confirmed by 2 separate blood and 24 hr urine tests between the last dose of combination therapy and the first dose of the mobilizing agent.

- Plasma from peripheral blood draws will be used to quantify the activity of CDA using an HPLC method (see under 5.5.3)
- Progression-free survival will be measured from study entry to progression as defined by international response criteria (see under 5.9.1) or death of any cause, whichever comes first.
- Overall survival will be measured from study entry to death from any cause.
- CD34+ cell yield will be calculated based on flow cytometry of mononuclear cells harvested following stem cell mobilization. Time to neutrophil ($\geq$ 1,000/mm^3) and platelet ($\geq$ 100,000/mm^3) recovery will be counted from the day of stem cell infusion (=day 0)
- Promoter demethylation and gene reactivation will be measured at least at the HTLD / HTLD-CKD level using the Illumina® HumanMethylation27 BeadChip array on CD138 purified and CD34 purified cells obtained from bone marrow aspirates within 7 days before treatment start and at the end of cycle #1.RNA will be harvested from the same cell populations to confirm reactivation of selected genes identified on the array. Since the HTLD / HTLD-CKD is identified retrospectively DNA and RNA will be isolated from purified cell populations from all bone marrow aspirations before and after cycle #1.
- The RNA harvested from myeloma cells before and after the first cycle of therapy at the HTLD / HTLD-CKD level will furthermore be used to identify changes in global gene expression using the Illumina® HT12 array.

STUDY DURATION: 24 months	**TOTAL SAMPLE SIZE:** up to 44 patients

DRUG SUPPLIES:

For study participants, Celgene Corporation will provide lenalidomide at no charge through the RevAssist® program and azacitidine, also at no cost, via the Cleveland Clinic Taussig Cancer Center chemotherapy pharmacy or the pharmacy of the University Hospital Seidman Cancer Center.

DOSING REGIMEN(S):

Combination therapy (six 28-day cycles)
GFR $\geq$ 60ml/min (initial study cohort):

All dose levels (**during phase I portion of the study**):

- Lenalidomide 25mg p.o. d1-21 every 28 d
- Dexamethasone 40mg p.o. weekly

Azacitidine dose levels:

1. 30mg/m^2 s.c. weekly
2. 40mg/m^2 s.c. weekly

3. 30mg/m^2 s.c. twice a week
4. 40mg/m^2 s.c. twice a week
5. 50mg/m^2 s.c. twice a week

GFR 30-59 ml/min (amendment 7):

All dose levels (**during phase I portion of the study**):

- Lenalidomide 10mg p.o. d1-21 every 28 d
- Dexamethasone 40mg p.o. weekly

Azacitidine dose levels:

CKD -1. 30mg/m^2 s.c. twice a week

CKD 1. 40mg/m^2 s.c. twice a week

CKD 2. 50mg/m^2 s.c. twice a week

Maintenance therapy (

Lenalidomide at last tolerated dose until disease progression.

2 Schedule of Study Assessments *

Procedure	Screening	Cycle 1				Cycle 2		Cycle 3,4,5,6		**Maintenance**	**Discontinuation From Protocol Therapy**	**Follow-Up Phase**
	≤ 7 days before day 1, cycle 1, unless indicated otherwise	Day **1**	Day **8**	Day **15**	Day **22**	Day **1**	**Weekly**	Day **1**	**Weekly**	**Monthly during first 6 cycles, then every 2 months**		**Every 3 months for 3 years from entry**
Record prior medications, treatments	X											
Record prior anti-cancer therapies	X											
Physical examination, vital signs, weight	X	X[1]	X	X	X	X		X		X	X	
ECOG performance status	X	X[1]	X	X	X	X		X		X	X	
Skeletal survey [2]	X											
Bone marrow [3]	X	X[1]				X						
ECG	X											
CBC with differential	X	X[1]	X	X	X	X	X	X	X	X	X	
Research blood sample (10mL EDTA, 10mL Heparin)	X	X	X	X	X	X		X				
Calculated creatinine clearance (by Cockcroft-Gault)[4]	X	X[1]				X[4]		X[4]		X[4]		
TSH[5]	X[5]	X[1]						X[5]		X[5]	X[5]	
SPEP w m-spike, 24hr urine w m-spike, serum and urine immunofixation, serum quantitative immunoglobulins, serum free light chain assay, beta-2 microglobulin,	X	X[1]				X		X		X	X	
Complete metabolic panel	X	X[1]	X	X	X	X	X	X	X	X	X	
LDH, phosphorus, uric acid	X	X[1]										
Pregnancy testing[7]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]		X[8]		X[8]	X[8]	
Register patient into RevAssist® program[9]	X[9]											
Prescribe lenalidomide via RevAssist® [10]		X[10]				X[10]		X[10]		X[10]		
Azacitidine administration		X[11]	X[11]	X[11]	X[11]	X[11]	X[11]	X[11]	X[11]			
Dexamethasone prescription		X[12]				X[12]		X[12]				
Response assessment [13]						X		X		X	X	
Record adverse events[14]			X	X	X	X		X		X	X[14]	
Record concomitant therapies/procedures	X	X				X		X		X	X	
Obtain Follow-Up anti-cancer treatments												X
Obtain Follow-Up survival information												X

* Variations of ± 3 days of the scheduled visit are permitted.
If Physical examination, vital signs, weight and ECOG performance status were done within 7 days of Day 1, they do not need to be repeated at Study Day 1. An unscheduled visit can occur at any time during the study. Source must be maintained for these unscheduled visits. The date for the visit and any data generated must be recorded on the appropriate CRF. Source documents for these unscheduled visits must also be maintained.

[1] If screening assessments were done within 7 days of Day 1, they do not need to be repeated at Study Day 1.

[2] A skeletal survey may be obtained up to 28 days before day 1, cycle 1. It will be repeated every 12 months or earlier if symptoms suggest bony progression.

[3] With each bone marrow aspiration two 20mL samples, each containing 2mL of heparin, will be obtained for correlative studies in addition to routine clinical analyses as indicated. In addition to defined times, a bone marrow examination is required to confirm complete response and before high dose melphalan in patients who choose to undergo autologous stem cell transplantation. In patients who have measurable disease only in the bone marrow, the bone marrow biopsy and aspiration will be performed every three cycles (+/- 7 days) while on combination therapy and every six cycles (+/- 7 days) while on maintenance.

[4] Calculated creatinine clearance (by Cockcroft-Gault) at baseline and before dosing at the start of each cycle until cycle 12, then every two cycles.

[5] To include Thyroid Stimulating Hormone (TSH) at screening, at least every 16 weeks and at treatment discontinuation. To facilitate monitoring in the maintenance phase it is recommended to obtain TSH at day 1 of cycle 1, 4, 8, 12, 16, and so on. T3 and T4 levels may be assessed as clinically indicated.

[6] If patients undergo stem cell transplantation before completion of six cycles, SPEP w m-spike, 24hr urine w m-spike, serum and urine immunofixation, and serum free light chain assay should be repeated before mobilization begins for confirmation of their pre-transplant response.

[7] Pregnancy tests 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).

[8] Pregnancy tests must occur within 10 – 14 days and again within 24 hours prior to prescribing lenalidomide (prescriptions must be filled within 7 days). FCBP with regular or no menstruation must have a pregnancy test 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 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 I: Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods).

[9] Lenalidomide must be prescribed through and in compliance with the RevAssist® program of Celgene Corporation. Prescriptions must be filled within 7 days.

[10] Consideration should be given to prescribing lenalidomide 5 to 7 days in advance of Day 1 of each cycle to allow time for required patient and prescriber surveys, and drug shipment to patient. Any unused Revlimid® (lenalidomide) should be returned to the patient for disposition in accordance with the RevAssist® program. Lenalidomide will be taken by patients from day 1-21 of the 28 day cycle as outlined under 5.6.

[11] Azacitidine will be administered at the Taussig Cancer Institute and Case Medical Center as outlined under 5.6, with weekly dosing for dose levels 1 and 2, and twice-a-week administration for dose levels 3 to 5.

[12] Dexamethasone will be taken by patients weekly during cycle 1 to 6 as outlined under 5.6. At day one of each cycle it should be ascertained that patients have an active prescription.

[13] Response according international uniform response criteria, outlined in section 7. If PD is suspected, response may be re-assessed through repeat of relevant lab tests at any time before the next planned assessment for confirmation.

[14] An additional safety assessment will be done 28 days (+/- 2 days) following the last dose of protocol therapy.

3 Background and Rationale

3.1 Lenalidomide

3.1.1 Introduction

Lenalidomide is a proprietary IMiD™ compound of Celgene Corporation. IMiD™ compounds have both immunomodulatory and anti-angiogenic properties which could confer anti-tumor and anti-metastatic effects. Lenalidomide has been demonstrated to possess anti-angiogenic activity through inhibition of bFGF, VEGF and TNF-alpha induced endothelial cell migration, due at least in part to inhibition of Akt phosphorylation response to bFGF (16). In addition, lenalidomide has a variety of immunomodulatory effects. Lenalidomide stimulates T cell proliferation, and the production of IL-2, IL-10 and IFN-gamma, inhibits IL-1 beta and IL-6 and modulates IL-12 production(17). Upregulation of T cell derived IL-2 production is achieved at least in part through increased AP-1 activity(18).

Although the exact anti-tumor mechanism of action of lenalidomide is unknown, a number of mechanisms are postulated to be responsible for lenalidomide's activity against multiple myeloma. Lenalidomide has been shown to increase T cell proliferation, which leads to an increase in IL-2 and IFN-gamma secretion. The increased level of these circulating cytokines augment natural killer cell number and function, and enhance natural killer cell activity to yield an increase in multiple myeloma cell lysis(19). In addition, lenalidomide has direct activity against multiple myeloma and induces apoptosis or G1growth arrest in multiple myeloma cell lines and in multiple myeloma cells of patients resistant to melphalan, doxorubicin and dexamethasone(20).

3.1.2 INDICATIONS AND USAGE:

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

3.1.3 Adverse Events

Most frequently reported adverse events reported during clinical studies with lenalidomide in oncologic and non-oncologic indications, regardless of presumed relationship to study medication include: anemia, neutropenia, thrombocytopenia and pancytopenia, abdominal pain, nausea, vomiting and diarrhea, dehydration, rash, itching, infections, sepsis, pneumonia, UTI, upper respiratory infection, cellulites, atrial fibrillation, congestive heart failure, myocardial infarction, chest pain, weakness, hypotension, hypercalcemia, hyperglycemia, back pain, bone pain, generalized pain, dizziness, mental status changes, syncope, renal failure, dyspnea, pleural effusion, pulmonary embolism, deep vein thrombosis, CVA, convulsions, dizziness, spinal cord compression, syncope, disease progression, death not specified and fractures.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

3.2 Azacitidine (Vidaza®)

3.2.1 Introduction

Azacitidine is an azanucleoside analogon, which was developed in the early 1960s as a cytotoxic agent(21). A number of studies have looked at different parenteral doses and schedules of azacitidine, finding maximum tolerated doses of up to 500 mg/m2 when administered weekly(22). Almost 20 years after the original development, azacitidine was found to inhibit the methylation of newly synthesized DNA by inhibiting DNA methyltransferase (DNMT)(7-9), leading to differentiation of human cell lines(23) and reversing oncogenic silencing of tumor suppressor genes(24). Early trials used high doses of the drug that predominantly had nucleoside analogue-type cytotoxic effects but in recent years lower doses, that can induce DNA demethylation in vivo (25)have been used successfully in MDS and AML, improving survival compared to conventional care regimens in high risk MDS and WHO AML patients in a recent phase III trial(10).

3.2.2 INDICATIONS AND USAGE

Azacitidine is indicated for treatment of patients with the following French-American-British (FAB) myelodysplastic syndrome subtypes: refractory anemia (RA) or refractory anemia with ringed sideroblasts (if accompanied by neutropenia or thrombocytopenia or requiring transfusions), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMMoL).

3.2.3 Adverse Events

The most frequently reported adverse events reported during clinical studies with azacitidine, regardless of presumed relationship to study medication include: Gastrointestinal events (nausea, vomiting, diarrhea, constipation, and anorexia), neutropenia, febrile neutropenia, thrombocytopenia, injection site events, arthralgia, dizziness, dyspnea, cough, and myalgia.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

3.3 Rationale for Use in Relapsed or Refractory Multiple Myeloma

With an incidence of 5.6/100,000 multiple myeloma is the second most common hematologic malignancy after Non-Hodgkin lymphomas (SEER 2001-2005). It remains an incurable disease and in the setting of second line or later treatment the progression-free survival ranged between 6-12 months with an overall survival between 1.5 and 3 years in recent trials(1). One of the most successful regimens combined lenalidomide at 25mg on 21 days of a 28 day cycle with dexamethasone in pulse fashion at 40mg day 1-4, 9-12, 17-20 of the first 4 cycles followed by administration from day 1-4 only in US

patients (11). The majority of patients in this trial had been treated with at least 2 prior regimens. The median time to progression was 11.1 months in patients treated with the combination compared to 4.7 month with dexamethasone alone. Although the majority of dexamethasone-only treated patients received lenalidomide at relapse, the median overall survival was superior in the group randomly assigned to combination therapy (29.6 months versus 20.2 months). In Europe and Australia this regimen yielded similar results(26) leading to approval of lenalidomide in combination with dexamethasone for patients with myeloma who have been treated with at least one prior regimen in the US and Europe and for relapsed or refractory myeloma in Australia. A more recent trial randomly assigned newly diagnosed myeloma patients to a lower dexamethasone dose (40mg weekly) or to pulsed dexamethasone in combination with lenalidomide (Rd vs. RD, respectively) and found an improved 2 year overall survival (p=0.006) of 87% (Rd) vs. 75% (RD) at the most recently published analysis(12), suggesting at least equivalent outcome with a trend for lower toxicity, especially regarding venous thromboembolism(27). This finding was rapidly translated into clinical practice in the front-line and relapsed setting and is the regimen used in ongoing clinical trials (FIRST trial, SO777).

To further improve the outcome of patients with relapsed and/or refractory multiple myeloma, resistance mechanisms towards available therapies and immune surveillance need to be overcome. In the course of myeloma progression genetic and epigenetic changes accumulate(2-4, 6, 28-34). The latter are most stable if mediated by DNA methylation of promoter regions which confers heritable silencing of tumor suppressor genes favoring apoptosis resistance, cell cycle dysregulation, and immune escape(2-6, 28-32, 35-41). Azacitidine can reverse such silencing and has been shown to have anti-myeloma activity in vitro and in mouse xenografts as a single agent and in combination therapy by overcoming apoptosis resistance as exemplified in a myeloma cell line with a silenced pro-apoptotic gene, called XAF-1(35, 42). The genome wide activity of DNA methyltransferase I (DNMT1) inhibitors like azacitidine offers a mechanism to overcome apoptosis resistance mediated by silencing of diverse tumor suppressor genes in different myeloma clones of individual patients and patient populations as illustrated by in vitro studies of resistance to interferon-induced apoptosis in kidney cancer cell lines(37). Reactivation of tumor antigens and immunomodulatory genes by DNMT1 inhibition (37) may further increase the activity of immunomodulatory drugs and intrinsic immune responses as suggested by an encouraging 31% response rate of melanoma patients treated with decitabine, the in vivo DNA demethylating metabolite of azacitidine, and interleukin-2 in a phase I trial(43). After prolonged exposure to low doses of aza nucleosides in vitro, cancer cells may undergo cell death via differentiation, senescence, or direct apoptosis, likely depending on the methylation profile. Interestingly, healthy human hematopoietic stem cells can be expanded in vitro by decitabine(44, 45). Indirect evidence for expansion of the healthy stem cell pool in vivo comes from the observation that overall survival can be extended independent of a complete response in MDS(10), a disease that can be fatal due to a lack of normal hematopoietic stem cells. Such an expansion of healthy stem cells would have added benefit in myeloma patients who undergo hematopoietic stem cell harvesting for high dose chemotherapy and autologous stem cell rescue.

In MDS azacytidine at 75mg/m^2 d1-5 every 28 days or 50mg/m^2 d1-5 and 8-12 every 28 days was relatively well tolerated in combination with lenalidomide 5-10mg daily and showed promising activity with an overall response rate of 71% and a complete response rate of 41%, suggesting the two drugs can be combined synergistically without excess toxicity(46). The FDA approved regimen of azacitidine, 75mg/m^2 day 1-7 every 28 days for the treatment of MDS leads to maximal blood concentrations that, while lower than in the original studies, are still directly cytotoxic in vitro(47) which could limit the tumor suppressor gene reactivating function by inhibiting DNA duplication which is required for DNA demethylation. It furthermore likely contributes to side effects that make a treatment interruption of three weeks necessary to allow for recovery when the standard MDS schedule is used (75mg/m^2 d1-7 every 28 days). Unfortunately this also leads to DNA re-methylation before a new cycle begins (25, 48, 49). Furthermore, in diseases with a lower proliferative rate, like multiple myeloma, only a minority of cells would be expected to reactivate tumor suppressor genes if the drug was only given on one out of four weeks. In vitro, non-toxic single low doses of azacitidine (1μM) can reactivate tumor suppressor genes in myeloma cell lines for 3-7 days (unpublished observations). Doses higher than 2-5μM, depending on the cell type, can exert immediate toxicity likely related to nucleoside analogue properties of the drug. Pharmacokinetic data in humans suggest that a single dose of 50mg/m^2 subcutaneously reaches concentrations that should be sufficient for DNA demethylation but could cause classical nucleoside analogue toxicity in some patients (2.89μM +/- 1.35 μM SD), higher doses like 75mg/m2 reach 4.88 μM +/- 1.4μM SD and thus almost certainly exert classic nucleoside analogue toxic effects (13). In this study we aim to avoid pronounced nucleoside analogue side effects while maximally exploiting DNA demethylating effects of azacitidine. We will therefore assess the safety of combination therapy with azacitidine given at low but increasing doses up to 50mg/m^2 twice a week, together with standard dose lenalidomide and low dose dexamethasone in relapsed or refractory myeloma patients in a phase I/II study with 10 additional patients enrolled at the highest tolerated low azacitidine dose to obtain an initial impression of efficacy and to evaluate whether this dose exerts the expected pharmacodynamic effects in myeloma cells before a larger phase II trial may be conducted.

4 Study Objectives and Endpoints

4.1 Objectives

4.1.1 Primary Objective

- Define the highest tolerated low doses (HTLD and HTLD-CKD for GFR > 60 ml/min and 30-59ml/min, respectively) and safety of azacitidine given at low but increasing doses up to 50mg/m^2 twice a week concurrently with GFR-adjusted lenalidomide doses and low dose dexamethasone in patients with relapsed or refractory multiple myeloma.

4.1.2 Secondary Study Objectives

- Response according to international response criteria (≥PR) and clinical benefit response (≥minor response according to adapted EBMT criteria), see under 7
- Correlate response with plasma activity of the azacitidine inactivating enzyme cytidine deaminase (CDA)
- Progression-free survival and overall survival
- Peripheral blood hematopoietic progenitor (CD34+) yield and time to neutrophil and platelet recovery in patients undergoing autologous stem cell transplantation
- Promoter demethylation and gene reactivation in myeloma cells and hematopoietic progenitors treated at the HTLD / HTLD-CKD level after cycle 1
- Changes in global gene expression in myeloma cells treated at the HTLD / HTLD-CKD level after cycle 1

4.2 Endpoints

4.2.1 Primary Endpoint

- The highest tolerated low dose (HTLD and HTLD-CKD for GFR > 60 ml/min and 30-59ml/min, respectively) of azacitidine in combination with GFR-adjusted lenalidomide will be defined as the highest dose level studied (up to 50mg/m^2 twice a week) at which dose limiting toxicity (DLT) occurs during the first 28-day cycle in ≤ 1 out of 6 patients. If one out of the first 3 patients with GFR ≥ 60ml/min develops DLT, up to three additional patients are treated at the same dose level. In patients with GFR 30-59ml/min each dose level will enroll 6 patients unless more than 1 patient develops DLT.. Any dose level evaluated as the possible HTLD / HTLD-CKD must include 6 patients assessed for DLT. DLT is defined in Section 5.1. Toxicity according to CTCAE v.4.0 will be assessed with weekly CBC w. Diff and CMP during the first 6 cycles, then before each cycle, weekly history and physical during the first 28-day cycle, then before each cycle until cycle 12.

4.2.2 Secondary Endpoints

- Response will be determined monthly during the first 12 cycles and every two months thereafter with serum and 24hr urine protein electrophoresis, and as appropriate, supplemented by immunofixation, serum free light chain assay, and bone marrow examination. Response before high dose melphalan and autologous stem cell transplant will also be confirmed by two separate blood and 24 hr urine tests between the last dose of combination therapy and the first dose of the mobilizing agent.

- Plasma from peripheral blood draws will be used to quantify the activity of CDA using an HPLC method (see under 5.5.3)
- Progression-free survival will be measured from study entry to progression as defined by international response criteria (see under 5.9.1) or death of any cause, whichever comes first.
- Overall survival will be measured from study entry to death from any cause.
- CD34+ cell yield will be calculated based on flow cytometry of mononuclear cells harvested following stem cell mobilization. Time to neutrophil ($\geq$ 1,000/mm^3) and platelet ($\geq$ 100,000/mm^3) recovery will be counted from the day of stem cell infusion (=day 0)
- Promoter demethylation and gene reactivation will be measured at least at the HTLD / HTLD-CKD level using the Illumina® HumanMethylation27 BeadChip array on CD138 purified and CD34 purified cells obtained from bone marrow aspirates within 7 days before treatment start and at the end of cycle #1.RNA will be harvested from the same cell populations to confirm reactivation of selected genes identified on the array. Since the HTLD / HTLD-CKD is identified retrospectively DNA and RNA will be isolated from purified cell populations from all bone marrow aspirations before and after cycle #1.
- The RNA harvested from myeloma cells before and after the first cycle of therapy at the HTLD / HTLD-CKD level will furthermore be used to identify changes in global gene expression using the Illumina® HT12 array.

5 Investigational Plan

5.1 Overall Design

This phase I/II trial will use a classical 3x3 design to determine the highest tolerated low dose (HTLD) for patients with GFR $\geq$ 60 ml/min (Cockroft-Gault) and a modified 3x3 design to identify the highest tolerated low dose for patients with GFR 30-59 ml/min (HTLD-CKD) with additional enrollment of 10 patients at the highest tolerated low doses (HTLD / HTLD-CKD) of azacitidine.

Dose levels are outlined under 5.6.2.3.

Dose limiting toxicity (DLT) is defined as one of the following drug-related toxicities occurring during the first 28-day cycle (if a DLT is attributed to progressive disease, it will not be counted as a DLT):

- Any CTCAE v.4.0 grade $\geq$3 non hematologic toxicity (including febrile neutropenia)
    - For nausea, vomiting, or diarrhea, subjects must have a Grade 3 or 4 event that persists at this level despite the use of optimal symptomatic treatment, in order for these events to be considered a DLT

- Grade 4 transaminitis (serum transaminase > 20 x upper limit of normal [ULN]) is a DLT, while Grade 3 transaminitis (serum transaminase > 5 x and ≤ 20 x ULN) must be present for ≥ 7 days to be considered a DLT
- Grade 3 or 4 venous thromboembolic events are not considered to be DLTs as long as anticoagulant therapy can be administered (see Section 6.5.3 and 6.6.2)
- Grade 3 or 4 hypokalemia, hypophosphatemia, hypomagnesemia or hyponatremia that responds to electrolyte supplementation within 7 days would not qualify as a DLT.

- CTCAE v.4.0 grade ≥ 4 neutropenia or thrombocytopenia that does not resolve to grade ≤ 3 within seven days of holding azacitidine and lenalidomide. While platelet transfusions are allowed at the discretion of the treating physician to maintain 100% dose level, any grade ≥ 4 thrombocytopenia that is followed by platelet transfusion prior to resolution to grade ≤ 3 will be counted as DLT.

Dose escalation for patients with GFR ≥ 60ml/min:
At least 3 patients will be treated in each dose level tested beginning with dose level 1. At any dose level, if none of the first 3 evaluable patients experience DLT in the first 28 day cycle, the next three patients will be treated at the next dose level. At any dose level, if DLT occurs in one out of three patients during the first 28 day cycle, up to 3 additional patients will be treated at the same dose level. If no DLT occurs in the additional 3 patients, subsequent 3 patients will be treated at the next dose level. Once DLT occurs in 2 patients treated at a given dose level no further patients will be accrued at this dose level but patients already on treatment will continue according to study protocol. If DLT occurs in 2 patients treated at a given dose level, the next lower dose level will be evaluated as the possible HTLD, and if only 3 patients had been enrolled at this dose level, an additional 3 patients will enrolled to properly assess this dose level as the possible HTLD. The dose level at which DLT occurs in ≤ 1 out of 6 patients will be defined as the highest tolerated low dose (HTLD) and ten more patients will be accrued at this dose level. Six evaluable patients must be assessed for DLT at any dose level that is being considered as the HTLD. Patients experiencing a DLT during their first 28-day cycle may remain on study with appropriate dose reduction (5.7.3).

Dose escalation for patients with GFR 30-59ml/min:
Only two dose levels of azacitidine will be tested in combination with fixed dose lenalidomide. Starting with dose level "CKD 1" (DL CKD1), 3 patients will initially be enrolled and if no more than 1 experience DLT, 3 additional patients will be enrolled at the same dose level. If no more than 1 out of 6 patients treated at DL CKD1 develop DLT, subsequent patients will be enrolled at DL CKD2. Once 2 of 3 or 2 of up to 6 patients treated at a given dose level develop DLT, no further patients will be enrolled at that dose level. If this occurs at DL CKD1, subsequent patients will be enrolled at DL CKD-1. If it occurs at DL CKD2, DL CKD1 will be defined as HTLD-CKD. If 2 of 3 or 2 of up to 6 patients treated at DL CKD-1 develop DLT, the tested azacitidine / lenalidomide / dexamethasone regimen will be considered intolerable for patients with

GFR 30-59 ml/min. The highest dose level that causes DLT in no more than 1 out of 6 evaluable patients will be considered the HTLD-CKD and once this is defined additional patients treated at this dose level may contribute to the 10 patient phase II extension. Patients experiencing a DLT during their first 28-day cycle may remain on study with appropriate dose reduction (5.7.3).

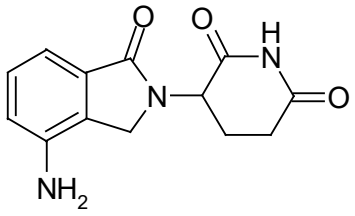
Patients that are not evaluable for DLT throughout the first 28 day cycle of the dose escalation portion of the study due to progressive disease or for other reasons, will be replaced.

5.1.1 Protocol Therapy

5.1.1.1 Lenalidomide Description

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

Chemical Structure of Lenalidomide



3-(4-amino-1-oxo 1,3-dihydro-2*H*-isoindol-2-yl) piperidine-2,6-dione

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

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

5.1.1.2 CLINICAL PHARMACOLOGY

Mechanism of Action:

The mechanism of action of lenalidomide remains to be fully characterized. Lenalidomide possesses immunomodulatory and anti-angiogenic properties. Lenalidomide inhibited the secretion of pro-inflammatory cytokines and increased the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. Lenalidomide inhibited cell proliferation with varying effectiveness (IC50s) in some but not all cell lines. Of cell lines tested, lenalidomide was effective in inhibiting growth of

Manawa cells (a human B cell lymphoma cell line with a deletion of one chromosome 5) but was much less effective in inhibiting growth of KG-1 cells (human myeloblastic cell line, also with a deletion of one chromosome 5) and other cell lines without chromosome 5 deletions. Lenalidomide inhibited the expression of cyclooxygenase-2 (COX-2) but not COX-1 in vitro.

5.1.1.3 Pharmacokinetics and Drug Metabolism:

Absorption:

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

Pharmacokinetic analyses were performed on 15 multiple myeloma patients treated in the phase I studies. Absorption was found to be rapid on both Day 1 and Day 28 with time to maximum blood levels ranging from 0.7 to 2.0 hours at all dose levels (5mg, 10mg, 25mg, and 50mg). No plasma accumulation was observed with multiple daily dosing. Plasma lenalidomide declined in a monophasic manner with elimination half-life ranging from 2.8 to 6.1 hours on both Day 1 and 28 at all 4 doses. Peak and overall plasma concentrations were dose proportional over the dosing range of 5mg to 50mg(50). Exposure (AUC) in multiple myeloma patients is 57% higher than in healthy male volunteers(51).

Pharmacokinetic Parameters:

Distribution:

In vitro (^{14}C)-lenalidomide binding to plasma proteins is approximately 30%.

Metabolism and Excretion:

The metabolic profile of lenalidomide in humans has not been studied. In healthy volunteers, approximately two-thirds of lenalidomide is eliminated unchanged through urinary excretion. The process exceeds the glomerular filtration rate and therefore is partially or entirely active. Half-life of elimination is approximately 3 hours. Renal impairment increases the area under the curve (AUC) and increases half-life to 9-10 hours with moderate to severe renal dysfunction and to more than 15hours in ESRD making dose adjustments necessary(52). A study presented at ASH 2011 determined lenalidomide AUC concentrations in 37 multiple myeloma patients with various glomerular filtration rates (Cockroft-Gault) who were treated with lenalidomide dosing schedules predicted to yield equal AUCs based on pharmacokinetic data obtained in patients without cancer diagnosis but with chronic kidney disease (CKD)(15). Ten

myeloma patients with GFR > 80ml/min were treated with standard lenalidomide 25mg day 1-21 every 28 days and served as comparison for patients with GFR 50-80 ml/min (n=10), GFR 30-49 ml/min (n=7), GFR < 30 ml/min (n=5), and patients on chronic hemodialysis (n=5), who received 25mg daily, 10mg daily, 15mg every other day, and 5mg daily, respectively, from day 1-21 every 28 days. All patients received dexamethasone 40mg once a week but no other antimyeloma drugs. Average daily AUC values of patients with CKD were between 103-149% of the ones in patients with GFR > 80 ml/min and essentially identical for patients with GFR 30-49 (103%). The renal function remained stable in all patients. Severe adverse events occurred in 60% of patients on dialysis and 45% of remaining patients (p=0.3), overall comparable to reported results(11, 26), as were response rates of around 70% in these patients who had received a minimum of one prior myeloma regimen. In summary, GFR-adjusted lenalidomide dosing achieved appropriate drug exposure and was safe and effective(15).

5.1.1.4 Supplier(s)

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

5.1.1.5 Dosage form

Lenalidomide will be supplied as capsules for oral administration.

5.1.1.6 Packaging

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

5.1.1.7 Storage

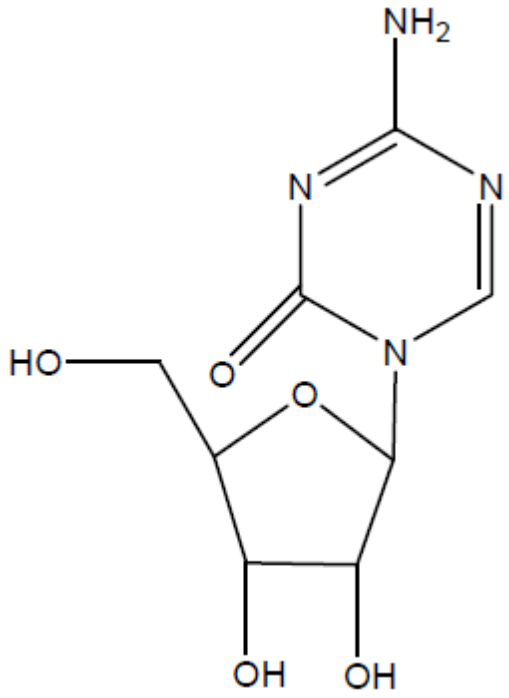
Lenalidomide should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

5.1.1.8 Prescribing Information

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

5.1.1.9 Azacitidine Description

VIDAZA (azacitidine for injection) contains azacitidine, which is a pyrimidine nucleoside analog of cytidine. Azacitidine is 4-amino-1-β-D-ribofuranosyl-s-triazin-2(1H)-one. The structural formula is as follows:



The empirical formula is C8H12N4O5. The molecular weight is 244. Azacitidine is a white to off-white solid. Azacitidine was found to be insoluble in acetone, ethanol, and methyl ethyl ketone; slightly soluble in ethanol/water (50/50), propylene glycol, and polyethylene glycol; sparingly soluble in water, water saturated octanol, 5% dextrose in water, N-methyl-2-pyrrolidone, normal saline and 5% Tween 80 in water; and soluble in dimethylsulfoxide (DMSO).

The finished product is supplied in a sterile form for reconstitution as a suspension for subcutaneous injection or reconstitution as a solution with further dilution for intravenous infusion. Vials of VIDAZA contain 100 mg of azacitidine and 100 mg mannitol as a sterile lyophilized powder.

5.1.1.10 CLINICAL PHARMACOLOGY

Mechanism of Action:

VIDAZA is a pyrimidine nucleoside analog of cytidine. VIDAZA is believed to exert its anti-neoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The concentration of azacitidine

required for maximum inhibition of DNA methylation *in vitro* does not cause major suppression of DNA synthesis. Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms. Non-proliferating cells are relatively insensitive to azacitidine(51).

5.1.1.11 Pharmacokinetics and Drug Metabolism:

The pharmacokinetics of azacitidine were studied in 6 MDS patients following a single 75 mg/m^2 subcutaneous (SC) dose and a single 75 mg/m^2 intravenous (IV) dose. Azacitidine is rapidly absorbed after SC administration; the peak plasma azacitidine concentration of 750 ± 403 ng/ml occurred in 0.5 hour. The bioavailability of SC azacitidine relative to IV azacitidine is approximately 89%, based on area under the curve. Mean volume of distribution following IV dosing is 76 ± 26 L. Mean apparent SC clearance is 167 ± 49 L/hour and mean half-life after SC administration is 41 ± 8 minutes(52). In a more recent trial the cytidine deaminase inhibitor tetrahydrouridine (THU) was added to collected blood samples to avoid enzymatic breakdown of azacitidine in vitro. This trial suggested that maximal azacitidine blood concentrations after s.c. administration were about 50% higher, 1192.8 +/- 342.4 ng/mL(13).

While deamination by cytidine deaminase in plasma appears to be the main mode of inactivation, urinary excretion is the primary route of elimination of azacitidine and its metabolites. Following IV administration of radioactive azacitidine to 5 cancer patients, the cumulative urinary excretion was 85% of the radioactive dose. Fecal excretion accounted for <1% of administered radioactivity over 3 days. Mean excretion of radioactivity in urine following SC administration of ^{14}C-azacitidine was 50%. The mean elimination half-lives of total radioactivity (azacitidine and its metabolites) were similar after IV and SC administrations, about 4 hours(52).

Special Populations

The effects of renal or hepatic impairment, gender, age, or race on the pharmacokinetics of azacitidine have not been studied prospectively but investigators at MD Anderson Cancer Center have recently reviewed their experience with the use of azacitidine and decitabine in 41 patients with myeloid malignancies and kidney dysfunction(14). The GFR was between 30 and 59ml/min in 29 patients and below 30ml/min in 12 patients. Eleven patients were treated with standard-dose azacitidine (75mg/m^2 for 7 days every 28 days), 10 patients SC, one IV, and three of the eleven patients had GFRs below 30ml/min. Overall, adverse events to decitabine and azacitidine were not felt to be more frequent than in historical controls for patients with GFR $\geq$ 30ml/min but more severe kidney dysfunction appeared to increase risk for hematologic and non-hematologic toxicities leading to more frequent dose reduction or treatment delay. Response rates and survival was similar to historical controls. Results thus support the use of azacitidine and decitabine at standard doses, at least in patients with GFR $\geq$ 30ml/min(14).

Drug-Drug Interactions

Drug interaction studies with azacitidine have not been conducted.

An *in vitro* study of azacitidine incubation in human liver fractions indicated that azacitidine may be metabolized by the liver. Whether azacitidine metabolism may be affected by known microsomal enzyme inhibitors or inducers has not been studied.

The potential of azacitidine to inhibit cytochrome P450 (CYP) enzymes is not known. *In vitro* studies with human cultured hepatocytes indicate that azacitidine at concentrations of
1.0 μM to 100 μM does not induce CYP 1A2, 2C19, or 3A4/5.

5.1.1.12 NONCLINICAL TOXICOLOGY

Carcinogenesis, Mutagenesis, Impairment of Fertility

The potential carcinogenicity of azacitidine was evaluated in mice and rats. Azacitidine induced tumors of the hematopoietic system in female mice at 2.2 mg/kg (6.6 mg/m^2, approximately 8% the recommended human daily dose on a mg/m^2 basis) administered IP three times per week for 52 weeks. An increased incidence of tumors in the lymphoreticular system, lung, mammary gland, and skin was seen in mice treated with azacitidine IP at 2.0 mg/kg (6.0 mg/m^2, approximately 8% the recommended human daily dose on a mg/m^2 basis) once a week for 50 weeks. A tumorigenicity study in rats dosed twice weekly at 15 or 60 mg/m^2 (approximately 20-80% the recommended human daily dose on a mg/m^2 basis) revealed an increased incidence of testicular tumors compared with controls.

The mutagenic and clastogenic potential of azacitidine was tested in *in vitro* bacterial systems Salmonella typhimurium strains TA100 and several strains of trpE8, Escherichia coli strains WP14 Pro, WP3103P, WP3104P, and CC103; in *in vitro* forward gene mutation assay in mouse lymphoma cells and human lymphoblast cells; and in an *in vitro* micronucleus assay in mouse L5178Y lymphoma cells and Syrian hamster embryo cells. Azacitidine was mutagenic in bacterial and mammalian cell systems. The clastogenic effect of azacitidine was shown by the induction of micronuclei in L5178Y mouse cells and Syrian hamster embryo cells.

Administration of azacitidine to male mice at 9.9 mg/m^2 (approximately 9% the recommended human daily dose on a mg/m^2 basis) daily for 3 days prior to mating with untreated female mice resulted in decreased fertility and loss of offspring during subsequent embryonic and postnatal development. Treatment of male rats 3 times per week for 11 or 16 weeks at doses of 15-30 mg/m^2 (approximately 20-40%, the recommended human daily dose on a mg/m^2 basis) resulted in decreased weight of the testes and epididymides, and decreased sperm counts accompanied by decreased pregnancy rates and increased loss of embryos in mated females. In a related study, male rats treated for 16 weeks at 24 mg/m^2 resulted in an increase in abnormal embryos in mated females when examined on day 2 of gestation.

5.1.1.13 Adverse Events in Clinical Trials

In addition to hematologic toxicities the most frequent side effects and discomforts reported for azacitidine are nausea, vomiting and diarrhea. Infrequent side effects include neuromuscular aches, liver enzyme abnormalities, and renal tubular acidosis (the kidneys excrete too much bicarbonate and/or not enough hydrogen ions).

The azacitidine injection may result in redness and inflammation at the injection site.

5.1.1.14 Supplier(s)

Celgene Corporation will supply VIDAZA® (azacitidine) to study participants at no charge through the Cleveland Clinic Taussig Cancer Center chemotherapy pharmacy or the pharmacy of the University Hospital Ireland Cancer Center.

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5.1.1.15 Dose Preparation and Storage of Azacitidine (VIDAZA®)

VIDAZA is a cytotoxic drug and, as with other potentially toxic compounds, caution should be exercised when handling and preparing VIDAZA suspensions (see 5.1.1.24).

If reconstituted VIDAZA comes into contact with the skin, immediately and thoroughly wash with soap and water. If it comes into contact with mucous membranes, flush thoroughly with water.

The VIDAZA vial is single-use and does not contain any preservatives. Unused portions of each vial should be discarded properly. See **Handling and Disposal (5.1.1.24).** Do not save any unused portions for later administration.

5.1.1.16 Storage

Store unreconstituted vials at 25º C (77º F); excursions permitted to 15º-30º C (59º-86º F). There is no need to protect azacitidine from exposure to light.

5.1.1.17 Dosage Form

Azacitidine will be supplied as a lyophilized powder in 100mg single-use vials for subcutaneous administration.

5.1.1.18 Preparation for Subcutaneous Administration

VIDAZA should be reconstituted aseptically with 4 mL sterile water for injection. The diluent should be injected slowly into the vial. Vigorously shake or roll the vial until a uniform suspension is achieved. The suspension will be cloudy. The resulting suspension will contain azacitidine 25 mg/mL.

5.1.1.19 Preparation for Immediate Subcutaneous Administration

Doses greater than 4 mL should be divided equally into 2 syringes. The product may be held at room temperature for up to 1 hour, but must be administered within 1 hour after reconstitution.

5.1.1.20 Preparation for Delayed Subcutaneous Administration

The reconstituted product may be kept in the vial or drawn into a syringe. Doses greater than 4 mL should be divided equally into 2 syringes. The product must be refrigerated immediately, and may be held under refrigerated conditions (2ºC–8ºC, 36ºF–46ºF) for up to 8 hours. After removal from refrigerated conditions, the suspension may be allowed to equilibrate to room temperature for up to 30 minutes prior to administration.

5.1.1.21 Subcutaneous Administration

To provide a homogeneous suspension, the contents of the syringe must be re-suspended by inverting the syringe 2–3 times and vigorously rolling the syringe between the palms for 30 seconds immediately prior to administration.

VIDAZA suspension is administered subcutaneously. Doses greater than 4 mL should be divided equally into 2 syringes and injected into 2 separate sites. Rotate sites for each injection (thigh, abdomen, or upper arm). New injections should be given at least 1 inch from an old site and never into areas where the site is tender, bruised, red, or hard.

5.1.1.22 Suspension Stability

VIDAZA reconstituted for subcutaneous administration may be stored for up to 1 hour at 25°C (77°F) or for up to 8 hours between 2°C and 8°C (36°F and 46°F).

5.1.1.23 Packaging

Azacitidine will be shipped to the Cleveland Clinic Foundation Taussig Cancer Center chemotherapy pharmacy and the pharmacy of the University Hospital Ireland Cancer Center.

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5.1.1.24 Handling and Disposal

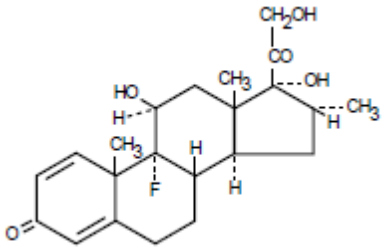
Procedures for proper handling and disposal of anticancer drugs should be applied.

5.1.1.25 Prescribing Information

Azacitidine (VIDAZA®) will be provided to research subjects for the duration of their participation in this trial at no charge to them or their insurance providers.

5.1.1.26 Dexamethasone Description

Glucocorticoids are adrenocortical steroids, both naturally occurring and synthetic, which are readily absorbed from the gastrointestinal tract. Dexamethasone, a synthetic adrenocortical steroid, is a white to practically white, odorless, crystalline powder. It is stable in air. It is practically insoluble in water. It is designated chemically as 9-fluoro-11β, 17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione. The structural formula is represented below:



$C_{22}H_{29}FO_5$ MW 392.47

The tablets, for oral administration are available at 0.25 mg, 0.5 mg, 0.75 mg, 1.5 mg, 4 mg or 6 mg strength of dexamethasone. The 4mg tablet will be used in this study.

Each tablet contains the following inactive ingredients: Anhydrous lactose, croscarmellose sodium, magnesium stearate, microcrystalline cellulose and stearic acid.

5.1.1.27 CLINICAL PHARMACOLOGY

Pharmacokinetics and Mechanism of Action

Glucocorticoids, naturally occurring and synthetic, are adrenocortical steroids that are readily absorbed from the gastrointestinal tract. Glucocorticoids cause varied metabolic and immune effects after binding to cytoplasmic glucocorticoid receptors with subsequent translocation into the nucleus, where they affect gene expression. Naturally occurring glucocorticoids (hydrocortisone and cortisone), which also have sodium-retaining properties, are used as replacement therapy in adrenocortical deficiency states. At equipotent anti-inflammatory doses, the synthetic analogue Dexamethasone almost completely lacks the sodium-retaining property of hydrocortisone and closely related derivatives of hydrocortisone. Dexamethasone, when used at relatively high doses, can directly inhibit growth of myeloma cells and cause apoptosis. In numerous clinical trials increases of response rate have been observed by the addition of dexamethasone to standard chemotherapeutics and novel myeloma drugs.

5.1.1.28 Expected Side Effects:

Common side effects of dexamethasone include anxiety, mood alteration/lability, potassium loss, hypokalemic alkalosis, peptic ulcer with possible perforation and hemorrhage, impaired wound healing, menstrual irregularities, hyperglycemia, increased intraocular pressure, weight gain, nausea, headache, insomnia, peripheral edema, myopathy (with chronic use), acne, hirsutism.

No drug interactions that raise additional concerns are known or postulated between azacitidine, lenalidomide and dexamethasone.

5.1.1.29 Dosage Form

Dexamethasone will be prescribed as tablets of 4mg strength for oral administration.

5.1.1.30 Storage

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

5.1.1.31 Prescribing Information

Dexamethasone will be prescribed according to standard practices and charged to the patient's insurance.

5.2 Screening and Eligibility

The Investigator is responsible for keeping a record of all subjects who sign an Informed Consent Form for entry into the study. All subjects will be screened for eligibility. Screening procedures are outlined in Section 2, Schedule of Study Assessments and unless otherwise specified, must take place within 28 days prior to initiation of therapy.

Approximately 100 subjects with relapsed or refractory myeloma will be screened for enrollment and must meet the eligibility criteria below.

5.2.1 Inclusion Criteria

Subjects must meet the following inclusion/exclusion criteria to be eligible for the study.

Inclusion Criteria

1. Understand and voluntarily sign an informed consent form.
2. Age ≥18 years at the time of signing the informed consent form.
3. Able to adhere to the study visit schedule and other protocol requirements.
4. Refractory or relapsed multiple myeloma (see under 5.3.)
5. Measurable disease defined as at least one of the following: Serum m-spike ≥ 1g/dL, urine m-spike ≥ 200mg/24hrs, serum free light chains ≥ 100mg/L (provided the kappa/lambda ratio is abnormal), or bone marrow plasma cells ≥ 30%.
6. Previous therapy with IMiD™ compounds (thalidomide, lenalidomide, pomalidomide), proteasome inhibitors (bortezomib, carfilzomib), and corticosteroids must be discontinued at least 14 days before entry onto this study.
7. Previous cytotoxic chemotherapy (e.g. alkylating chemotherapy, anthracyclines, and vinca alkaloids), radiation therapy to the pelvis, and any experimental therapy other than carfilzomib or pomalidomide must have been discontinued at least 28 days prior to entry onto this study.
8. ECOG performance status of ≤ 2 at study entry (see Appendix II).
9. Laboratory test results within these ranges:
   - Absolute neutrophil count ≥ 1,500 /mm³
   - Platelet count ≥ 75,000/mm³
   - Calculated creatinine clearance (Cockroft-Gault) ≥ 30ml/min.
   - Total bilirubin ≤ 1.5 x ULN
   - Serum glutamic-oxaloacetic transaminase (SGOT) (aspartate aminotransferase [AST]) and serum glutamic-pyruvic transaminase (SGPT) (alanine aminotransferase [ALT]) levels ≤2 x ULN
10. All study participants must be registered into the mandatory RevAssist® program, and be willing and able to comply with the requirements of RevAssist®.
11. Females of childbearing potential (FCBP)[†] must have a negative serum or urine pregnancy test with a sensitivity of at least 50 mIU/mL within 10 – 14 days prior to and again within 24 hours of prescribing lenalidomide (prescriptions must be filled within 7 days) and must either commit to continued abstinence from

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

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

12. Able to take aspirin (81 or 325 mg) daily as prophylactic anticoagulation (patients intolerant to ASA may use warfarin or low molecular weight heparin) if no additional risk factor for VTE other than myeloma diagnosis according to IMW guidelines(55) and as specified for this study under Section 5.6.2.5.
13. Able to take low molecular weight heparin or warfarin if $\geq$1 additional risk factor for VTE according to IMW guidelines(55) and as specified for this study under Section 5.6.2.5.

5.2.2 Exclusion Criteria

1. Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form.
2. Pregnant or breast feeding females. (Lactating females must agree not to breast feed while taking lenalidomide or azacitidine).
3. Any condition, including the presence of laboratory abnormalities, which places the subject at unacceptable risk if he/she were to participate in the study or confounds the ability to interpret data from the study.
4. Use of any experimental drug or therapy other than carfilzomib and pomalidomide within 28 days of treatment start on this protocol.
5. Neuropathy > Grade 2
6. Known hypersensitivity to thalidomide, lenalidomide, azacitidine, or mannitol.
7. The development of erythema nodosum if characterized by a desquamating rash while taking thalidomide or lenalidomide drugs.
8. Concurrent use of other anti-cancer agents or treatment or concurrent radiation to the pelvis. Palliative radiation to areas outside the pelvis is allowed.
9. Previous inability to tolerate full-dose lenalidomide, adjusted to creatinine clearance (CrCl) according to Cockroft-Gault at the time of previous lenalidomide treatment (25mg day 1-21 every 28 days if CrCl $\geq$ 60ml/min, 10mg lenalidomide d1-21 every 28 days if CrCl < 60mL/min but $\geq$ 30mL/min, lenalidomide 15mg every 48 h d1-21 every 28 days if CrCl < 30mL/min but not requiring dialysis, lenalidomide 5mg daily, day 1-21 every 28 days if CrCl < 30mL/min and requiring dialysis).

5.3 Definition Relapsed Myeloma and Refractory Myeloma

A recent ASH/FDA panel has recommended to include clinical criteria to define relapsed myeloma and to separately analyze data for the relapsed as opposed to the refractory group of patients (56). In accordance with this consensus statement, International Myeloma Working Group diagnostic criteria (57), and the international uniform response criteria (58) the following definitions will be used:

5.3.1 Refractory Myeloma.

Biochemical or clinical evidence of loss of disease control within 90 days after the last therapy. Any of the following defines refractory myeloma:

1. Progressive disease (see 5.8.1) on prior therapy
2. Best response to prior therapy was stable disease (see under 7)
3. Progressive disease (see 5.8.1) within 90 days of the last therapy

5.3.2 Relapsed Myeloma.

Not fulfilling criteria for refractory disease and at least one biochemical ***and*** at least one clinical criterion fulfilled indicating recurrence of disease more than 90 days after the last therapy:

<u>One or more biochemical criteria for recurrent disease:</u>

An **increase of 25% from lowest response value** in **any one or more** of the following, **verified on two consecutive measurements**:

1. Serum M-component (absolute increase must be $\geq$0.5 g/100 ml) *
2. Urine M-component (absolute increase must be $\geq$200mg per 24 h)
3. Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be >100 mg/l)
4. Bone marrow plasma cell percentage (absolute % must be $\geq$10%)

<u>AND one or more clinical criteria for recurrent disease:</u>
Any of the following:

1. Definite development of new bone lesions or soft tissue plasmacytomas
2. Definite increase in the size of existing bone lesions or soft tissue plasmacytomas. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of each measurable lesion
3. Development of hypercalcemia (corrected serum calcium >11.5mg / 100ml) that can be attributed solely to the plasma cell proliferative disorder
4. Decrease of hemoglobin by at least 2g/dL below the best independent value (without ESA or blood transfusion for at least 28 days) since the end of previous

therapy or new decrease in HgB below 10g/dL or to 2g/dL below the lower limit of normal

5. New increase in serum creatinine to > 2mg/dL, or increase by 2mg/dL attributed to myeloma progression

*: If the starting serum m-protein level is ≥5g/dL an absolute increase of ≥1g/dL, verified on two consecutive measurements, is sufficient as a biochemical criterion for recurrent disease.

5.4 Visit Schedule and Assessments

Screening assessments and all on study scheduled visits and assessments are outlined in Section 2 Table of Study Assessments.

At treatment discontinuation, subjects will undergo off study evaluations per the Schedule of Assessments, Section 2. In addition, a safety assessment will be done approximately 28 days post the last dose of protocol therapy. Follow-Up contact with the subjects should occur at a minimum of every three months for three years from study entry.

5.5 Correlative Science

Azacitidine has been shown to reactivate expression of tumor suppressor genes silenced by DNA methylation of their promotors in multiple cell lines, animal experiments, and clinical trials, but so far this has not been demonstrated in myeloma cells of patients treated with azacitidine. Lenalidomide has pleiotropic effects that can include selective reactivation of SPARC in 5q- but not cytogentically normal erythroid progenitors (59). The two drugs might therefore act in concert, reactivating tumor suppressor genes silenced in myeloma cells. With the proposed studies we will investigate baseline DNA methylation and gene expression of myeloma cells and if silenced tumor suppressor genes can be reactivated using the proposed regimen. Effects on normal CD34+ hematopoietic progenitors will be investigated as well.

5.5.1 Sample Handling and Processing

All samples obtained at the Cleveland Clinic will be picked up and processed by staff of Dr. Reu's laboratory (R4-035) the same day they are obtained. The personnel obtaining the sample (bone marrow technicians and phlebotomists) will be given a sheet (appendix V, VI) specifying what tubes are to be used and who to page (81077) upon sample acquisition.

Samples obtained at University Hospitals will be shipped by same-day courier service at room temperature to:

Frederic J. Reu, MD

Cleveland Clinic Taussig Cancer Institute, R4-035

9500 Euclid Ave

Cleveland, OH 44195

Phone: 216 636 0200; Cell: 216 312 9596; Email: reuf@ccf.org

Please either page or email Dale Grabowski when sample is shipped. Email: grabowd@ccf.org

Paging: 216-444-4000 – 81077 followed by # sign – enter call back number followed by # sign

Bone marrow samples from University Hospitals will also be processed the same day by staff of Dr. Reu's laboratory, peripheral blood samples may be stored at 4-8°C and processed the following day if the sample arrives late.

5.5.2 Bone Marrow Examinations

Two 20mL syringes, each pre-filled under sterile conditions with 2mL heparin will be used to obtain 40mL bone marrow aspirate on day 1 (or up to 7 days prior) of the first cycle and on day 1 of the second cycle, each time before drug administration. After isolation of mononuclear cells using the Ficoll ™ method, CD34+ cells and CD138+ cells will be purified using 2 purification steps with Miltenyi columns followed by flow cytometric assessment of achieved purity. Samples of at least 70% purity will be used to assess DNA demethylation and mRNA reactivation. DNA and RNA of purified cells will be harvested using the Qiagen AllPrep DNA/RNA mini kit ™, which allows for column-based isolation of DNA and RNA from the same sample. Isolated RNA and DNA will be stored at -80°C. Within 30 days (once a month) RNA samples will be transcribed into cDNA using Superscript III (Invitrogen ™), then stored at -80°C until further analysis. The Illumina® HT12 array will be used to analyze global gene expression in CD138+ cells. DNA methylation profiling will be performed using the Illumina® HumanMethylation27 BeadChip array in CD138+ and CD34+ cells according to the manufacturer's instructions including bisulfite modification using Zymo™ kits. For approximately 10 selected sample genes, changes in expression after cycle one will be confirmed using real-time RT-PCR (TaqMan, Applied Biosystems ™).

5.5.3 Blood Studies

EDTA blood samples (10mL purple top) obtained at day one of week 1,2,3,4 and day one of cycle 2,3,4,6 will be used to obtain buffy coats for DNMT1 immunohistochemistry with Q-dot® (Invitrogen ™) analysis, according to a protocol that has been validated in samples of AML and MDS patients treated with decitabine in the laboratory of Dr. Sauntharajah. Briefly, cytospin slides of buffy coats will be prepared and cells fixed and permeabilized with 10% formalin and 0.25% triton. Non specific binding sites will be blocked with 10% normal goat serum and 6% BSA. Slides will be incubated overnight with mouse anti-DNMT1 antibody (Abcam catalog number ab13537) (diluted 1:500 in blocking solution), followed by a 655 nm Quantum DotsTM-conjugated goat anti-mouse antibody (Invitrogen catalog number Q11022MP) (diluted 1:500). Finally, cells will be stained with 3 μM DAPI for 5 min before dehydration in graded alcohols and toluene before mounting on Qmount™ Qdot™ mounting media (Invitrogen), which provides

Qdot emission stability for up to 8 months. Images will be obtained the next working day.

Heparin tubes (10mL) from the same time points (day one of week 1,2,3,4 and day one of cycle 2,3,4,6) will be used to isolate whole blood DNA using the DNA blood and tissue mini kit® from Qiagen ™. This DNA will be stored at -80°C and may later be used to investigate whether genes identified as hypermethylated on the methylation array can be detected in the peripheral blood using pyrosequencing of bisulfite modified DNA . A PyroMark Q24® sequencer in Dr Reu's laboratory will be used for these studies.

Plasma from heparin tubes will be collected and frozen at -80°C for later determination of the activity of the azacitidine inactivating enzyme cytidine deaminase (CDA). This will be performed by an HPLC method optimized in Dr. Saunthararajah's laboratory, which quantifies deamination of added cytidine. The enzymatic activity is determined by comparison of cytidine deamination achieved by plasma samples with deamination achieved by incubation of cytidine with dilutions of pure CDA enzyme standards.

5.5.4 Data Analysis

Promoter demethylation after cycle 1 in bone marrow cells enriched either for CD138 or for CD34 expression will be measured using Illumina ™ array technology. The Illumina™ bead array software assigns each CpG region tested a number between 0 and 1.0 representing the degree of DNA methylation from 0 to 100%. The number of genes changing their methylation status significantly according to Illumina ™ Bead Studio software, version 3 and the number of genes changing their methylation score by at least 0.2 points absolute will be analyzed and will be made publicly available. Dr. Mohammed Orloff will assist in the analysis and if necessary, additional help will be obtained from the Cleveland Clinic department of quantitative health sciences. If differences in purity occur between pre-and post-treatment samples this will be taken into account in the statistical analysis.

Changes in global gene expression (Illumina® HT12 array) after cycle one of therapy at the HTLD / HTLD-CKD in bone marrow cells enriched for CD138 will analyzed by the Illumina ™ Bead Studio software, version 3. Genes statistically significantly up- or downregulated at least 2, 5, and 10 fold will be analyzed for each patient and will be made publicly available. Dr. Mohammed Orloff will assist in the analysis and if necessary, additional help will be obtained from the Cleveland Clinic department of quantitative health sciences. If differences in purity occur between pre-and post-treatment samples this will be taken into account in the statistical analysis.

To confirm reactivation of about 10 selected sample genes in CD138+ and CD34+ cells, real-time RT-PCR will be performed in triplicates and reported for each gene analyzed with mean and standard deviation of expression after compared to before cycle one.

DNMT1 suppression will be analyzed after above outlined immunohistochemistry protocol. Image files will be loaded into the Image-Pro Plus environment and individual cells will be segmented for quantification of fluorescence signal with the "count/size" function of Image-Pro Plus. Precise segmentation will be achieved by setting a lower threshold just above background emission. A filter will then be set for an area of whole

cells to exclude fluorescent fragments from the measurement. Image-Pro Plus generates a high-content array of measurements that include event number, area, mean of pixel intensities, and maximum and minimum pixel intensities. These scores will be integrated into a mean intensity fluorescence (MIF) score using the Image-Pro Plus software. The mean and standard deviation of reduction in MIF scores after treatment with azacitidine at the studied time points will be reported for all tested dose levels.

5.6 Drug Administration

5.6.1 Treatment Assignments

The first three patients will be treated at dose level 1. Subsequent increases in dose level will occur as outlined under 5.1. after approval by the PI.

5.6.2 Dosing Regimen

The trial will use a classical 3x3 phase I design for patients with GFR > 60ml/min and a modified 3x3 design for patients with GFR 30-59ml/min with enrollment of ten additional patients at the highest tolerated low dose (HTLD / HTLD-CKD) of azacitidine as outlined under 5.1. The dosing schedule is outlined below. Assigned combination treatment with lenalidomide, azacitidine, and dexamethasone will be continued for six 28-day cycles followed by maintenance treatment with lenalidomide single agent at the last tolerated dose until disease progression with adjustment of dose as outlined under 5.7.1 and 5.7.3.

Peripheral blood stem cell harvesting and high dose chemotherapy therapy may be performed at any point after treatment cycle #2. In such patients protocol therapy will be permanently discontinued at least five days before stem cell mobilization begins but in addition to the basic follow up outlined under 2, schedule of study assessments, their peripheral blood stem cell yield and engraftment will be documented.

5.6.2.1 Special Handling Instructions

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

5.6.2.2 Record of administration

Accurate records will be kept in the source documents of all drug administration (including prescribing and dosing).

5.6.2.3 Combination Therapy

Dosing Schedule for Patients with GFR ≥ 60ml/min (Cockroft-Gault)

Dose level	Azacitidine (s.c.)* Starting day 1	Lenalidomide (p.o.) Starting day 1	Dexamethasone (p.o.)

1	30mg/m^2 once a week[1]	25mg d1-21 every 28d[3]	40mg p.o. once a week[4]
2	40mg/m^2 once a week[1]	25mg d1-21 every 28d[3]	40mg p.o. once a week[4]
3	30mg/m^2 s.c. twice a week[2]	25mg d1-21 every 28d[3]	40mg p.o. once a week[4]
4	40mg/m^2 s.c. twice a week[2]	25mg d1-21 every 28d[3]	40mg p.o. once a week[4]
5	50mg/m^2 s.c. twice a week[2]	25mg d1-21 every 28d[3]	40mg p.o. once a week[4]

* After highest tolerated low dose (HTLD) of azacitine determination, azacitidine starting dose will be at the HTLD

Dosing Schedule for Patients with GFR 30-59ml/min (Cockroft-Gault)

Dose level	Azacitidine (s.c.)* Starting day 1	Lenalidomide (p.o.) Starting day 1	Dexamethasone (p.o.)
-1	30mg/m^2 s.c. twice a week[2]	10mg d1-21 every 28d[3]	40mg p.o. once a week[4]
1	40mg/m^2 s.c. twice a week[2]	10mg d1-21 every 28d[3]	40mg p.o. once a week[4]
2	50mg/m^2 s.c. twice a week[2]	10mg d1-21 every 28d[3]	40mg p.o. once a week[4]

* After highest tolerated low dose (HTLD-CKD) of azacitidine determination, azacitidine starting dose will be at the HTLD-CKD

[1] Ideally, azacitidine is administered on the same day each week, but +/- up to two days is allowed, individual doses should be at least 5 days apart. Rotation of injection sites is obligatory.

[2] Azacitidine should be administered on the same days each week, +/- one day is allowed but there should be at least 48hrs between doses. The following schedules are recommended: Monday-Thursday, Monday-Friday, Tuesday-Friday, Tuesday-Saturday. Rotation of injection sites is obligatory

[3]: Lenalidomide dosing will be in the evening at approximately the same time each day. Prescriptions must be filled within 7 days. 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.

[4]: Dexamethasone dosing, independent of age, will be in the morning with breakfast. Dexamethasone should be taken at least 24h after and not less than 24h before azacitidine to maximize the s-phase specific epigenetic effects of azacitidine.

5.6.2.4 Lenalidomide Maintenance Treatment and Guidelines for Re-Introduction of Azacitidine and Dexamethasone

After six 28-day cycles on combination therapy, dexamethasone and azacitidine will be discontinued and lenalidomide will be continued as a single agent at the last tolerated dose until disease progression. At physician discretion, for patients with response < PR after six 28-day cycles on combination therapy, the dose of lenalidomide may be

escalated during maintenance treatment to a maximum dose of 25mg daily on Days 1-21 of each 28-day cycle. Lenalidomide dose titration may only occur at the start of a new cycle of maintenance one dose level at a time, and only in patients who completed the previous treatment cycle without dose modifications or treatment delays due to toxicity. Lenalidomide dose titration may occur NOT more frequently than once every 28 days. After 12 months since initiation of protocol therapy, no further lenalidomide dose titration is permitted. Also for patients with response < PR after six 28-day cycles on combination therapy, dexamethasone and/or azacitidine may be continued or restarted (if it had been discontinued) at physician discretion. In particular, patients with response < PR after six 28-day cycles on combination therapy that have not shown sufficient signs of end organ improvement should be considered for lenalidomide dose titration and/or additional dexamethasone and/or azacitidine as outlined above. Similarly, patients who have achieved disease control (stable disease or better) during the first six 28-day cycles of combination therapy and who's myeloma progresses on maintenance therapy may be restarted on lenalidomide, azacitidine, and dexamethasone combination therapy at the last tolerated dose at physician discretion.

5.6.2.5 Anticoagulation

Throughout the study, patients who receive lenalidomide (not while the drug is held for high dose chemotherapy and ASCT), who have none of the following risk factors for VTE will receive aspirin 81-325mg daily. If patients have one or more risk factors as outlined below, more intensive prophylaxis should be considered with enoxaparin, 40mg s.c. daily if their GFR (according to Cockroft-Gault, Appendix IV) is $\geq$ 30ml/min and 30mg s.c. daily if it is < 30ml/min. Alternatively, full anticoagulation with warfarin to a target INR of 2.0-3.0 may be used if $\geq$ 1 risk factor is present. Enoxaparin or warfarin at above doses may also be used for patients with no risk factor if aspirin is not tolerated.

Risk factors:

- Previous venous thromboembolism
- Central venous catheter
- Cardiac pacemaker
- Chronic kidney disease as defined by a GFR < 30ml/min lasting > 28 days
- Symptomatic coronary artery disease or heart failure with a left ventricular ejection fraction < 30%
- Diabetes mellitus (unless controlled with diet alone)
- Acute infection, necessitating hospitalization
- Immobility with bed rest > 50% of daytime (ECOG PS $\geq$3) due to any reason while on study
- General surgery or anesthesia

- Treatment with erythropoiesis stimulating agents
- Hereditary hypercoagulability
- Symptomatic hyperviscosity

Subjects experiencing adverse events may need study treatment modifications (See section 5.7).

5.7 Dose Continuation, Modification and *Interruption*

Subjects will be evaluated for AEs at each visit with the NCI CTCAE v.4.0 (Appendix III: NCI CTCAE v.4.0) used as a guide for the grading of severity. Sections below describe recommended dose reduction steps, instructions for initiation of a new cycle of therapy and dose modifications during a cycle of therapy. Clinical judgment may be used when deciding which drug to hold or modify in scenarios that are not covered below. Patients encountering a dose-limiting toxicity during their first cycle may remain on study with appropriate reduction steps.

5.7.1 Dose Modification Steps and Dose Re-Escalation Guidelines

5.7.1.1 Dose Modification Steps

Table 1: Lenalidomide dose modification steps for all toxicities listed in Table 5 EXCEPT for renal impairment or toxicities in the presence of renal impairment*

Current Lenalidomide Dose	One Level Dose Reduction
25 mg daily on Days 1-21 every 28 days	20 mg daily on Days 1-21 every 28 days
20 mg daily on Days 1-21 every 28 days	15 mg daily on Days 1-21 every 28 days
15 mg daily on Days 1-21 every 28 days	10 mg daily on Days 1-21 every 28 days
10 mg daily on Days 1-21 every 28 days	5 mg daily on Days 1-21 every 28 days
5 mg daily on Days 1-21 every 28 days	Discontinue lenalidomide

* Renal impairment is defined as calculated creatinine clearance ≥ 30mL/min but < 60mL/min (see Table 2 below for lenalidomide dosing)

Table 2: Lenalidomide dose modification steps for renal impairment (see Table 5) and for other toxicities which occur after dose reduction for renal impairment*	
Current Lenalidomide Dose	**One Level Dose Reduction**
25 mg daily on Days 1-21 every 28 days	10 mg daily on Days 1-21 every 28 days
10 mg daily on Days 1-21 every 28 days	15 mg every 48 hours during Days 1-21 every 28 days
15 mg every 48 hours during Days 1-21 every 28 days	5 mg daily on Days 1-21 every 28 days
5 mg daily on Days 1-21 every 28 days	Discontinue lenalidomide

* Renal impairment is defined as calculated creatinine clearance ≥ 30mL/min but < 60mL/min

Table 3: Azacitidine Dose Adjustment Steps	
Dose Level +2	100 mg/m^2 SC twice a week (only for patients on twice a week azacitidine who tolerated azacitidine 75mg/m^2 SC twice a week but did not respond, see under 5.7.1.2)
Dose Level +1	75 mg/m^2 SC twice a week (only for patients on twice a week azacitidine who tolerated their full azacitidine starting dose but did not respond, see under 5.7.1.2)
Dose Level 0	Starting azacitidine dose (differs depending on cohort during phase I part, see 5.6.2.3)
Dose Level -1	Reduce dose by 50%, maintain frequency of administration
Dose Level -2	Discontinue azacitidine

Table 4: Dexamethasone Dose Reduction Step	
Dose Level – 1	Reduce each dexamethasone dose by 50%
Dose Level – 2	Discontinue dexamethasone

5.7.2 Intra-Individual Dose Escalation of Azacitidine Beyond Starting Dose

In patients treated with twice a week azacitidine we have measured the plasma activity of the azacitidine inactivating enzyme cytidine deaminase (CDA) by an HPLC method that quantifies deamination of added cytidine. Responders had significantly lower plasma CDA activities than non-responders suggesting that destruction of azacitidine precluded response (submitted to ASH 2012). Patients who do not achieve a reduction of monoclonal proteins to MR range after the first cycle or to PR range after the second cycle and who have tolerated the previous cycle at their full starting dose of azacitidine twice a week without dose reduction or delay due to toxicities may therefore be treated with 75mg/m^2 azacitidine in the next cycle and if an entire cycle at this dose level is

tolerated without the need for dose reduction or delay due to toxicities but monoclonal proteins are still not in MR range after one cycle or PR range after two cycles at this azacitidine dose, the dose of azacitidine may further be increased to 100mg/m^2. There will be no intra-individual dose escalation of azacitidine beyond 100mg/m^2.

5.7.3 Dose Re-Escalation Guidelines

Dose Re-Escalation Upon Recovery from Hematologic Adverse Events

If the dose of lenalidomide or azacitidine was reduced due to dose limiting hematologic toxicity and continued treatment results in improvement of bone marrow function, dose increases by one dose level and drug at a time may be performed if the ANC is ≥ 1000 /mm^3 and platelets are ≥ 50,000 /mm^3 at day one of the respective cycle if the previous cycle was completed without dose modifications due to toxicity (regardless of type). If patient's myeloma has previously been refractory to lenalidomide consideration should be given to increase azacitidine first, otherwise lenalidomide should be increased first. In general, drug dosing increases should alternate between lenalidomide and azacitidine until target doses of both drugs are reached or toxicity occurs but the treating physician may use clinical judgment and first increase one drug to target dose. After 12 months since initiation of protocol therapy, no further lenalidomide dose titration is permitted.

Dose Re-Escalation Upon Recovery of Kidney Function

If the patient's GFR increases from 30-59ml/min at enrollment to ≥ 60ml/min at the start of a cycle and the previous cycle was tolerated without need for dose reduction of lenalidomide due to any adverse event and no G-CSF or platelet transfusions were required, the dose of lenalidomide may be increased by one dose level according to table 2. If the dose of lenalidomide or azacitidine was reduced due to renal impairment on study, and continued treatment results in calculated creatinine clearance ≥ 60ml/min (by Cockroft-Gault), the dose of lenalidomide and azacitidine can be increased by one dose level each at the start of each cycle until the target dose of both drugs is reached provided the calculated creatinine clearance remains ≥ 60ml/min (by Cockroft-Gault) and no relationship between study drugs and renal impairment was suspected. If a relationship between study drugs and renal impairment is considered possible, drug increases should be done one drug at a time and alternating between lenalidomide and azacitidine, starting with lenalidomide. Doses may be increased only in patients who completed the previous treatment cycle without dose modifications or treatment delays due to toxicity (regardless of type). Dose increases may occur NOT more frequently than once every 28 days. If the current dose of lenalidomide is 15 mg every 48 hours during Days 1-21 every 28 days, or 5 mg daily on Days 1-21 every 28 days, the first dose increase would be to 10 mg daily on Days 1-21 every 28 days. Additional dose increases if warranted would be in 5 mg increments. After 12 months since initiation of protocol therapy, no further lenalidomide dose titration is permitted.

5.7.4 Instructions for Initiation of a New Cycle

A new course of treatment may begin on the scheduled Day 1 of a new cycle if:

- The ANC is ≥ 1000/mm³ (G-CSF is allowed to achieve this threshold)
- The platelet count is ≥ 50,000/mm³ (During the first 4 cycles platelet transfusions are allowed to achieve this threshold)
- Any new drug-related neuropathy or rash other than injection site reaction that may have occurred has resolved to ≤ grade 1 severity;
- Any bicarbonate decrease below 20mEq/L that may have occurred has resolved to baseline.
- Calculated creatinine clearance ≥ 30ml/min (by Cockroft-Gault), after 12 cycles on therapy, determining the creatinine clearance every 2 months will suffice
- Any other drug-related adverse events that may have occurred have resolved to ≤ grade 2 severity. Physician discretion may be used in initiating the next cycle of treatment if the only toxicities that have not resolved to ≤ grade 2 severity are dexamethasone-related toxicities.

If these conditions are not met on Day 1 of a new cycle, the subject will be evaluated weekly and a new cycle of treatment will not be initiated until the toxicity has resolved as described above. If criteria to initiate a new cycle are not met by 21 days after the planned start of a new cycle patients go off study. If a lenalidomide or azacitidine dose reduction was taken during the previous cycle, and the cycle was completed without requiring further dose modification, then the next cycle will start at the same reduced dose. **If lenalidomide and / or azacitidine dosing was omitted for the remainder of the previous cycle or if the new cycle is delayed due to toxicity newly encountered on the scheduled Day 1**, then the new cycle will be started with a one-level dose reduction of lenalidomide and/or azacitidine as appropriate. Dose reductions should generally be taken one drug at a time based on attribution of toxicity to one of the drugs unless otherwise indicated in Section 5.7.3.

5.7.5 Instructions for Dose Modifications or Interruption During a Cycle

Table 5 outlines instructions for dose reductions of study drugs for specific toxicities. Study drugs may be temporarily or permanently dose reduced using doses outlined in table 1-4 or temporarily discontinued in other situations where their continued administration according to protocol is felt unsafe by the treating physician.

Table 5: Dose Modifications Azacitidine and Lenalidomide	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Grade 3 neutropenia associated with fever (temperature ≥ 38.5º C) or Grade 4 neutropenia	• Hold (interrupt) lenalidomide and, while on combination, also azacitidine dose. • Follow CBC weekly. • During combination therapy with lenalidomide and azacitidine: o If neutropenia has resolved to ≤ grade 2 prior to Day 21 of the current cycle, restart azacitidine at the next lower dose level and continue lenalidomide at the same dose level if the patient's myeloma has not previously been refractory to lenalidomide, otherwise maintain azacitidine dose level and reduce lenalidomide by one dose level (if the patient is only on one of these drugs reduce its dose by one level) and continue treatment through the scheduled end of the cycle. Otherwise, omit treatment for the remainder of the cycle and reduce dose levels in the same way at the start of the next cycle. Omitted doses are not made up. o If neutropenia is the only toxicity for which a dose reduction is required (no fever or other reason to reduce doses), G-CSF should be considered instead of a dose reduction. • During maintenance therapy with lenalidomide: o If neutropenia has resolved to ≤ grade 2 prior to Day 21 of the current cycle, restart lenalidomide at the next lower dose level, and continue through the scheduled end of the cycle. Otherwise, omit lenalidomide for the remainder of the cycle and reduce lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.

Table 5: Dose Modifications Azacitidine and Lenalidomide	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Thrombocytopenia ≥Grade 3 (platelet count < 50,000/mm³)	• During the first 4 cycles platelet transfusions may be used at the discretion of the treating physician to achieve platelet counts ≥ 50,000/mm^3 on post-transfusion CBCs and thereby maintain the current dose level of lenalidomide and azacitidine. If platelet transfusions are administered, the CBC should be monitored at least twice a week. • For thrombocytopenia ≥ grade 3 (< 50,000/mm^3) after platelet transfusion or if platelet transfusions are not used hold (interrupt) lenalidomide and, while on combination, also azacitidine dose. • If thrombocytopenia ≥ grade 4 (< 25,000/mm^3) on a pre-platelet transfusion CBC or if platelet transfusions are not used hold prophylactic anticoagulation • Follow CBC weekly, if given platelet transfusions twice a week. • If thrombocytopenia resolves to ≤ grade 4 (≥ 25,000/mm^3) and last platelet transfusion ≥ 7 days ago resume prophylactic anticoagulation. • During combination therapy with lenalidomide and azacitidine: o If thrombocytopenia resolves to ≤ grade 2 (with or without the use of platelet transfusions) prior to Day 21 of the current cycle, restart azacitidine at the next lower dose level and lenalidomide at the same dose level if the patient's myeloma has not previously been refractory to lenalidomide, otherwise maintain azacitidine dose level and reduce lenalidomide by one dose level (if the patient is only on one of these drugs reduce its dose by one level). Otherwise, omit treatment for remainder of cycle and reduce dose levels in the same way at the start of the next cycle. Omitted doses are not made up. • During maintenance therapy with lenalidomide: o If thrombocytopenia has resolved to ≤ grade 2 prior to Day 21 of the current cycle, restart lenalidomide at the next lower dose level, and continue through the scheduled end of the cycle. Otherwise, omit lenalidomide for the remainder of the cycle and reduce lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.
Non-blistering rash **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide and, while on combination, also azacitidine dose. • Follow weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21 of the current cycle, restart lenalidomide at next lower lenalidomide dose level and continue azacitidine at the same dose level through the scheduled end of the cycle. Otherwise, omit treatment for remainder of cycle and reduce the lenalidomide dose by 1 dose level and maintain the current azacitidine dose at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide and azacitidine. Remove patient from study.

Table 5: Dose Modifications Azacitidine and Lenalidomide	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Desquamating (blistering) rash- any Grade	• Discontinue lenalidomide and azacitidine. Remove patient from study.
Injection site reaction **Grade 1 (pain, itching, erythema)** **Grade 2 (pain or swelling with inflammation or phlebitis)** **Grade 3 (ulceration or necrosis that is severe, operative intervention indicated)**	• Unless ≥ Grade 3 maintain azacitidine dose • For Grade 1-2 use post-injection hydrocortisone cream (recommended strength: 2.5%) until resolution. Make sure injection sites are rotated. • If grade 3 discontinue azacitidine. Remove patient from study.
Neuropathy **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose and, while on combination, also azacitidine dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21 of the current cycle, restart lenalidomide at next lower lenalidomide dose level, restart azacitidine at the current dose and continue treatment through the scheduled end of the cycle. Otherwise, omit treatment for remainder of cycle and reduce the dose of lenalidomide by 1 lenalidomide dose level and maintain the current azacitidine dose at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide. Remove patient from study.
Venous thrombosis/embolism ≥ Grade 3	• Hold (interrupt) lenalidomide and start anticoagulation; restart lenalidomide at investigator's discretion (maintain dose level). • Omit lenalidomide for remainder of cycle. See Anticoagulation (Section 5.8.1.2)
Hyperthyroidism or hypothyroidism	• Omit lenalidomide for remainder of cycle, evaluate etiology, and initiate appropriate therapy. • See Instructions for Initiation of a New Cycle and reduce the dose of lenalidomide by 1 lenalidomide dose level.
Bicarbonate - low (CO_2 below 20mEq/L)	• If unexplained hold azacitidine and lenalidomide • Check weekly • Upon recovery to baseline restart azacitidine at next lower dose level and restart lenalidomide at the current dose. If recovery to baseline occurs prior to Day 21 of the current cycle, restart as instructed above and continue treatment through the scheduled end of the cycle. Otherwise, omit treatment for remainder of cycle and reduce the dose of azacitidine by 1 dose level and maintain the current dose of lenalidomide at the start of the next cycle. Omitted doses are not made up.

Table 5: Dose Modifications Azacitidine and Lenalidomide	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Renal impairment **(Calculated creatinine clearance ≥ 30ml/min < 60ml/min)**	• Maintain current dosing schedule, but reduce lenalidomide by 1 dose level (see Section 5.7.1, Table 2). If lenalidomide dose had been ≥ 15mg, reduce to 10mg daily on Days 1-21 of each 28-day cycle.
(Calculated creatinine clearance < 30ml/min)	• Hold lenalidomide and, while on combination, also azacitidine • Check weekly • Upon recovery to calculated creatinine clearance ≥ 30ml/min restart at next lower dose level for both lenalidomide and azacitidine (see Section 5.7.1, Table 2 and Table 3). If lenalidomide dose had been ≥ 15mg, reduce to 10mg daily on Days 1-21 of each 28-day cycle. If recovery occurs prior to Day 21 of the current cycle, restart as instructed above and continue treatment through the scheduled end of the cycle. Otherwise, omit treatment for remainder of cycle and reduce the dose of azacitidine by 1 dose level and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle (see Section 5.7.1, Table 2 and Table 3). If lenalidomide dose had been ≥ 15mg, reduce to 10mg daily on Days 1-21 of each 28-day cycle. Omitted doses are not made up.
other non-hematologic toxicity ≥ Grade 3	• In general hold (interrupt) lenalidomide and, while on combination, also azacitidine dose but clinical discretion may be used to only hold the most likely causative agent. • Follow at least weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21 of the current cycle, restart lenalidomide and azacitidine and continue through the scheduled end of the cycle. Otherwise, omit treatment for remainder of cycle. Omitted doses are not made up. For toxicity attributed to lenalidomide, reduce the lenalidomide dose by 1 lenalidomide dose level when restarting lenalidomide. For toxicities attributed to azacitidine, reduce dose by one azacitidine dose level when restarting azacitidine.

Table 6: Dose Modifications Dexamethasone	
NCI CTC Toxicity Grade	**Dose Modification Instructions**
Dyspepsia Grade 1-2	• Maintain dose • Add proton pump inhibitor or H2 blocker
Dyspepsia Grade > 3	• Hold dose • Add proton pump inhibitor or H2 blocker • When symptoms controlled decrease one dose level and restart. Omitted doses are not made up
Edema ≥ Grade 3	• Use diuretics as needed and decrease by one dose level
Confusion or mood alteration ≥ Grade 2	• Hold dose until symptoms resolve. When restarting decrease one dose level.
Muscle weakness (steroid myopathy) ≥ Grade 2	• Hold dose until muscle weakness < Grade 1. When restarting decrease one dose level.
Hyperglycemia ≥ Grade 3	• Decrease dose by one dose level. Treat with insulin or oral hypoglycemic agent.
Acute pancreatitis	• Discontinue dexamethasone

5.7.6 Treatment Compliance

Research center personnel will review the dosing instructions with subjects. Subjects will be asked to maintain a diary to record the drug administration (appendix VII). Subjects will be asked to bring any unused drug and empty drug containers to the research center at their next visit. Research personnel will count and record the number of used and unused drug at each visit and reconcile with the patient diary. Azacitidine will be administered at the Taussig Cancer Center and at University Hospital Ireland cancer Center, compliance will be assessed by review of medication administration reports.
Any unused Revlimid® (lenalidomide) should be returned to the patient for disposition in accordance with the RevAssist® program.

5.8 Concomitant Therapy

5.8.1 Recommended Concomitant Therapy

Subjects should receive full supportive care, including transfusions of blood and blood products, antibiotics, and antiemetics when appropriate.

5.8.1.2 Anticoagulation

Lenalidomide increases the risk of thrombotic events in patients who are at high risk or with a history a thrombosis, in particular when combined with other drugs known to cause thrombosis. When lenalidomide is combined with other agents such as steroids

(e.g. dexamethasone, prednisone), anthracyclines (Doxil, Adriamycin) and erythropoietin the risk of thrombosis is increased.

Prophylactic anticoagulation is mandatory on this trial according to guidelines outlined under 5.6.2.5 with holding and restarting parameters as per Table 5 (hold for plts < 25,000/mm^3, restart once plts $\geq$ 25,000/mm^3 and platelet transfusion independent for > 7 days).

In case of documented deep venous thrombosis or embolism standard anticoagulation with subcutaneous low molecular weight heparin or intravenous heparin with conversion to warfarin will be started. Full anticoagulation will be maintained for at least 12 months while patients are on lenalidomide and at least 6 months if they have gone off trial and the DVT was below the knee (distal to the popliteal vein).

5.8.1.3 Concomitant Therapy with Azacitidine

5HT3 serotonin receptor antagonists should be administered at least 30min prior to each azacitidine dose. If constipation or headache limits the use of HT3 antagonists, metoclopramide may be used.

Rotation of azacitidine injection sites is obligatory. Patients who nevertheless experience grade 1-2 injection site reactions should be treated with topical hydrocortisone (2.5% cream recommended) until resolution.

5.8.2 Prohibited Concomitant Therapy

Concomitant use of other anti-cancer therapies, thalidomide, or other investigational agents is not permitted while subjects are receiving protocol therapy on this study. Palliative radiation (with the exception of pelvic radiation) is permitted.

5.9 Discontinuation of Study Treatment Including Progressive Disease Definition

Treatment will be discontinued in case of:

- Progressive Disease (see under 5.9.1)
- Adverse event(s) that, in the judgment of the Investigator, may cause severe or permanent harm or which rule out continuation of the treatment regimen.
- Delay of the start of a new cycle by more than 21 days due insufficient recovery from toxicity
- Permanent discontinuation of lenalidomide for any reason
- Major violation of the study protocol
- Withdrawal of consent
- Lost to follow up
- Death
- Suspected pregnancy or positive pregnancy

5.9.1 Progressive Disease

Progressive disease (PD) is defined according to the international uniform response criteria (Durie et al. Leukemia 20:1467-73, 2006, ERRATUM Durie et al. Leukemia 21:1134, 2007) and requires any of the following, confirmed on two consecutive assessments:

An **increase of 25% from lowest response value** in **any one or more** of the following:

- Serum M-component (absolute increase must be ≥0.5 g/100 ml) *
- Urine M-component (absolute increase must be ≥200mg per 24 h)
- Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be >100 mg/l)
- Bone marrow plasma cell percentage (absolute % must be ≥10%)

And / or:

- Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas
- Development of hypercalcemia (corrected serum calcium >11.5 mg/100 ml) that can be attributed solely to the plasma cell proliferative disorder

*: If the starting serum m-protein level is ≥5g/dL an absolute increase of ≥1g/dL, verified on two consecutive measurements, is sufficient to define progressive disease.

The decision as to when to repeat the assessment to document progressive disease will be left at the discretion of the treating physician, there is no need to wait until the next scheduled response assessment.

5.10 Follow-Up

Subjects who discontinue treatment for any reason, will be followed every 3 months for 3 years from study entry (if patients are no longer treated at this Institution attempts will be made to obtain information over the phone as per Schedule of Assessments, Section 2). Patients, who stay on protocol therapy will be followed at least every 2 months according to schedule of study assessments (2) for 3 years from study entry. At treatment discontinuation, off study evaluations per the Schedule of Assessments, Section 2, will be done. In addition, subjects will undergo a safety assessment approximately 28 days post the last dose of protocol therapy.

6 Adverse Events

6.1 Serious Adverse Event (SAE) Definition

A serious adverse event is one that at any dose (including overdose):

- Results in death
- Is life-threatening[1]

- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity[2]
- Is a congenital anomaly or birth defect
- Is an important medical event[3]
- Pregnancy

[1]"Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2]"Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3]Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

6.2 Adverse Drug Reaction Reporting

Toxicity will be scored using CTCAE Version 4.0 for toxicity and adverse event reporting. A copy of the CTCAE Version 4.0 can be downloaded from the CTEP homepage (HTTP://CTEP.INFO.NIH.GOV). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.0. All Grade 3 and 4 adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to the test drug and for its seriousness.

The investigator must appraise all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test drug and about the patient's outcome.

6.2.1 Dose Limiting Toxicity Reporting

Dose limiting toxicity (DLT) is defined as one of the following drug-related toxicities occurring during the first 28-day cycle (if a DLT is attributed to progressive disease, it will not be counted as a DLT):

- Any CTCAE v.4.0 grade ≥3 non hematologic toxicity (including febrile neutropenia)
    - For nausea, vomiting, or diarrhea, subjects must have a Grade 3 or 4 event that persists at this level despite the use of optimal symptomatic treatment, in order for these events to be considered a DLT
    - Grade 4 transaminitis (serum transaminase > 20 x upper limit of normal [ULN]) is a DLT, while Grade 3 transaminitis (serum transaminase > 5 x and ≤ 20 x ULN) must be present for ≥ 7 days to be considered a DLT
    - Grade 3 or 4 venous thromboembolic events are not considered to be DLTs as long as anticoagulant therapy can be administered (see Section 6.5.3 and 6.6.2)
    - Grade 3 or 4 hypokalemia, hypophosphatemia, hypomagnesemia or hyponatremia that responds to electrolyte supplementation within 7 days would not qualify as a DLT.

- CTCAE v.4.0 grade ≥ 4 neutropenia or thrombocytopenia that does not resolve to grade ≤ 3 within seven days of holding azacitidine and lenalidomide.

The PI has to be informed about the occurrence of DLT within one working day after the sub investigator or nurse becomes aware of it. The PI may not allow further accrual at a given dose level once two patients have developed DLT. The PI has to inform Celgene within 5 working days of any DLT with a brief description of the nature of the DLT.

6.2.2 Pregnancies

Pregnancy of a female subject or the female partner of a male subject occurring while the subject is on lenalidomide or azacitidine or within 4 weeks after the subject's last dose of lenalidomide or azacitidine are considered expedited reportable events. If the subject is on lenalidomide or azacitidine, both drugs are 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 or azacitidine 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 or azacitidine 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.

6.2.3 Celgene Drug Safety Contact Information:

Celgene Corporation
Drug Safety
86 Morris Avenue
Summit, N.J. 07901

Toll Free: **(800)-640-7854**
Phone: **(908) 673-9667**
Fax: **(908) 673-9115**
e-mail: drugsafety@celgene.com

6.3 Investigator Reporting Responsibilities

The conduct of the study will comply with all FDA safety reporting requirements.

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.

6.3.1 Expedited Reporting by Investigator to Celgene

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 within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours/1 business 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-0507) 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.

SAEs occurring in patients treated at a participating study site must be reported to Celgene by the respective site investigator within 24h of awareness as outlined above. Copies should be sent at the same time to the principal investigator.

6.3.2 Report of Adverse Events to the Institutional Review Board

The principal Investigator is required to notify his/her Institutional Review Board (IRB) of a serious adverse event according to institutional policy.

6.4 Adverse Event Updates/IND Safety Reports

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

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

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

The Investigator must keep copies of all AE information, including correspondence with Celgene and the IRB/EC, on file (see Section 11.4 for records retention information).

7 Response Criteria

Baseline assessments must occur within ≤ 7 days of protocol therapy initiation as indicated in Section 2, Schedule of Study Assessments.

Efficacy assessments are scheduled to occur monthly, beginning at Cycle 3 Day 1, during the first 12 cycles, then every two months.

All response categories (60)require two consecutive blood and urine assessments (bone marrow biopsies do not need to be repeated) and no evidence for progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required for response assessment but should be obtained when there is clinical suspicion for progressive bone disease.

Complete response (CR)	Negative immunofixation of serum and urine (or normal sFLC ratio if the only measurable disease outside of the bone marrow is by sFLCs) and disappearance of any soft tissue plasmacytomas, and <5% plasma cells in bone marrow
Stringent CR (sCR)	CR as defined above plus normal sFLC ratio and absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence
Very good partial response (VGPR)	Serum and urine M-component detectable by immunofixation but not on electrophoresis or ≥90% or greater reduction in serum M-component plus urine M-component <100mg per 24 h or >90% reduction in the difference between involved and uninvolved serum free light chains if this is the only measurable disease outside of the bone marrow
Partial response (PR)	≥50% reduction of serum M protein and reduction in 24-h urinary M protein by ≥90% or to <200mg per 24 h, if the serum and urine M protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved sFLC levels is required in place of the M protein criteria, if the only measurable disease is in the bone marrow, a ≥50% reduction in bone marrow plasma cells is required, provided the baseline percentage was ≥30%. In addition to the above criteria, if present at baseline, a ≥50% reduction in the size of soft tissue plasmacytomas is required.
Minor response (MR)	≥25% but ≤49% reduction of serum M protein and reduction in 24 h urine M protein by 50–89%, which still exceeds 200mg per 24 h. In addition to the above criteria, if present at baseline, 25–49% reduction in the size of soft tissue plasmacytomas is also required. No increase in size or number of lytic bone lesions (development of compression fracture does not exclude response).

Stable disease (SD)	Not meeting criteria for sCR, CR, VGPR, PR, MR, or progressive disease (see under 5.9.1).

8 Protocol Amendments/Deviations

8.1 Protocol Amendments

Any amendment to this protocol must be agreed to by the Principal Investigator and reviewed by Celgene. Amendments should only be submitted to IRB/EC after consideration of Celgene review. Written verification of IRB/EC approval will be obtained before any amendment is implemented.

8.2 Protocol Deviations

When an emergency occurs that requires a deviation from the protocol for a subject, a deviation will be made only for that subject. A decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. In addition, the Investigator will notify the IRB/EC in writing of such deviation from protocol.

Non-emergency minor deviations from the protocol will be permitted with approval of the Principal Investigator.

9 Data Management

9.1 Analyses and Reporting

Oncore will be used for data entry and analysis. Data will be analyzed and reported after the last patient has been on trial for at least three 28 day cycles. All subsequent data collected will be analyzed and reported in a follow-up clinical report.

9.2 Data Safety Monitoring Plan

Twice a month the data safety and toxicity committee of the joint Comprehensive Cancer Center of the Cleveland Clinic Foundation and Case Western Reserve University Cancer Centers reviews all hematologic and oncologic trials run at either institution to ensure the safety of patients, validity of data, and appropriate termination of trials should undue risks be discovered or if the trial can not be successfully completed.

9.3 Study Auditing

9.3.1 Investigator Responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

Investigators must enter study data onto CRFs or other data collection system. The Investigator will permit study-related audits by Celgene or its representatives, IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

The Investigator, or a designated member of the Investigator's staff, must be available at some time during audits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to each visit and be made available to the Celgene representative so that the accuracy and completeness may be checked.

10 Biostatistical Analysis

10.1 Overview

The phase I 3x3 design with 10 additional patients at the highest tolerated low dose (HTLD / HTLD-CKD) of azacitidine as a small phase II extension will lead to a patient accrual of up to 44 patients if the highest dose level is reached. Safety, efficacy, and mechanism of action will be analyzed. Dr. Paul Elson will perform the statistical analysis of all clinical endpoints outlined below. The correlative science will be analyzed utilizing the Illumina Bead Studio ™ software version 3 with support from Dr. Mohammed Orloff.

10.2 Datasets to be Analyzed

10.2.1 Dose Limiting Toxicity

Dose limiting toxicity is defined as any one of the following drug-related toxicities occurring during the first 28-day cycle (if a DLT is attributed to progressive disease, it will not be counted as a DLT):

- Any CTCAE v.4.0 grade $\geq$3 non hematologic toxicity (including febrile neutropenia)
    - For nausea, vomiting, or diarrhea, subjects must have a Grade 3 or 4 event that persists at this level despite the use of optimal symptomatic treatment, in order for these events to be considered a DLT
    - Grade 4 transaminitis (serum transaminase > 20 x upper limit of normal [ULN]) is a DLT, while Grade 3 transaminitis (serum transaminase > 5 x and $\leq$ 20 x ULN) must be present for $\geq$ 7 days to be considered a DLT
    - Grade 3 or 4 venous thromboembolic events are not considered to be DLTs as long as anticoagulant therapy can be administered (see Section 6.5.3 and 6.6.2)

- Grade 3 or 4 hypokalemia, hypophosphatemia, hypomagnesemia or hyponatremia that responds to electrolyte supplementation within 7 days would not qualify as a DLT.

- CTCAE v.4.0 grade $\geq$ 4 neutropenia or thrombocytopenia that does not resolve to grade $\leq$ 3 within seven days of holding azacitidine and lenalidomide

Dose limiting toxicity will be assessed weekly during the first cycle. The highest tolerated low dose will be determined as described in Section 5.1.

10.2.2 Safety

Data from all subjects who receive any protocol therapy will be included in the safety analyses. Subjects who entered the study and did not receive any protocol therapy and had this confirmed, will not be evaluated for safety. Adverse events will be recorded during the first 12 cycles, graded according to CTCAE v.4.0 whenever possible, and reported for the time points day 1 of cycle 2, 3, 6, 9, and 12 according to dose level treated. Additionally, worst overall toxicity will be reported at each publication.

10.2.3 Response

The response rate (RR= sCR+CR+VGPR+PR) and clinical benefit response rate (CBRR=sCR+CR+VGPR+MR) will be analyzed for the entire study group, and for the 16-22 patients treated at the HTLD / HTLD-CKD according to whether patients had relapsed or refractory disease at study entry. All response categories require two consecutive assessments but the time a response is first reached will be used to calculate response rates after 6 and 12 cycles. Additionally, best response will be reported. If one or more patients on this trial elect to undergo stem cell transplantation, response before mobilization will be reported.

10.2.4 Progression-free Survival

Progression-free survival will be calculated from the time of enrollment until the development of progressive disease or death from any cause, whichever occurs first. Patients who elect to undergo high dose chemotherapy followed by autologous stem cell transplantation will not be included in an analysis of progression-free survival.

10.2.5 Overall Survival

Overall survival will be calculated from the time of study entry until the date of death from any cause. Patients who elect to undergo high dose chemotherapy followed by autologous stem cell transplantation will not be included in an analysis of overall survival.

10.2.6 Autologous Stem Cell Transplantation

In patients who elect to undergo stem cell harvesting, high dose chemotherapy, and autologous peripheral blood stem cell transplantation, CD34+ yield and time to neutrophil and platelet engraftment (counted from the day of stem cell infusion = day 0) will be reported.

10.2.7 Pharmacodynamics

See under correlative science (5.5).

10.3 Statistical Methodology

The Kaplan-Meier method will be used to report progression free survival and overall survival for patients treated at the HTLD / HTLD-CKD and for the entire study cohort but excluding patients who elect to undergo high dose chemotherapy and autologous stem cell transplantation. For statistical methods pertaining to pharmacodynamic analyses please see under correlative science (5.5).

11 Regulatory Considerations

11.1 Institutional Review Board/Ethics Committee Approval

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/EC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The Investigator will be responsible for preparing documents for submission to the relevant IRB/EC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

Any amendments to the protocol after receipt of IRB/EC approval must be submitted by the Investigator to the IRB/EC for approval. The Investigator is also responsible for notifying the IRB/EC of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Any advertisements used to recruit subjects for the study must be reviewed and approved by the IRB/EC prior to use.

11.2 Informed Consent

The Investigator must obtain informed consent of a subject or his/her designee prior to any study related procedures as per GCPs as set forth in the CFR and ICH guidelines.

Documentation that informed consent occurred prior to the subject's entry into the study and the informed consent process should be recorded in the subject's source documents. The original consent form signed and dated by the subject and by the person consenting the subject prior to the subject's entry into the study, must be maintained in the Investigator's study files.

11.3 Subject Confidentiality

Celgene affirms the subject's right to protection against invasion of privacy. In compliance with United States federal regulations, Celgene requires the Investigator to permit representatives of Celgene Corporation and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study in accordance with local laws.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

11.4 Study Records Requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the protocol therapy, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; SAE reports, pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiches; photographic negatives, microfilm, or magnetic media; x-rays; subject files; and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study; documents regarding subject treatment and drug accountability; original signed informed consents, etc.]) be retained by the Investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The Investigator agrees to adhere to the document/records retention procedures by signing the protocol.

11.5 Premature Discontinuation of Study

The Principal Investigator, institution and Celgene have the right to discontinue this study at any time for reasonable medical or administrative reasons. Possible reasons for termination of the study could be but are not limited to:

- Unsatisfactory enrollment with respect to quantity or quality.
- Inaccurate or incomplete data collection.
- Falsification of records.

- Failure to adhere to the study protocol.

Any possible premature discontinuation would be documented adequately with reasons being stated, and information would have to be issued according to local requirements (e.g., IRB/EC, regulatory authorities, etc.).

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Appendices

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

Risks Associated with Pregnancy

The use of lenalidomide in pregnant females and nursing mothers has not been studied nor has the effect of the lenalidomide on human eggs and sperm. The risks to a fetus are not known. However, because lenalidomide is related to thalidomide, and thalidomide is known to cause severe birth defects, the following requirements must be observed.

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

Females of childbearing potential (FCBP)[†] 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) while participating in the study; and 3) for at least 28 days after discontinuation from the study. The two methods of reliable contraception must include one highly effective method (i.e. intrauterine device (IUD), hormonal [birth control pills, injections, or implants], tubal ligation, partner's vasectomy) and one additional effective (barrier) method (i.e. latex condom, diaphragm, cervical cap). FCBP must be referred to a qualified provider of contraceptive methods if needed.

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

Before starting lenalidomide:

Female Subjects:

- 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

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

must be performed within 24 hours prior to prescribing lenalidomide (prescriptions must be filled within 7 days). The subject may not receive lenalidomide until the Investigator has verified that the results of these pregnancy tests are negative.

Male Subjects:

- Must agree to use a latex condom during sexual contact with females of childbearing potential while participating in the study and for at least 28 days following discontinuation from the study even if he has undergone a successful vasectomy.

During study participation and for 28 days following discontinuation from the study:

All Subjects:

- If pregnancy or a positive pregnancy test does occur in a study subject or the partner of a male study subject during study participation, lenalidomide must be immediately discontinued.

Female Subjects:

- FCBP with regular or no menstrual cycles must agree to have pregnancy tests weekly for the first 28 days of study participation and then every 28 days while on study, at study discontinuation, and at day 28 following discontinuation from the study. If menstrual cycles are irregular, the pregnancy testing must occur weekly for the first 28 days and then every 14 days while on study, at study discontinuation, and at days 14 and 28 following discontinuation from the study.
- In addition to the required pregnancy testing, the Investigator must confirm with FCBP that she is continuing to use two reliable methods of birth control at each visit.
- Pregnancy testing and counseling must be performed if a subject misses her period or if her pregnancy test or her menstrual bleeding is abnormal. Lenalidomide treatment must be discontinued during this evaluation.

Male Subjects:

- Must agree to use a latex condom during sexual contact with females of childbearing potential while participating in the study and for at least 28 days following discontinuation from the study even if he has undergone a successful vasectomy.

Appendix II: ECOG Performance Status Scale

SCORE	DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Appendix III : NCI CTC Version 4.0

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

Appendix IV : Cockroft-Gault estimation of CrCl

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

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

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

Appendix V: Bone marrow aspirations RV0507

Date:

Patient study number:

Aspiration sequence

1. Two 20mL heparin syringes, each pre-filled with 2mL of heparin
2. One 10mL heparin syringe pre-filled with 1mL heparin

Distribution

1. Page 81077 for pick-up of the two 20mL syringes and this sheet
2. Send the 10mL syringe to pathology for cytogenetics and FISH (both Mayo send-out, sent by our pathology department)

Outside CCF:

Please either page or email Dale Grabowski when sample is shipped:

Email: grabowd@ccf.org

Paging: 216-444-4000 – 81077 followed by the # sign – enter call back number followed by # sign

Appendix VI: Blood draws for correlative science RV0507

Date:

Patient study number:

Obtain 10mL heparin (green top tube) and 10mL EDTA (two purple top tubes)

Page 81077 for sample pickup

Outside CCF:

Please either page or email Dale Grabowski when sample is shipped:

Email: grabowd@ccf.org

Paging: 216-444-4000 – 81077 followed by the # sign – enter call back number followed by # sign

Appendix VII: Lenalidomide and dexamethasone drug administration diary

Cycle # :

Date of day 1 of above cycle:

Patient study number:

Revlimid® (lenalidomide) should be taken from day 1-21 and dexamethasone on day 1, 8, 15, and 22. On the table "R" represents Revlimid® and "D" represents dexamethasone.

Please call you study nurse when you take the 21st dose of Revlimid®, to allow time for the next order and ensure you have Revlimid® on time for the next cycle.

Please cross out the R if you took Revlimid® and cross out the D if you took dexamethasone on the indicated days. Please leave blank if you did not take the indicated drugs on the respective day, irrespective of the reason, whether you were advised by your physician or simply forgot.

Day 1 R	**Day 2** RD	**Day 3** R	**Day 4** R	**Day 5** R	**Day 6** R	**Day 7** R
Day 8 R	**Day 9** RD	**Day 10** R	**Day 11** R	**Day 12** R	**Day 13** R	**Day 14** R
Day 15 R	**Day 16** RD	**Day 17** R	**Day 18** R	**Day 19** R	**Day 20** R	**Day 21** R (call)
Day 22	**Day 23** **D**	**Day 24**	**Day 25**	**Day 26**	**Day 27**	**Day 28**

Received by study nurse on: (date as MM/DD/YY)

Name of study nurse: Signature of study nurse: