NCT01178814

Protocol Title:

A prospective trial of Revlimid® in transfusion dependent patients with **non-del(5q)** low/Intermediate-1 risk Myelodysplastic Syndrome

Celgene Tracking #: RV-MDS-PI-388

DATE FINAL: 10-October-2008

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Amendment 3: 8-May-2010

Amendment 4: 26-October-2010

Amendment 5: 23-April-2013

Amendment 6: 29-October-2013

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator:	Mark Heaney, M.D., Ph.D.	
	Signature of Investigator	Date
	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 prospective trial of Revlimid® in transfusion dependent patients with **non-del(5q)** low/Intermediate-1 risk Myelodysplastic Syndrome	
DATE PROTOCOL FINAL:	26-OCT-2010
INDICATION:	MDS
STUDY PHASE:	

BACKGROUND AND RATIONALE: The myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by the presence of variable cytopenias in the presence of a hypercellular bone marrow (BM) (1). These disorders are comprised of several distinct syndromes (2), with varying incidence of transformation to acute myeloid leukemia (AML). No etiologic agent has been identified so far, and the disease appears to predominate in the elderly. Most of the clinical problems in the low risk group of patients are related to the consequences of cytopenias, while transformation to acute leukemia is the most serious threat in higher risk patients. A number of therapeutic strategies have been tried with limited and essentially palliative success. Treatment options range from supportive care to stem cell transplantation (SCT), with supportive care being the most commonly used therapy at this time (3-6). SCT is the only potentially curative therapy for this disease; unfortunately it is an option for <5% patients due to the advanced age of the majority of cases.

STUDY OBJECTIVES:

Primary:

To conduct a prospective trial testing the Revlimid® (lenalidomide) response signature in patients who have transfusion dependent, non-del(5q), low and intermediate-1 risk Myelodysplastic syndromes (MDS) in order to confirm the predictive value of the signature and to establish the boundaries of the z-score which can be used to pre-select patients in future clinical studies.

Secondary:

- The primary clinical efficacy parameter will be transfusion independence
- The secondary efficacy parameters will be the remaining criteria as defined by the IWG
- Time to disease progression
- Overall and progression-free survival

STUDY DESIGN:

In the present study, only the non-del(5q) transfusion dependent, low and intermediate-1 risk MDS patients will be treated with single agent Revlimid®. All patients will have their pre-therapy bone marrows studied by **gene expression microarray, Luminex bead assay, and real-time PCR.**

STUDY ENDPOINTS

Primary:

- To conduct a prospective trial testing the Revlimid® (lenalidomide) response signature in patients who have transfusion dependent, non-del(5q), low and intermediate-1 risk myelodysplastic syndromes (MDS) in order to confirm the predictive value of the signature and to establish the boundaries of the z-score which can be used to pre-select patients in future clinical studies.
- Assessment of pre-therapy expression profile associated with Revlimid® responsiveness

Secondary:

- Response will be evaluated **every 4 weeks during the first 12 weeks** of therapy for each of the 56 transfusion-dependent, non-del(5q) low/Int-1 risk MDS patients. Treatment will be stopped and the patient will be taken off the protocol in case of no response or stable disease by 12 weeks. International Working Group **(IWG 2006) criteria** will be used for response evaluation. The proportion of TI will be calculated and an exact 95% confidence interval of TI proportion will be estimated.
- The percent of major and minor responses in erythroid, platelets, neutrophils will also be calculated as the secondary endpoints.

STUDY DURATION: 12 weeks. Patients responding to treatment will continue on Revlimid® until disease progression or unmanageable toxicities.	**TOTAL SAMPLE SIZE:** 56
DOSING REGIMEN(S): The starting dose of REVLIMID® will be 10 mg days 1-28 with the first dose reduction going to 5 mg days 1-28 of a 28 day cycle. Therapy will cease if there is no response after 12 weeks	**STUDY DRUG SUPPLIES:** For study participants, Celgene Corporation will provide lenalidomide at no charge through the RevAssist® program.

2 Schedule of Study Assessments*

Procedure	Screening ≤ 28days from Baseline (First day study drug administration)	Cycle 1 Day 1[14]	Cycle 1 Day 8[14]	Cycle 1 Day 15[14]	Cycle 1 Day 22[14]	Cycle 2 Day 1[15]	Cycle 3[9] Day 1[15]	Cycle 4 and higher[12] Day 1	Discontinuation From Study Drug	30 Days (+/- 7 days) following last dose of study drug[6]	Follow-Up Phase Every 3 months
Informed Consent	X										
Reconfirm Eligibility		X									
Medical history (including signs/symptoms/diagnoses)	X										
Record prior medications, treatments	X										
Record prior anti-cancer therapies	X										
Physical examination, vital signs, weight	X					X	X	X[11,]	X	X	
Transfusion History	X	X[7]				X	X	X	X	X	
ECOG performance status	X					X	X	X[11]	X	X	
Bone marrow biopsy and aspirate[1]	X[1]								X[10]		
ECG	X								X		
Hematology[2]	X	X	X	X	X	X[2]	X[2]	X[2]	X[2]	X[2]	
Serum chemistry[3]	X	X		X		X[3]	X[3]	X[13]	X[3]	X[3]	
MDS-specific tests (Serum B12, erythropoietin)	X										
TSH[3]	X							X[3]	X[3]		
Pregnancy testing[4]	X[5]	X	X	X	X	X[5]	X[5]	X[5]	X[5]	X[5]	
Register patient into RevAssist® program	X										
Prescribe lenalidomide via RevAssist® [8]		X[8]					X[8]	X[8]	X[8]		
Record adverse events	X	X[7]	X		X	X	X	X[11]	X	X	
Record concomitant therapies/procedures		X[7]	X		X	X	X	X[11]	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 during Cycles 1 to 3, and ± 7 days of scheduled visits are permitted thereafter.
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.

[1]Include testing for expression assays. Within 6 weeks of starting therapy.
[2]Hgb, Hct, RBC indices: RBC count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, WBC w/diff, platelet count, at screening and then weekly during Cycles 1 to 3, and then monthly (i.e., at Day 1 of each cycle) thereafter.

[3] To include creatinine, BUN, total bilirubin, alkaline phosphatase, ALT, AST, LDH, total protein at screening, on Days 1 and 15 during Cycle 1 and on Day 1 of Cycles 2 and 3, and then every other cycle on Day 1(Cycles 4, 6, 8, 10, etc.), study drug discontinuation visit, and 30 days post-study drug discontinuation visit. Thyroid Stimulating Hormone (TSH) at screening, Cycle 4, and then every 16 weeks (Cycle 8, 12, 16, etc.), and at treatment discontinuation. T3 and T4 levels may be assessed as clinically indicated.

[4]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).

[5]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).

[6]This safety assessment will be done 30 days (+/- 7 days) following the last dose of study drug.

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

[8]Lenalidomide must be prescribed through and in compliance with Celgene's RevAssist® program. Prescriptions must be filled within 7 days. Any unused Revlimid® (lenalidomide) should be returned to the patient for disposition in accordance with the RevAssist® program.

[9]Subject will follow this schedule if determined to have a response after the first 12 weeks of treatment for subsequent visits.

[10]To document complete response, as needed.

[11]Performed every other cycle starting at Cycle 4 (i.e., Cycles 4, 6, 8, 10, etc.)

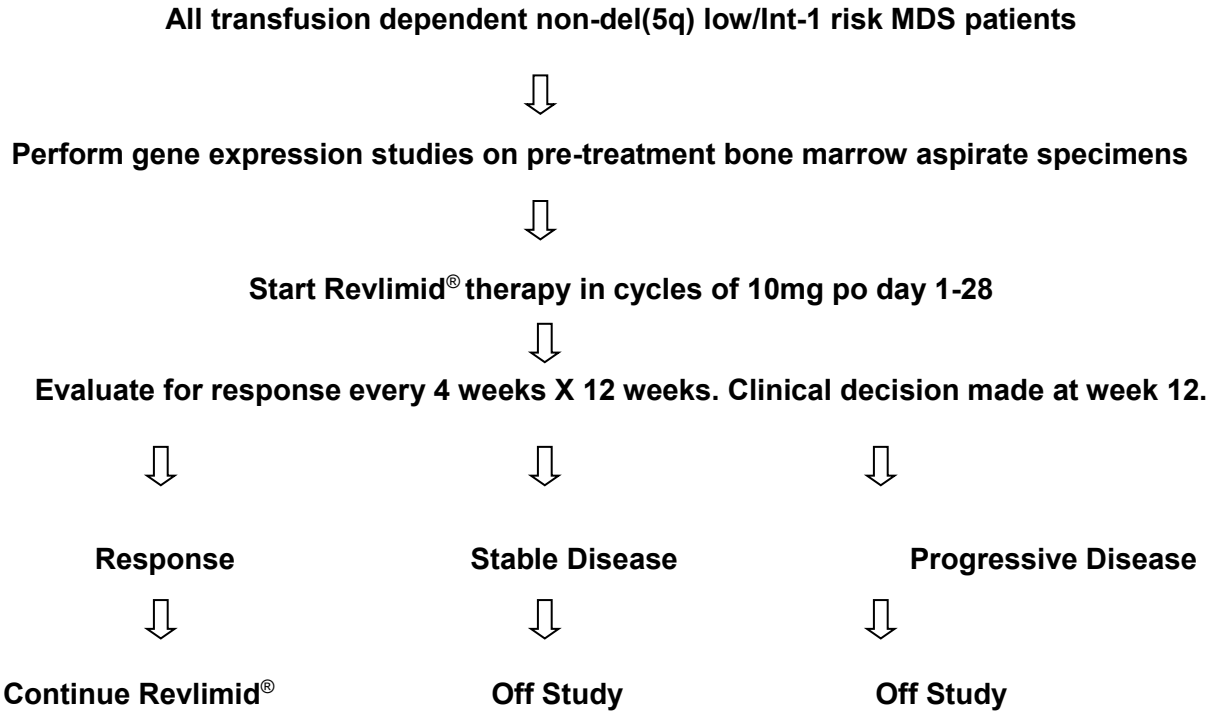
[12]After cycle 4, subjects may have Day 1 evaluations of odd number cycles performed by their local oncologist. Day 1 of even numbered cycles must be performed at Columbia University Medical Center.

[13]After cycle 4, perform only as clinically indicated

[14] Subjects who do not reside locally, may have this visit performed by their local oncologist

[15]Cycle 2 Day 1, Cycle 3 Day 1, and Cycle 4 Day 1 visits must be performed at Columbia University Medical Center. Other visits during Cycles 2 and 3 may be performed by subject's local oncologist.

3 Schema:



All responders should continue therapy until disease progression

4 Background and Rationale

4.1

The myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by the presence of variable cytopenias in the presence of a hypercellular bone marrow (BM) (1). These disorders are comprised of several distinct syndromes (2), with varying incidence of transformation to acute myeloid leukemia (AML). No etiologic agent has been identified so far, and the disease appears to predominate in the elderly. Most of the clinical problems in the low risk group of patients are related to the consequences of cytopenias, while transformation to acute leukemia is the most serious threat in higher risk patients. A number of therapeutic strategies have been tried with limited and essentially palliative success. Treatment options range from supportive care to stem cell transplantation (SCT), with supportive care being the most commonly used therapy at this time (3-6). SCT is the only potentially curative therapy for this

disease; unfortunately it is an option for <5% patients due to the advanced age of the majority of cases.

4.2

Recent biological insights show that cytopenias in MDS are most probably the result of excessive apoptosis of hematopoietic cells in the BM (7-10). This apoptosis appears to be both cytokine mediated as well as the result of an increased propensity of CD34+ BM stem cells to undergo premature programmed cell death (11). Biochemical pathways of apoptosis appear to involve both Fas/FasL and caspases (12-14). Our group was the first to demonstrate that an excessive apoptosis of hematopoietic cells in the bone marrow accounts for the quintessential variable cytopenias in MDS and that a suppression of apoptosis by anti-cytokine therapies can produce clinical improvement (15-18). We also showed that there are at least three major biochemical pathways leading to this excessive cell death. First, there appears to be a sequential activation of caspases 1 and 3 (19). Second, there is upregulation of the Fas/FasL system (20). Finally, there is an attenuation of Fap-1 pathway, which is normally responsible for initiating an anti-apoptosis effect (21). In addition to the increase in apoptosis of maturing hematopoietic cells, we have also demonstrated the propensity of early CD34+ progenitor cells to die prematurely (22).

4.3

The bone marrows of MDS patients also demonstrate markedly increased neo-angiogenesis and higher than normal levels of vascular endothelial growth factor (VEGF) (19). In MDS/AML myeloblasts and myelo-monocytic cells, production of VEGF along with co-expression of one or more cognate receptor tyrosine kinases has been demonstrated (23). In addition, increased microvessel density correlates with blast percentage. VEGF activity through promotion of adhesion via beta-1 and beta-2 integrins could actually explain the central clustering of ALIPs (abnormally localized immature precursors) in more advanced cases of MDS (24). Matrix metalloproteinases (MMP) produced by the malignant clone as well as the stroma appear to liberate membrane bound TNF and fas ligand which have been associated with the excessive intramedullary apoptosis of hematopoietic cells (25). In other words, both autocrine and paracrine angiogenic molecules potentiate self-renewal and promote neo-vascularity, inflammatory cytokine generation and ineffective hematopoiesis. Agents which antagonize the elaboration of angiogenic molecules thus potentially have considerable therapeutic value in MDS.

4.4

Finally, a more intrinsic immune defect may also exist in some MDS patients and suppression of T-cell function by using anti-thymocyte globulin (ATG) and cyclosporin is associated with improvement in the cytopenias (26, 27). Thalidomide was considered a potentially useful drug for MDS patients. It is both an immune-modulatory agent with anti-cytokine activities (28-32), and anti-angiogenic effects (33-35). Based on this rationale, a pilot study was conducted to test the efficacy of thalidomide in improving the ineffective hematopoiesis seen in patients with myelodysplastic syndromes. Thalidomide was administered to 83 patients with myelodysplastic syndrome (MDS) starting at 100mg po daily and increasing to 400mg as tolerated. Thirty-two patients stopped therapy before 12 weeks (minimum period for response evaluation), and 51 have completed 12 weeks of therapy. International Working Group (IWG) response criteria for MDS were used to evaluate responses. Intent-to-treat (ITT) analysis classified all off study patients as non-responders. Off-study patients belonged to a higher risk category (p=0.002) and had a higher percentage of blasts in their pre-therapy bone marrows (BM) than patients who completed 12 weeks of therapy (p=0.003). No cytogenetic or complete responses were seen, but 16 showed hematologic improvement, ten previously transfusion dependent patients becoming transfusion independent. Responders had lower pre-therapy blasts (p=0.016), lower duration of pre-therapy platelet transfusions (p=0.013) and higher pre-therapy platelets (p=0.003). Among responders, 9 had refractory anemia (RA), 5 had RA with ringed sideroblasts, and 2 had RA with excess blasts. By ITT analysis, 19% patients responded (16/83), and by analyzing only evaluable patients, 31% (16/51) responded. We concluded from this pilot study that thalidomide, as a single agent, is effective in improving cytopenias of some MDS patients, especially those who present without excess blasts

4.5

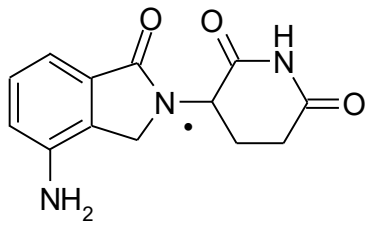
Celgene has now synthesized several analogs of thalidomide that appear to be more potent and less toxic than the parent drug. Two of these (Revlimid® and CC-1088) have been undergoing clinical trials in hematologic malignancies, and the background for one of these (Revlimid®) will now be provided in detail. During the development of thalidomide analogs such as Revlimid®, chemical modifications of the thalidomide template were made in an effort to specifically optimize the anti-TNF-α activity. Two discrete sub-structures of the thalidomide molecule were altered, namely the phthaloyl and glutarimide portions of the molecule. Both families of compounds were tested for TNF-α inhibition and further structural modifications were made to optimize this activity. The primary hydrolysis products of thalidomide were also used as subsidiary templates even

though they showed poor potency as TNF-α modulators in their unmodified form. This strategy led to the development of two primary families of compounds showing enhanced TNF-α inhibition properties: substituted phthaloyl analogs with intact glutarimide functionality and molecules which retain the basic phthaloyl moiety, but have substituted β-amino acid functionality in place of the glutarimide ring of thalidomide, such as Revlimid®. The finding that these varied structural modifications could yield the same enhanced potency in TNF-α inhibition suggests the presence of two different pharmacophores that most likely have different pharmacological targets, but are both capable of TNF-α downregulation.

Revlimid® is a thalidomide analog. In vitro studies have shown that REVLIMID® is more potent than thalidomide in inhibiting TNF-α production and multiple myeloma cell proliferation. Furthermore, in preliminary non-clinical and clinical studies conducted to-date, Revlimid® appears to lack the sedative and teratogenic activity of thalidomide.

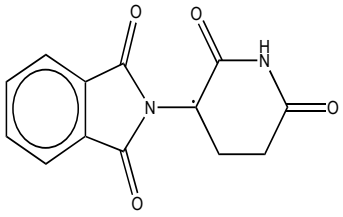
The structures of Revlimid® and thalidomide are illustrated below.

REVLIMID®



MW=259.26

Thalidomide



MW=258.2

4.6

Lenalidomide is a proprietary IMiD™ compound of Celgene Corporation. IMiD™ compounds have both immunomodulatory and anti-angiogenic properties which could confer antitumor and antimetastatic effects. Lenalidomide has been demonstrated to possess anti-angiogenic activity through inhibition of bFGF, VEGF and TNF-alpha induced endothelial cell migration, due at least in part to inhibition of Akt phosphorylation response to bFGF.[1] 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.[37] Upregulation of T cell derived IL-2 production is achieved at least in part through increased AP-1 activity. [38]

Although the exact antitumor mechanism of action of lenalidomide is unknown, a number of mechanisms are postulated to be responsible for lenalidomide's activity against multiple myeloma. Lenalidomide has been shown to increase T cell proliferation, which leads to an increase in IL-2 and IFN-gamma secretion. The increased level of these circulating cytokines augment natural killer cell number and function, and enhance natural killer cell activity to yield an increase in multiple myeloma cell lysis [(39)]. 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 [(40)].

4.7

Clinical experience in myelodysplastic syndromes (MDS) with lenalidomide

Phase I/II study of REVLIMID® in transfusion dependent MDS patients: At the University of Arizona Cancer Center, a pilot study was conducted in transfusion dependent MDS patients by List et al (41) to show that the most tolerable dose/schedule was 10mg po q day X 3 weeks on and 1 week off in repeated cycles (syncopated schedule). Among 36 evaluable patients treated with Revlimid® in a safety and efficacy trial, 24 (67%) experienced an erythroid response according to International Working Group (IWG) criteria, with 21 patients experiencing sustained transfusion independence. Response rate varied by cytogenetic pattern and was highest among patients with a chromosome 5q(31.1) deletion (91%) compared to a normal karyotype (68%) or other chromosome abnormality (17%) [*P* = 0.009]. Similarly, patients with lower risk International Prognostic Scoring System (IPSS) categories experienced a higher frequency of erythroid response compared to patients with higher risk disease (72% vs 25%). Unlike cytokine therapy, cytogenetic remissions were common, with 65% of informative patients experiencing 50% or greater reduction in abnormal metaphases, including 10 (57%) complete cytogenetic remissions. Major cytogenetic response occurred most commonly in patients with a chromosome 5(q31.1) interstitial deletion (9 of 11 patients). Perhaps of greater importance, responses appear durable. After a median follow-up of 81 weeks, median duration of transfusion-independence had not been reached (48+; range, 13+ to > 101 weeks) with median sustained hemoglobin of 13.2 g/dL (range, 11.5–15.8 g/dL).

Multi-institutional experience (MDS 002 and 003 trials):

Revlimid® in del(5q) patients: The List experience described above was followed by a multicenter Phase II study (42) evaluating the efficacy of REVLIMID® in transfusion dependent (TD)-MDS pts with del5q31. A multicenter Phase II trial (MDS 003) for transfusion dependent low to Int-1 del(5q) patients

showed remarkable results. Among 148 pts with del(5q31), 111 had this as an isolated cytogenetic abnormality, and 37 patients had additional chromosomal changes. The median age was 71 years (range, 37-95) with 66% females and 146 patients had a confirmed transfusion dependence. In an intent-to-treat analysis, transfusion independence was achieved in 99 patients (67%) with a median 5.3/dl hemoglobin increase (range: 1.1-11.4/dl). The median time to response was 4 weeks (range: 3.6-5.3 weeks). The transfusion independence rate was greater in patients with isolated del(5q) abnormality (69% versus 49%; p=0.003). Cytogenetic response (≥50 decrease in abnormal metaphases) was achieved in 70% of transfusion independent patients with 44% achieving a complete cytogenetic remission. Pathologic complete remission was documented in 32/110 (29%) evaluable patients. After a median follow-up of 9.3 months (range: 4.2 to 14.8+ months), the median response duration is not reached with only 10 responders (9%) failing.

Revlimid® in non-del(5q) patients: The results of Revlimid® treatment of transfusion dependant low to Int-1 patients without the del(5q) has also shown that Revlimid® is an effective treatment option (43). A total of 214 patients were treated with Revlimid® (MDS 002). Overall, 56 (26%) of 214 enrolled patients achieved transfusion independence. Median time to response was 5 weeks, median duration of response was 41.0 weeks (range, 8.0-136.4), and median Hgb increase achieved was 3.2 g/dL (range, 1.0-9.8). An additional 36 patients experienced a ≥50% reduction in RBC transfusions (hematological improvement in 92 [43%] patients). Of 47 (22%) patients who had an abnormal karyotype at baseline, 9 (19%) patients achieved a cytogenetic response (4 complete). These early findings indicate that Revlimid® has significant erythroid activity in patients with low- to int-1-risk MDS.

4.8

Results of the expression analysis suggesting a signature profile associated with Revlimid® responsiveness (44):

Clinical response to a specific agent is one way of unifying seemingly unrelated subsets of MDS patients who carry a common therapeutic target. The most important observation from the Revlimid® trials is that the majority of del(5q) patients as well as a small but definite subset of non-del(5q) patients experience equally good hematologic responses. The precise mechanism of response remains unknown. Thalidomide is thought to be effective because of its anti-TNF, anti-angiogenic and immune-modulatory effects. Revlimid® shares these signature thalidomide activities, except with greater potency (and less toxicity). This would suggest that MDS patients are responding to Revlimid® because of its broad spectrum of activity both on the clone of cells as well as the bone marrow microenvironment. The exceptionally high response rate in the del(5q) patients on the other hand suggests a more specific target. Supporting this specificity is

the observation that some of the non-del(5q) patients have equally striking responses as the del(5q) patients. We used these clinical observations as a means of defining an expression profile associated with Revlimid® responsiveness. Our hypothesis was that the responders share a common genetic abnormality/molecular signature.

Microarray technology offers a chance to explore disease pathology in greater detail, yet only a limited number of these studies have been reported so far in MDS, mainly due to the marrow cellular heterogeneity. An inherent problem in MDS is that a single cell type is not rapidly proliferating and becoming the dominant cell population in the marrow. Several strategies have been tried to overcome this challenge, yet no approach appears to be perfectly suited for expression profiling in MDS. While some investigators based their work on selecting cell types (CD34+, AC133+, neutrophils), IPSS (high versus low risk) or cytogenetics (monosomy 7 versus trisomy 8), our hypothesis is that differences between individual marrow samples may be minimized by using clinical response as a means of identifying uniform groups of patients. For example, responders to Revlimid® whether they have the del(5q) abnormality or not, may share some common genetic and/or expression profiles which makes them sensitive to the drug. We propose that a bedside to bench approach as evidenced by response to a given drug can serve as the initial guideline in defining patient subgroups with relatively similar biology and natural history of the disease. A total of 51 patients were enrolled by our group, with 28 participating in the multi-center MDS-002 and 23 in MDS-003 trials. Under an independent IRB protocol for Tissue Repository, we collected and stored BM aspirates from the patients enrolled in these two studies. These samples have been used for the following expression studies (35). Both DNA and RNA were isolated from the pre-therapy bone marrow cells of 33 MDS patients that were enrolled in the Revlimid® protocols. The RNA was amplified and used to generate biotin labeled anti-sense DNA that was fragmented and hybridized to Affymetrix chips.

Initial microarray data was available on 8 responders and 8 non-responders who did <u>not</u> have the del(5q). (the "training" set). There was a very distinct pattern of gene expression that separated the responders from non-responders. Equally exciting was the observation that among the 100 ranked genes of interest in the heat-map were groups of genes that have previously been shown to be associated with MDS pathology. It seems that a number of genes involved in erythropoiesis are clearly under-expressed in patients, including all of the responders. For example HBA1 (alpha1 globin), ankyrin1 erythrocytic, dematin or EPB 49 (erythrocyte membrane protein band 49), EPB3 (red cell anion exchanger), ferrochelatase, hemoglobin alpha 2/DEF (human alpha globin gene with flanks), human sickle cell beta globin, Kidd blood group (SLC14A1) are genes that are under-expressed in the cells of the responders. This expression signature can be used for predicting which patients have a chance to respond versus those who definitely will not benefit. However, the DNA microarray technology cannot be easily adapted for prospective clinical testing in a timely fashion. Two simpler assays to measure gene expression currently used by

clinical labs are quantitative real time PCR and the Luminex bead hybridization assay. The relative ease of use and cost of these assays will allow them to be easily implemented in a clinical setting in the future. This bead based technology is being rapidly developed for clinical, research and diagnostic purposes. It is less complex than microarray analysis, and can analyze the expression of up to a hundred genes in a single well, making it easier to use in the clinical diagnostic setting. The results with the Luminex assay gave essentially the same results as the Affymetrix chips, and better results than the TaqMan. Identical methodology for analysis of Affymetrix, TaqMan qPCR and Luminex data were used. Thus Luminex **may** be adaptable to the broader clinical setting.

Validation of the Microarray Profiles (the "test set"): An independent set of 26 Revlimid® treated patients enrolled in the national trials patients was used to validate the microarray data (35). A predictive analysis of the "test" set that including patients with del(5q) and those without del(5q) with known clinical outcomes, showed that the expression profile on pre-treatment marrow aspirates may be a useful tool in the clinical management of MDS patients. The ability to avoid potentially toxic side effects in patients unlikely to respond to Revlimid® would be of great benefit to the patients.

4.8.1 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.

4.9 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.

Tumor flare reaction (TFR) has been reported frequently in CLL patients treated with lenalidomide. Tumor lysis syndrome (TLS) has been reported in CLL patients treated with lenalidomide. Precautions must be taken to prevent TLS including proper selection of patients with regard to renal function, correction of electrolyte abnormalities, and TLS prophylaxis and monitoring. Lenalidomide has been shown to increase the level of digoxin in the blood in some patients.

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

4.10 Rational for Treatment in this Setting

Selection of patients: The first Revlimid® study reported by List et al included all transfusion dependent MDS patients, but given the early experience which showed a better response in del(5q) patients and those with lower risk disease, more patients belonging to this category were entered on the trial. The subsequent multi-center studies were focused entirely on the low/Int-1 patients who were transfusion dependent. In the present study, we would like to recapitulate the initial study by only treating the transfusion dependent, low and intermediate-1 risk MDS patients without deletion (5q). Since the predictive assay is most applicable for the non-del(5q) patients, and to avoid confusion resulting from an admixture of patients with del(5q) for whom the drug is already FDA approved, we have elected to restrict this clinical trial to only the non-del(5q) patients.

Current Protocol: In the present study, only the non-del(5q) transfusion dependent, low and intermediate-1 risk MDS patients will be treated with single agent Revlimid®. All patients will have their pre-therapy bone marrows studied by gene expression **microarray, Luminex bead assay, and real-time PCR.**

5 Study Objectives and Endpoints

5.1 Objectives

5.1.1 Primary Objective

To conduct a prospective trial testing the Revlimid® (lenalidomide) response signature in patients who have transfusion dependent, non-del(5q), low and intermediate-1 risk myelodysplastic syndromes (MDS) in order to confirm the predictive value of the signature and to establish the boundaries of the z-score which can be used to pre-select patients in future clinical studies.

5.1.2 Secondary Objectives

- The primary clinical efficacy parameter for these patients will be transfusion independence.
- The secondary efficacy parameters for the study will be the remaining criteria as defined by the IWG. The following parameters also will be assessed:
    - Time to disease progression
    - Overall and progression-free survival

5.2 Endpoints

5.2.1 Primary Endpoint

- Assessment of pre-therapy gene expression profile associated with Revlimid® responsiveness.

5.2.2 Secondary Endpoints

- Response will be evaluated **every 4 weeks during the first 12 weeks** of therapy for each of the 56 transfusion-dependent, non-del(5q) low/Int-1 risk MDS patients. Treatment will be stopped and the patient will be taken off the protocol in case of no response or stable disease by 12 weeks. International Working Group **(IWG 2006) criteria** will be used for response evaluation. The proportion of TI will be calculated and an exact 95% confidence interval of TI proportion will be estimated.
- The percent of major and minor responses in erythroid, platelets, neutrophils will also be calculated as the secondary endpoints.

6 Investigational Plan

6.1 Overall design

6.1.1 PRE, INTRA, and POST-THERAPY STUDIES:

- Bone marrow (BM) aspirate and biopsy
    - All patients must have a BM aspirate and biopsy examination performed within 6 weeks before starting therapy. Routine diagnostic studies include morphology, histochemistry, flow cytometry, immuno-phenotyping, karyotyping, fluorescence in situ hybridization (FISH), cytogenetics. A marrow sample will be saved for the gene

expression microarray, Luminex bead assay, and real-time PCR.
    - A repeat bone marrow aspirate and biopsy will be done at discontinuation from study drug to document complete response, if needed.

- **Medical/Medication History:** Obtained at screening. Medical history to include diagnoses and any signs/symptoms within prior 28 days. Medication history within prior 28 days and all prior treatment for MDS.

- **Physical Exam:** Screening, monthly at Day 1 of Cycles 2, 3, and 4 and at Day 1 of every other cycle thereafter, and at discontinuation from study drug, and 30 days (+/- 7 days) following the last dose of study drug.

- **ECOG Status:** At screening, Day 1 of Cycles 2, 3, and 4, and every other cycle thereafter.

- **Transfusion History:** Screening, monthly at Day 1 of Cycles 1, 2, 3, each Day 1 study visit thereafter, at discontinuation from study drug, and 30 days (+/- 7 days) following the last dose of study drug.

- **Hematology** (hemoglobin, hematocrit, RBC indices: RBC count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration; WBC count with differential; and platelet count) at screening, weekly during cycles 1 to 3, and then monthly (i.e., at Day 1 of each cycle) thereafter, at discontinuation from study drug, and 30 days (+/- 7 days) following the last dose of study drug.

- **Clinical Chemistry** (creatinine, BUN, total bilirubin, alkaline phosphatase, ALT, AST, LDH, total protein) at screening, on Cycle 1 Day 1 and Cycle 1 Day 15 and on Day 1 of Cycles 2, 3, 4 and then as clinically indicated after cycle 4, at discontinuation from study drug, and 30 days (+/- 7 days) following the last dose of study drug. Thyroid stimulating hormone (TSH) will be done at screening Cycle 4 and then every 16 weeks (i.e., Cycles 8, 11, 16, etc.), and at discontinuation from study drug. T3 and T4 levels may be assessed as clinically indicated.

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

- Pregnancy test for women: 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. 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).

- 12-Lead EKG will be performed prior to therapy.

- **MDS-Specific Tests:** at baseline and as clinically appropriate thereafter (serum B_{12} and erythropoietin). Optional depending on clinical situation: serum iron, total iron-binding capacity (TIBC), ferritin; flow cytometry for CD34.

- **Adverse Events, Toxicity, Concomitant Medications/Procedures:** at each study visits per Schedule of Study Assessments (Section 2), assessed by NCI Common Terminology Criteria for Adverse Events, Ver.3 Note: After Cycle 4, these assessments will be obtained every 8 weeks.

- Patient blood counts, chemistries, transfusion requirements will be reviewed at 4-week intervals with decision for continued therapy at week 12. At the discretion of the Principal Investigator, therapy will be discontinued in the event of a serious adverse event.

- Transfusion criteria: Packed red blood cells (PRBC) will be transfused in case hemoglobin falls below 8.0 Gm/dL or at a higher level in a symptomatic patient. Platelet transfusions will be given only if the patient is showing evidence of bleeding or if at risk of bleeding.

6.1.1.2 Patient Entry Procedure

†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).

- Once an eligible candidate has been identified for the study, the Research Study Specialist should be called to bring the IRB approved informed consent form and HIPAA Authorization to the Investigator and potential participant.

- The Investigator will consent the participant and verify the eligibility criteria of the candidate. No study related procedures will be performed prior to the participant signing the consent form.

- Anticoagulated bone marrow and peripheral blood specimens and bone marrow biopsies will be placed on ice and sent to Dr. Naomi Galili's lab for gene expression assays required for this study.

6.1.1.3

The starting dose of REVLIMID® will be 10 mg days 1-28 with the first dose reduction going to 5 mg days 1-28 of a 28 day cycle. Therapy will cease if there is no response after 12 weeks. Patients responding to treatment will continue on Revlimid® until disease progression or unmanageable toxicities.

- Based on previous trials in del 5q MDS patient one hundred percent of patients reported at least one adverse event; 89% (131/148) experienced at least one grade 3/4 adverse event. Eighty percent required a dose delay and/or reduction for toxicity during the study. Thirty-four percent required a second dose delay/reduction. Dose adjustment recommendations for neutropenia and thrombocytopenia are provided in product labeling. Patients should have complete blood counts monitored weekly for the initial 12 weeks and at least monthly thereafter. Thromboembolic events were rare in the Revlimid® studies in MDS patients with deletion 5q cytogenetic abnormalities. However, in recently reported trials conducted in multiple myeloma, a significantly increased risk of deep venous thrombosis and pulmonary embolism was observed in patients treated with Revlimid® combination therapy. An elevated risk of thrombosis has also been reported when erythropoietin is used during Revlimid® treatment. The role of prophylactic anticoagulation and/or antiplatelet therapy with Revlimid® has not been adequately assessed. Any prophylactic measures should be prescribed after a careful assessment of individual risk factors.
- Patients with serum creatinine above 2.5 mg/dl were excluded from the studies. Because Revlimid® is predominately excreted by the kidney, renal function should be carefully monitored.
- Females should be advised to avoid pregnancy while taking Revlimid®. Revlimid is an analogue of thalidomide, a known human teratogen that causes severe human birth defects. Additional

reproductive toxicity studies will be performed to assess any potential Revlimid® teratogenicity. Only prescribers and pharmacists registered under the RevAssist® program can prescribe or dispense Revlimid®. Patients must agree to comply with the RevAssist® program requirements.

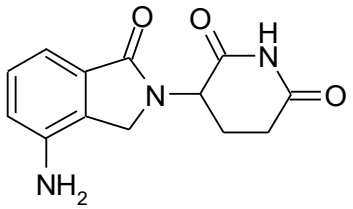
- Clinical toxicity has been infrequent with administration of REVLIMID® (see section 4.9). Cases of neutropenia and thrombocytopenia have been reported in the phase I/II experience in the majority of patients and appear to be dose and/or duration dependent. MDS patients with existing neutropenia and/or thrombocytopenia should be monitored closely during treatment for potential exacerbation that may merit a treatment hiatus and dose reduction as described below.

6.1.2 Study Drug

6.1.2.1 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.

6.1.2.2 CLINICAL PHARMACOLOGY

Mechanism of Action:

The mechanism of action of lenalidomide remains to be fully characterized. Lenalidomide possesses immunomodulatory and antiangiogenic properties. Lenalidomide inhibited the secretion of pro-inflammatory cytokines and increased

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

6.1.2.3 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 sampling in myelodysplastic syndrome (MDS) patients was not performed. In multiple myeloma patients maximum plasma concentrations occurred between 0.5 and 4.0 hours post-dose both on Days 1 and 28. AUC and Cmax values increase proportionally with dose following single and multiple doses. Exposure (AUC) in multiple myeloma patients is 57% higher than in healthy male volunteers.

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.

6.1.2.4 Supplier(s)

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

6.1.2.5 Dosage form

Lenalidomide will be supplied as capsules for oral administration.

6.1.2.6 Packaging

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

6.1.2.7 Storage

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

6.1.2.8 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. 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**.

6.2 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 56 of subjects with transfusion-dependent, **non-del(5q)** low/Intermediate-1 risk Myelodysplastic Syndrome will be screened for enrollment and must meet the eligibility criteria below.

6.2.1 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 ≥ 21 years at the time of signing the informed consent form.
3. Able to adhere to the study visit schedule and other protocol requirements.
4. A confirmed diagnosis (using standard FAB criteria) of a myelodysplastic syndrome with low/Int-1 risk and with a non-del(5q) karyotype must be established.
5. Patients must have transfusion dependence (at least 2 units within 8 weeks prior to starting therapy).
6. All transfusion dependent **non-**del(5q) low/Int-1 risk patients will be eligible for treatment with Revlimid®
7. Newly diagnosed as well as previously treated patients will be eligible
8. Patients with primary de novo or secondary MDS will be eligible
9. All previous cancer therapy, including radiation, hormonal therapy and surgery, must have been discontinued at least 4 weeks prior to treatment in this study.
10. ECOG performance status of 0-2 at study entry (see Appendix II).
11. Laboratory test results within these ranges:
    - Absolute neutrophil count > 250/uL
    - Platelet count > 30,000/uL
    - Serum creatinine ≤ 2.0 mg/dL
    - Total bilirubin ≤ 1.5 mg/dL
    - AST (SGOT) and ALT (SGPT) ≤ 3 x ULN
    - BUN ≤ 2 x ULN
12. Disease free of prior malignancies for ≥ 2 years with exception of currently treated basal cell, squamous cell carcinoma of the skin, or carcinoma “in situ” of the cervix or breast.
13. All study participants must be registered into the mandatory RevAssist® program, and be willing and able to comply with the requirements of RevAssist®.

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

6.2.2 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).
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. Any clinically significant cardiac disease, including congestive heart failure
5. Liver function studies including SGOT/SGPT > 3 x ULN
6. Clinically significant renal disease.
7. Any previous chemotherapy, hematopoietic growth factors, erythropoietin, or cytokines within 4 weeks of starting treatment. Note: prior therapy with G-CSF within 4 weeks is allowed.
8. Use of any other experimental drug or therapy within 28 days of baseline.
9. Known hypersensitivity to thalidomide.
10. The development of erythema nodosum if characterized by a desquamating rash while taking thalidomide or similar drugs.

† 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).

11. Any prior use of lenalidomide. (Patients with prior therapy with thalidomide will be eligible as long as at least 4 weeks have elapsed between end of the drug and accrual on the present trial)
12. Concurrent use of other anti-cancer agents or treatments.
13. Known positive for HIV or infectious hepatitis, type B or C.
14. Life expectancy < 3 months

6.3 Visit schedule and assessments

Screening Assessments and all on study scheduled visits and assessments are outlined in Section 2 Table of Study Assessments, and Section 6.1.1.

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 30 days (±7 days) post the last dose of study drug.

6.4 Drug Administration

6.4.1 Treatment assignments

Transfusion-dependent, **non-del(5q)** low/intermediate-1 risk myelodysplastic syndrome patients will be eligible for this study.

6.4.2 Dosing regimen

The planned dose of lenalidomide for investigation is 10 mg/day, orally on days 1-28 for a 28 day cycle. Dosing will be in the morning 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.

Subjects experiencing adverse events may need study treatment modifications (See section 6.5).

6.4.3 Special Handling Instructions

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

6.4.4 Record of administration

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

6.5 Dose Continuation, Modification and *Interruption*

Patients will be evaluated for adverse events at each visit with the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) used as a guide to the grading of severity (See Appendix III). Dosing should be modified per the dose reduction steps in Section 6.5.1 for toxicities as outlined Section 6.5.2 and in the dose modification table in Section 6.5.3 (Table 2).

6.5.1 Dose Reduction Steps

Table 1: LENALIDOMIDE Dose Reduction Steps

	10 mg daily on Days 1-28 every 28 days
Starting Dose	
Dose Level – 1	5 mg daily on Days 1-28 every 28 days
Dose Level – 2	5 mg every other day on Days 1-28 every 28 days
Dose Level – 3	5 mg q 3 days on Days 1-28 every 28 days

6.5.2 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 ≥ 250/ul or at least half of baseline
- The platelet count is ≥ 30,000/ul or at least half of baseline
- Any drug-related rash or neuropathy that may have occurred has resolved to ≤ grade 1 severity;
- Any other drug-related adverse events that may have occurred have resolved to ≤ grade 2 severity.

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. Resolution of the toxicity must occur within 4 weeks or the patient will be removed from study. If lenalidomide dosing was halted during the previous cycle and was restarted with a one-level dose reduction without requiring an interruption for the remainder of the cycle, then that reduced dose level will be initiated on Day 1 of the new cycle. **If lenalidomide 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.

6.5.3 Instructions for dose modifications or interruption during a cycle.

Table 2: Dose Modifications	
NCI CTC Toxicity Grade (unless otherwise noted)	**Dose Modification Instructions**
<u>If baseline ANC ≥ 1000/ mm³:</u> Grade 3 neutropenia associated with fever (temperature ≥ 38.5º C) or Grade 4 neutropenia	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If neutropenia has resolved to ≤ grade 2 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. If neutropenia is the only toxicity for which a dose reduction is required, G-CSF may be used and the lenalidomide dose maintained
<u>If baseline ANC < 1000/ mm³:</u> Grade 3 neutropenia associated with fever (temperature ≥ 38.5º C) or ANC < 50% of baseline	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If ANC has recovered to **≥**50% of baseline and fever has resolved prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. If neutropenia is the only toxicity for which a dose reduction is required, G-CSF may be used and the lenalidomide dose maintained
<u>If baseline platelet count ≥ 50,000/mm³:</u> Thrombocytopenia ≥Grade 3 (platelet count < 50,000/mm³)	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If thrombocytopenia has recovered to ≤ grade 2 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.
<u>If baseline platelet count < 50,000/mm³:</u> Platelet count < 50% of baseline	• Hold (interrupt) lenalidomide dose. • Follow CBC weekly. • If platelet count resolves to **≥** 50% of baseline prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up.

Table 2: Dose Modifications	
NCI CTC Toxicity Grade (unless otherwise noted)	**Dose Modification Instructions**
Non-blistering rash **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose. Follow weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level at the start of the next cycle. Omitted doses are not made up. • If Grade 4, discontinue lenalidomide. Remove patient from study.
Desquamating (blistering) rash- any Grade	• Discontinue lenalidomide. Remove patient from study.
Neuropathy **Grade 3** **Grade 4**	• If Grade 3, hold (interrupt) lenalidomide dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 1 prior to Day 21, restart lenalidomide at next lower dose level and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle and reduce the dose of lenalidomide by 1 dose level 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 Consideration (Section 6.6.1.2)
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 dose level.
other non-hematologic toxicity ≥ Grade 3	• Hold (interrupt) lenalidomide dose. Follow at least weekly. • If the toxicity resolves to ≤ grade 2 prior to Day 21, restart lenalidomide and continue through the scheduled end of the cycle. Otherwise, omit for remainder of cycle. Omitted doses are not made up. For toxicity attributed to lenalidomide, reduce the lenalidomide dose by 1 dose level when restarting lenalidomide.

6.5.4 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. Subjects will be asked to bring any unused study drug and empty study drug containers to the research center at their next visit. Research personnel will count and record the number of used and unused study drug capsules at each visit and reconcile with the patient diary.

Any unused Revlimid® (lenalidomide) should be returned to the patient for disposition in accordance with the RevAssist® program.

6.6 Concomitant therapy

6.6.1 Recommended concomitant therapy

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

6.6.1.2 Anticoagulation Consideration

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.

Consideration should be given to the requirement or optional use of aspirin (81 or 325 mg) or some other form of prophylaxis as deemed appropriate. Low molecular weight heparin may be utilized in patients that are intolerant to ASA. Coumadin should be used with caution and close monitoring of INR.

6.6.2 Prohibited concomitant therapy

G-CSF is permitted for neutropenic fever and/or when clinically indicated.

Concomitant use of sargramostim (GM-CSF), other anti-cancer therapies, including radiation, thalidomide, or other investigational agents is not permitted while subjects are receiving study drug during the treatment phase of the study.

6.7 Discontinuation of Study Treatment

Treatment will continue for 12 weeks of treatment or the occurrence of any of the following events.

- Disease progression as defined by IWG
- 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.
- Toxicity that delays the start of the next cycle by more than 4 weeks.
- Discontinuation of lenalidomide for any reason.
- Major violation of the study protocol.
- Withdrawal of consent

- Lost to follow up
- Death
- Suspected pregnancy

6.8 Follow-Up

Subjects who discontinue treatment for any reason will have a discontinuation from study drug visit and a 30 days (± 7 days) post-drug discontinuation visit evaluations per the Schedule of Assessments, Section 2. In addition, off study follow-up evaluations for anti-cancer treatments and survival will be obtained every 3 months per the Schedule of Assessments, Section 2.

7 Adverse events

7.1 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]
- Suspected positive 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.

7.2 Adverse Drug Reaction Reporting

Toxicity will be scored using CTCAE Version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE Version 3.0 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 3.0. All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to 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.

Severity Ratings:

The investigator will evaluate the severity of each adverse experience using the following definitions:

- **Mild:** event may be noticeable to the patient, does not influence daily activities, usually does not require intervention.
- **Moderate:** event may be of sufficient severity to make subject uncomfortable; performance of daily activities may be influenced; intervention may be needed.
- **Severe:** event may cause severe discomfort; usually interferes with daily activities; subject may not be able to continue in the study; treatment or other intervention usually needed.

Relationship to Study Drug:

- **Probably Related**

  An adverse event which might be due to the use of the drug. The relationship in time is suggestive. An alternative explanation is less likely, e.g. concomitant drug(s), concurrent diseases(s).

- **Possibly Related**

  An adverse event which might be due to the use of the drug. The relationship in time is reasonable; therefore, a casual relationship cannot be excluded. An alternative explanation is inconclusive, e.g. concomitant drug(s), concurrent disease(s).

- **Not Related**

An adverse event which is judged to be clearly due only to extraneous causes (disease, environment, etc.). The cause must be noted on the AE CRF.

7.2.1 Pregnancies

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

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

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

Any suspected fetal exposure to lenalidomide must be reported to Celgene within 24 hours of being made aware of the event. The patient should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling.

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

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

7.2.2 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

7.3 Investigator Reporting Responsibilities

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

IND Annual Reports

If the FDA has granted an IND number, it is a requirement of 21 CFR 312.33, that an annual report is provided to the FDA within 60-days of the IND anniversary date. 21 CRF 312.33 provides the data elements that are to be submitted in the report. The Annual Report should be filed in the study's Regulatory Binder, and a copy provided to Celgene Corporation as a supporter of this study as follows.

Celgene Corporation
Attn: Medical Development
86 Morris Avenue
Summit, NJ 07901
Tel: (908) 673-9000

All adverse experience reports must include the patient number, age, sex, weight, severity of reaction (mild, moderate, severe), relationship to study drug (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.

7.3.1 Expedited reporting by investigator to Celgene

Serious adverse events (SAE) are defined above. The investigator must inform Celgene in writing with a Celgene SAE form or MEDWATCH 3500A of any SAE within 24 hours of being aware of the event. The date of awareness should be noted on the report. This written report must be completed and supplied to Celgene by facsimile within 24 hours/1 business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the

adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required. The Celgene tracking number (RV-MDS-PI-388) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) 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.

7.3.2 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.

7.3.3 Investigator Reporting to the FDA

Adverse drug reactions that are **Serious, Unlisted/unexpected, and at least possibly associated to the drug,** and that have not previously been reported in the Investigators brochure, or reference safety information document should be reported promptly to the Food and Drug Administration (FDA) in writing by each investigator/physician engaged in clinical research. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

The investigator/physician shall notify the FDA by telephone or by fax of any unexpected fatal or life threatening experience associated with the use of the drug. As soon as possible, but no later than 7 calendar days after the sponsors initial receipt of the information. Each phone call or fax shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND if applicable.

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7.4 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 study 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).

8 Response Criteria

8.1 The International Working Group (IWG 2006) Response Criteria for Myelodysplastic Syndromes will be used for efficacy assessments.

The primary efficacy parameter for these patients will be transfusion independence.

8.2 The secondary efficacy parameters for the study will be the remaining criteria as defined by the IWG. For each cohort, the parameters that were primary in one cohort will be secondary in the other; the following parameters also will be assessed:

- Time to disease progression;
- Overall and progression-free survival

8.3 Complete remission (CR):

Bone marrow evaluation: Repeat BM showing less than 5% myeloblasts with normal maturation of all cell lines, with no evidence of dysplasia (8 weeks duration). When erythroid precursors constitute less than 50% of BM nucleated cells, the % blasts is based on all nucleated cells; when there are 50% or more erythroid cells, the % blasts should be based on the non-erythroid cells.

Peripheral blood evaluation: (absolute values must last at least 2 months).

- Hemoglobin: greater than 11gm/dL (untransfused patient not on erythropoietin).
- Neutrophils: 1000/ul or more (not on a myeloid growth factor).
- Platelets: 100,000 ul or more (not on a thrombopoietic agent)
- Blasts: 0%.
- No dysplasia.

8.4 Partial remission (PR): (absolute values must last at least 2 months).

All the CR criteria (if abnormal before treatment), except:
Bone marrow evaluation-blasts decrease by 50% or more over pretreatment but still >5%. Cellularity or morphology is not relevant.

Marrow CR

Bone marrow blasts < or = 5% myeloblasts and decreased by 50% or more over pretreatment

Stable disease:
Failure to achieve at least a PR, but with no evidence of progression for at least 8 weeks

8.5 Failure:
Death during treatment or disease progression characterized by worsening of cytopenias, increase in the % BM blasts, or progression to an MDS FAB sub-type more advanced than pre-treatment.

8.6 Disease transformation:
Transformation to AML, 20% or more blasts.

8.7 Cytogenetic response: Requires 20 analyzable metaphases using conventional cytogenetic techniques.

Complete: No detectable cytogenetic abnormality, if pre-existing abnormality was present.
Partial: 50% or more reduction in abnormal metaphases.

8.8 Pre-therapy assessments: Baseline complete blood count (CBC) to which improvements will be compared was standardized using a mean value of the 4 weeks prior to the start of therapy for all patients.

8.9 During therapy: Responses will be assessed at 12 weeks of therapy. With regard to packed red blood cell transfusions (PRBC) and transfusion independence, the same 4 week time period was used prior to treatment to determine transfusion dependence and to obtain a baseline monthly requirement. Subsequent transfusions will be reviewed at 16 weeks, and 1-year time points.

8.10 Hematologic Improvement or HI:
All improvements must last at least 8 weeks. For a designated response (CR, PR HI) all relevant response criteria must be noted on at least two successive determinations at least one week apart after appropriate period following therapy.

8.11 Erythroid response (HI-E):

Response: For patients with pre-treatment Hb less than 11 Gm/dL, greater than1.5Gm/dL increase in Hb; relevant reductions in units of RBC transfusion by an absolute number of at least 4 RBC transfusions/8wks compared with pre-treatment. Only RBC transfusions given for Hgb of < or = to 9.0g/dL pre-treatment will be count for transfusion response evaluation

8.12 Platelet response (HI-P):

Response: For patients with a pretreatment platelet count more than 200,000/ul, an absolute increase of 30,000/ul or more; for patients with a pretreatment platelet count less than 200,000/ul, a 100% or more increase in platelet count to more than 200,000/ul

8.12 Absolute neutrophil response (HI-ANC):

Response: For ANC less than 1000/ul before therapy at least a 100% increase or an absolute increase of 500/ul, whichever is greater.

9 Protocol Amendments/Deviations

9.1 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.

9.2 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.

10 Data Management

10.1 Data Collection:

Study-specific case report forms will be created to capture protocol information.

10.2 Analyses and Reporting

Data will be analyzed and reported after accrual is completed. All subsequent data collected will be analyzed and reported in a follow-up clinical report.

10.3 Data Monitoring Committee

The Data Monitoring Committee (DMC) will be composed of medical and statistical independent reviewers and will meet to review the efficacy and safety data and determine a risk/benefit analysis in this subject population. The purpose of the DMC is to advise on serious safety considerations, lack of efficacy and any other considerations within the charge to the Committee. The DMC may request additional meetings or safety reports as deemed necessary upon discussion with Celgene and its representatives. The DMC may stop the study following review of results from each interim analysis. The first interim analysis will examine only safety information; the second interim, conducted when the database is more mature, will examine both safety and efficacy. Appropriate efficacy and safety data summaries will be provided to the DMC after each interim analysis.

10.4 Study monitoring and auditing

10.4.1 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 audit visits 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.

11 Biostatistical Analysis

11.1 Overview

The aim of the study is to compare the diagnostic ability of the gold standard microarray assay to the Luminex Bead assay and to quantitative real-time PCR assay in order to detect Revlimid®-response in patients with MDS. Earlier studies have shown microarray assay to identify responders with a sensitivity of 94% and a specificity of 86%(44) It is hypothesized that the less expensive and more clinically useful Luminex Bead assay will predict MDS Revlimid® responders with a sensitivity of 75% and a specificity of 70% compared to criterion gold standard microarray assay. However, it may be possible that real-time PCR will also be equally useful clinically and an easier assay to perform in the clinical lab setting as many pathology laboratories are now equipped with the necessary instrumentation. We will thus compare both the Luminex and real-time PCR assays, assuming the same level of sensitivity and specificity for each, to the gold standard microarray assay. These levels would be 80% of the sensitivity and specificity of the gold standard.

To test this hypothesis, a paired design will be used with each subject being tested with both diagnostic tests [microarray versus either Luminex or PCR; (written “Luminex” in the formula)]. The null hypothesis is that the difference in sensitivity rates will be more than 0.80, and the alternative hypothesis will be that the difference in rates will be less or equal to 0.80. Using the sample size: formula:

$$n = \left(\frac{Z^{(1-\beta)} + Z^{(1-\alpha)}}{log\left(\gamma/\delta\right)} \right)^2 \left(\frac{(\gamma+1)TPR(luminex) - 2(concordance\ prob}{\gamma(TPR\ luminex)^2} \right.$$

Where 1-β is the power (0.80) and α is the Type I error rate (0.95), δ is the percentage difference between the microarray assay true positive rate (TPR) and the luminex TPR. The concordance probability is the estimated percentage of both tests having concordant true positives. Using the estimated concordance of 50%, an estimated 100 subjects will be required. With concordance set at 60%, an estimated 59 subjects will be required. (Alonzo, T Sample size calculations for comparative studies of medical tests for detecting presence of disease. Stats in medicine 2002: 21:835-852)

11.2 Analysis of Clinical Response

- Response will be evaluated **every 4 weeks during the first 12 weeks** of therapy for each of the 56 transfusion-dependent low/Int-1 risk MDS patients. Treatment will be stopped and the patient will be taken off the protocol in case of no response or stable disease by 12 weeks. International Working Group **(IWG 2006) criteria** will be used for response evaluation. The proportion of TI will be calculated and an exact 95% confidence interval of TI proportion will be estimated. The percent of major and minor responses in erythroid,

platelets, neutrophils, bone marrow and cytogenetic will also be calculated as the secondary endpoints.

11.3 Assessment of pre-therapy expression profile associated with Revlimid® responsiveness

Bone marrow samples will be collected and stored in the laboratory of Dr. Naomi Galili for gene expression analysis.

11.4 Microarray analysis

Microarray analysis will be performed on RNA purified from mononuclear cells using Trizol (Invitrogen). Linear amplification of 20 ng of total RNA will be performed using the Ovation Biotin RNA Amplification and Labeling System (Nugen, Inc.). Fragmented, labeled cDNA will be hybridized to Affymetrix HG_U133 Plus 2.0 oligonucleotide microarrays as described by the manufacturer. Raw expression values will be normalized using Robust Multiarray Averaging (RMA) (41). The genes to be analyzed in this trial are the same genes that were identified in Plos Medicine (44).

Ligation-mediated amplification and Luminex detection: All MDS patients enrolled in the Revlimid® protocol described below will have their pre-therapy bone marrow mononuclear cells stored in Dr. Naomi Galili's laboratory at Columbia University Medical Center. Total RNA from bone marrow mononuclear cells preserved in Trizol will be used for the Luminex assay the expression of 32 genes, which includes the 24 genes of interest and controls will be evaluated using multiplexed ligation mediated amplification (LMA).

For each transcript, two LMA probes have been designed. The upstream probe contains a T7 universal primer sequenced, one of 32 different barcodes (Tm Bioscience, www.universalarray.com), and a 20 nucleotide gene specific sequence. The downstream probes are 5' phosphorylated, and contain a 20 nucleotide sequence contiguous with the gene-specific fragment of the upstream probe and a T3 universal primer site. Gene specific probe sequences are unique to the target gene when compared to the Human RefSeq database, have similar base composition, and target the 3' end of the target genes.

Prior to LMA, 100 ng/well of total RNA will be applied to oligo-dT coated plates (GenePlateHT, RNAture) in Lysis Buffer (RNAture). Bound, poly(A) RNA will be reverse transcribed using Superscript II (Invitrogen). LMA probes will be annealed to their target cDNAs and ligated by *Taq* DNA ligase (New England Biolabs). Ligation products, all 104 nucleotides in length, will be amplified by 34 cycles of PCR using T3 and 5'-biotinylated T7 primers. Amplicons will be detected using fluorescent microspheres of 32 different colors (xMAP Multi-Analyte COOH Microspheres, Luminex). Microspheres of each color are covalently coupled to a unique capture probe complementary to one of the 32 gene-specific barcodes. Following labeling of the biotinylated amplicons with

streptavidin-coated phycoerythrin, the labeled amplicons will be annealed to capture probe-linked fluorescent microspheres. Microspheres are detected by flow cytometry and captured labeled amplicons are quantified (Luminex) by subtracting background from the mean fluorescent intensity of each bead.

Real-time PCR: Total RNA from bone marrow mononuclear cells preserved in Trizol will be used for the Real-time PCR assay for the expression of 32 genes, which includes the 24 genes of interest and controls. Primers for each gene will be designed (Roche Biosystems) such that only the RNA transcript and no genomic DNA will be measured by the Roche Light Cycler quantitative PCR assay . The Sybergreen kit (Roche) which measures relative amount of gene specific transcript will be used. Results will be analyzed with the Roche Light Cycler analysis package.

11.5 Safety evaluation

Data from all subjects who receive any study drug will be included in the safety analyses. Subjects who entered the study and did not take any of the study drug(s) and had this confirmed, will not be evaluated for safety.

The severity of the toxicities will be graded according to the NCI CTCAE v3.0 whenever possible.

12 Regulatory Considerations

12.1 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.

12.2 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.

12.3 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.

12.4 Study records requirements

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the study drug, 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 study 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.

12.5 Premature discontinuation of study

12.5.1 Single center

The responsible local clinical Investigator as well as Celgene have the right to discontinue this study at any time for reasonable medical or administrative reasons in any single center. 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.

12.5.2 Study as a whole

Celgene reserves the right to terminate this clinical study at any time for reasonable medical or administrative reasons.

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.

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 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.

† 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).

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 3.0

TOXICITY WILL BE SCORED USING NCI CTC VERSION 3.0 FOR TOXICITY AND ADVERSE EVENT REPORTING. A COPY OF THE NCI CTC VERSION 3.0 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