

Supplementary Appendix

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Hematologic and Cytogenetic Response to Lenalidomide in Myelodysplastic Syndrome with Chromosome 5q Deletion

Running Title: Lenalidomide in myelodysplastic syndrome

Alan List, M.D.¹, Gordon Dewald, PhD,² John Bennett, M.D.³, Aristotle Giagounadis, M.D.⁴, Azra Raza, M.D.⁵, Eric Feldman, M.D.⁶, Bayard Powell, M.D.⁷, Peter Greenberg, M.D.⁸, Deborah Thomas, M.D.⁹, Richard Stone, M.D.¹⁰, Craig Reeder, M.D.¹¹, Kenton Wride, MS¹², John Patin, MS¹², Michele Schmidt, RN, C¹², Jerome Zeldis, MD¹², and Robert Knight, MD¹² for the MDS-003 Study Investigators¹³

From the Department of Interdisciplinary Oncology, University of South Florida College of Medicine and the H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL¹; Mayo Clinic, Rochester, MN²; University of Rochester, Rochester, NY³; St. Johannes Hospital, Duisberg, DE⁴; University of Massachusetts, Worcester, MA⁵; New York Cornell Medical Center, NY⁶; Wake Forest University, Winston Salem, NC⁷; Stanford U, Stanford, CA⁸; MD Anderson Cancer Center, Houston, TX⁹; Dana Farber Cancer Center, Boston, MA¹⁰; Mayo Clinic, Scottsdale, AZ¹¹; and Celgene Corporation, Warren, NJ¹²; MDS-003 investigators and institutional affiliations are listed in Appendix 1¹³.

Correspondence: Alan List, M.D.
Malignant Hematology Division, SRB -4
H. Lee Moffitt Cancer Center and Research Institute
12902 Magnolia Drive
Tampa, FL 33612-9497
Phone: 813-745-6086
Fax: 813-745-3727
Email: ListAF@Moffitt.usf.edu

Abstract

Background

Myelodysplastic syndrome (MDS) associated with chromosome 5q31 deletion is characterized by severe anemia that is often refractory to cytokine therapy. We investigated whether lenalidomide (CC5013; Revlimid™) would restore erythropoiesis in red blood cell (RBC) transfusion-dependent patients and suppress the abnormal clone.

Methods

One hundred forty-eight patients received treatment with lenalidomide 10mg daily or for 21 days every four weeks. Hematologic, bone marrow and cytogenetic response were assessed after twenty-four weeks of treatment using an intention-to-treat analysis.

Results

One hundred twelve patients (76%; 95 percent confidence interval, 68% to 82%) had a transfusion-response, and 99 patients (67%; 95 percent confidence interval, 59% to 74%) achieved transfusion-independence regardless of karyotype complexity. Response to lenalidomide was rapid (median, 4.6 weeks; range, 1 to 49 weeks) and sustained, with the median duration of transfusion-independence not reached after 104 weeks median follow-up. Maximum response hemoglobin reached a median of 13.4 g/dl (range, 9.2 to 18.6 g/dl) with a corresponding median hemoglobin rise of 5.4 g/dl (range, 1.1 to 11.4 g/dl) compared to the baseline pre-transfusion hemoglobin nadir. Seventy-three percent of evaluable patients experienced cytogenetic improvement, which included complete cytogenetic remission in 45% of patients. Improvement in bone marrow morphology was common, with 36% of patients achieving a complete histological response. Moderate to severe neutropenia (55%) or thrombocytopenia (44%) were the most common reasons for treatment interruption or dose adjustment.

Conclusions

Lenalidomide is highly active in MDS patients with chromosome 5q31 deletion, inducing durable hematologic and cytogenetic responses in the majority of patients.

Introduction

Interstitial deletions involving the long arm of chromosome 5 are among the most common cytogenetic abnormalities in patients with myelodysplastic syndrome (MDS), with detection frequencies ranging from 16-28%^{1,2}. Though the size of interstitial deletions varies, deletion mapping has shown that the common deleted region (CDR) involves a 1.5 Mb segment extending from band 5q31 to 5q32³. Although a tumor suppressor gene is postulated to reside in the CDR, no specific gene with pathogenetic relevance has been identified. Patients with deletion 5q display distinct clinical and pathological features that include a hypo-proliferative anemia accompanied by dysplastic bone marrow megakaryocytes. Endogenous erythropoietin production is generally elevated, and the majority of patients become red blood cell (RBC) transfusion-dependent¹. The World Health Organization recognizes a subset of patients with deletion 5q as a distinct syndrome that was first described by Van den Berghe et al in 1974^{4,5}. The so-called '5q- syndrome' represents a minor subset of patients with deletion 5q, and is characterized by an isolated chromosome 5q31 deletion accompanied by severe hypoplastic anemia, normal or elevated platelet count, atypical marrow megakaryocytes with less than 5% myeloblasts, and a comparatively indolent clinical course.

In a preliminary study of lenalidomide (CC-5013, Revlimid™; Celgene Corporation, Summit, NJ) in MDS patients that were insensitive to treatment with recombinant erythropoietin, 10 of 12 patients (83%) with a deletion 5q achieved red blood cell transfusion-independence accompanied by a complete or partial cytogenetic response, whereas the response rate in patients with alternate karyotypes was 39%⁶. To evaluate the clinical benefit of lenalidomide in RBC transfusion-dependent patients with deletion 5q, we performed a multicenter international study to characterize the frequency of RBC transfusion response and cytogenetic response.

Methods

Patients

Eligible patients had a confirmed histological diagnosis of primary MDS according to French-American-British (FAB) criteria and a chromosome 5q31 deletion

that was either isolated or accompanied by additional cytogenetic abnormalities, Low or Intermediate-1 risk disease according to the International Prognostic Scoring System (IPSS), and RBC transfusion-dependent anemia (i.e., ≥ 2 units within 8 weeks of study registration)^{7,8}. Transfusion frequency and pre-transfusion hemoglobin values within the 8 weeks preceding study treatment served as reference for response assessment. Patients with severe neutropenia ($< 500/\mu\text{l}$), thrombocytopenia ($< 50,000/\mu\text{l}$), proliferative chronic myelomonocytic leukemia (leukocytes $> 12,000/\mu\text{l}$), treatment-related MDS, known hypersensitivity to thalidomide, or clinically significant co-morbid medical illnesses were excluded.

Study Design

All registered patients gave written informed consent. The trial was designed, monitored and analyzed by the principal investigator along with the cytogenetic and pathology reviewers in consultation with Celgene Corporation. The manuscript was authored by A.F.L. with editorial revision by co-authors without sponsor limitations. Lenalidomide was supplied in 5mg capsules and administered at a dose 10mg daily for 21 days of every 28-day cycle. The study was activated July 21, 2003 and the treatment schedule subsequently amended to 10mg daily owing to the longer interval to response observed in the pilot study. Treatment was interrupted for adverse effects \geq grade 3 and resumed after toxicity resolution at a dose of 5mg/day or 5mg every other day according to tolerance. Complete blood profiles were obtained weekly during the first eight weeks, followed by every two weeks thereafter. Bone marrow aspirate, biopsy and marrow cytogenetics were repeated after 24 weeks of treatment. Responding patients continued lenalidomide until disease progression, treatment failure or limiting toxicity. RBC transfusions were administered according to pre-study clinical indicators with the following guidelines: hematocrit $< 25\%$, 2 units; hematocrit $< 21\%$, 3 units; hematocrit $< 18\%$, 4 units. Myeloid growth factors were the only cytokines permitted for management of neutropenia.

Assessment of Response and Toxicity

The primary endpoint of the study was the frequency of transfusion-independence; secondary analyses included the duration of RBC transfusion-independence, frequency of minor erythroid response, cytogenetic and pathologic response, and the safety of study treatment. Hematologic response was assessed according to modified International Working Group (IWG) criteria in which response required sustained improvement for ≥ 8 consecutive weeks⁹. Transfusion-independence was analyzed using a more rigid definition of 56-consecutive days free from transfusion with a ≥ 1 g/dl hemoglobin rise; minor response was defined as $\geq 50\%$ reduction in transfusions compared to baseline. Hemoglobin rise in patients achieving transfusion-independence was calculated as the difference between the maximum hemoglobin response and the minimum pre-transfusion hemoglobin during the 8 weeks pre-study. Classification of cytogenetic response was determined by standard metaphase analysis before and after treatment for those patients with ≥ 20 evaluable metaphases in sequential specimens. Deletion of a Y chromosome in males was not considered abnormal. Complete cytogenetic remission was defined as absence of metaphases containing any abnormal clone. Partial cytogenetic response was defined as $\geq 50\%$ reduction in the proportion of abnormal metaphases after treatment. Patients with cytogenetic results based on less than 20 metaphases before and/or after treatment were considered unevaluable for cytogenetic response. Cytogenetic progression was defined as acquisition of a new clonal chromosome abnormality. Initial study eligibility was determined according to the local institutional pathology and cytogenetic assessment. Pre-treatment and week-24 bone marrow pathology and karyotype were reviewed for assignment of FAB category and cytogenetic pattern by J.B. and G.D., respectively. Adverse events were graded using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0¹⁰.

Statistical Analysis

A one-stage response-focused trial design required 30 patients, however, the target enrollment was extended to >90 patients to provide sufficient safety data and improve precision of response estimates. Extension of total enrollment to 148 patients was permitted following interim analysis by the data monitoring committee due to

favorable efficacy and safety results. All response and toxicity analyses include the 148 registered patients regardless of final eligibility and reflect data collected from patient visits through July 15, 2005.

The time to achievement of transfusion-independence represents the number of days from initiation of study treatment until the day after the date of the last RBC transfusion preceding the first 8-week response period. Duration of transfusion-independence was calculated from the day after the date of the last RBC transfusion until one day prior to the date of the next transfusion. Survival duration according to karyotype complexity was calculated from the date of MDS diagnosis until death. Median survival adjusted for left truncation and duration of transfusion-independence were estimated using the Kaplan and Meier method¹¹. Homogeneity of survival curves over stratification variables was tested using the Cox proportional hazards model. Univariate comparisons were performed by Fisher's exact test, a two-sample independent t test or a Wilcoxon rank sum test. All reported *p* values are two-sided. Summary statistics (N, standard deviation, median, and minimum, maximum) are reported as appropriate. Multivariate analysis of response variables was performed using logistic regression techniques. Efficacy endpoint frequency estimates were accompanied by Exact 95% confidence intervals (CI).

Results

Between July 21, 2003 and May 21, 2004 148 patients were registered and received lenalidomide treatment. Forty-five patients initiated treatment on the 21-day schedule, and 103 patients received continuous dosing. Ninety-five patients (64%) had either refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS), and 120 (81%) had Low or Intermediate-1 risk IPSS scores and were considered study eligible (Table 1). In 20 patients, IPSS category could not be assigned because of either inadequate specimen to confirm the FAB category, a diagnosis other than MDS, or inadequate metaphases to estimate karyotype complexity. The median number of RBC transfusions administered in the 8 weeks pre-study was 6 (range 0-18 units), with 105 patients (71%) receiving ≥ 4 units. Overall, 108 patients (73%) received prior erythropoietin treatment, 58 (39%) had received cytotoxic chemotherapy, and 55 (37%)

were receiving iron chelation therapy. Moderate to severe neutropenia and/or thrombocytopenia was present in 44 (27%) and 28 (17%) patients, respectively. All patients had a chromosome 5q31 deletion identified by either standard metaphase analysis [N=147] or fluorescence in situ hybridization (FISH) [N=1] (Table 2). One hundred ten patients (74%) had an isolated deletion 5q [del(5q)], whereas only 40 patients (27%) fulfilled criteria for the 5q- syndrome. In addition to del(5q), 37 patients (25%) had one or more additional cytogenetic abnormalities.

Hematologic Response

One hundred twelve patients (76%) responded to lenalidomide treatment (Table 3), among whom 99 patients (67%) achieved RBC transfusion-independence by week-24; whereas thirteen patients had a 50% or greater reduction in transfusions. There was no significant difference in response rate according to assigned treatment schedule ($p=0.26$). The median time to transfusion-independence was 4.6 weeks, and ranged from 1 to 49 weeks. The median peak hemoglobin level achieved in transfusion-independent patients was 13.4g/dl (range, 9.2 – 18.6g/dl), with a corresponding median hemoglobin rise from baseline of 5.4g/dl (range, 1.1 – 11.4g/dl). Four responding patients experienced excessive erythrocytosis reaching a hemoglobin exceeding 17g/dl. With 104 weeks median follow-up, 53 responders remained transfusion-free with none lost to follow-up. Therefore, the Kaplan-Meier estimate of the median duration of transfusion-independence could not be estimated (range, 8.6 to 89+ weeks) (Figure 1). Duration of transfusion-independence for at least 1 year was reached by 61 of 99 (62%) responders. No significant difference was observed in response rate by age, gender, FAB type, IPSS category or cytogenetic pattern (Table 4). Patients with baseline thrombocytopenia achieved RBC transfusion-independence at a significantly lower rate (39% versus 73%; $P=0.001$). The lower response rate in thrombocytopenic patients was associated with a shorter time on study drug treatment (N=28; median=22 weeks) compared to all other patients (N=120; median=63 weeks) ($P=0.004$). Multivariate analysis showed that among the above variables only thrombocytopenia (odds ratio, 4.53; $P=0.003$) and high transfusion burden (odds ratio, 3.59; $P=0.01$) adversely affected transfusion response to lenalidomide.

Cytogenetic Response

Eighty-five patients with ≥ 20 analyzable metaphases at baseline and at week-24 were evaluable for cytogenetic response. Thirty-eight patients (45%) achieved complete cytogenetic remission [95% C.I., 34%-56%], and 24 patients (28%) had a partial cytogenetic response [95% C.I., 19%-39%]. An additional 7 patients with < 20 analyzable metaphases at baseline had at least 20 metaphases analyzed at follow-up in which no abnormal metaphases were detected. Fifteen patients had their partial or complete remission confirmed using FISH. Cytogenetic responses closely correlated with RBC transfusion response, with 61 of 62 partial and complete responders achieving transfusion-independence. There was no significant difference in the frequency of cytogenetic response ($P=0.27$) or the frequency of complete cytogenetic remission ($P=0.93$) by karyotype complexity (Table 5). Overall, 77% of patients with an isolated del(5q) had a cytogenetic response, compared to 67% of patients with del(5q) and one other cytogenetic abnormality and 50% of patients with del(5q) and two or more other chromosome anomalies. Multivariate analysis showed that only thrombocytopenia (odds ratio, 4.78; $P=0.02$) and age ≤ 60 (odds ratio, 2.99; $P=0.07$) were associated with a lower probability of cytogenetic response. One hundred nineteen patients had at least one cytogenetic assessment on study treatment and were evaluable for cytogenetic progression. Among the 62 cytogenetic responders, 17 (27%) had either return of the initial cytogenetic pattern or an increase in the proportion of abnormal metaphases ($n=13$; 9 complete, 5 partial responders); 4 patients acquired a new clonal chromosome abnormality accompanying del(5q). Six additional patients experienced cytogenetic progression characterized by appearance of a new clone in the absence of del(5q). With a median follow-up from MDS diagnosis of 3.8 years, the estimated median survival was 7.6 years for patients with an isolated del(5q) and 5.6 years for patients with one or more cytogenetic abnormalities ($P=0.64$) (Figure 2).

Overall, twenty-three patients acquired new chromosome abnormalities during study treatment, thirteen of whom were cytogenetic non-responders. Baseline karyotype in patients experiencing cytogenetic evolution included 13 patients with isolated del(5q), one patient with an independent non- del(5q) clone, 6 patients with one additional

abnormality, and 4 patients with a complex karyotype. In two patients the new abnormality emerged coincident with complete suppression of the del(5q) clone, including one patient with isolated del(5q) and one patient with a discordant clone at baseline. Although acquired chromosome abnormalities varied widely and were not observed in more than one patient, structural or numerical abnormalities involving chromosome 7 were detected in only one patient.

Pathologic Response

One hundred six patients had sequential marrow specimens adequate for central pathology review, among which 82% were transfusion-independence responders (Table 6). Thirty-eight patients (36%) achieved a complete histological response after 24 weeks of treatment with resolution of cytological dysplasia in all hematopoietic lineages. Bone marrow myeloblast percentage returned to the normal range (<5%) in 74% of patients with excess blasts (baseline median, 7%±2.4% [range, 5-14%]; week 24, 1%±1.8% [range, 0-3%]) as did ringed sideroblasts in 64% of evaluable RARS patients (baseline median, 40%±10% [range, 30-50%]; week 24, 0%±2.8% [range, 0-3%]). Sixteen patients experienced progression to a more advanced MDS FAB type [n=8] or AML [n=8]. Multivariate analysis of variables associated with disease progression, including >5% myeloblasts, karyotype complexity, neutropenia, thrombocytopenia, lactate dehydrogenase, 5q- syndrome, and transfusion response, duration of disease and cytogenetic response, showed that leukemia or MDS progression was associated only with a lower frequency of cytogenetic response (13% versus 45%; P=0.05).

Toxicity and Dose Adjustment

Neutropenia and thrombocytopenia were the most common treatment-associated adverse events (Table 7). NCI-CTC grade 3 or 4 neutropenia (<1,000/ μ l) and thrombocytopenia (<50,000/ μ l) were reported in 54.7% and 43.9% of patients, respectively, and were the most common reasons for dose adjustment. Grade 4 neutropenia (<500/ μ l) was more common in patients treated with the continuous dosing regimen compared with the 21-day schedule (44.1% versus 17.4%, P<0.001), whereas grade 4 thrombocytopenia (<10,000/ μ l) was less frequent with continuous dosing (6.9% versus 15.2%, P=0.05). Severe myelosuppression generally occurred early in the

treatment course, with 62% of grade 3 or 4 hematologic adverse events occurring within the initial 8 weeks of treatment. Fever complicated neutropenia in only 4.1% of patients. Most other adverse events were of low or moderate severity and included pruritus, rash, diarrhea and fatigue. There were 11 deaths on the study or within 30 days of last dose of lenalidomide through the analysis date of July 15, 2005; 3 deaths attributed to neutropenic infection were judged possibly treatment-related by the treating investigator. All other deaths were considered unrelated and included CHF [n=3], ischemic colitis [n=1], AML [n=1], procedure associated intestinal perforation [n=1], subarachnoid hemorrhage [n=1], and sudden death [n=1]. The case of subarachnoid hemorrhage was trauma-related and occurred in a responding patient with stable moderate thrombocytopenia (platelet count >75,000/ μ l; baseline mean, 99,000/ μ l) during a 2-week hiatus from study treatment for unexplained dyspnea.

Lenalidomide dose-adjustment was required in 124 patients (84%) during the treatment course, including 93 patients (91%) receiving the continuous schedule and 31 patients (67%) receiving the 21-day schedule. The distribution of week-24 treatment doses was: 10mg daily, 32%; 5 mg daily, 44%; and 5 mg every other day, 24%. The median interval to dose adjustment was 22 days (range, 2-468 days). Twenty-nine patients (20%) discontinued lenalidomide treatment prematurely due to adverse events, including 18 patients (18%) initially assigned to the continuous schedule and 12 patients (26%) on the 21-day regimen. Toxicities included thrombocytopenia or neutropenia [n=10], rash [n=5], AML [n=2], anemia [n=1], facial edema [n=1], congestive heart failure [n=1], urticaria [n=1], diarrhea [n=1], weight loss [n=1], renal insufficiency [n=1], cerebrovascular accident [n=1], dementia [n=1], dyspnea [n=1], pyrexia [n=1], and pneumonia [n=1].

Discussion

Chronic anemia adversely affects both the quality of life and the clinical course of disease in patients with MDS. Hemodynamic compensation contributes to hypertrophic cardiac remodeling over time, the risk for which is reduced with each graded sustained elevation in hemoglobin¹². Dependence upon RBC transfusions, in particular, is

associated with reduced survival in lower risk MDS owing to consequences of iron loading, cardiac failure and AML progression, suggesting that sustained improvement in erythropoiesis might positively impact disease outcome¹³. In this study of transfusion-dependent patients with chromosome 5q deletion, the majority of patients failed prior treatment with recombinant erythropoietin and had a heavy transfusion burden (median, 3 units/month). Overall, 76% of the patients experienced a transfusion response to lenalidomide treatment, and 67% became transfusion-free with a corresponding rise in hemoglobin to a near normal range (Table 3). Response to treatment was rapid (median, 4.6 weeks) and durable, with 61 (62%) responders remaining transfusion-free for at least one year and the median duration of transfusion-independence not reached after a 2-year median follow-up (Figure 1). Moreover, the results of this study confirm initial observations that lenalidomide suppresses the del(5q) clone⁶. Seventy three percent of patients experienced a cytogenetic response and 45% achieved a complete cytogenetic remission after 24 weeks of therapy that was associated with sustained transfusion-independence and improvement in bone marrow morphology (Tables 5, 6). Overall, 36% of evaluable patients achieved a complete histological response without discernable residual dysplasia. In patients with an excess of bone marrow myeloblasts or ringed sideroblasts, the cytologic lineage abnormality returned to the normal range in the majority of patients. These findings are consistent with preclinical observations that lenalidomide is selectively cytotoxic to del(5q) clones, and thereby restores RBC production in part by suppressing the ineffective myelodysplastic clone (List A.F., unpublished data). By comparison, in a recently completed multicenter study evaluating transfusion response to lenalidomide in 215 lower risk, transfusion-dependent MDS patients without del(5q), only 26% of patients achieved transfusion-independence in an intention-to-treat analysis, indicating that the remitting activity of lenalidomide in MDS is karyotype-dependent¹⁴.

Analysis of response variables showed that thrombocytopenia was the most important variable associated with a lower probability of achieving either transfusion independence (P=0.003) or cytogenetic response (P=0.02). The number of consecutive days of drug treatment was significantly lower (P=0.004) in patients with baseline thrombocytopenia owing to recurring treatment interruption for intervening

myelosuppression. This observation suggests that the duration of time on study drug is a critical determinant of the potential for clonal suppression and consequent hematologic improvement. We found no difference in the frequency of transfusion or cytogenetic response according to karyotype complexity, or among patients with isolated del(5q) either with or without the ‘5q- syndrome’. The presence of one or more additional chromosome abnormalities is associated with a more aggressive clinical course and considerably inferior overall survival in MDS patients with del(5q)^{1,2,15}. Our findings that survival among patients with additional chromosome abnormalities did not differ from that for patients with isolated del(5q) suggests that lenalidomide may extend survival in higher risk patients (Figure 2). Furthermore, our observations that cytogenetic response to lenalidomide in patients with greater karyotype complexity is accompanied by disappearance of the additional chromosome abnormalities and morphologic features of disease is consistent with the leukemogenesis model proposed by Pedersen-Bjergaard, J. et al., in which del(5q) represents an initiating event responsible for clonal propagation, whereas acquisition of additional aberrations is secondary¹⁶. Cellular mechanisms of resistance responsible for recurrence of the initial clone remain undefined, but may relate to emergence of target mutations or amplification, analogous to the experience with imatinib treatment in patients with chronic myeloid leukemia¹⁷.

Myelosuppression, manifested by moderate to severe neutropenia and thrombocytopenia, occurred routinely and generally early in the treatment course (Table 7), findings that are consistent with the agent’s suppressive effect on the del(5q) clone. Overall, approximately two-thirds of patients required dose reduction and 24% of patients required a second dose adjustment later in the treatment course. Three patients succumbed to neutropenic infection, emphasizing the need for close laboratory monitoring and consideration of myeloid growth factors during the initial weeks of treatment. Nonetheless, infectious complications were otherwise infrequent, particularly during the initial period of clonal suppression. There was no apparent response rate or toxicity advantage to either of the treatment schedules evaluated.

We conclude that lenalidomide has remarkable erythroid and cytogenetic remitting activity in lower-risk transfusion-dependent MDS patients with del(5q). Based on the results of this pivotal efficacy study, lenalidomide was approved by the Food and

Drug Administration on December 27, 2005 for the management of transfusion-dependent anemia in MDS patients with del(5q).

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Table 1. Clinical and Hematologic Features

Characteristic	Number (%)
No. Patients	148
Median Age [range]	71 [37-95]
Male: Female	51:97 (66)
Median years MDS duration [range]	2.5 [0.1-20.7]
Median RBCs/8wk [range]	6 units [0-18]
Patients with ≥ 2 Units/month	105 (71)
IPSS Risk Category	
Low Risk	55(37)
Intermediate -1	65 (44)
Intermediate-2/High	8 (5)
Unclassified	20 (14)
FAB Type	
RA	77 (52)
RARS	18 (12)
RAEB	30 (20)
CMML	3 (2)
AML	1 (1)
Atypical CML	3 (2)
Inadequate specimen	16 (11)
Neutropenia < 1500/ μ l	44 (30)
Thrombocytopenia < 100,000/ μ l	28 (19)

Note: CMML denotes chronic myelomonocytic leukemia; RAEB, RA with excess blasts; CML, chronic myeloid leukemia.

Table 2. Cytogenetic Features at Baseline

Parameter	No. Patients (%)
Specimens	148
5q Deletion Segment	
(q13q33)	59 (40)
(q14q34)	28 (19)
(q13q31)	19 (13)
(q15q33)	15(10)
Other	26 (18)
Unknown*	1
Isolated deletion 5q	110 (74)
Deletion 5q + 1 additional abnormality	25 (17)
Complex [≥ 3 abnormalities]	12 (8)
5q- Syndrome	40 (27)

* 4 normal metaphases analyzed; 85% of 100 interphase nuclei lacked EGR1 signal by FISH at 5q31.

Table 3. Erythroid Response to Lenalidomide

Parameter	Daily Dose Schedule10 mg/day	21 Day Schedule 10 mg/day x 21	Total (%) [95% Confidence Interval]
Number of Patients	102	46	148
Erythroid Response			
Transfusion-independent ≥50% ↓ transfusions	71 (70) 8 (8)	28 (61) 5 (11)	99 (67) [59-74] 13 (9) [5-15]
Overall transfusion response	79 (77)	33 (72)	112 (76) [68-82]
Time to response (weeks)			
Median	4.7	4.3	4.6
Range	1-34	1-49	1-49
Median hemoglobin, <i>range</i>			
Baseline [1]	7.7, 5.3-10.4	8.0, 5.6-10.3	7.8, 5.3-10.4
Response [2]	13.4, 9.2-18.6	13.5, 9.3-16.9	13.4, 9.2-18.6
Median hemoglobin change↑, <i>range</i>	5.4, 2.2-11.4	5.4, 1.1-9.1	5.4, 1.1-11.4

[1] minimum hgb during baseline period.

[2] maximum hgb during transfusion independence response period.

Table 4. Frequency of Transfusion-Independence by Clinical and Pathological Features

Feature	No. of Patients	Transfusion-Independent	P-value [1]
		<i>No. (%)</i>	
Age (years)			0.711
≤65	48	31 (65)	
>65	100	68 (68)	
Gender			0.274
Male	51	31 (61)	
Female	97	68 (70)	
RBC Transfusions			0.002
< 4 units/8 weeks	43	37 (86)	
≥ 4 units/8 weeks	105	62 (59)	
IPSS Risk Category			0.154
Low Risk	55	40 (73)	
Intermediate -1	65	43 (66)	
Intermediate-2	6	2 (33)	
High	2	1 (50)	
FAB Type			0.927
RA	77	56 (73)	
RARS	18	9 (50)	
RAEB	30	19 (63)	
CMML	3	2 (67)	
AML	1	0	
Atypical CML	3	3 (100)	
Inadequate	16	10 (63)	
Karyotype Complexity			0.068
Isolated 5q deletion	110	79 (72)	
Deletion 5q + 1 additional	25	12 (48)	
Complex [≥3]	12	8 (67)	
Neutrophil count			0.358
<1,000/μl	13	7 (54)	
≥1,000/μl	135	92 (68)	
Platelet Count			0.001
<100,000/μl	28	11 (39)	
≥100,000/μl	120	88 (73)	

[1] 2-sided Fisher's Exact test.

Table 5. Frequency of Cytogenetic Response According to Karyotype Complexity

Complexity	No. of Evaluable [1] Patients	No. Responding	No. Complete Cytogenetic Remission
	[N=85]	<i>No. (%)</i>	<i>No. (%)</i>
Isolated deletion 5q31	64	49 (77)	29 (45)
Deletion 5q + 1 additional	15	10 (67)	6 (40)
Complex [≥ 3 abnormalities]	6	3 (50)	3 (50)
P value		0.266	0.931

[1] At least 20 analyzable metaphases at baseline and at least one follow-up assessment.

Table 6. Bone Marrow Histologic Response

Feature	No. Patients (%)
No evaluable	106
RBC transfusion-independent	87 (82)
Complete histologic response	38 (36)
RAEB & RAEB-in transformation at baseline	19
<5% myeloblasts post-baseline	14 (74)
RARS	14
<15% ringed sideroblasts	9 (64)
Disease progression	16
MDS progression	8
AML	8

Table 7. Treatment-Related Adverse Events \geq Grade 3

Adverse Event	Grade 3(%)		Grade 4 (%)		Grade 3 or 4(%)
	10 mg/d [N=102]	10mg x21d [N=46]	10 mg/day [N=102]	10mg x21d [N=46]	Total [N=148]
Neutropenia	20 (19.6)	8 (17.4)	45 (44.1)	8 (17.4)	81 (54.7)
Thrombocytopenia	37 (36.3)	14 (30.4)	7 (6.9)	7 (15.2)	65 (43.9)
Anemia NOS	4 (3.9)	2 (4.3)	4 (3.9)	0 (0)	10 (6.8)
Leukopenia NOS	3 (2.9)	2 (4.3)	4 (3.9)	0 (0)	9 (6.1)
Rash	5 (4.9)	4 (8.7)	0 (0)	0 (0)	9 (6.1)
Febrile neutropenia	2 (2.0)	1 (2.2)	2 (2.0)	1 (2.2)	6 (4.1)
Pruritus	2 (2.0)	2 (4.3)	0 (0)	0 (0)	4 (3.4)
Fatigue	2 (2.0)	2 (4.3)	0 (0)	0 (0)	4 (2.7)
Muscle cramp	3 (2.9)	0 (0)	0 (0)	0 (0)	3 (2.0)
Pneumonia	1 (1.0)	2 (4.3)	1 (1.0)	0 (0)	4 (2.7)
Nausea	3 (2.9)	1 (2.2)	0 (0)	0 (0)	4 (2.7)
Diarrhea	4 (3.9)	0 (0)	0 (0)	0 (0)	4 (2.7)
DVT	3 (2.9)	1 (2.2)	0 (0)	0 (0)	4 (2.7)
Hemorrhagic events	1 (1.0)	2 (4.3)	1 (1.0)	1 (2.2)	4 (2.7)
Hypokalemia	1 (1.0)	1 (2.2)	0 (0)	0 (0)	2 (1.4)
Pyrexia	1 (1.0)	0 (0)	0 (0)	0 (0)	1 (0.7)

*Note: NOS denotes 'not otherwise specified'.

Figures

Figure 1. Kaplan-Meier estimate of duration of RBC Transfusion-Independence. Blue symbols are censored patients who remain transfusion-free at time of data cutoff (July 15, 2005) or at time of study discontinuation. Week 0 at the intercept reflects the day after the patients' last transfusion preceding response. After a median follow-up of 104 weeks, the median duration of transfusion-independence could not be estimated.

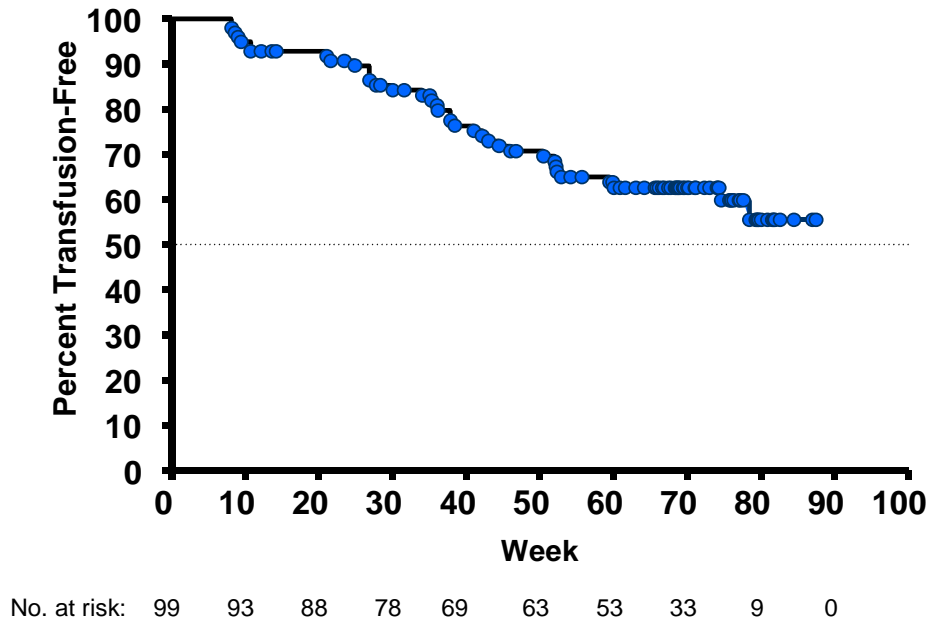
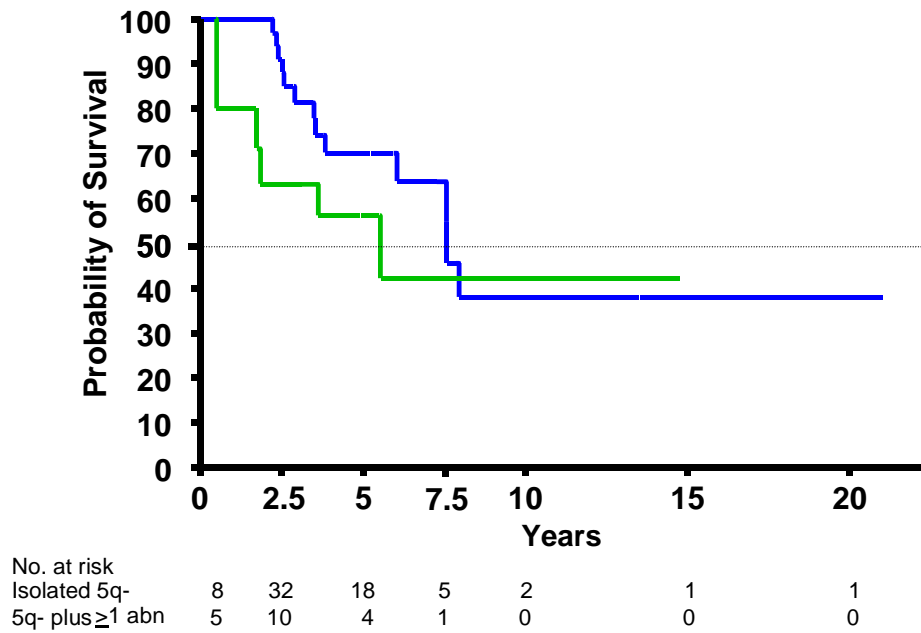


Figure 2. Kaplan-Meier estimate of overall survival according to karyotype complexity at baseline. Survival estimates reflect a data cutoff of July 15, 2005 and are adjusted for left truncation (time between MDS diagnosis and date of first lenalidomide dose). After a median follow-up of 3.8 years, the estimate of median survival from date of MDS diagnosis was 7.6 years for patients with isolated del(5q) at baseline and 5.6 years for patients with del(5q) plus one or more additional chromosome abnormality (P=0.64).



[a] Product-Limit survival estimates adjusted for left-truncation.

Appendix 1.

In addition to the authors, the following investigators participated in the MDS-003 study:

Investigator	Institution	City
Baer, Maria	Roswell Park Cancer Institute	Buffalo, NY
Curtin, Peter	Oregon Health & Science University	Portland, OR
Deeg, H. Joachim	Fred Hutchinson Cancer Research Center	Seattle, WA
Dreisbach, Luke	Desert Hematology Oncology	Rancho Mirage, CA
Feldman, Eric	New York Cornell/Medical Center	New York, NY
Fonseca, Gustavo	Cancer & Blood Disease Center	Lecanto, FL
Gordon, Michael	Arizona Cancer Center	Scottsdale, AZ
Gore, Steven D.	Johns Hopkins	Baltimore, MD
Gotlib, Jason	Stanford Cancer Center	Stanford, CA
Hermann, Robert	Northwest Georgia Oncology	Marietta, GA
Ifthikharuddin, J.	James P. Wilmot Cancer Center	Rochester, NY
Larson, Richard	University of Chicago Medical Center	Chicago, IL
Lian, Eric	Sylvester Cancer Center	Miami, FL
Maness, Lori	University of Nebraska Medical Center	Omaha, NE
Moreno, Alvaro	The Mayo Clinic	Jacksonville, FL
Nimer, Stephen	Memorial Sloan-Kettering Cancer Center	New York, NY
Sekeres, Mikkael	The Cleveland Clinic Foundation	Cleveland, OH
Shaddock, Richard	Western Pennsylvania Cancer Institute	Pittsburgh, PA
Shammo, Jamile	Rush-Presbyterian-St. Luke's	Chicago, IL
Silverman, Lewis	Mt. Sinai Medical Center	New York, NY
Tefferi, Ayalew	Mayo Clinic	Rochester, MN