

ORIGINAL ARTICLE

Immunotherapy with a Ragweed–Toll-Like Receptor 9 Agonist Vaccine for Allergic Rhinitis

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ABSTRACT

BACKGROUND

Conjugating immunostimulatory sequences of DNA to specific allergens offers a new approach to allergen immunotherapy that reduces acute allergic responses.

METHODS

We conducted a randomized, double-blind, placebo-controlled phase 2 trial of a vaccine consisting of Amb a 1, a ragweed-pollen antigen, conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA (AIC) in 25 adults who were allergic to ragweed. Patients received six weekly injections of the AIC or placebo vaccine before the first ragweed season and were monitored during the next two ragweed seasons.

RESULTS

There was no pattern of vaccine-associated systemic reactions or clinically significant laboratory abnormalities. AIC did not alter the primary end point, the vascular permeability response (measured by the albumin level in nasal-lavage fluid) to nasal provocation. During the first ragweed season, the AIC group had better peak-season rhinitis scores on the visual-analogue scale ($P=0.006$), peak-season daily nasal symptom diary scores ($P=0.02$), and midseason overall quality-of-life scores ($P=0.05$) than the placebo group. AIC induced a transient increase in Amb a 1–specific IgG antibody but suppressed the seasonal increase in Amb a 1–specific IgE antibody. A reduction in the number of interleukin-4–positive basophils in AIC-treated patients correlated with lower rhinitis visual-analogue scores ($r=0.49$, $P=0.03$). Clinical benefits of AIC were again observed in the subsequent ragweed season, with improvements over placebo in peak-season rhinitis visual-analogue scores ($P=0.02$) and peak-season daily nasal symptom diary scores ($P=0.02$). The seasonal specific IgE antibody response was again suppressed, with no significant change in IgE antibody titer during the ragweed season ($P=0.19$).

CONCLUSIONS

In this pilot study, a 6-week regimen of the AIC vaccine appeared to offer long-term clinical efficacy in the treatment of ragweed allergic rhinitis. (ClinicalTrials.gov number, NCT00346086.)

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STANDARD ALLERGEN IMMUNOTHERAPY has been a cornerstone of the allergist's therapeutic options since its introduction in 1911.¹ However, this approach is limited by the potential for systemic allergic reactions, including anaphylaxis, caused by the relatively large doses of allergen required for efficacy.²⁻⁴ Furthermore, standard allergen immunotherapy is logistically difficult to administer because it requires regular, frequent dosing, typically over a period of 3 to 5 years,^{5,6} and thus frequently results in noncompliance.^{7,8} Therefore, there is a need for new immunotherapeutic agents that have decreased risks of serious adverse events and involve shorter regimens that are more easily followed.

One possibility is an immunotherapeutic compound in which the ragweed-pollen antigen Amb a 1 is conjugated to a phosphorothioate oligodeoxyribonucleotide immunostimulatory sequence of DNA containing a CpG motif.⁹ The immunostimulatory sequence binds to toll-like receptor 9 (TLR9), which is predominantly expressed in plasmacytoid dendritic cells, and this interaction is associated with the inhibition of immune responses mediated by type 2 helper T (Th2) cells.^{9,10} In addition, when peripheral-blood mononuclear cells from patients who are allergic to ragweed are exposed to the Amb a 1-immunostimulatory oligodeoxyribonucleotide conjugate (AIC) in vitro, production of the Th2 cytokines interleukin-4 and interleukin-5 is decreased,¹¹ suggesting a potential therapeutic role for AIC in these patients. We conducted a small, placebo-controlled clinical trial in which patients who were allergic to ragweed were immunized with a six-injection regimen of the AIC vaccine before the first ragweed season and evaluated during the next two ragweed seasons.

METHODS

PATIENTS

Twenty-five patients who were 23 to 60 years of age and had a history of seasonal (fall) allergic rhinitis, a positive puncture skin test (as reflected by erythema [sum of the longest diameter and the orthogonal diameter intersecting the midpoint of the longest diameter] ≥ 30 mm) in response to a licensed, standardized ragweed extract (Greer Laboratories), and an immediate positive response to ragweed nasal challenge (three sneezes more

than were elicited in response to the diluent nasal challenge and a doubling of the albumin level) were enrolled at the Johns Hopkins Asthma and Allergy Center. Further inclusion and exclusion criteria are given in the Supplementary Appendix, available with the full text of this article at www.nejm.org. The protocol was approved by the institutional review board of Johns Hopkins Bayview Campus, and all patients provided written informed consent.

STUDY DESIGN

The study was a randomized, double-blind, placebo-controlled, phase 2 clinical trial of AIC in patients with ragweed-induced seasonal allergic rhinitis. At enrollment, an attempt was made to balance the numbers of patients in the treatment groups according to their response to the ragweed puncture skin test, with eligible patients assigned to receive either AIC or placebo according to a random block design. Study personnel who were unaware of patients' treatment assignments administered a total of six injections at weekly intervals before the onset of the first ragweed season (2001). The planned sizes of the six doses were 0.06, 0.3, 1.2, 3.0, 6.0, and 12.0 μ g of AIC; these doses were adjusted in the event of a local or systemic reaction to an injection or a missed injection. After undergoing a clinical and immunologic evaluation during the first ragweed season, the patients were asked to give written informed consent to continue in the study, and 17 of the 25 did so. No additional study injections were administered, and the patients were followed through the subsequent ragweed season (2002) and evaluated for serious adverse events, symptom scores, medication use, and immune responses.

STUDY MATERIALS

AIC was prepared by Primedica for Dynavax Technologies. The placebo was phosphate-buffered saline. The same lot of standardized extract of ragweed (*Ambrosia artemisiifolia*), containing approximately 300 μ g of Amb a 1 per milliliter, was used throughout the study. Ragweed-pollen counts were measured in Baltimore with the use of a standard rotorod sampling device. The patients were given medication to be used for relief of moderate-to-severe nasal or eye symptoms that they were unwilling to tolerate (see the Supplementary Appendix). Dynavax Technologies sup-

plied the study medication and helped design the trial and reviewed the manuscript but did not collect or analyze the data or fund the trial.

CLINICAL AND MECHANISTIC ASSESSMENTS

The primary end point of the study was the effect of treatment on change from baseline in the albumin level in nasal-lavage fluid after nasal provocation and was based on previous observations that the level of albumin, a marker of vascular leakage and inflammation, is lowered by standard allergen immunotherapy.¹² Ragweed provocation procedures were conducted before the injections were begun and 2 weeks and 2 months after the injections were finished. After the challenge, the number of sneezes was counted and the degree of nasal congestion, rhinorrhea, postnasal drip and itchiness of the ears, nose, and throat were also scored on a scale of 0 (no symptoms) to 100 (the worst possible symptoms).

Other secondary clinical end points recorded during the ragweed season included the rhinitis visual-analogue score, which was self-reported and ranged from 0 mm (no symptoms) to 100 mm (the worst possible symptoms)¹³; the daily nasal symptom diary score, ranging from 0 (no symptoms) to 5 (very severe symptoms)¹⁴; the use of relief medication^{14,15} (see the Supplementary Appendix for details on scoring); the score on the standardized Juniper rhinoconjunctivitis quality-of-life questionnaire, with scores ranging from 0 (best quality) to 6 (worst)¹⁶; adverse events; and skin-test sensitivity (see the Supplementary Appendix).

Serum Amb a 1-specific and ragweed-specific IgG and IgE levels were measured by enzyme immunoassays¹⁷ at baseline, after treatment, before the first ragweed season (in 2001), 2 weeks and 2 months after the end of the first ragweed season, before the second ragweed season (in 2002), and at the end of the second ragweed season. To follow the antibody response to treatment, we used a functional antigen presentation (FAP) assay that measures the ability of postimmunotherapy serum to inhibit the binding of allergen-IgE complexes to B cells at the same time points.¹⁸ At various times, the levels of interleukin-4 in T cells and basophils and the intracellular levels of interferon- γ and interleukin-10 as well as the secreted levels were measured to assess immune responses.^{19,20} Similarly, peripheral-blood mono-

nuclear cells were analyzed by TaqMan (Applied Biosystems) reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay for immune response genes.²¹ Standard safety blood assays were performed at screening (or baseline) and during treatment (see the Supplementary Appendix).

STATISTICAL ANALYSIS

Peak ragweed season was defined as the period starting when ragweed pollen counts were at least 11 grains per cubic meter on 2 consecutive days and ending on the last day before ragweed pollen counts were less than 11 grains per cubic meter for 2 consecutive days (that were not followed by 2 days of higher pollen counts). Differences between the groups in post-treatment albumin levels in nasal-lavage fluid, the primary end point, were assessed on an intention-to-treat basis (see the Supplementary Appendix) by regression modeling adjusted for baseline values, with a P value of less than 0.05 for the primary outcome and less than 0.01 for all secondary outcomes used to indicate statistical significance; the P values were not adjusted for multiple comparisons. Descriptive statistics for daily nasal symptom diary scores and seasonal rhinitis visual-analogue scores are based on the patients' median scores during the specified seasonal periods (preseason and peak season).

Repeated-measures analysis was used to examine differences between the AIC and the placebo groups in symptoms, medication use, and scores on the rhinoconjunctivitis quality-of-life questionnaire (2002 results) and was used to test for main effects of treatment group, time, and interactions between treatment and time. An analysis-of-covariance model that included the preseason score as a covariate was also used to analyze midseason scores on the rhinoconjunctivitis quality-of-life questionnaire (2001 results). The Wilcoxon rank-sum test was used to compare median scores at given times for serum immune responses, some cellular immune responses, and nasal challenge outcomes, including both the raw scores and the difference from baseline. The Wilcoxon signed-rank test was used for within-group comparisons of serum immune responses. The chi-square test was used to analyze basophil results. Analyses were performed by the Emmes Corporation, Johns Hopkins University, and the Immune Tolerance Network. Additional information is provided in the Supplementary Appendix.

RESULTS

PATIENTS

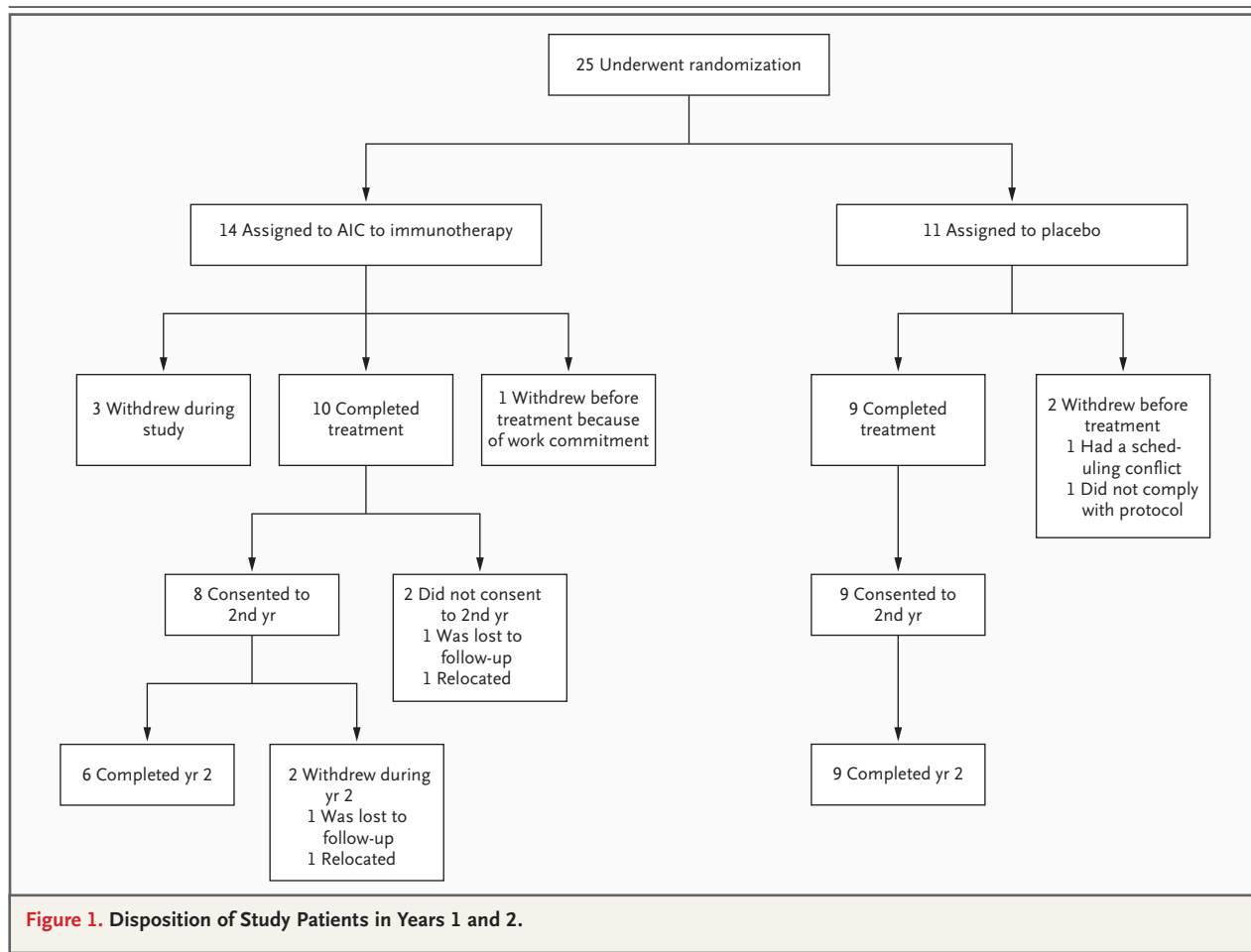
Twenty-five patients who were allergic to ragweed were enrolled in the trial in May and June of 2001 (Fig. 1). At the time of randomization, there were no significant differences between the study groups in age or in sensitivity to ragweed, as determined by the puncture skin test (Table 1). Of the 14 patients assigned to AIC treatment, 1 voluntarily withdrew after a serious adverse event related to previous surgery and unrelated to treatment, 1 relocated, 1 was lost to follow-up, and 1 withdrew because of a work commitment before beginning immunotherapy. Of the 11 patients assigned to placebo, 2 dropped out before the first ragweed season: 1 because of noncompliance with the study protocol and 1 because of scheduling conflicts. Of the 19 patients in the study at the end of the first ragweed season, 17 consented to continue in the study, and 15 of these patients (9 assigned to

placebo and 6 to AIC) remained in the trial through the end of the second ragweed season. Despite dropouts, there continued to be no significant differences in demographic characteristics between the AIC and placebo groups (see Table 2 in the Supplementary Appendix).

NASAL ALLERGEN CHALLENGE

AIC failed to affect the primary end point of the study, the increase in albumin levels in nasal secretions after post-treatment ragweed challenge ($P=0.47$). Furthermore, such increases were not diminished by AIC in the two postseason challenges, nor were there post-treatment differences in histamine levels in nasal-lavage fluids.

Several clinical end points of nasal allergen challenge were improved at the post-therapy challenge. The median number of sneezes increased from baseline by 3 in the placebo group and decreased from baseline by 1.5 in the AIC group ($P=0.03$ for the difference between the groups



in the change from baseline). As compared with the placebo group, the AIC group also had improvements in the total post-challenge symptom score, including the sneeze count (median, 37 vs. 70; $P=0.03$); the total post-challenge nasal symptom score, excluding the sneeze count (median, 33 vs. 60; $P=0.02$); and the postnasal drip score (median, 24 vs. 57; $P=0.007$).

CLINICAL OUTCOMES DURING THE FIRST RAGWEED SEASON (2001)

Rhinitis symptoms, as measured by the visual-analogue scale, were significantly improved in the AIC group as compared with the placebo group (Fig. 2A). The mean peak-season rhinitis visual-analogue score in the AIC group was one third of that in the placebo group (13.2 vs. 40.8, $P=0.006$ by repeated-measures analysis of the interaction between treatment and time). The difference in least-squares means on the visual-analogue scale was significant for the third week of the peak season ($P=0.007$), with treatment differences for the second and fourth weeks of the peak season ($P=0.02$ and $P=0.04$, respectively). When the data for the full ragweed season were analyzed, a similar pattern was observed: the mean score was 13.8 in the AIC group as compared with 35.1 in the placebo group ($P=0.01$ for the interaction between treatment and time).

The peak-season daily nasal symptom diary scores were also lower in the AIC group than in the placebo group (mean, 1.8 vs. 4.0; $P=0.02$ by repeated-measures analysis), with a similar treatment effect observed for the full season ($P=0.03$ by repeated-measures analysis) (Fig. 2B). In addition, the mean (weighted) use during the peak season was lower in the AIC group (median, 0.1 vs. 0.6) (see the Supplementary Appendix). At mid-season, the total score on the rhinoconjunctivitis quality-of-life questionnaire (adjusted for baseline) was lower in the AIC group than in the placebo group (raw mean, 0.5 vs. 1.6; $P=0.05$ by analysis of covariance), as were the subscores for eye symptoms (0.5 vs. 1.8, $P=0.03$) and sleep (0.3 vs. 2.1, $P=0.04$).

CLINICAL OUTCOMES DURING THE SECOND RAGWEED SEASON (2002)

Although the patients in the AIC group received no further treatment after 2001, the improvements in clinical outcome measures in this group as compared with the placebo group were maintained

Table 1. Demographic Characteristics of the Patients.*

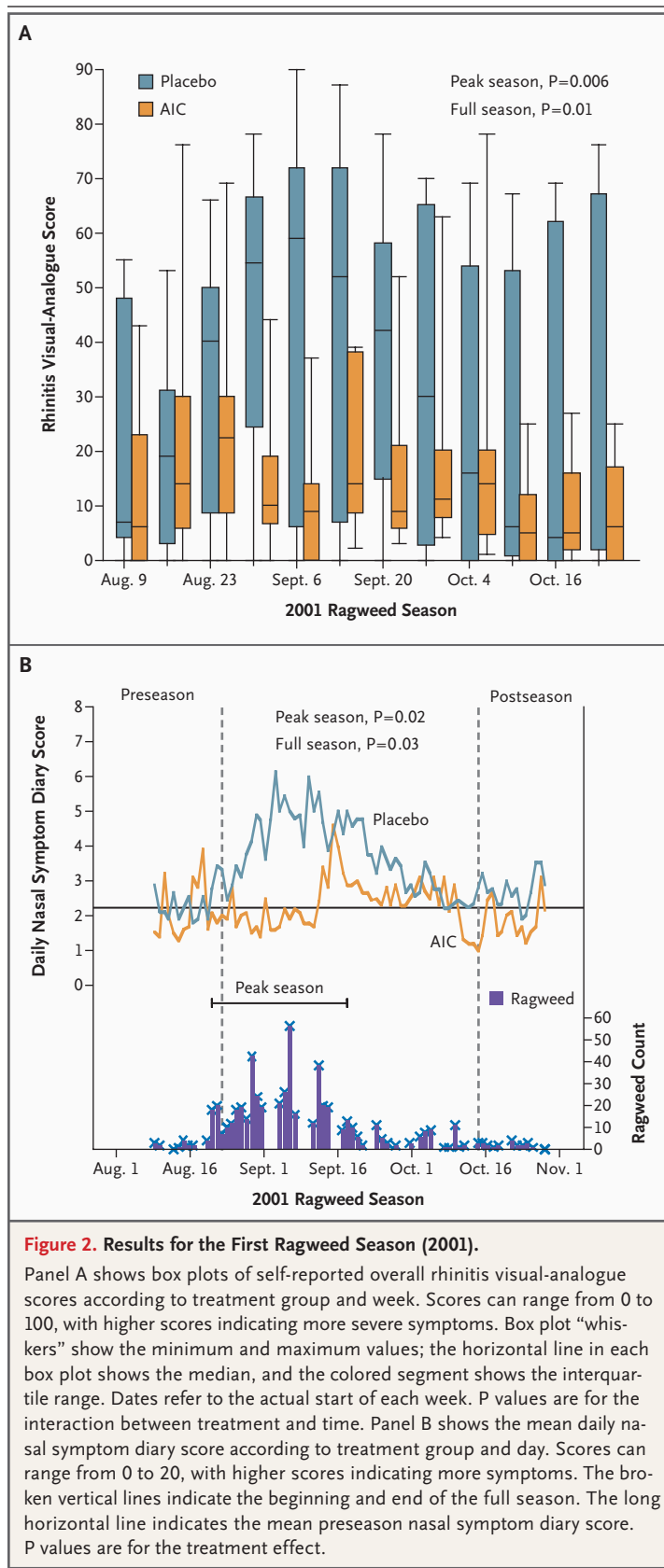
Characteristic	AIC (N=14)	Placebo (N=11)
Male sex — no. (%)	6 (43)	4 (36)
Race or ethnic group — no. (%)†		
White	9 (64)	7 (64)
Black	4 (29)	4 (36)
Mixed	1 (7)	0
Age — yr		
Mean	37.6±2.7	41.7±2.6
Median	38	39
Range	23–58	33–60
No. of years with seasonal rhinitis	26±4	33±3
Ragweed IgE — U/ml		
Mean	12.0±2.8	9.8±3.5
Median	9.0	7.0
Range	0.2–30.1	0.4–33.9
Puncture skin test for ragweed sensitivity — mm of erythema‡	72	72
Sensitivity to other allergens — no. (%)		
Grass pollen	11 (79)	11 (100)
Tree pollen	11 (79)	11 (100)
Dust mites	10 (71)	6 (55)
Mold	10 (71)	6 (55)
Cat dander	9 (64)	7 (64)
Dog dander	6 (43)	4 (36)
Cockroaches	1 (7)	0
Coexisting conditions — no. (%)		
Asthma	4 (29)	2 (18)
Conjunctivitis	13 (93)	11 (100)
Sinusitis	2 (14)	4 (36)
Eczema	3 (21)	1 (9)

* Plus-minus values are means ±SE.

† Race or ethnic group was self-reported.

‡ Millimeters of erythema was defined as the sum of the longest diameter and the orthogonal diameter intersecting the midpoint of the longest diameter.

through the subsequent 2002 ragweed season. During the peak ragweed season of 2002, the mean rhinitis visual-analogue score in the AIC group was approximately one third of that in the placebo group (13.9 vs. 49.4, $P=0.02$ by repeated-measures analysis) (Fig. 3A). During 6 of the 8 weeks of the full ragweed season in 2002, the weekly mean rhinitis visual-analogue scores reported by AIC-treated patients were lower than the mean score for the baseline week.



In 2002, the mean nasal symptom diary score during the peak season was reduced by 53% in the AIC group, as compared with the placebo group (2.8 vs. 5.9, $P=0.02$ by repeated-measures analysis), a result similar to that observed in 2001. The nasal symptom diary scores for the full season showed a similar pattern, but the difference was not significant ($P=0.06$ by repeated-measures analysis) (Fig. 3B). As compared with placebo, AIC treatment also lowered 2002 peak-season scores for the use of relief medication (median, 0 vs. 0.6; $P=0.08$ by repeated-measures analysis), and resulted in fewer days of antihistamine use (median, 0 vs. 8 days) and decongestant use (median, 0 vs. 4 days). The rhinoconjunctivitis quality-of-life questionnaire scores were improved in the AIC group in 2002, with a treatment effect in the activity subscore ($P=0.04$ by repeated-measures analysis), the nasal subscore ($P=0.03$ by repeated-measures analysis), the trends-in-practical-problems subscore ($P=0.06$ by repeated-measures analysis), and the total score ($P=0.06$ by repeated-measures analysis).

SAFETY

There was no pattern of vaccine-associated systemic adverse reactions or clinically significant laboratory abnormalities. No new formation or increase in the titers of antinuclear, anti-double-stranded DNA or anti-single-stranded DNA was observed. Patients in both groups reported local reactions to injections: erythema ranging from 0 to 75 mm in diameter and wheals from 0 to 44 mm in diameter in the AIC group, and erythema from 0 to 27 mm in diameter and wheals from 0 to 6 mm in diameter in the placebo group. Erythema or wheals occurred in response to AIC in 45 of 71 injections (63%). All local reactions were self-limited, and none required medication or a change in treatment dose. One AIC-treated patient had severe itching at the injection site. One patient in the placebo group had systemic symptoms (rhinitis) after an injection.

Overall, 135 adverse events were observed in 23 patients during the first year: 73 (54%) in the AIC group and 62 (46%) in the placebo group. Of these, 112 (83%) were mild or moderate and 23 (17%) were severe; 12 of the severe adverse events occurred in the AIC group, and 11 in the placebo group. Ten of the 135 adverse events (5 in each group) were classified as possibly related to study medication. Two serious adverse events were reported in the AIC group during the first year:

Figure 3. Results for the Second Ragweed Season (2002).

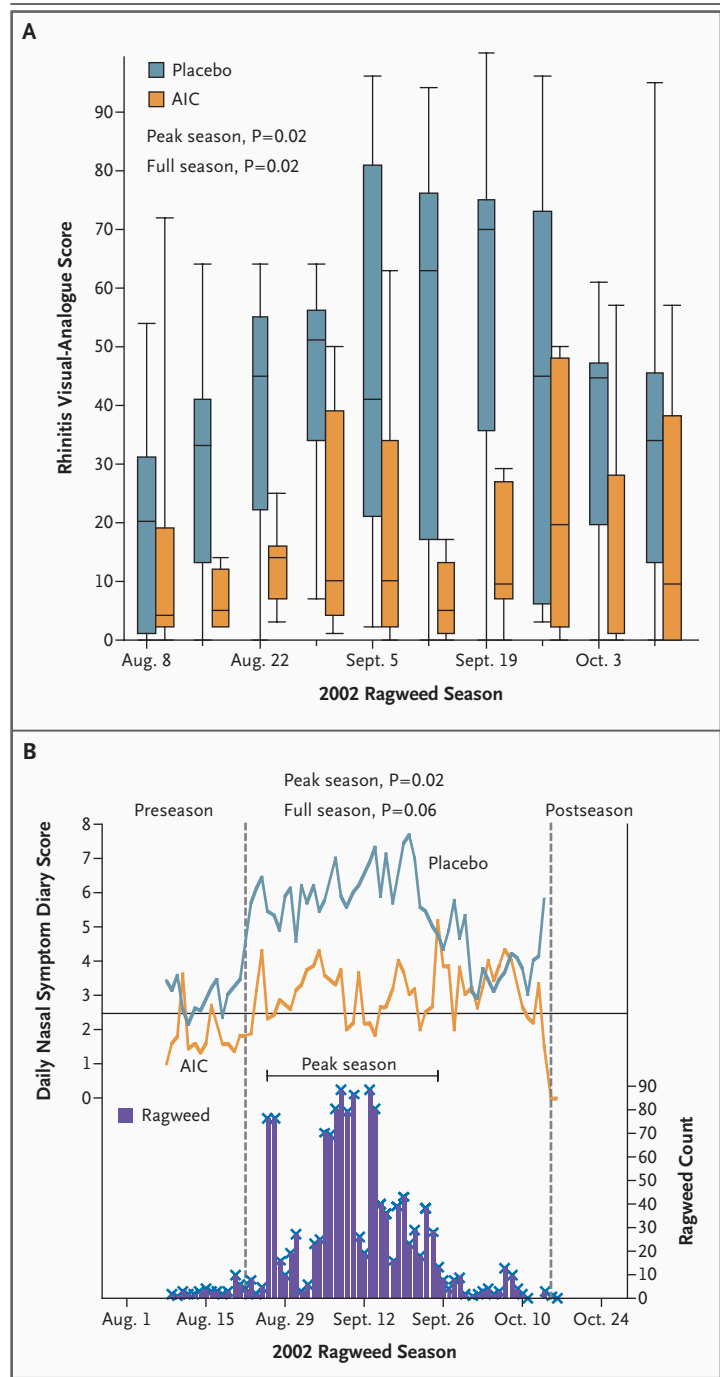
Panel A shows box plots of self-reported overall rhinitis visual-analogue scores according to treatment group and week. Scores can range from 0 to 100, with higher scores indicating more severe symptoms. Box plot “whiskers” show the minimum and maximum values; the horizontal line in each box plot shows the median, and the colored segment shows the interquartile range. Dates refer to the actual start of each week. P values are for the interaction between treatment and time. Panel B shows the mean daily nasal symptom diary score according to treatment group and day. Scores can range from 0 to 20, with higher scores indicating more severe symptoms. The broken vertical lines indicate the beginning and end of the full season. The long horizontal line indicates the mean preseason nasal symptom diary score. P values are for the treatment effect.

a postsurgical abdominal skin infection occurred in one patient, who was withdrawn from the study, and another patient had a right ovarian cyst removed. Both of these events were considered to be unrelated to treatment. One serious adverse event was reported in the placebo group during the second year. The dose was modified or temporarily stopped in two placebo recipients because of severe allergic rhinitis and fatigue in one patient and in the other, two episodes of mild upper respiratory tract infection: one with asthmatic bronchitis and one with postnasal drip and cough. The dose was modified in two AIC recipients because of a mild, influenza-like syndrome in one and a moderate upper respiratory tract infection or allergic rhinitis with asthma flare in the other.

ANTIBODY TITERS

Standard allergen immunotherapy results in a significant increase in IgG antibody against an immunized allergen.^{22,23} The patients were immunized against the major protein moiety of ragweed (Amb a 1). AIC induced a transient increase in Amb a 1-specific and ragweed-specific IgG. No post-treatment increases in Amb a 1-specific or ragweed-specific IgG titers were observed in the placebo group. Furthermore, the FAP assay showed no evidence that IgG in serum from AIC-treated patients inhibited the binding of the ragweed allergen-IgE complex to B cells (see the Supplementary Appendix).

AIC induced a rise in the mean ($\pm$ SE) level of Amb a 1-specific IgE antibody from a baseline value of 94.3 ± 34.3 U per milliliter to 162.8 ± 70.1 U per milliliter after treatment; the median difference was 10.3 U per milliliter ($P=0.008$). The post-



treatment titers in the placebo group were unchanged. AIC treatment suppressed the increase in Amb a 1-specific IgE that usually is observed during the ragweed allergy season: the mean IgE levels were 180.0 ± 90.2 U per milliliter before the 2001 ragweed season and 97.0 ± 37.0 U per milliliter 2 months after the season.^{24,25} Patients in the placebo group had a typical seasonal rise in Amb a 1-specific IgE, from 60.4 ± 22.0 U per mil-

liliter before the 2001 ragweed season to 105.7 ± 38.5 U per milliliter 2 weeks after the season, with a median difference of 18.0 U per milliliter ($P=0.008$) (Fig. 4). Similar results were seen in 2002 during the second season, with a rise in the placebo group from 66.8 ± 25.8 U per milliliter before the season to 87.3 ± 28.0 U per milliliter after the season ($P=0.02$), but no such rise in the AIC group (82.1 ± 35.2 and 52.4 ± 26.9 U per milliliter before and after the season, respectively; $P=0.19$) (Fig. 4). Measurements of whole ragweed-specific IgE antibodies in the placebo group produced similar results (data not shown).

SKIN-TEST SENSITIVITY

Suppression of the immediate skin-test responses (at 15 minutes) and the late-phase responses (at 24 hours), measured as a decrease in either puncture or intradermal skin-test reactivity, also serves as a marker of successful standard allergen immunotherapy.^{15,26-29} AIC decreased immediate intradermal skin-test reactivity: the mean decrease in wheal size after treatment with AIC was 8 mm,

as compared with 3 mm with placebo ($P=0.04$ for the difference from baseline). The AIC group also had a larger reduction in immediate (puncture) and late-phase (intradermal) skin-test erythema (see the Supplementary Appendix).

CELLULAR IMMUNE RESPONSES

No clear correlations were detected between AIC treatment and intracellular levels of interleukin-4 or interferon- γ in activated CD4+CD69+ T cells, as measured by flow cytometry or an enzyme-linked immunosorbent assay. Interferon- γ was detected in the positive control samples (peripheral-blood mononuclear cells stimulated with tetanus toxoid) by both methods. Increased levels of interleukin-10 were spontaneously secreted by peripheral-blood mononuclear cells cultured in medium alone for 16 hours, but the levels did not differ significantly between cells from patients in the AIC group and cells from patients in the placebo group. This effect appeared to be driven by seasonal allergen exposure, since interleukin-10 secretion was most evident in peripheral-blood mononuclear cells

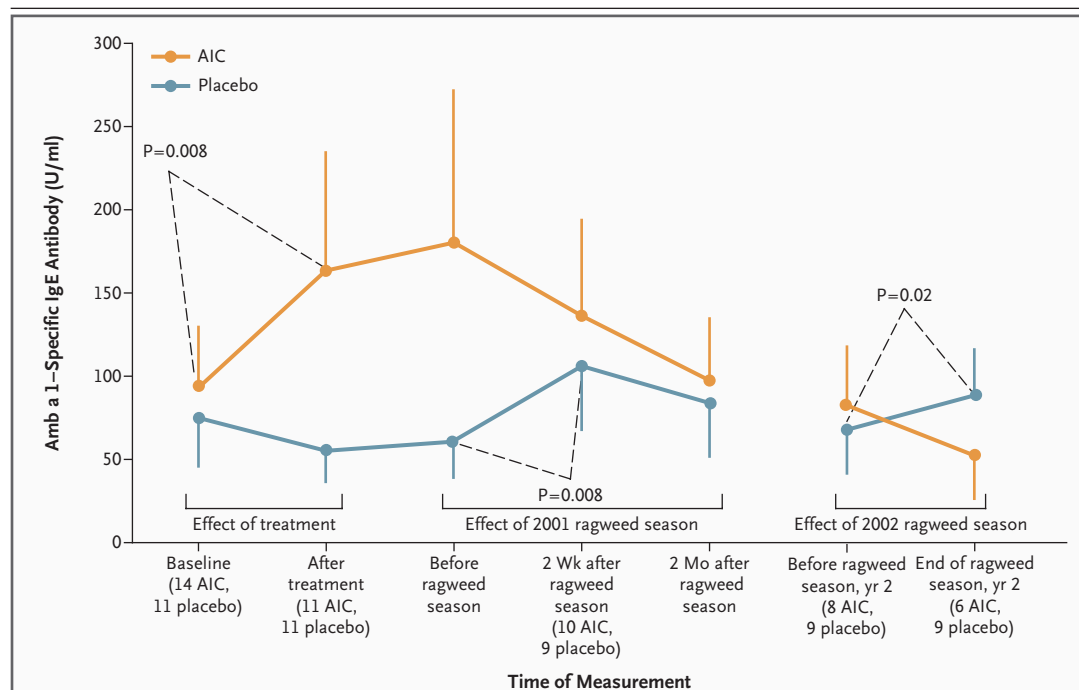


Figure 4. Effect of Treatment on Amb a 1-Specific IgE Antibody.

Antibody measurements were performed at defined times over the 2-year study period: 2 weeks (near November 1) and 2 months (near December 15) after the end of the 2001 ragweed season, and at the end of the 2002 ragweed season (near October 14). There was a significant rise in Amb a 1-specific IgE in the placebo group in each ragweed season. Although AIC induced a rise in the titer of Amb a 1-specific IgE, the increase was transient. AIC treatment blunted the usual seasonal rise in IgE during each ragweed season.

taken from patients in either group 2 weeks after the end of the ragweed season (see the Supplementary Appendix). Similarly, the levels of expression of 184 cytokine, chemokine, and immune-response genes analyzed by a TaqMan RT-PCR assay showed no consistent differences before and after treatment (see the Supplementary Appendix).

Two weeks after the end of the 2001 ragweed season, the placebo group had a mean increase from baseline in the frequency of interleukin-4-positive basophils by a factor of 5.0 ± 2.7 ($P=0.02$) (see the Supplementary Appendix). In contrast, this effect was not observed in half the AIC-treated patients. This difference between the two groups was not significant ($P=0.06$). However, there was a correlation between diminished frequencies of interleukin-4-positive basophils and lower rhinitis visual-analogue scores for the two groups ($r=0.49$, $P=0.03$).

DISCUSSION

Although we did not demonstrate that AIC affected the primary end point (nasal vascular permeability as assessed by nasal-lavage albumin), we did demonstrate that a once-weekly, six-injection regimen of AIC substantially reduced allergic rhinitis symptoms during the ragweed season. Furthermore, the protection afforded by the administration of AIC before the first ragweed season was sustained during the second ragweed season. In contrast, several studies of standard allergen immunotherapy (consisting of 14 to 27 injections administered before the beginning of the season) only showed an improvement of 35 to 40% in ragweed seasonal symptom scores,^{30,31} with no lasting benefit,^{5,32,33} and a long-lasting benefit persisting after the discontinuation of standard allergen immunotherapy has been shown only after 3 or 4 years of treatment.³⁴

In our study, no serious adverse events attributable to AIC occurred, no injections were discontinued because of local reactions, and dose adjustments were similar in frequency and type in the AIC and placebo groups. In contrast, systemic reactions occur in 1 to 20% of patients receiving standard allergen immunotherapy²⁻⁴ and in up to 73% of patients receiving "cluster" or "rush" immunotherapy.^{35,36} In standard allergen immunotherapy, a slow buildup (two injections per week for 10 to 12 weeks) is generally used to minimize

the risk of allergic reactions to injections.^{5,37} AIC may therefore also offer a safer route of allergen administration that does not sacrifice efficacy.

The limitations of our study include the small number of patients enrolled, the lack of effect of AIC on the primary end point, and the unknown long-term safety of the treatment. Hence, additional clinical trials with longer-term follow-up are needed to assess the safety and clinical effectiveness of AIC.

The mechanism by which AIC induces clinical tolerance of an inhaled allergen is incompletely understood. Like standard allergen immunotherapy, AIC blunts the seasonal rise in ragweed-specific and Amb a 1-specific IgE that is generally observed in persons with atopy.^{23,25,38} Our finding that the six-injection regimen suppressed the rise in ragweed-specific IgE during the subsequent allergy season provides evidence that this regimen had immunomodulatory effects associated with a clinical benefit lasting through two ragweed seasons. The data are consistent with our observation that AIC inhibits the cutaneous response to challenge with ragweed, a defined hallmark of successful immunotherapy.^{15,26,29} In contrast to standard allergen immunotherapy, successful vaccination with AIC appears to be independent of the formation of allergen-specific IgG, since AIC induced only a moderate and transient rise in Amb a 1-specific and ragweed-specific IgG antibody and did not inhibit allergen-IgE complexes from binding to B cells.^{5,13,18,39}

In conclusion, this study provides preliminary evidence that a six-injection regimen of AIC reduces allergic rhinitis symptoms during the ragweed season. Furthermore, the clinical effects of AIC appear to be associated with the induction of long-lasting immune modulation. Although the mechanisms underlying the clinical benefit require further investigation, AIC vaccine has properties that make it qualitatively superior to standard allergen immunotherapy. Large-scale phase 3 studies will be needed to determine the role of AIC as a therapeutic option in ragweed-induced allergic disease.

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Greer Laboratories; and having served as a consultant to Curalogic. Dr. Coffman reports being an employee of Dynavax Technologies, owning equity and stock options in the company, and receiving consulting fees from the National Institute of Allergy and Infectious Diseases Board of Scientific Counselors. Dr. Eiden, a former employee of Dynavax Technologies, reports owning equity and stock options in the company. Dr. Broide reports having received grant support from the National Institutes of Health Immune Tolerance Network and royalties from the University of California Office of Technology Transfer for being a coinventor of methods and devices for immunizing a host. No

other potential conflict of interest relevant to this article was reported.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: List A, Dewald G, Bennett J, et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med 2006;355:1456-65.

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

Running Title: Lenalidomide in myelodysplastic syndrome

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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, $7\% \pm 2.4\%$ [range, 5-14%]; week 24, $1\% \pm 1.8\%$ [range, 0-3%]) as did ringed sideroblasts in 64% of evaluable RARS patients (baseline median, $40\% \pm 10\%$ [range, 30-50%]; week 24, $0\% \pm 2.8\%$ [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 ($<1,000/\mu\text{l}$) and thrombocytopenia ($<50,000/\mu\text{l}$) were reported in 54.7% and 43.9% of patients, respectively, and were the most common reasons for dose adjustment. Grade 4 neutropenia ($<500/\mu\text{l}$) 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 ($<10,000/\mu\text{l}$) 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]	No. (%)	No. (%)
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.

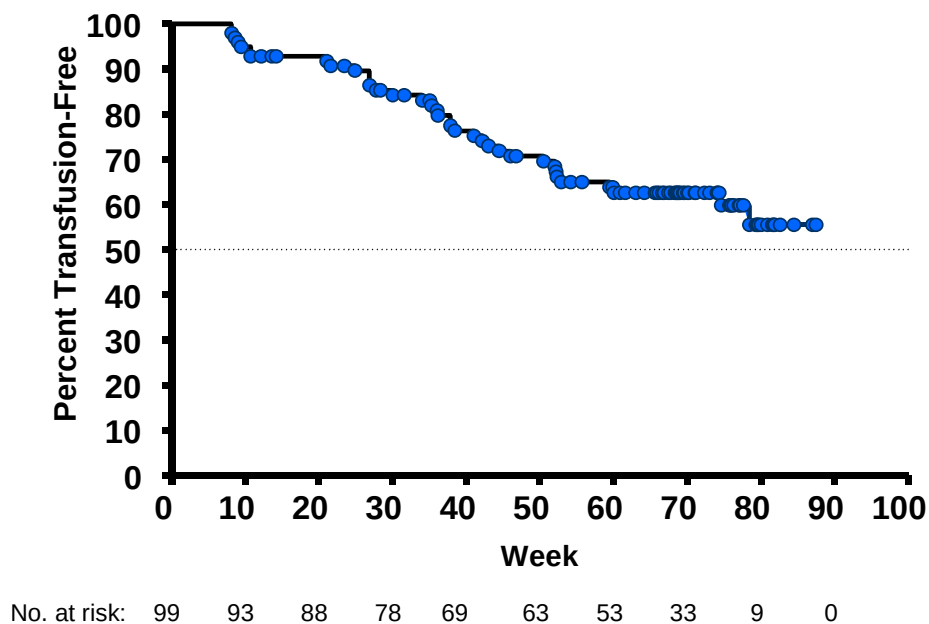
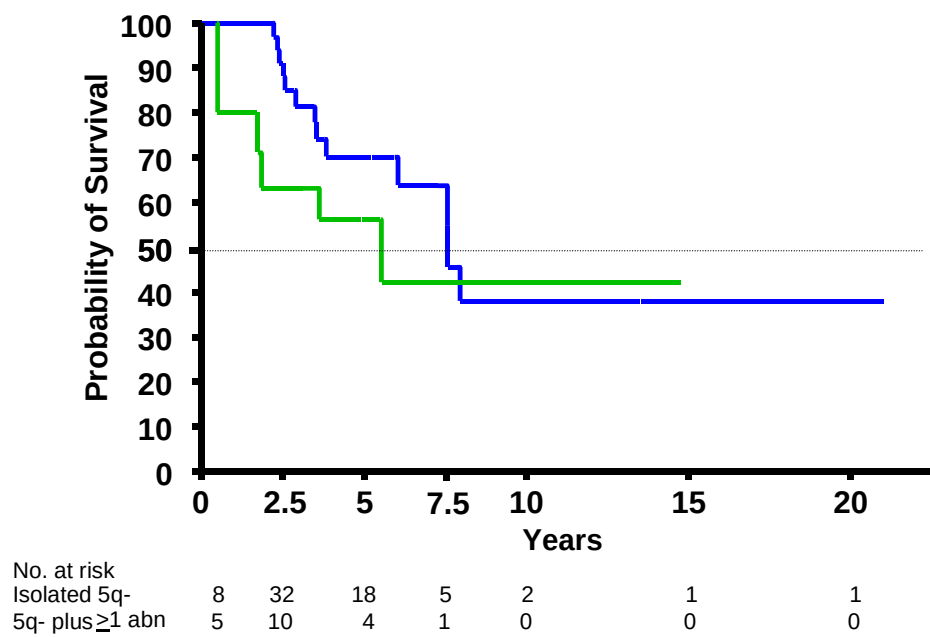


Figure 2. Kaplan-Meier estimate of overall survival according to karyotype complexity at baseline. Survival estimates reflect a data cutoff of July 15, 2005 and are adjusted for left truncation (time between MDS diagnosis and date of first lenalidomide dose). After a median follow-up of 3.8 years, the 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
Shadduck, 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