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Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia

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ABSTRACT

BACKGROUND

A single-group, phase 1–2 study indicated that eltrombopag improved the efficacy of standard immunosuppressive therapy that entailed horse antithymocyte globulin (ATG) plus cyclosporine in patients with severe aplastic anemia.

METHODS

In this prospective, investigator-led, open-label, multicenter, randomized, phase 3 trial, we compared the efficacy and safety of horse ATG plus cyclosporine with or without eltrombopag as front-line therapy in previously untreated patients with severe aplastic anemia. The primary end point was a hematologic complete response at 3 months.

RESULTS

Patients were assigned to receive immunosuppressive therapy (Group A, 101 patients) or immunosuppressive therapy plus eltrombopag (Group B, 96 patients). The percentage of patients who had a complete response at 3 months was 10% in Group A and 22% in Group B (odds ratio, 3.2; 95% confidence interval [CI], 1.3 to 7.8; P=0.01). At 6 months, the overall response rate (the percentage of patients who had a complete or partial response) was 41% in Group A and 68% in Group B. The median times to the first response were 8.8 months (Group A) and 3.0 months (Group B). The incidence of severe adverse events was similar in the two groups. With a median follow-up of 24 months, a karyotypic abnormality that was classified as myelodysplastic syndrome developed in 1 patient (Group A) and 2 patients (Group B); event-free survival was 34% and 46%, respectively. Somatic mutations were detected in 29% (Group A) and 31% (Group B) of the patients at baseline; these percentages increased to 66% and 55%, respectively, at 6 months, without affecting the hematologic response and 2-year outcome.

CONCLUSIONS

The addition of eltrombopag to standard immunosuppressive therapy improved the rate, rapidity, and strength of hematologic response among previously untreated patients with severe aplastic anemia, without additional toxic effects. (Funded by Novartis and others; RACE ClinicalTrials.gov number, NCT02099747; EudraCT number, 2014-000363-40.)

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Acquired aplastic anemia is a disease that involves primary bone marrow failure and manifests with pancytopenia. The best evidence of an autoimmune pathogenesis comes from the patients' response to immunosuppressive treatment and from laboratory studies.[1] The introduction of antithymocyte globulin (ATG) in the late 1970s[2-4] and the addition of cyclosporine to ATG in the 1980s[5] led to considerable improvements in hematopoietic recovery and longer survival among patients with severe or very severe aplastic anemia (a distinction based on the neutrophil count and thus on susceptibility to infection).

Overall, two thirds of patients have a response to standard immunosuppressive treatment with horse ATG plus cyclosporine.[5-7] The quality and timing of hematologic response are the best predictors of long-term survival.[8] Over the past three decades, many studies have shown the failure of methods to improve the results of standard therapy; the tested methods included replacing horse ATG with rabbit ATG, alemtuzumab, or cyclophosphamide; adding a third immunosuppressive drug such as mycophenolate mofetil or sirolimus; and adding hematopoietic growth factors to standard therapy.[7,9-15] Moreover, the development of myeloid cancers remains a troublesome complication after immunosuppressive therapy, and it accounts for 10 to 15% of late treatment failures.[8,16]

Eltrombopag, an oral thrombopoietin-receptor agonist, initially was shown to have efficacy in patients with aplastic anemia that was refractory to immunosuppressive therapy.[17-19] An open-label, nonrandomized, phase 1–2 study showed that eltrombopag in combination with standard horse ATG plus cyclosporine had efficacy in untreated patients with severe or very severe aplastic anemia.[20] We report the results of a phase 3, prospective, investigator-led, multicenter, open-label, randomized trial comparing horse ATG plus cyclosporine with or without eltrombopag as first-line therapy in patients with severe or very severe aplastic anemia.

Methods

Trial Design and Oversight

RACE (Randomized, Multicenter Trial Comparing Horse ATG plus Cyclosporine with or without Eltrombopag as First-Line) was conducted at 24 sites in six European countries by the European Society for Blood and Marrow Transplantation (EBMT). Ethics committees at the participating institutions approved the trial, and an independent data and safety monitoring board provided oversight. Patients were randomly assigned to receive either horse ATG plus cyclosporine or horse ATG plus cyclosporine and eltrombopag. Randomization was stratified according to age (≥15 to <40 years or ≥40 years), disease severity (severe or very severe), and center. Adverse events were classified according to the Common Terminology Criteria for Adverse Events, version 4.03.[21]

The authors wrote the manuscript without assistance, gathered and analyzed the data, and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol, available with the full text of this article at NEJM.org. All the patients or their legal guardians provided written informed consent. The first and last authors conceived of the trial, which was designed in collaboration with the other authors. No one who is not an author contributed to the writing of the manuscript. Pfizer provided horse ATG, and Novartis provided eltrombopag; both companies also provided research support to EBMT under a Cooperative Research and Development Agreement but had no role in writing the manuscript.

Patients

From July 2015 through April 2019, a total of 285 patients who were 15 years of age or older, who had a new diagnosis of acquired severe or very severe aplastic anemia,[22] and who were not eligible for front-line hematopoietic stem-cell transplantation underwent screening. Of these patients, 205 untreated patients were enrolled. After enrollment, 2 patients died and 6 were later found to have diagnoses other than aplastic anemia, leaving 197 patients with a confirmed diagnosis of severe or very severe aplastic anemia (Fig. S1 in the Supplementary Appendix, available at NEJM.org).

Treatment

Patients in Group A received standard immunosuppressive therapy consisting of horse ATG (Atgam, Pfizer) administered at a dose of 40 mg

per kilogram of body weight per day on 4 consecutive days and oral cyclosporine at a dose of 5 mg per kilogram of body weight per day from day 1 for a minimum of 12 months; cyclosporine was subsequently tapered during the following 12 months and was discontinued by 24 months (Supplementary Appendix). Patients in Group B received experimental therapy consisting of standard immunosuppressive therapy plus eltrombopag administered orally at a dose of 150 mg per day from day 14 through 6 months or through 3 months in patients who had a complete response (defined below) at 3 months. All patients in Group B who had a partial response (defined below) at 3 months continued to receive eltrombopag through 6 months, in accordance with the protocol.

ANALYSES OF SOMATIC MUTATIONS

Samples of bone marrow were obtained at baseline, 6 months, and 2 years in order to analyze the frequency and variant allele frequency of somatic myeloid cancer–associated mutations. A 31-gene targeted molecular bar-coded panel was used (Table S1).

END POINTS

The primary end point of the trial was a hematologic complete response at 3 months, defined as a hemoglobin level greater than 10 g per deciliter, an absolute neutrophil count greater than 1000 per cubic millimeter, and a platelet count greater than 100,000 per cubic millimeter in patients who had not received transfusions.[7] The criteria for a partial response were transfusion independence (both red cells and platelets), with a blood lineage that did not meet the criteria of severe aplastic anemia but was insufficient for a complete response. Secondary end points included overall response (defined as a complete response or partial response); the time to first response, best response, and complete response; overall survival; event-free survival; relapse; clonal evolution; hemolytic paroxysmal nocturnal hemoglobinuria; discontinuation of immunosuppression; and quality of life as reported by the patient (Supplementary Appendix).

STATISTICAL ANALYSIS

The trial was based on the hypothesis that the hematologic complete response rate at 3 months would be 3 times as high in the experimental group (estimated at 21%) as in the standard-therapy group (estimated at 7%).[13] We estimated that a sample of 96 patients in each treatment group would provide the trial with 80% power (two-sided test) to reject the null hypothesis at a 5% significance level; this sample was increased to 100 patients to compensate for patients with data that could not be evaluated. The cutoff date for analysis was March 1, 2020. All efficacy end points were evaluated on an intention-to-treat basis. The comparison of the complete response rate and the overall response rate was performed with the Mantel–Haenszel pooled odds ratio, stratified according to the factors used for randomization (age, severity of aplastic anemia, and center) (Table S2).

RESULTS

PATIENTS

No significant differences between the two groups with respect to demographic and clinical features were noted (Table 1). The median follow-up among the patients in both groups was 24 months (95% confidence interval [CI], 23 to 24).

HEMATOLOGIC RESPONSE

The percentage of patients with a complete response at 3 months was 10% in Group A and 22% in Group B (pooled odds ratio, 3.2; 95% CI, 1.3 to 7.8; P=0.01), which represented a significant between-group difference in the primary end point (Table 2). The overall response rate at 3 months was lower in Group A (31%) than in Group B (59%).

Of the 70 patients in Group A who did not have a response at 3 months, 14 had an overall response at 6 months (4 had a complete response, and 10 had a partial response). Of the 39 patients in Group B who did not have a response at 3 months, 11 had an overall response at 6 months (4 had a complete response, and 7 had a partial response). At 6 months, the overall response rate was 41% in Group A and 68% in Group B. Better responses were observed in Group B than in Group A at each time point and in all strata (i.e., the severity of aplastic anemia and age) (Table 2). The superiority of the experimental therapy over standard therapy was also confirmed when National Institutes of Health

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Group A: Horse ATG–Cyclosporine (N=101)	Group B: Horse ATG–Cyclosporine–Eltrombopag (N=96)	All Patients (N=197)
Follow-up — mo			
Median	24	23	24
95% CI	23–24	19–24	23–24
Age — yr			
Median	52	55	53
Range	15–81	16–77	15–81
Age category — no. (%)			
≥15 to <18 yr	7 (7)	2 (2)	9 (5)
≥18 to <40 yr	29 (29)	27 (28)	56 (28)
≥40 to <65 yr	43 (43)	43 (45)	86 (44)
≥65 yr	22 (22)	24 (25)	46 (23)
Sex — no. (%)			
Male	52 (52)	56 (58)	108 (55)
Female	49 (48)	40 (42)	89 (45)
Severity of aplastic anemia — no. (%)			
Severe	67 (66)	62 (65)	129 (66)
Very severe	34 (34)	34 (35)	68 (34)
Laboratory values			
GPI-deficient neutrophils ≥1.0% — no./total no. (%)	44/100 (44)	33/93 (36)	77/193 (40)
Reticulocyte count — per mm^3			
Median	20,000	23,300	21,000
IQR	8,900–36,000	12,000–46,800	10,000–38,000
Neutrophil count — per mm^3			
Median	300	500	400
IQR	100–700	100–1000	100–800
Lymphocyte count — per mm^3			
Median	1,400	1,400	1,400
IQR	1,000–1,800	1,000–1,700	1,000–1,800
Platelet count — per mm^3			
Median	18,000	15,000	17,000
IQR	10,000–32,000	10,000–29,000	10,000–30,000
Cytogenetic abnormalities — no./total no. (%)			
Normal	64/86 (74)	61/84 (73)	125/170 (74)
Abnormal karyotype†	7/86 (8)	6/84 (7)	13/170 (8)
Karyotypic analysis failed	15/86 (17)	17/84 (20)	32/170 (19)
Somatic myeloid mutations — no. of patients/total no. evaluated (%)‡	23/78 (29)	24/78 (31)	47/156 (30)

* ATG denotes antithymocyte globulin, CI confidence interval, GPI glycophosphatidylinositol, and IQR interquartile range.
† The category of abnormal karyotype included 7 patients with deletion Y (3 in Group A and 4 in Group B), 2 patients with trisomy 8 in Group A, 1 patient with deletion 20q in Group B, and 3 patients (1 in Group A and 2 in Group B) with other abnormalities (Table S12).
‡ In 41 patients, mutations were missing at baseline for the following reasons: 9 minors (<18 years of age) could not be included according to the King's College London Haemato-Oncology Tissue Bank policy, 8 patients did not consent to biosampling, 2 samples were lost during transit to the central laboratory, and 22 samples were not included for other reasons, mainly because the analysis had not been performed at the time of data lock.

Table 2. Hematologic Response, According to Treatment Group.

Cohort and Response	Response at 3 Mo				Response at 6 Mo	
	Group A: Horse ATG–Cyclosporine (N=101)	Group B: Horse ATG–Cyclosporine–Eltrombopag (N=96)	Odds Ratio (95% CI)*	P Value	Group A: Horse ATG–Cyclosporine (N=101)	Group B: Horse ATG–Cyclosporine–Eltrombopag (N=95)†
All patients — no. (%)						
Complete response‡	10 (10)	21 (22)	3.2 (1.3–7.8)	0.01	20 (20)	30 (32)
Partial response	21 (21)	36 (38)			21 (21)	35 (37)
No response	70 (69)	39 (41)			60 (59)	30 (32)
Overall response§	31 (31)	57 (59)			41 (41)	65 (68)
Patients with severe aplastic anemia — no./total no. (%)						
Complete response	10/67 (15)	17/62 (27)			15/67 (22)	20/62 (32)
Partial response	17/67 (25)	27/62 (44)			16/67 (24)	26/62 (42)
No response	40/67 (60)	18/62 (29)			36/67 (54)	16/62 (26)
Patients with very severe aplastic anemia — no./total no. (%)						
Complete response	0/34	4/34 (12)			5/34 (15)	10/33 (30)
Partial response	4/34 (12)	9/34 (26)			5/34 (15)	9/33 (27)
No response	30/34 (88)	21/34 (62)			24/34 (71)	14/33 (42)
Patients ≥15 to <40 yr — no./total no. (%)						
Complete response	6/36 (17)	6/29 (21)			11/36 (31)	15/29 (52)
Partial response	6/36 (17)	14/29 (48)			7/36 (19)	8/29 (28)
No response	24/36 (67)	9/29 (31)			18/36 (50)	6/29 (21)
Patients ≥40 yr — no./total no. (%)						
Complete response	4/65 (6)	15/67 (22)			9/65 (14)	15/66 (23)
Partial response	15/65 (23)	22/67 (33)			14/65 (22)	27/66 (41)
No response	46/65 (71)	30/67 (45)			42/65 (65)	24/66 (36)

* The pooled odds ratios for Group B as compared with Group A and 95% confidence intervals were obtained with the use of the Mantel–Haenszel test, stratified according to the factors used at randomization (age, severity of aplastic anemia, and center).
† One patient in Group B did not have follow-up to the 6-month evaluation and did not have a competing event at the last follow-up.
‡ At 6 months, 4 of the patients who had had a complete response at 3 months (1 patient in Group A and 3 patients in Group B) had loss of response (i.e., they moved from complete response to no response) and 7 patients (all in Group B) had a downgrade in response from complete response to partial response.
§ The overall response corresponded to the percentage of patients who had a partial or complete response.

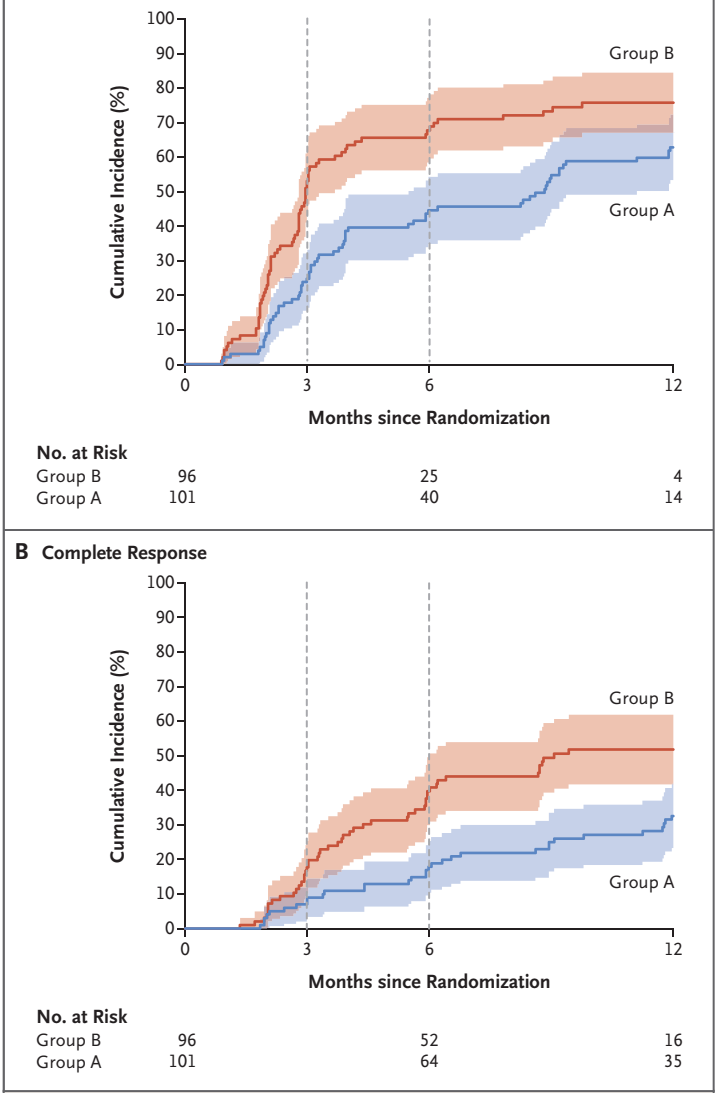

Figure 1. Kinetics of Hematologic Response.

The cumulative incidences of a response are plotted according to treatment group. The shaded areas indicate 95% confidence intervals. Competing events were hematopoietic stem-cell transplantation, any additional treatment for aplastic anemia, clonal evolution, and death. Panel A shows the time to first response. The cumulative incidence of a first response at 3 months was 25% (95% CI, 16 to 33) in Group A and 53% (95% CI, 43 to 63) in Group B; at 6 months, the incidences were 45% (95% CI, 35 to 54) and 68% (95% CI, 58 to 77), respectively. Panel B shows the time to complete response. The median time to complete response was 9.1 months in Group B, whereas in Group A less than 50% of the patients had a complete response during the trial observation period.

(NIH) criteria for partial response (which do not include transfusion independence[7]) were used, with an overall response rate of 66% in Group A and 77% in Group B at 3 months and 66% and 79%, respectively, at 6 months (Tables S3 and S4).

The median time to a first response was 8.8 months in Group A and 3.0 months in Group B (Fig. 1A). At 12 months, the complete response rate was 33% in Group A and 52% in Group B (Fig. 1B). The time from partial response to complete response was 5.1 months in Group A (32 patients) and 2.7 months in Group B (43 patients). The median time to best response was 8.9 months in Group A and 3.9 months in Group B.

Among the patients who had a response, the time to platelet transfusion independence was 68 days (interquartile range, 34 to 151) in Group A and 40 days (interquartile range, 20 to 80) in Group B. The time to red-cell transfusion independence was 140 days (interquartile range, 62 to 252) in Group A and 51 days (interquartile range, 23 to 122) in Group B.

PREDICTORS OF RESPONSE

Details of the univariate and multivariable analyses are provided in Table S5A and S5B. In the multivariable analysis, randomization group, age, and disease severity were the only three factors associated with a response. Patients in Group B had a higher probability of a complete response at 3 months and an overall response at 6 months. More severe disease (very severe vs. severe) was a negative predictor for both a complete response at 3 months and an overall response at 6 months. Older age (≥40 years) was associated with a lower overall response rate at 6 months but not with a lower complete response rate at 3 months.

ADVERSE EVENTS

One patient in Group A who died prematurely did not begin to receive horse ATG according to the protocol; all other patients received horse ATG. Six patients (3 in each group) had an interrupted course of horse ATG because of safety reasons or the physician's decision. Cyclosporine was permanently discontinued within the first 6 months in 18 patients (11 in Group A and 7 in Group B), predominantly because of renal toxicity (data not shown). All patients who were randomly assigned to Group B received eltrombopag, which was discontinued before 6 months in 10 patients (Table S6A) because of elevated liver enzyme levels (in 4 patients); a slight in-

crease in reticulin deposition in the bone marrow (in 2 patients) (Table S6B); or other reasons (in 4 patients). The incidence of all adverse events, including infectious and hepatic complications, was similar in the two groups (Tables S7 through S10).

PATIENT-REPORTED OUTCOMES

Outcomes reported by the patients were assessed with the use of the European Organization for Research and Treatment of Cancer core Quality of Life of Cancer Patients questionnaire at baseline and at 6, 12, and 24 months after randomization. Scores improved from baseline over time with respect to global health status, as well as on physical, social, and emotional scales, with minimal differences between the groups (Table S11).

KARYOTYPIC ABNORMALITIES, MYELOID CANCERS, AND SOMATIC MUTATIONS

Only three confirmed karyotypic abnormalities met the definition of karyotypic evolution according to the protocol (1 patient in Group A had monosomy 7 and 2 patients in Group B had del[13q]). There was no morphologic evidence of myelodysplastic syndrome.

Next-generation sequencing was available at the time of analysis for 156 patients at baseline, 121 patients at 6 months, and 53 patients at 24 months of follow-up. At baseline, with the exclusion of *PIGA* mutations, 47 patients (30%) had somatic mutations; 36 patients (23%) had 1 mutation, 10 patients (6%) had 2 mutations, and 1 patient (<1%) had more than 2 mutations (Fig. 2A). The most frequently mutated genes were *DNMT3A*, *BCOR*, *BCORL1*, and *PIGA*. Overall, patients with mutations were older, had severe aplastic anemia (as compared with very severe aplastic anemia), and had a higher neutrophil count than those without mutations. The median age of patients with *PIGA*, *BCOR*, and *BCORL1* mutations was lower than that of patients with other mutations (41 years vs. 59 years).

At baseline, the frequency of mutations, the mutated genes, and the median variant allele frequency did not differ significantly between the two treatment groups (Fig. 2B). Baseline mutations were not significantly associated with overall survival (Fig. S3) or response. Complete response rates at 3 months were 14% among patients without mutations and 21% among those with mutations. The overall response rate at 6 months was 49% among patients without mutations and 60% among those with mutations (Table S13).

During the disease course, the frequency of mutations in patients increased from approximately 30% in both groups at baseline to 66% in Group A and 55% in Group B at 6 months, and to 77% and 52%, respectively, at 24 months (Fig. 2B). Fluctuations in the variant allele frequency of mutations were noted in both groups at three different time points (Fig. 2C).

Irrespective of baseline mutations, at 6 months, new or additional mutations were acquired in 30 patients (53%) in Group A and in 22 patients (39%) in Group B. At 24 months, new or additional mutations were acquired in 16 patients (62%) in Group A and in 6 patients (27%) in Group B (Table S15). These mutations did not correlate with hematologic response or with overall survival (Fig. S5).

LONG-TERM OUTCOMES

The 2-year overall survival was similar in Group A (85%; 95% CI, 78 to 92) and Group B (90%; 95% CI, 82 to 97) (Fig. S2). Twenty-two patients died during the trial — 14 in Group A and 8 in Group B (Table S17). A total of 23 patients underwent hematopoietic stem-cell transplantation — 12 in Group A and 11 in Group B; details of other additional treatments according to group are provided in Table S18. The cumulative incidence of hemolytic paroxysmal nocturnal hemoglobinuria at 24 months was 7% in Group A and 1% in Group B (Table S19). The cumulative incidence of relapse 18 months after response did not differ significantly between Group A (11%; 95% CI, 2 to 20) and Group B (19%; 95% CI, 9 to 29).

In the multivariable analysis, the two groups had similar overall survival (hazard ratio for death in Group B as compared with Group A, 0.57; 95% CI, 0.24 to 1.37) and relapse risk (hazard ratio, 1.32; 95% CI, 0.55 to 3.21). Older age was the only factor associated with worse overall survival and relapse risk. At 2 years, more events had occurred in Group A than in Group B, which resulted in an inferior event-free survival in Group A (34%; 95% CI, 24 to 44) than in Group B (46%; 95% CI, 36 to 57) (Fig. 3A). The

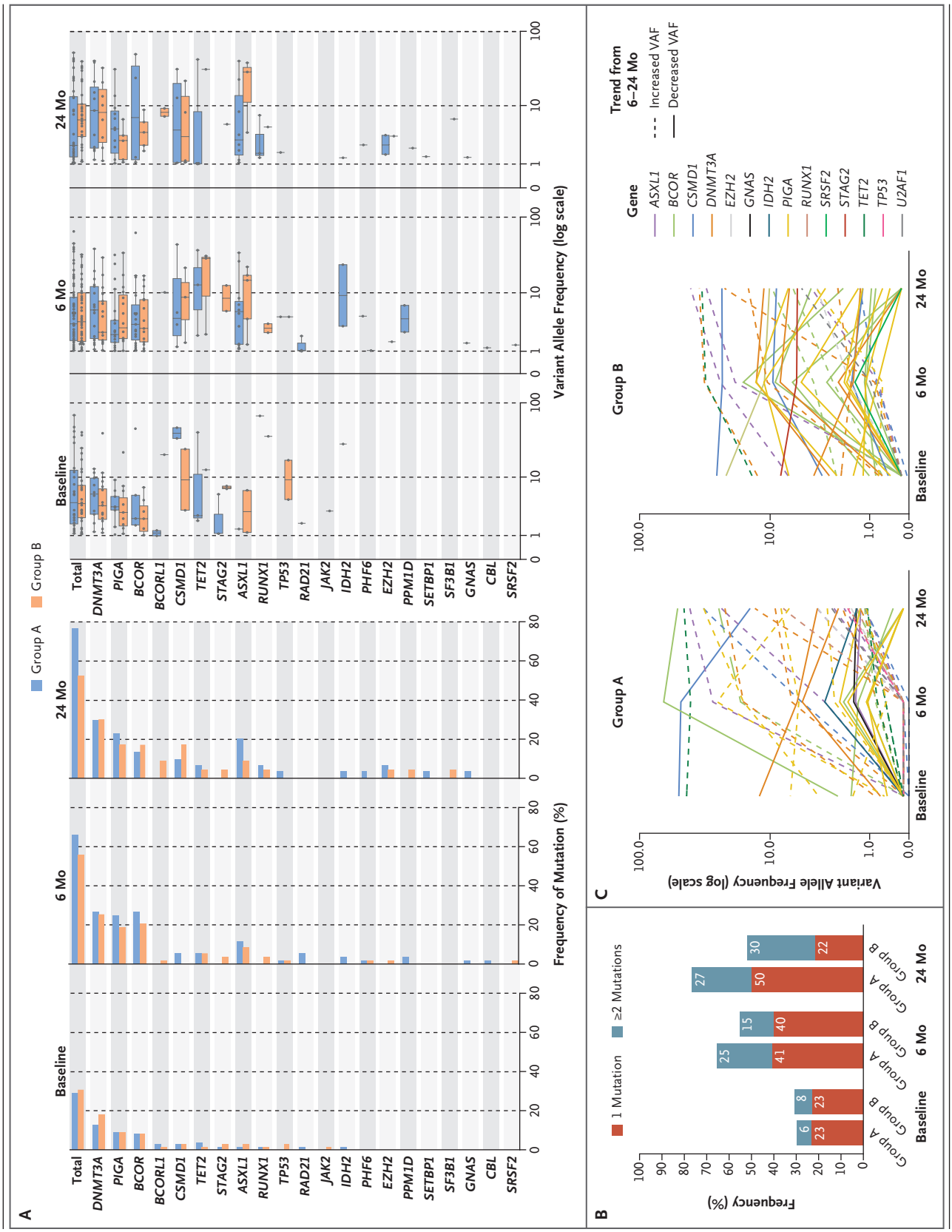

Figure 2 (facing page). Somatic Mutations.

Panel A shows the frequency and variant allele frequency (VAF) of mutations at baseline, 6 months, and 24 months. The variant allele frequency of the mutations is shown on a logarithmic scale. The box-and-whisker plots of the specific gene mutations are shown; the whiskers indicate the range, the sides of the boxes indicate the interquartile range, and the vertical line within each box indicates the median. The gray dots indicate individual mutations, and the vertical lines over some of the gray dots indicate the range (minimum and maximum). Panel B shows the frequency of mutations as a measure of 1 or 2 or more mutations in each group at different time points. Panel C shows the variant allele frequency of mutations in each group in patients who were screened (45 patients) and had detectable mutations (34 patients) at all three time points.

most common treatment failure events were no response in Group A and no response and the use of additional treatment in Group B (Fig. 3B). In the multivariable analysis, risk among patients in Group B was reduced in the first 6 months (hazard ratio for treatment failure events, 0.42; 95% CI, 0.25 to 0.72). Older age and disease severity were confirmed as risk factors. At 24 months, 19% of the patients in Group A and 28% of those in Group B had a cyclosporine-independent response.

DISCUSSION

Over the past 30 years, efforts to improve the results of standard therapy (horse ATG plus cyclosporine) in patients with severe aplastic anemia have been largely unsuccessful.[7,9-15] In this prospective, randomized, multicenter trial, hematologic complete and overall responses at 3 months were significantly better with eltrombopag added to standard therapy than with standard therapy alone, and the quality and speed of hematologic recovery were better with the addition of eltrombopag as well, with no excess of toxic effects. In this trial, eltrombopag was started at day 14 (to prevent possible cumulative toxicity from concomitant administration of ATG), with no detrimental effect on the early hematologic response; in contrast, the phase 2 NIH trial initially suggested that the efficacy of standard therapy was improved with the addition of eltrombopag, with the best results when eltrombopag was introduced at day 1.[20]

The mechanisms of action of eltrombopag in aplastic anemia warrant further investigation. Previous studies have shown that eltrombopag stimulates hematopoiesis despite high levels of endogenous thrombopoietin.[23] However, it is not clear whether this action is exerted at the level of hematopoietic stem cells or on more mature progenitor cells (i.e., by increasing the ratio of progenitor cells to stem cells). Thus, independent of its molecular mechanisms, eltrombopag appears to sustain hematopoiesis, buying time for immunosuppression to curb the immune attack on hematopoietic stem cells. Furthermore, in addition to its direct stimulatory action on hematopoiesis, eltrombopag might contribute to the immunosuppressive effect of ATG plus cyclosporine. A recent study showed that by binding to the transmembrane domain of the thrombopoietin receptor, eltrombopag prevents the inhibitory effect of interferon-γ by interrupting the interaction between endogenous thrombopoietin and its cognate receptor (i.e., serving as a decoy receptor).[24]

We evaluated the hematologic complete response as a primary end point because survival and long-term outcomes are associated with the quality of hematologic response at 3 months and with the presence of early recovery after the administration of ATG.[7,8] In our trial, the median times to first response and complete response were shorter with immunosuppressive therapy plus eltrombopag than with immunosuppressive therapy alone; these faster response times accounted for the achievement of earlier red-cell and platelet transfusion independence in the experimental group. At 6 months, the overall response rate increased from 41% to 68%, with transfusion independence as a prerequisite for partial response. On the basis of the NIH criteria for partial response (i.e., improvement in blood counts but no need for transfusion independence), the overall response rate at 6 months with immunosuppressive therapy plus eltrombopag was also significantly better than with immunosuppressive therapy alone (79% and 66%). Even if the difference in the response rate decreases over time, this earlier hematologic recovery may translate into fewer patients having to switch to early hematopoietic stem-cell transplantation.

The ability to identify patients who have a higher probability of hematologic response is important. In our trial, less severe aplastic ane-

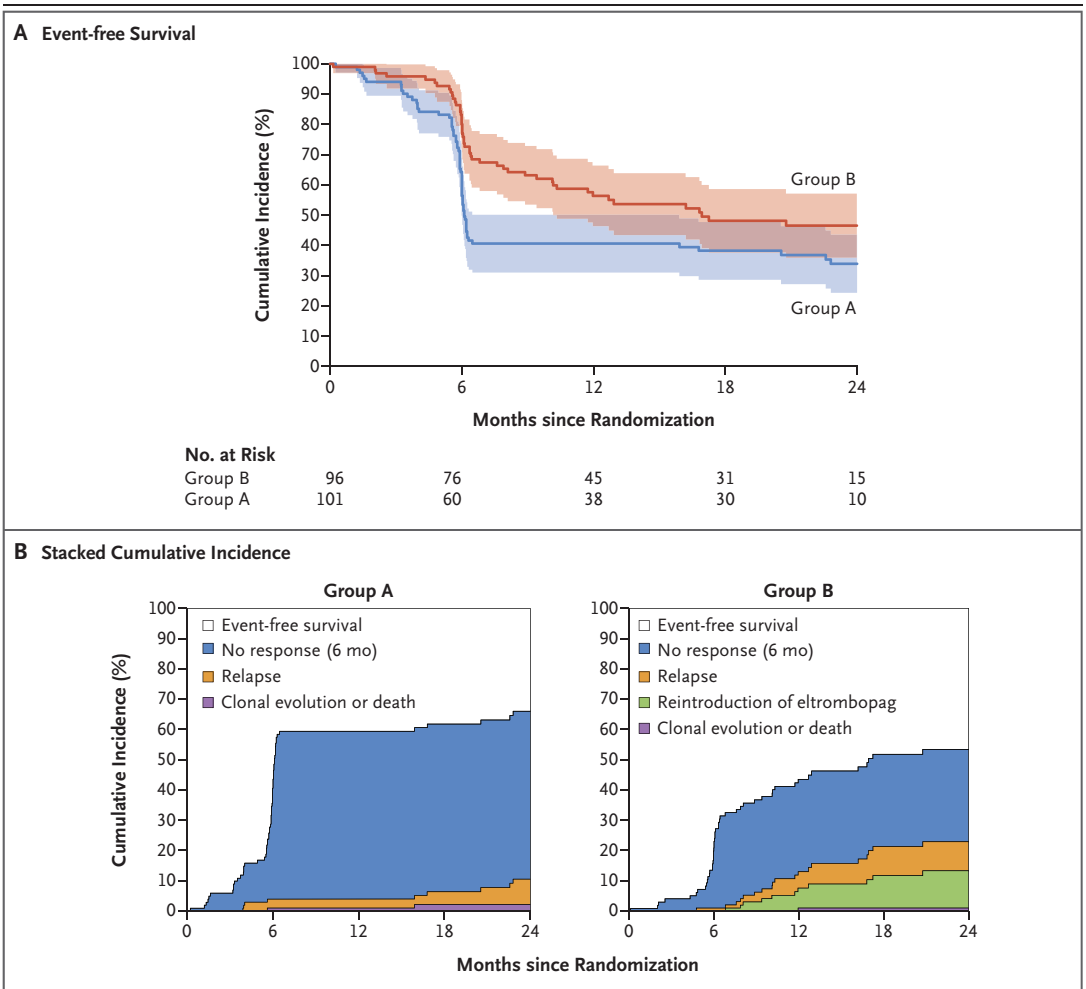

Figure 3. Event-free Survival and Stacked Cumulative Incidence Curves.

Panel A shows Kaplan–Meier curves for event-free survival according to treatment group. The shaded areas indicate 95% confidence intervals. In Group A, event-free survival at 6 months was 59% (95% CI, 50 to 69); at 12 months, 41% (95% CI, 31 to 50); and at 24 months, 34% (95% CI, 24 to 44); in Group B, these rates were 79% (95% CI, 71 to 87), 56% (95% CI, 46 to 66), and 46% (95% CI, 36 to 57), respectively. Events were no response at 6 months, hematopoietic stem-cell transplantation, any additional treatment for aplastic anemia, clonal evolution, relapse, or death. Panel B shows stacked cumulative incidence curves according to event. The overall cumulative incidence of an event (1 minus the probability of event-free survival) is shown according to the type of event that occurred. In Group B, reintroduction of eltrombopag was defined according to the protocol in case of relapse or a weak hematologic response (see the Eltrombopag Dosing and Adjustments section in the Supplementary Appendix).

mia (severe vs. very severe) and younger age (<40 years) were associated with a better response. Thus, both factors remain the two main clinical predictors, even in triple therapy for aplastic anemia.[20] None of the previously reported baseline hematologic characteristics[25] were associated with the overall response rate in our trial.

The results of a prespecified overall safety analysis that included infectious and hepatic complications did not differ significantly between the two groups. Two patients discontinued eltrombopag because of focal grade 1 reticulin deposition in the trephine biopsy that reversed on discontinuation of eltrombopag; these findings are consistent with the long-term follow-up of the use of a thrombopoietin-recep-

tor agonist in immune thrombocytopenia.[26] The outcomes reported by the patients showed overall improvement in both treatment groups, with no significant differences between the groups.

The addition of eltrombopag to standard immunosuppressive therapy did not result in significantly improved overall survival, which was expected considering the additional effect of rescue treatment. The 85 to 90% 2-year overall survival rate is higher than most rates observed in multicenter studies involving patients with severe aplastic anemia. Nevertheless, eltrombopag added to standard immunosuppressive therapy significantly increased event-free survival from 34% to 46% at 2 years through the reduction in initial refractoriness to immunosuppression. However, the reintroduction of eltrombopag was the most common event in the experimental group; this finding provides justification for further studies with longer follow-up to improve long-term treatment in patients with aplastic anemia.

With the advent of next-generation sequencing, the presence of somatic mutations emerged as a common finding in patients with aplastic anemia; these mutations had a possible effect on progression to myeloid cancers and on long-term outcomes.[27,28] This finding was an obvious concern because of eltrombopag-associated stem-cell stimulatory properties.[29] In this prospective trial, we found that the prevalence of somatic mutations was not higher in the eltrombopag group (Group B) than in the standard-therapy group (Group A). We were surprised to find, however, that the percentage of patients with mutations increased from 29% at baseline to 66% at 6 months in Group A and from 31% at baseline to 55% at 6 months in Group B. Thus, we prospectively found that hematologic recovery after immunosuppressive therapy with or without eltrombopag was likely to be oligoclonal. However, clones (at baseline or later) did not negatively affect the response or 2-year outcomes. The dominance of these clones, as tracked by variant allele frequency, was largely unpredictable, with no common patterns across patients, suggesting genetic drift more than an active selection process.[30] Similarly, despite the prevalence of the *PIGA* mutation, eltrombopag did not selectively induce paroxysmal nocturnal hemoglobinuria clonal expansion, as compared with standard immunosuppressive therapy.

Clonal hematopoiesis associated with hematopoietic recovery[31,32] was frequent but, as shown in paroxysmal nocturnal hemoglobinuria,[30,33] it should not be confused with "clonal evolution," which is considered to be progression to a myeloid cancer.[16] A long-term follow-up of this trial is planned to explore the clinical relevance of this oligoclonal hematopoiesis and to evaluate the risk of myeloid malignant transformation, which usually appears in 10 to 15% of patients 5 to 10 years after diagnosis.[8,16] However, treating physicians should not overinterpret the presence of somatic mutations; therapeutic decisions (i.e., commitment to hematopoietic stem-cell transplantation) should be made only in the presence of clear clinical indications.

This prospective randomized trial showed that the addition of eltrombopag to horse ATG plus cyclosporine, as compared with horse ATG plus cyclosporine alone, was beneficial in patients with severe aplastic anemia. The addition of eltrombopag induced a response that was of higher quality and occurred faster without increasing toxic effects.

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APPENDIX

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