ORIGINAL ARTICLE

Telomerase Inhibitor Imetelstat in Patients with Essential Thrombocythemia

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ABSTRACT

BACKGROUND

Imetelstat, a 13-mer oligonucleotide that is covalently modified with lipid extensions, competitively inhibits telomerase enzymatic activity. It has been shown to inhibit megakaryocytic proliferation in vitro in cells obtained from patients with essential thrombocythemia. In this phase 2 study, we investigated whether imetelstat could elicit hematologic and molecular responses in patients with essential thrombocythemia who had not had a response to or who had had unacceptable side effects from prior therapies.

METHODS

A total of 18 patients in two sequential cohorts received an initial dose of 7.5 or 9.4 mg of imetelstat per kilogram of body weight intravenously once a week until attainment of a platelet count of approximately 250,000 to 300,000 per cubic millimeter. The primary end point was the best hematologic response.

RESULTS

Imetelstat induced hematologic responses in all 18 patients, and 16 patients (89%) had a complete hematologic response. At the time of the primary analysis, 10 patients were still receiving treatment, with a median follow-up of 17 months (range, 7 to 32 [ongoing]). Molecular responses were seen in 7 of 8 patients who were positive for the *JAK2* V617F mutation (88%; 95% confidence interval, 47 to 100). *CALR* and *MPL* mutant allele burdens were also reduced by 15 to 66%. The most common adverse events during treatment were mild to moderate in severity; neutropenia of grade 3 or higher occurred in 4 of the 18 patients (22%) and anemia, headache, and syncope of grade 3 or higher each occurred in 2 patients (11%). All the patients had at least one abnormal liver-function value; all persistent elevations were grade 1 or 2 in severity.

CONCLUSIONS

Rapid and durable hematologic and molecular responses were observed in patients with essential thrombocythemia who received imetelstat. (Funded by Geron; ClinicalTrials.gov number, NCT01243073.)

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N Engl J Med 2015;373:920-8.
DOI: 10.1056/NEJMoa1503479

Essential thrombocythemia, a myeloproliferative neoplasm, is a clonal disorder of a multipotent hematopoietic progenitor cell.[1,2] The disease is associated with an increased risk of thrombotic complications, hemorrhagic complications, or both, and can evolve into myelofibrosis or, in rare cases, can transform to acute leukemia.[3] Common mutations associated with essential thrombocythemia are found in the Janus kinase 2 (*JAK2*) gene, the gene encoding the thrombopoietin receptor (*MPL*), and the calreticulin (*CALR*) gene.[4-8] Current standard therapies for high-risk patients with essential thrombocythemia induce nonspecific reductions in platelet counts but do not typically eliminate or alter the biologic characteristics of the disease.[9-12]

We have reported that telomerase activity in malignant cells obtained from patients with essential thrombocythemia and induced telomerase activity in cells isolated from healthy donors were inhibited by the telomerase inhibitor imetelstat.[13] However, imetelstat inhibited spontaneous proliferation of megakaryocytic colonies obtained from patients with essential thrombocythemia but did not inhibit cytokine-induced megakaryocytic colonies from healthy donors. These findings suggest an intrinsic sensitivity of essential-thrombocythemia megakaryocytes to telomerase inhibition.[13]

Imetelstat, a 13-mer thiophosphoramidate oligonucleotide that is covalently modified with lipid extensions, inhibits telomerase enzymatic activity[14] (see the Supplementary Background section in the Supplementary Appendix, available with the full text of this article at NEJM.org). In this phase 2 study, we investigated whether imetelstat could elicit hematologic and molecular responses in patients with essential thrombocythemia who had disease that was refractory to prior therapies or who had had unacceptable side-effects from previous therapies.

METHODS

ELIGIBILITY CRITERIA

Patients who were 18 years of age or older and had received a diagnosis of essential thrombocythemia defined according to World Health Organization criteria[15] (Table S1 in the Supplementary Appendix) were eligible for enrollment if they required cytoreduction because of a platelet count higher than 600,000 per cubic millimeter and if they had not had a response to or had had unacceptable side effects from at least one prior therapy or if they declined to receive standard therapy. Additional inclusion and exclusion criteria are described in the Supplementary Appendix.

STUDY DESIGN

This was a phase 2, open-label study that was conducted at seven sites in the United States, Germany, and Switzerland; enrollment occurred during the period from January 2010 through January 2013. Patients were enrolled into sequential cohorts that received weekly doses of 7.5 or 9.4 mg of imetelstat per kilogram of body weight, intravenously, until a platelet count of approximately 250,000 to 300,000 per cubic millimeter was achieved or toxic effects occurred. Maintenance dosing at a reduced frequency began after a patient had a complete or partial hematologic response (Table S2 in the Supplementary Appendix), with doses (7.5 to 11.7 mg per kilogram) adjusted according to the patient's response and the side-effect profile of the drug. Additional details regarding the study design are provided in the Supplementary Appendix.

The primary end point of the study was the best overall hematologic response, which was defined according to the platelet response component of the 2009 European LeukemiaNet (ELN) response criteria[16] (Table S2 in the Supplementary Appendix). Secondary end points included the frequency and severity of adverse events, the duration of hematologic response, the ELN "clinicohematologic" response, and the molecular response in patients with *JAK2* V617F mutations (Table S2 in the Supplementary Appendix), *MPL* W515L or *MPL* W515K mutations, or *CALR* mutations.

All patients were assessed for *JAK2* V617F, *MPL* W515L, *MPL* W515K, and *CALR* mutations at baseline and were reassessed every 12 weeks (±4 weeks) (see the Supplementary Methods section in the Supplementary Appendix). Patients who were enrolled in Europe underwent testing of spontaneous growth inhibition of megakaryocyte colony-forming units (CFUs) (see the Supplementary Methods section in the Supplementary Appendix).

SAFETY ASSESSMENT

Data were collected on the extent of imetelstat exposure and on all adverse events. Adverse events were summarized according to terms used in the *Medical Dictionary for Regulatory Activities*, and severity was assessed with the use of the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE),[17] version 4.03. For events with varying severity, the maximum reported grade was used in summaries. Laboratory abnormalities were defined according to normal laboratory ranges from each institution and converted to CTCAE grades.

STUDY OVERSIGHT

This phase 2 study was approved by the institutional review board at each participating site. The study was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines. All patients provided written informed consent.

The sponsor, Geron, provided the study drug and in collaboration with the authors designed the study and analyzed the data. The first author wrote the initial draft, and subsequent drafts were written by all authors with assistance from medical writers paid by Janssen Research and Development. All the authors made the decision to submit the manuscript for publication and vouch for the accuracy and completeness of the data and analyses and for adherence to the study protocol, which is available at NEJM.org. Clinical-trial agreements between the sponsor and the authors' institutions included confidentiality provisions.

STATISTICAL ANALYSIS

The analysis of the primary end point of hematologic response included all patients who received at least one dose of imetelstat. The analysis of the duration of response included all patients who had a response. The analysis of molecular response included patients who received at least one dose of imetelstat and who had a *JAK2* V617F, *MPL* W515L, *MPL* W515K, or *CALR* mutant allele burden at baseline. For response end points, exact 95% confidence intervals were calculated. The duration of hematologic response was estimated with the use of the Kaplan–Meier method. Patients who were lost to follow-up, discontinued the study, or began to receive other essential thrombocythemia–directed therapy before a hematologic response was documented and confirmed were counted as not having had a response. Safety analyses included all patients who received at least one dose of imetelstat.

RESULTS

PATIENTS

A total of 18 patients were enrolled in the study, and all had received one or more previous treatments; 17 had received hydroxyurea (94%), 13 had received anagrelide (72%), and 4 had received interferon (22%) (Table 1). Nine of the 18 patients (50%) had disease that was resistant to at least one prior therapy, and 14 (78%) had had unacceptable side effects from prior therapy. The median time from the initial diagnosis of essential thrombocythemia to enrollment was 7.2 years (range, 0.3 to 24.9), and the median baseline platelet count was 788,000 per cubic millimeter (range, 521,000 to 1,359,000). Fifteen patients had baseline mutant allele burdens (8 with *JAK2* V617F mutations, 2 with *MPL* W515L or *MPL* W515K mutations, and 5 with *CALR* mutations) (Table 1). Table S3 in the Supplementary Appendix describes additional baseline characteristics of the patients.

PRIMARY EFFICACY END POINT AND KEY SECONDARY EFFICACY END POINT

Of the 18 patients who received imetelstat, 7 received an initial dose of 7.5 mg per kilogram and 11 received 9.4 mg per kilogram intravenously. The overall rate of hematologic response was 100% (95% confidence interval [CI], 83 to 100), and 16 of the 18 patients (89%) had a complete hematologic response (95% CI, 65 to 99) (Fig. 1A). In the 2 patients with a partial hematologic response, the lowest platelet counts were 261,000 per cubic millimeter at 23 weeks and 56,000 per cubic millimeter at 18 weeks, but neither response was sustained. Among the 16 patients who had a complete hematologic response, the median time to a complete response was 6.1 weeks (range, 5.1 to 12.1); the median time to a complete response was similar in *JAK2* V617F–positive and *JAK2* V617F–negative patients. A trend was observed toward a more rapid response in patients in whom the initial dose was 9.4 mg per kilogram than in those in whom the initial dose was 7.5 mg per kilogram (median, 6.1 vs. 12.1

weeks), although the difference was not significant (P=0.71).

During maintenance dosing, intermittent dosing was attempted in most patients and the frequency of dosing generally decreased with time; all the patients who had a complete hematologic response received a dose every 2 weeks or less frequently. At the time of the primary analysis, with a median follow-up of 17 months, 10 of the 16 patients with a complete hematologic response (62%) were still receiving treatment, and the median duration of response had not been reached (follow-up range, 5 to 30 months).

While receiving the study treatment, one patient had grade 2 bilateral retinal ischemia and was recovering, and another had grade 2 left hemiparesis and recovered; these thromboembolic events were assessed by the study investigators as being potentially related to underlying essential thrombocythemia. The durations of response ended at the reported onset of these conditions, no further clinical follow-up information was obtained, and neither patient discontinued imetelstat. Three patients had disease progression from essential thrombocythemia to secondary myelofibrosis, either during the study treatment period or during the safety follow-up period.

Table 1. Baseline Characteristics of the Patients.

Variable	Patients (N=18)
Age >60 yr — no. (%)	8 (44)
Sex — no. (%)	
Female	10 (56)
Male	8 (44)
Time since initial diagnosis — yr	
Median	7.2
Range	0.3–24.9
Platelet count — per mm^3*	
Median	788,000
Range	521,000–1,359,000
History of thrombosis — no. (%)	5 (28)
Splenomegaly — no. (%)	1 (6)
Bone marrow findings — no. (%)	
Reticulin fibrosis: grade 1+ or 2+	6 (33)
Megakaryocyte hyperplasia	14 (78)
Previous treatment for essential thrombocythemia — no. (%)	
Hydroxyurea	17 (94)
Anagrelide	13 (72)
Interferon	4 (22)
>1 prior therapy	13 (72)
Response to previous therapy — no. (%)	
Resistant to ≥1 therapy	9 (50)
Unacceptable side effects from ≥1 therapy	14 (78)
Mutation — no. (%)†	
JAK2 V617F	8 (44)
MPL W515L or *MPL* W515K	2 (11)
CALR	5 (28)

* One patient did not meet the inclusion criteria for the minimum baseline platelet count, but the investigator thought that cytoreductive therapy was clinically indicated.
† Three patients had no mutation.

OTHER SECONDARY EFFICACY END POINTS

Molecular Response

A partial molecular response was detected in seven of eight patients with *JAK2* V617F mutations at baseline (88%; 95% CI, 47 to 100) (Fig. S1 in the Supplementary Appendix). The median *JAK2* V617F mutant allele burden was reduced by 71% at month 3 after the initiation of treatment and remained reduced by 59% at month 12 despite less frequent maintenance dosing (Fig. 1B).

MPL W515L, *MPL* W515K, and *CALR* mutant allele burdens were also reduced, by 15 to 66%. Further information is provided in Table S4 in the Supplementary Appendix.

Inhibition of Proliferation

A megakaryocyte CFU assay was performed in five patients. It showed substantial inhibition of spontaneous proliferation at 1 month after initiation of imetelstat treatment, with a median 92% reduction from baseline (Table S5 in the Supplementary Appendix).

ELN "Clinicohematologic" Response

Results with respect to the secondary end point of a "clinicohematologic" response according to ELN criteria[16] (see Table S2 of the Supplementary Appendix) were similar to those of the primary end point. A complete ELN clinicohematologic response was observed in 17 of the 18 patients (94%), and a partial response was observed in 1 patient (6%). In 1 patient, splenomegaly that was palpable at 3 cm at baseline became nonpalpable after the first cycle of treatment. None of

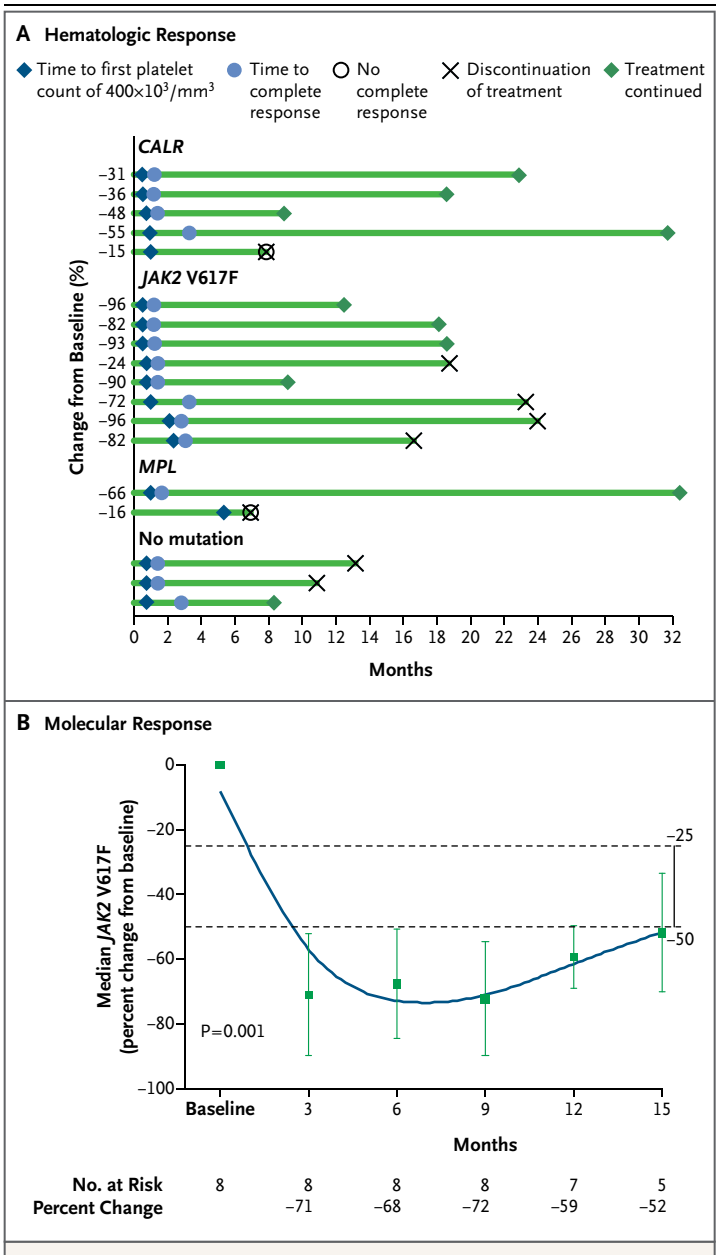

Figure 1. Hematologic and Molecular Responses in Patients Who Received Imetelstat.

Panel A shows the hematologic response according to mutation status (calreticulin [*CALR*], Janus kinase 2 [*JAK2*] V617F, *MPL* W515L or *MPL* W515K mutations, or no mutation). A complete hematologic response was observed in 16 of the 18 patients in the study (89%), and a partial hematologic response ("no complete response") was observed in 2 patients (11%). The median time to a complete hematologic response was 1.4 months. Percentages shown are the best percent change in the mutant allele burden from baseline. Panel B shows the molecular response in *JAK2* V617F–positive patients after treatment with imetelstat. The median *JAK2* V617F mutant allele burden was reduced by 71% at month 3 and by 59% at month 12 despite less frequent maintenance dosing. I bars indicate 95% confidence intervals. The percent change shown is the median percent change in the *JAK2* V617F mutant allele burden from baseline.

the 18 patients had constitutional symptoms at baseline.

Telomerase Activity, Telomere Length, and Bone Marrow Response

Figure S3 in the Supplementary Appendix shows telomerase activity. Assessments of telomere length and bone marrow response are described in the text of the Supplementary Appendix.

SAFETY

The most frequent adverse events that occurred during treatment (those that occurred in 50% or more of the patients and selected clinically significant adverse events) are listed in Table 2. All adverse events are listed in Table S6 in the Supplementary Appendix. Adverse events were reported in all patients, and 15 of the 18 patients (83%) reported at least one event of grade 3 or higher. Eighteen events of grade 3 or higher were attributed to imetelstat by the investigators, including neutropenia, headache, anemia, and a syncopal episode due to an infusion reaction (a second syncopal episode in the same patient was deemed to be unrelated to imetelstat). One grade 4 adverse event (a femoral-neck fracture) was reported by the treating clinician as being unrelated to imetelstat (Table S6 in the Supplementary Appendix). Eight of the 18 patients (44%) discontinued the study (Table S7 in the Supplementary Appendix).

The most common nonlaboratory adverse events attributed to imetelstat were fatigue (in 15 of the 18 patients), nausea (in 12), diarrhea (in 11), and headache (in 8) (Table S8 in the Supplementary Appendix). Infections occurred during treatment in 17 of the 18 patients (94%); 15 of these 17 patients (88%) had infections of grade 2 or lower. The most common infections were upper respiratory infections (in 39% of the patients), urinary tract infections (in 22%), and influenza (diagnosed on the basis of clinical and laboratory findings), nasopharyngitis, and rash (each in 17%). The infections lasted less than 3 weeks in 81% of the patients and were not typically associated with cumulative imetelstat exposure or with concurrent neutropenia or lymphopenia.

At least one increase in grade, from baseline, in a liver-function value was observed in all 18 patients (Table 3). The majority of patients had grade 1 increases in alanine aminotransferase levels, with accompanying grade 1 increases in aspartate aminotransferase levels (the latter per-

sisted with ongoing imetelstat treatment). Within 4 weeks after the initiation of imetelstat treatment, a transient increase in the alanine aminotransferase level of 5 to 7 times the standard upper limit of the normal range (grade 3) and concurrent increases in the aspartate aminotransferase level of 2 to 6 times the standard upper limit of the normal range (grades 1 to 3) were observed. These abnormalities resolved with dose reduction and did not recur with continued treatment. With a longer duration of treatment, persistent (≥6 weeks) grade 1 increases in alkaline phosphatase levels were observed in 14 of the 18 patients; these increases were associated with grade 1 or 2 unconjugated hyperbilirubinemia in 4 patients (Table 3).

In post-study follow-up safety assessments, among the 14 patients who had persistent (≥6 weeks) abnormalities in liver-function values, 11 (79%) had values that reversed to normal or baseline values. In 3 patients, the liver-function values did not return to the upper limit of the normal range; all hepatic biochemical abnormalities in 2 of these patients improved, with complete resolution of the abnormal values, and the 1 remaining patient whose condition was not improving at the end of follow-up had progression to secondary myelofibrosis. Among patients who underwent extended follow-up for persistent (≥6 weeks) abnormalities on liver-function tests, the median time to resolution after discontinuation of treatment was 12 weeks. The observed resolutions of these abnormalities followed permanent discontinuation of imetelstat in all patients as the result of a full clinical hold issued by the Food and Drug Administration (FDA) (see the Discussion section).

After the primary analysis was performed in October 2013, one patient who had received imetelstat for approximately 3 years died from bleeding esophageal varices approximately 2 months after discontinuation of treatment. Cirrhosis, secondary to hepatic steatosis, was the suspected underlying disease. Although the patient's age (83 years), multiple coexisting conditions (including a history of exposure to hepatitis B), and concomitant medications confounded assessment, imetelstat could not be conclusively excluded as a contributory agent.

Hematologic toxic effects, defined according to changes in laboratory results from baseline values, are listed in Table 4. Thrombocytopenia of less than grade 3 was seen in 9 of the 18 patients (50%). Neutropenia was observed in 15 patients (83%), including 7 with grade 3 neutropenia (39%) and 3 with grade 4 neutropenia (17%).

Table 2. Frequent Adverse Events among the 18 Patients Enrolled in the Study.*

Event	All Grades	Grade 3 or 4
	number of patients (percent)	
Fatigue	15 (83)	1 (6)
Diarrhea	14 (78)	0
Nausea	13 (72)	0
Dizziness	11 (61)	0
Increased alanine aminotransferase level	10 (56)	1 (6)
Increased aspartate aminotransferase level	10 (56)	1 (6)
Constipation	9 (50)	0
Cough	9 (50)	0
Epistaxis	9 (50)	1 (6)
Headache	9 (50)	2 (11)
Decreased neutrophil count	7 (39)	4 (22)
Anemia	6 (33)	2 (11)
Upper respiratory tract infection	6 (33)	1 (6)
Decreased white-cell count	6 (33)	1 (6)
Myalgia	4 (22)	1 (6)
Neutropenia	4 (22)	4 (22)
Hypokalemia	4 (22)	1 (6)
Syncope	3 (17)	2 (11)
Cellulitis	3 (17)	1 (6)

* Included are the adverse events that occurred in 50% or more of the patients and selected clinically significant adverse events, regardless of whether they were deemed to be related to the study drug. The grade of adverse event was defined on the basis of the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).

Table 3. Liver-Enzyme Levels in 18 Patients with Essential Thrombocythemia.*

Analyte	Grade 1	Grade 2	Grade 3
	number of patients (percent)		
Alanine aminotransferase	13 (72)	2 (11)	2 (11)
Aspartate aminotransferase	15 (83)	1 (6)	1 (6)
Alkaline phosphatase	14 (78)	1 (6)	0
Total bilirubin	2 (11)	4 (22)	0

* Laboratory abnormalities were defined on the basis of normal laboratory ranges at each institution and were graded according to the CTCAE. The maximum grade was calculated for patients who had an increase in grade, as compared with the baseline grade. There were no grade 4 increases.

Table 4. Hematologic Toxic Effects among 18 Patients Who Received Imetelstat.*

Effect	All Grades	Grade 3	Grade 4
	number of patients (percent)		
Neutropenia	15 (83)	7 (39)	3 (17)
Thrombocytopenia	9 (50)	0	0
Anemia	15 (83)	3 (17)	0
Leukopenia	16 (89)	5 (28)	0
Lymphopenia	6 (33)	1 (6)	0

* Laboratory abnormalities were defined on the basis of normal laboratory ranges at each institution and were graded according to the CTCAE. The maximum grade was calculated for patients who had an increase in grade, as compared with the baseline grade.

No cases of febrile neutropenia were reported, and no patients with grade 4 neutropenia had a concurrent infection. Lymphopenia was reported in 6 patients (33%), with one grade 3 event. Fifteen patients (83%) had anemia, and 3 (17%) had grade 3 anemia. Three patients required red-cell transfusions, including a patient who had a postsurgical hemorrhage. Three reported cases of cytopenia led to dose reductions: one for grade 1 thrombocytopenia, one for grade 3 anemia in a patient who required a red-cell transfusion, and one for grade 3 neutropenia; all three cases resolved.

DISCUSSION

In this study, imetelstat rapidly induced hematologic responses in patients with essential thrombocythemia who had disease that was refractory to conventional therapies or who had had unacceptable side effects from conventional therapies. All the patients had a hematologic response, and 89% had a complete hematologic response, a rate that exceeded rates observed with hydroxyurea, anagrelide, and interferon treatment.[12,18-21] Elsewhere in this issue of the *Journal*, Tefferi et al. report the results of a pilot study of imetelstat therapy showing the induction of complete or partial remissions, as well as molecular remissions, in a subgroup of patients with myelofibrosis.[22]

JAK2 V617F may provide a useful marker of neoplastic proliferation, and current therapies elicit molecular responses in only a minority of patients with this mutation.[12,20,23,24] In contrast, a rapid, substantial reduction in mutant allele burden was observed in all *JAK2* V617F–positive patients in this study; 88% of patients had a partial molecular response. Attenuation and loss of a molecular response later during therapy may be due in part to reduced-frequency maintenance dosing; this suggests that the duration of imetelstat treatment was insufficient to eliminate all mutated committed and clonal primitive hematopoietic progenitors. The decrease in the *CALR* and *MPL* mutant allele burdens provides support for the hypothesis that imetelstat can reduce the various malignant clones observed in myeloproliferative neoplasms. The decrease in the mutant allele burden appeared to be more pronounced for the *JAK2* V617F mutations than for the *CALR* mutations, possibly because of activation of telomerase reverse transcriptase by the Janus kinase–signal transducer and activator of transcription (JAK-STAT) signaling pathway in patients with essential thrombocythemia who had *JAK2* V617F mutations.[25,26]

Megakaryocyte CFU assays that were used to more directly measure inhibition of proliferation showed more than 90% inhibition of baseline proliferation activity within 1 month after the initiation of treatment in four of the five patients tested. These data suggest that imetelstat may have a selective inhibitory effect on the growth of the neoplastic clone or clones that drive essential thrombocythemia.

Although all the patients had hematologic responses, there were subsequent fluctuations above normal platelet levels in all patients. Responses were considered to be durable if intermittent dosing restored and maintained the platelet count (≤600,000 per cubic millimeter for a partial response or ≤400,000 per cubic millimeter for a complete response). Once an initial response was achieved, patients with platelet counts that were not controlled by maintenance dosing (i.e., platelet counts that were >600,000 per cubic millimeter in patients with a partial response or >400,000 per cubic millimeter in patients with a complete response) for at least 8 weeks were considered to have a loss of response. The reported complete hematologic responses were durable but required ongoing imetelstat therapy; interpatient variability was noted in the frequency of dosing required to manage platelet levels (every 2 weeks to every 4 weeks or more) (Fig. S2 in the Supplementary Appendix).

After discontinuation of treatment, increases in platelet counts were consistent with the maintenance interval during treatment. Patients with longer treatment intervals had more gradual increases after discontinuation of treatment, whereas patients who required more frequent dosing had more rapid increases.

At the time of the primary analysis, 10 patients were still receiving treatment, with a median follow-up of 17 months (range, 7 to 32 [ongoing]). Of the adverse events attributed to imetelstat, most were mild to moderate; 18 events were of grade 3 or higher (Table S8 in the Supplementary Appendix). Upper respiratory infections, which were reported in 6 of 18 patients (33%), were mostly mild to moderate and were not associated with myelosuppression or disease progression. Three patients had disease progression from essential thrombocythemia to secondary myelofibrosis, either during the study treatment period or during the safety follow-up period, and JAK inhibitor therapies were initiated. Among these 3 patients, one had an unrelated grade 2 upper respiratory tract infection and grade 3 osteomyelitis and another had grade 2 influenza.

In a study involving 1104 patients, the rates of progression from essential thrombocythemia and from early or prefibrotic primary myelofibrosis to overt myelofibrosis were 0.8% and 12.3%, respectively, at 10 years and 9.3% and 16.9%, respectively, at 15 years.[26] The prognostic variables associated with death or progression to leukemia or myelofibrosis were bone marrow histologic features (early or prefibrotic primary myelofibrosis vs. essential thrombocythemia), age (>60 years), history of thrombosis, leukocytosis, and anemia.[27] The 3 patients in our study who had disease that progressed to myelofibrosis had essential thrombocythemia for 12, 13, and 21 years and were 67, 61, and 56 years old, respectively, when myelofibrosis was diagnosed. Two of the 3 patients received the diagnosis near the date of their last dose of imetelstat, and 1 received the diagnosis 6 months after the last dose, during extended follow-up of liver function. At baseline, 2 of the 3 patients had 1+ and 2+ reticulin fibrosis in the bone marrow, and the hemoglobin level in all 3 patients was lower than 12 g per deciliter. The rate of progression to myelofibrosis in the current study (17%; 3 of 18 patients) is higher than historical estimates; however, the small number of patients in this study and the presence of prognostic factors make cross comparisons difficult.

Abnormal results of liver-function tests were observed frequently but were mostly mild in grade; one fatal event of bleeding esophageal varices occurred after the primary-analysis time point. On March 11, 2014, the FDA issued a full clinical hold on imetelstat, citing a lack of evidence of reversibility of hepatotoxicity, concern regarding a risk of chronic liver injury, and a lack of adequate follow-up in patients who had hepatotoxic effects. All the patients who were still receiving the study medication permanently discontinued active treatment and were observed for safety. Follow-up safety data showed that treatment-related abnormalities on liver-function tests resolved in most patients (Table S9 in the Supplementary Appendix), and the FDA lifted the clinical hold on October 31, 2014.

In conclusion, imetelstat, a telomerase inhibitor, had a clinically significant effect on disease burden in patients with essential thrombocythemia who had not had a response to previous treatment or who had had unacceptable side effects from conventional therapies. Neutropenia and abnormal liver-function tests were the main adverse events. Furthermore, molecular responses were observed in patients with mutated *JAK2* and *CALR* molecular signatures; this finding suggests therapeutic activity in the malignant clones that are the underlying source of essential thrombocythemia.

Supported by Geron.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients who volunteered to participate in this study and the study site staff who cared for them: Renata Bünter, Ursina Sager, Regula Jäggi, and Dr. Stephan Reichenbach at the Clinical Trials Unit of the University of Bern, Switzerland; Drs. Alexandre Theocharides, Gabi Vetsch, Giuseppe Colucci, and other members of the Clinical Hematology Team of the University Hospital of Bern; Dr. Monika Haubitz, Dr. Meike Dahlhaus, Ingrid Helsen, Barbara Hügli, Elisabeth Ischi, and other members of the Experimental Hematology, Molecular Diagnostics, and Stem Cell Laboratory of Hematology teams of the University of Bern and University Hospital of Bern; the staff of Geron who were involved in data collection and analyses, including Dr. Joi Ninomoto for contributions to study design, and Ted Shih, Neeru Batra, Dianne Morfeld, and Raheela Kauser Steiner of Geron for their contributions to data acquisition, review, and interpretation in previous studies and throughout the span of the trial; and Dr. Tracy T. Cao of Source One Technical Solutions and Dr. Namit Ghildyal of Janssen Research and Development, for editorial assistance with an earlier version of the manuscript.

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