ORIGINAL ARTICLE

A Pilot Study of the Telomerase Inhibitor Imetelstat for Myelofibrosis

Ayalew Tefferi, M.D., Terra L. Lasho, Ph.D., Kebede H. Begna, M.D., Mrinal M. Patnaik, M.D., Darci L. Zblewski, C.N.P., Christy M. Finke, B.Sc., Rebecca R. Laborde, Ph.D., Emnet Wassie, M.D., Lauren Schimek, B.S., Curtis A. Hanson, M.D., Naseema Gangat, M.D., Xiaolin Wang, Ph.D., and Animesh Pardanani, M.D., Ph.D.

From the Department of Internal Medicine, Division of Hematology (A.T., T.L.L., K.H.B., M.M.P., D.L.Z., C.M.F., R.R.L., E.W., L.S., N.G., A.P.), and Department of Laboratory Medicine, Division of Hematopathology (C.A.H.), Mayo Clinic, Rochester, MN; and Biometrics and Development Operations, Geron, Menlo Park, CA (X.W.). Address reprint requests to Dr. Tefferi at the Department of Internal Medicine, Division of Hematology, Mayo Clinic College of Medicine, 200 First St. SW, Rochester, MN 55905, or at tefferi.ayalew@mayo.edu.

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ABSTRACT

BACKGROUND

Current drugs for myeloproliferative neoplasm–associated myelofibrosis, including Janus kinase (JAK) inhibitors, do not induce complete or partial remissions. Imetelstat is a 13-mer lipid-conjugated oligonucleotide that targets the RNA template of human telomerase reverse transcriptase.

METHODS

We sought to obtain preliminary information on the therapeutic activity and safety of imetelstat in patients with high-risk or intermediate-2–risk myelofibrosis. Imetelstat was administered as a 2-hour intravenous infusion (starting dose, 9.4 mg per kilogram of body weight) every 1 to 3 weeks. The primary end point was the overall response rate, and the secondary end points were adverse events, spleen response, and independence from red-cell transfusions.

RESULTS

A total of 33 patients (median age, 67 years) met the eligibility criteria; 48% had received prior JAK inhibitor therapy. A complete or partial remission occurred in 7 patients (21%), with a median duration of response of 18 months (range, 13 to 20+) for complete responses and 10 months (range, 7 to 10+) for partial responses. Bone marrow fibrosis was reversed in all 4 patients who had a complete response, and a molecular response occurred in 3 of the 4 patients. Response rates were 27% among patients with a *JAK2* mutation versus 0% among those without a *JAK2* mutation (P=0.30) and 32% among patients without an *ASXL1* mutation versus 0% among those with an *ASXL1* mutation (P=0.07). The rate of complete response was 38% among patients with a mutation in *SF3B1* or *U2AF1* versus 4% among patients without a mutation in these genes (P=0.04). Responses did not correlate with baseline telomere length. Treatment-related adverse events included grade 4 thrombocytopenia (in 18% of patients), grade 4 neutropenia (in 12%), grade 3 anemia (in 30%), and grade 1 or 2 elevation in levels of total bilirubin (in 12%), alkaline phosphatase (in 21%), and aspartate aminotransferase (in 27%).

CONCLUSIONS

Imetelstat was found to be active in patients with myelofibrosis but also had the potential to cause clinically significant myelosuppression. (Funded by Geron; ClinicalTrials.gov number, NCT01731951.)

Allogeneic stem-cell transplantation is currently the only method of treatment for patients with myeloproliferative neoplasm–associated myelofibrosis that has been shown to induce long-term disease-free remission.[1] Unfortunately, allogeneic stem-cell transplantation is associated with a relatively high rate of treatment-related death and complications, including chronic graft-versus-host disease. Furthermore, many older patients are not eligible for this intervention. Other treatment strategies, including the use of Janus kinase (JAK) inhibitors, are palliative and lack selective anticlonal activity.[2] Ruxolitinib and other JAK inhibitors can alleviate constitutional symptoms and reduce spleen size, but they often cannot induce complete or partial remissions, reversal of bone marrow fibrosis, or molecular responses.[3-6]

Telomeres are protein-bound repetitive DNA sequences (TTAGGG in humans)[7] that constitute the natural end of linear chromosomes and that protect coding DNA from genetic damage and cells from replicative senescence.[8] Telomere length is genetically determined and shortens after each round of cell division, with age, and during neoplastic transformation.[9,10] Telomerase, a holoenzyme made up of human telomerase reverse transcriptase (hTERT), an RNA template, and specialized proteins (e.g., dyskerin), participates in the synthesis of telomeres and maintenance of telomere length in rapidly dividing cells.[11-13] Telomerase has been shown to be active in most cancer cells but not in normal somatic tissue.[14] In addition, studies suggest telomere-independent mechanisms by which hTERT might contribute to cancer development and progression, including modulation of Wnt–β-catenin[15] and nuclear factor κB[16] signaling and mitochondrial RNA processing.[17]

Imetelstat (GRN163L) is a 13-mer lipid-conjugated oligonucleotide that targets the RNA template of hTERT and has been shown to inhibit telomerase activity and cell proliferation in various cancer cell lines and tumor xenografts in mice.[18] Phase 1 studies involving patients with breast cancer, non–small-cell lung cancer, multiple myeloma, or chronic lymphocytic leukemia have provided information on drug pharmacokinetics and pharmacodynamics and helped establish 9.4 mg per kilogram of body weight (given as a 2-hour intravenous infusion) as the maximum dose associated with an acceptable side-effect profile, with reversible myelosuppression constituting the dose-limiting toxic effect.[19] A subsequent phase 2 study, reported in this issue of the *Journal*, involving patients with essential thrombocythemia showed platelet-lowering activity accompanied by a significant reduction in *JAK2* V617F and *CALR* mutant allele burdens.[20] Previously published data on short telomeres and up-regulated telomerase activity in myeloproliferative neoplasms provided additional rationale for conducting the current study.[21,22]

METHODS

STUDY DESIGN AND OVERSIGHT

The main objective of the study was to obtain preliminary information on the therapeutic activity and safety of imetelstat in patients with advanced myelofibrosis. This investigator-driven, single-center study was approved by the Mayo Clinic institutional review board. The protocol is available with the full text of this article at NEJM.org. The authors, who included employees of the sponsor, designed the study and gathered and analyzed the data. The manuscript was written without the assistance of anyone who is not an author. The authors vouch for the accuracy and completeness of the data and the fidelity of the study to the protocol, and they made the decision to submit the manuscript for publication. The first draft of the manuscript was prepared by the first author, who is the principal investigator of the study, without any assistance or payment from any source. The study drug and research funding were provided by Geron.

PATIENTS

Eligibility criteria included conventionally defined primary myelofibrosis[23] or post–polycythemia vera or post–essential thrombocythemia myelofibrosis[24] (see the Methods section in the Supplementary Appendix, available at NEJM.org); high-risk or intermediate-2–risk disease according to the Dynamic International Prognostic Scoring System (DIPSS) Plus, which categorizes patients in one of four risk groups (low, intermediate-1, intermediate-2, or high risk) on the basis of a number of risk factors[25]; a platelet count of at least 50×10^9 per liter; an absolute neutrophil count of at least 1×10^9 per liter; aspartate and alanine aminotransferase levels up to 2.5 times the upper limit of the normal range; a total bilirubin level of up to 3.0 mg per deciliter (51 μmol per liter); and a

serum creatinine level of up to 3.0 mg per deciliter (260 μmol per liter). Previous, but not concomitant, therapy with JAK inhibitors or other myelofibrosis drugs was allowed. Previous therapy had to be discontinued at least 14 days before initiation of the study drug.

At baseline, all study patients underwent bone marrow examination with cytogenetic studies; in addition, blood samples were obtained. Drug safety and adverse events were monitored with the use of the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). Recently revised international criteria were used to assign status with respect to complete remission and partial remission (see the Methods section in the Supplementary Appendix).[26] Bone marrow fibrosis was assessed according to the European consensus on the grading of bone marrow fibrosis and evaluation of cellularity.[27]

PROTOCOL TREATMENT

Imetelstat was administered in a 2-hour intravenous infusion at a starting dose of 9.4 mg per kilogram. Three different dose schedules were planned, with a view toward gathering information that would be useful to the design of subsequent multicenter trials; however, on the basis of preliminary safety data, only two were pursued: once every 3 weeks (group A) or weekly for 4 weeks, followed by once every 3 weeks (group B). Additional details regarding dosing and study sample size are available in the Methods section in the Supplementary Appendix. All the patients were scheduled to receive the study drug for nine 3-week cycles. Treatment beyond cycle 9 was allowed in patients who did not have disease progression or unacceptable drug-related toxic effects. The occurrence of grade 4 neutropenia or thrombocytopenia or grade 3 or higher nonhematologic toxic effects warranted dose interruption, followed by dose reduction to 7.5 mg per kilogram or 6 mg per kilogram after recovery from the drug-related toxic effects.

LABORATORY CORRELATIVE STUDIES

Laboratory correlative studies were performed to gain insight into the mechanism of drug action and to identify biomarkers of response. Mutation screening for prognostically relevant genes (*JAK2*, *CALR*, *MPL*, *ASXL1*, *EZH2*, *IDH1*, *IDH2*, and *SRSF2*) and phenotypically relevant genes (spliceosomal mutations including *SF3B1* and *U2AF1*) was performed in peripheral-blood granulocytes or bone marrow cells, according to methods described previously.[28,29] In addition, exome sequencing was performed to compare paired baseline and posttreatment samples in one of the patients who had an imetelstat-induced complete remission; matched genomic libraries were prepared from DNA extracted from granulocytes, and data were analyzed with the use of GeneSifter software (PerkinElmer). For analysis of telomere length, a Geron-developed assay was used (see the Methods section in the Supplementary Appendix).

STATISTICAL ANALYSIS

The primary end point was the overall response rate, determined according to conventional criteria of response during the first nine cycles of treatment. All patients meeting the eligibility criteria who had signed a consent form and had begun treatment were evaluated for overall response. The secondary end points were adverse events, spleen response, and independence from red-cell transfusions (in patients who had been dependent on such transfusions). All eligible patients who initiated treatment were evaluated for adverse events. Spleen size was measured by physical examination and a response defined as more than a 50% reduction in the distance below the left costal margin for at least 12 weeks.

RESULTS

CLINICAL AND LABORATORY FEATURES AT STUDY ENTRY

A total of 33 consecutive patients with myelofibrosis were enrolled and met eligibility criteria. The median age of the patients was 67 years; 67% were men. Of the 33 patients, 18 (55%) had primary myelofibrosis, 10 (30%) had post–polycythemia vera myelofibrosis, and 5 (15%) had post–essential thrombocythemia myelofibrosis.

A total of 26 patients (79%) had a *JAK2* mutation (*JAK2* V617F in all cases), 6 (18%) had a *CALR* mutation (type 1 [L367fs*46] in all cases), and 1 (3%) had an *MPL* mutation (W515S). In addition, 11 patients (33%) had an *ASXL1* mutation, 3 (9%) had an *IDH1* mutation, and 11 (33%) had a mutation in a spliceosome component gene (5 had a *U2AF1* mutation, 3 had an *SRSF2* mutation, and 3 had an *SF3B1* mutation; these mutations were

Table 1. Treatment-Related Adverse Events (of Any Grade) That Occurred in at Least Three Patients.*

Event	Group A (N=19)	Group B (N=14)	Total (N=33)	P Value†
	number of patients (percent)			
Thrombocytopenia				
All grades	10 (53)	5 (36)	15 (45)	0.48
Grade 3	8 (42)	1 (7)	9 (27)	
Grade 4	2 (11)	4 (29)	6 (18)	
Anemia				
All grades	8 (42)	5 (36)	13 (39)	1.0
Grade 2	3 (16)	0	3 (9)	
Grade 3	5 (26)	5 (36)	10 (30)	
Elevation in aspartate aminotransferase				
All grades	5 (26)	4 (29)	9 (27)	1.0
Grade 1	5 (26)	4 (29)	9 (27)	
Neutropenia				
All grades	3 (16)	6 (43)	9 (27)	0.12
Grade 3	3 (16)	2 (14)	5 (15)	
Grade 4	0	4 (29)	4 (12)	
Elevation in alkaline phosphatase				
All grades	5 (26)	2 (14)	7 (21)	0.67
Grade 1	4 (21)	2 (14)	6 (18)	
Grade 2	1 (5)	0	1 (3)	
Elevation in alanine aminotransferase				
All grades	4 (21)	2 (14)	6 (18)	1.0
Grade 1	4 (21)	2 (14)	6 (18)	
Fatigue				
All grades	5 (26)	1 (7)	6 (18)	0.21
Grade 1	2 (11)	0	2 (6)	
Grade 2	3 (16)	1 (7)	4 (12)	
Nausea				
All grades	6 (32)	0	6 (18)	0.03
Grade 1	5 (26)	0	5 (15)	
Grade 2	1 (5)	0	1 (3)	
Elevation in total bilirubin				
All grades	2 (11)	2 (14)	4 (12)	1.0
Grade 1	0	2 (14)	2 (6)	
Grade 2	2 (11)	0	2 (6)	
Infusion-related reaction				
All grades	2 (11)	2 (14)	4 (12)	1.0
Grade 1	1 (5)	2 (14)	3 (9)	
Grade 2	1 (5)	0	1 (3)	
Diarrhea				
All grades	2 (11)	1 (7)	3 (9)	1.0
Grade 1	0	1 (7)	1 (3)	
Grade 2	2 (11)	0	2 (6)	
Epistaxis				
All grades	2 (11)	1 (7)	3 (9)	1.0
Grade 1	1 (5)	1 (7)	2 (6)	
Grade 2	1 (5)	0	1 (3)	

* Shown are events that were considered by the treating physician and subsequently confirmed by the principal investigator to be at least possibly related to the study drug. Events are listed in order of decreasing frequency. Group A received a 2-hour intravenous infusion once every 3 weeks, and group B received a 2-hour intravenous infusion weekly for 4 weeks, followed by once every 3 weeks.
† P values, which were calculated with the use of Fisher's exact test, were for the comparison of group A with group B.

Table 2. Baseline Clinical and Laboratory Characteristics, including Mutational Status, of the Seven Patients Who Had a Complete or Partial Remission after Treatment with Imetelstat.*

Patient No.	Dosing Group†	Best Response	Age and Sex	Type of MF	Risk Status‡	Palpable Spleen Size	Hemoglobin	White-Cell Count	Platelet Count	Karyotype	*JAK2*, *CALR*, or *MPL*	*ASXL1*	*IDH1* or *IDH2*	*U2AF1*, *SF3B1*, or *SRSF2*
							g/dl	*×10⁻⁹/liter*						
1	A	CR	73-yr-old man	Primary	Intermediate-2	Spleen edge palpable	Transfusion-dependent	5.5	153	Normal	*JAK2* Mut	WT	WT	*U2AF1* Q157P
2	A	CR	53-yr-old woman	Post-PV	Intermediate-2	7 cm	12.5	12.1	848	Normal	*JAK2* Mut	WT	WT	WT
3	A	CR	73-yr-old man	Primary	Intermediate-2	Spleen edge palpable	Transfusion-dependent	9.6	286	Normal	*JAK2* Mut	WT	WT	*U2AF1* Q157-Y158insYE
4	B	CR	79-yr-old man	Post-ET	High	10 cm	10.2	26.3	585	Loss of Y chromosome	*JAK2* Mut	WT	WT	*SF3B1* K666E
5	A	PR	76-yr-old man	Primary	High	5 cm	Transfusion-dependent	15.1	337	Normal	*JAK2* Mut	WT	WT	*SRSF2* 283–306del
6	B	PR	67-yr-old man	Primary	Intermediate-2	Not palpable	9.0	12.8	2525	Del(9) (q13q22) +9	*JAK2* Mut	WT	WT	WT
7	B	PR	69-yr-old man	Primary	High	8 cm	11.3	32.9	766	Normal	*JAK2* Mut	WT	WT	WT

* The median time to the onset of response in these seven patients was 3.5 months (range, 1.4 to 7.2), and the median duration of response was 18 months (range, 13 to 20+) for complete responses (CR) and 10 months (range, 7 to 10+) for partial responses (PR). Details on treatment response in individual patients as of the data-cutoff date of December 5, 2014, are provided in the Supplementary Appendix. ET denotes essential thrombocythemia, MF myelofibrosis, Mut mutation, PV polycythemia vera, and WT wild-type.
† Patients in group A received imetelstat every 3 weeks, and patients in group B received imetelstat weekly for 4 weeks, followed by once every 3 weeks.
‡ Risk was classified according to the Dynamic International Prognostic Scoring System Plus, which categorizes patients in one of four risk groups (low, intermediate-1, intermediate-2, and high risk) on the basis of a number of risk factors.[25]

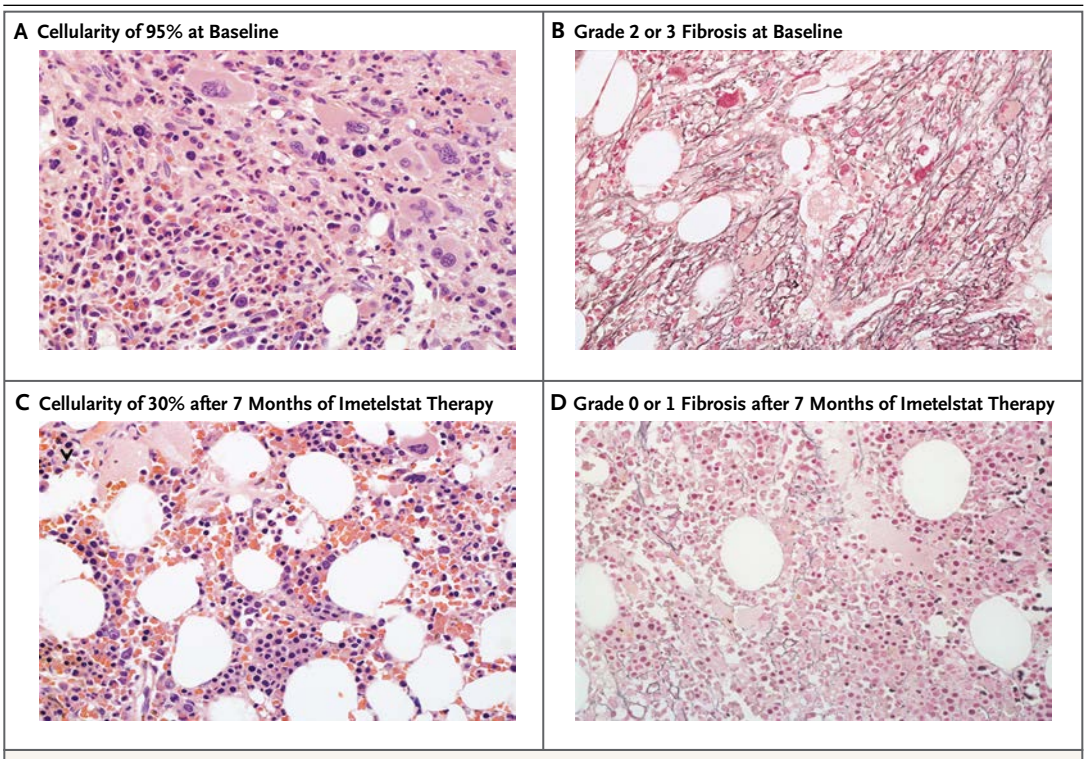

Figure 1. Reversal of Bone Marrow Fibrosis in Patient 1.

At baseline (December 17, 2012), the bone marrow–biopsy specimen (hematoxylin and eosin) was hypercellular, with hyperplasia in all myeloid lineages and clusters of large, atypical megakaryocytes (Panel A). Reticulin staining showed grade 2 or 3 fibrosis (Panel B). After 7 months of imetelstat therapy (July 15, 2013), the bone marrow–biopsy specimen was normocellular, with no megakaryocyte clusters (Panel C). Reticulin staining showed very focal grade 0 or 1 fibrosis (Panel D).

mutually exclusive). The specific *ASXL1*, *IDH1*, *U2AF1*, and *SF3B1* mutation variants are provided in the Results section in the Supplementary Appendix.

Approximately 52% of the patients had high-risk disease and 48% had intermediate-2–risk disease, according to the DIPSS Plus. A total of 13 patients (39%) were dependent on red-cell transfusions, 21 (64%) had constitutional symptoms, 23 (70%) had palpable splenomegaly (median, 15 cm below the left costal margin; range, 5 to 33), and 18 (55%) had an abnormal karyotype, including 6 (18%) with an unfavorable karyotype. Approximately 79% of the patients had received prior therapy, including 48% who had received JAK inhibitors.

TOXIC EFFECTS

At the data-cutoff date of December 5, 2014, treatment had been discontinued in 25 patients (76%); the median duration of treatment for all study patients was 8.6 months (range, 1.4 to 21.7). Causes of treatment discontinuation included insufficient response (16 patients); disease progression or relapse after an initial response (3 patients); death during the treatment period (2 patients), including 1 death due to intracranial hemorrhage that was attributed by the treating physician to drug-induced grade 4 thrombocytopenia after weekly dosing and 1 due to an upper gastrointestinal hemorrhage that was not considered to be drug-related; adverse events (2 patients) in the form of persistent thrombocytopenia; and other reasons (2 patients), including financial constraints in 1 patient and a preexisting condition (atrial fibrillation) in 1 patient.

Hematologic adverse events, liver-function abnormalities, and other adverse events that occurred during the treatment period, regardless of attribution, are shown in Tables S1, S2, and S3, respectively, in the Supplementary Appendix. The most clinically significant side effect of im-

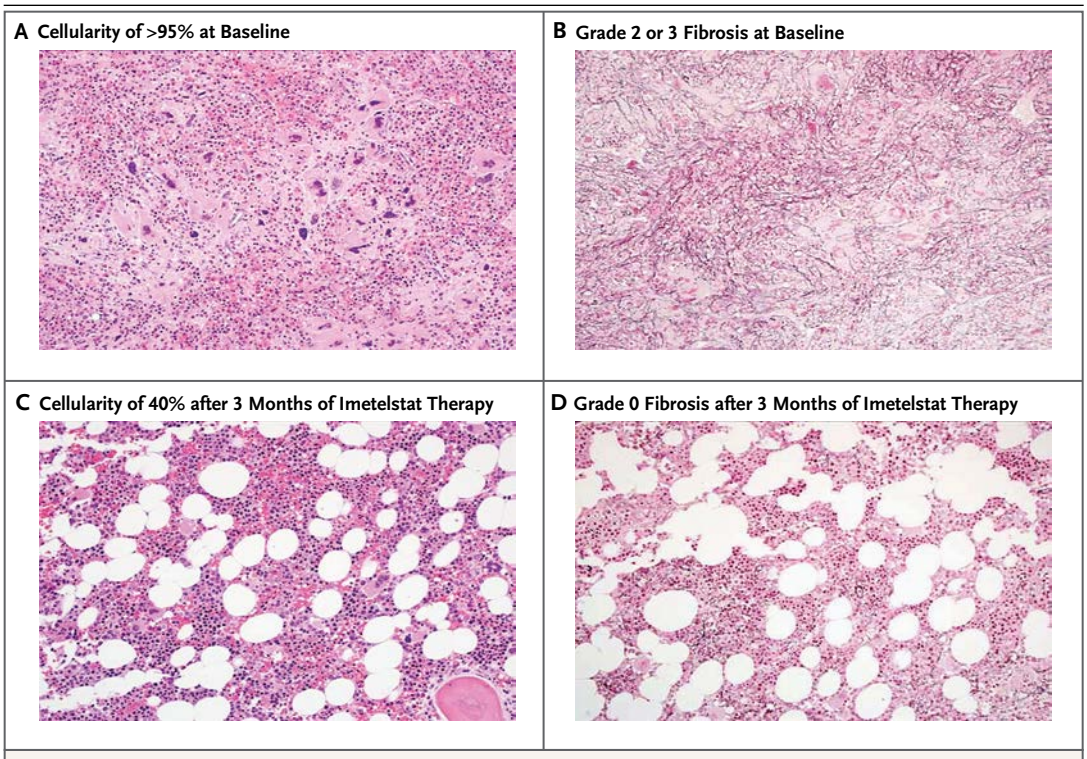

Figure 2. Reversal of Bone Marrow Fibrosis in Patient 4.

At baseline (April 29, 2013), the bone marrow–biopsy specimen (hematoxylin and eosin) was hypercellular, with hyperplasia in all myeloid lineages and prominent clusters of large, atypical megakaryocytes (Panel A). Reticulin staining showed grade 2 or 3 fibrosis (Panel B). After 3 months of imetelstat therapy (July 24, 1013), the bone marrow–biopsy specimen was normocellular, with no megakaryocyte clusters (Panel C). Reticulin staining showed no fibrosis (Panel D).

etelstat was myelosuppression, which was the primary reason for the protocol-mandated dose reduction that occurred in 22 patients (67%). Grade 4 thrombocytopenia, regardless of attribution to the study drug, occurred in 21% of the patients, grade 4 neutropenia in 18%, and grade 3 anemia in 52%. Febrile episodes from any cause occurred in 21% of the patients, epistaxis in 18%, bruising in 15%, hematoma in 6%, lung infection in 6%, skin infection in 3%, and upper gastrointestinal hemorrhage in 3%. Grade 3 or higher myelosuppression occurred in 22 patients (88%), of whom 18 (82%) had a return to grade 2 or lower myelosuppression or to baseline values; plots of drug reintroduction after resolution of grade 3 or 4 thrombocytopenia or neutropenia in individual patients are shown in Figures S1A, S1B, and S1C in the Supplementary Appendix.

Another notable side effect of imetelstat therapy in patients with myelofibrosis was low-grade elevation of liver-enzyme levels (Tables S2A and S2B in the Supplementary Appendix). A grade 3 or higher elevation of total bilirubin level, regardless of attribution, occurred in 3% of the patients, and a grade 3 or higher elevation of alkaline phosphatase level occurred in 6%. The rates of grade 1 or 2 increases in levels of total bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase that occurred during the treatment period, regardless of attribution, were 45%, 52%, 58% and 27%, respectively. None of the abnormalities in liver-enzyme levels were linked to clinically overt liver damage, and reversal of the changes to baseline values was documented in the majority of patients. Table S4 in the Supplementary Appendix lists all grade 3 or higher nonhematologic and nonhepatic adverse events.

Table 1 lists adverse events (of any grade) that occurred in at least three patients during the treatment period and were attributed as being at least possibly related to the study drug. None of

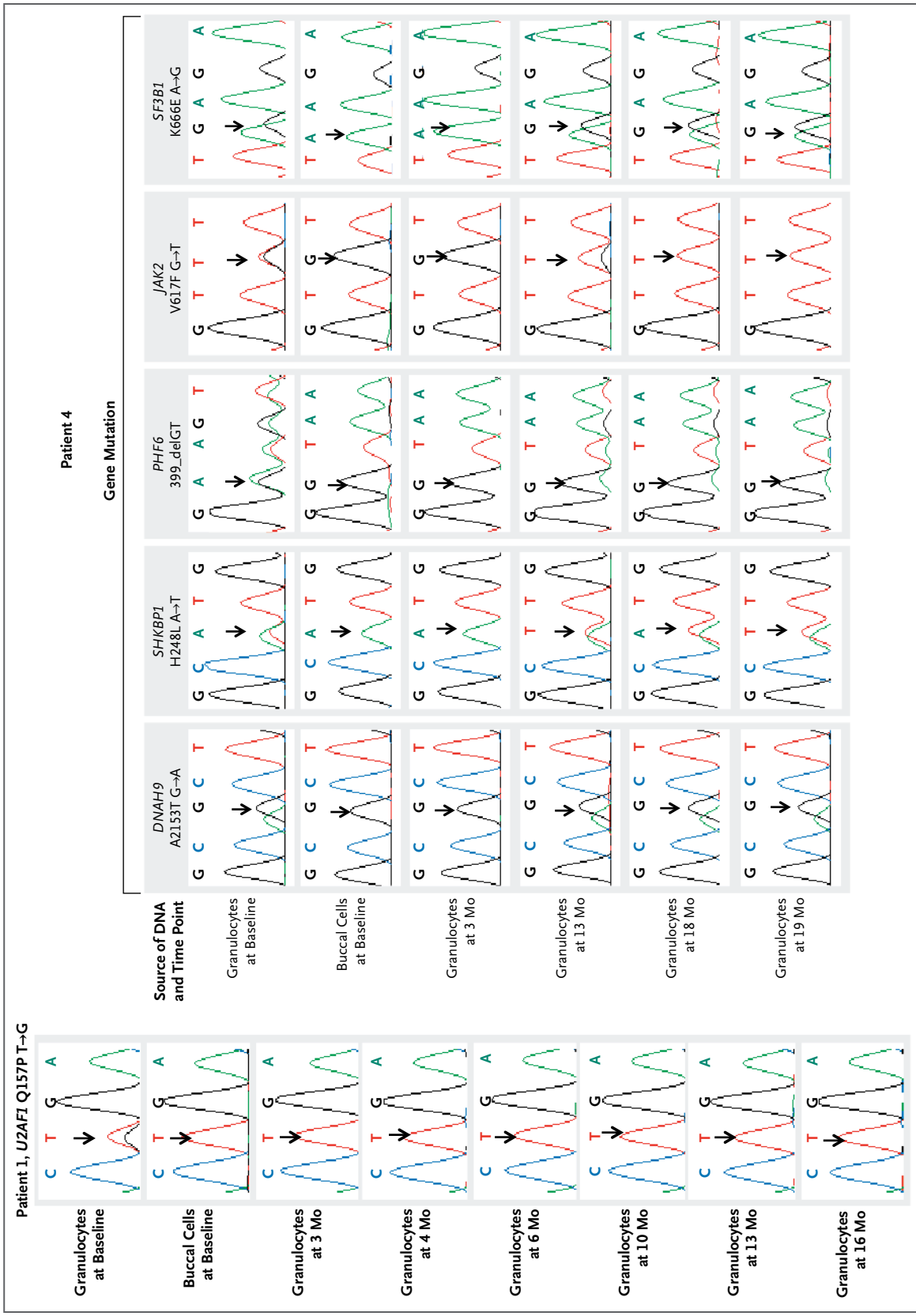

Figure 3. Mutation Screening at Baseline and during Follow-up in Two Patients with Myelofibrosis Who Had a Complete Remission after Treatment with Imetelstat.
Reemergence of the abnormal clone at the time of relapse (at 13 months) was noted in Patient 4.

Exome Sequencing of Matched PBMC and PMN at Baseline and PMN at 3 Mo

Type of Mutation	Gene ID	Chromosome	Position (hg19)	Reference	Alternative	Protein Change	% of PBMC	% of PMN at Baseline	% of PMN at 3 Mo	COSMIC ID
Insertion	*PHF6*	X	133511785	GGT	GGT/G		93.0	53.0	0.0	0
SNV	*JAK2*	9	5073770	G	T	V617F	93.0	57.0	2.0	29906
SNV	*SF3B1*	2	198267361	A	G	K666E	46.0	32.0	2.0	0
SNV	*SHKBP1*	19	41086741	A	T	H248L	40.0	42.0	0.0	0
SNV	*DNAH9*	17	11650930	G	A	A2153T	48.0	31.0	0.0	0

Validation of Relevant Mutations by Sanger Sequencing at Baseline and during Follow-up

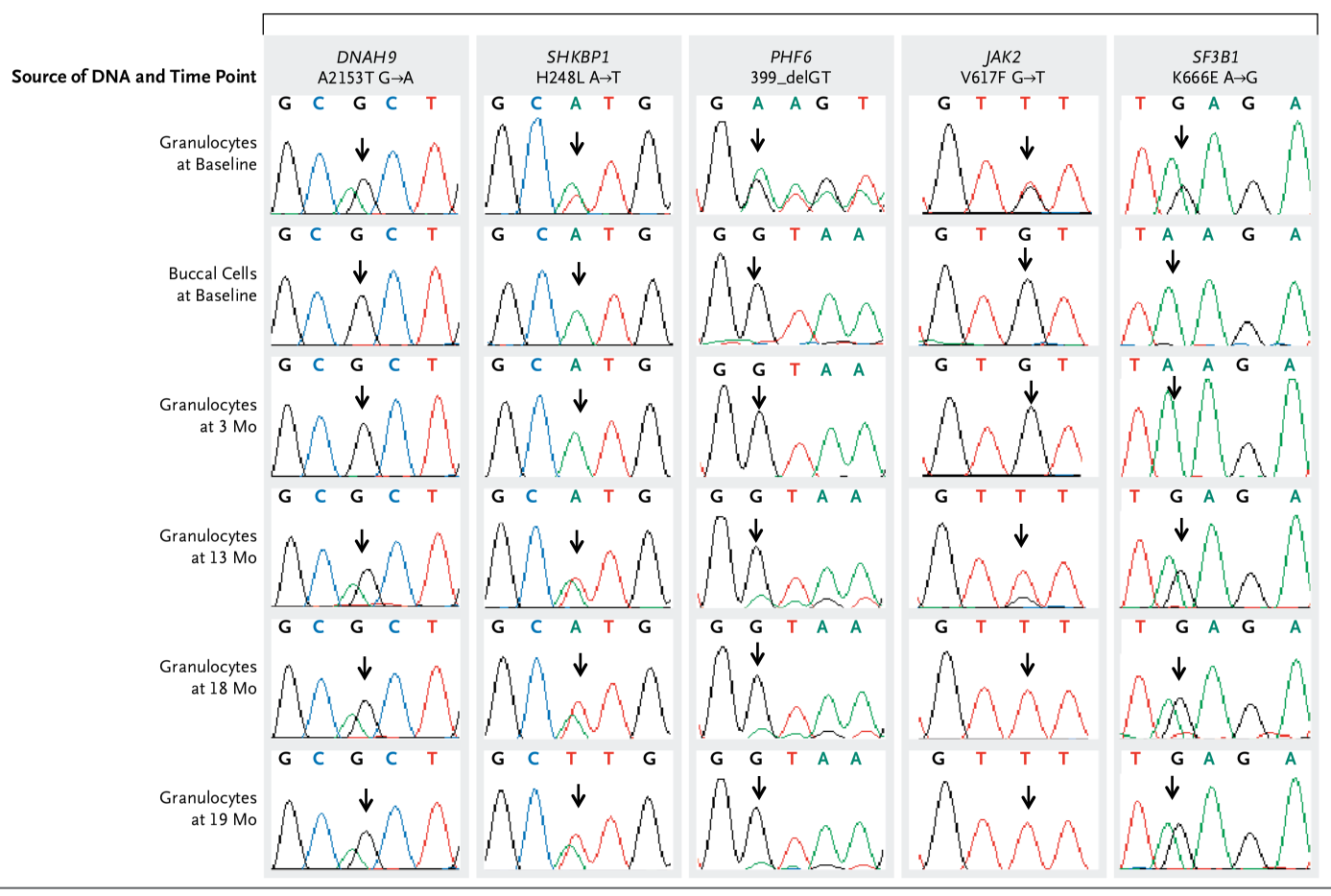

Figure 4 (facing page). Exome Sequencing and Validation by Sanger Sequencing at Baseline and during Follow-up in a Patient with Post–Essential Thrombocythemia Myelofibrosis (Patient 4) Who Had a Complete Remission after Treatment with Imetelstat.

A relapse occurred after 13 months of treatment. COSMIC denotes the Catalogue of Somatic Mutations in Cancer, PBMC peripheral-blood mononuclear cells, and PMN polymorphonuclear leukocytes.

the four infusion-related reactions (one instance each of flushing, itching, thrombophlebitis, and pain at the infusion site) were anaphylactic.

EFFICACY DATA

Seven patients (21%) had a complete response (four patients) or partial response (three patients); Table 2 lists their baseline characteristics, including their mutational status. The median time to the onset of response in these seven patients was 3.5 months (range, 1.4 to 7.2), and the median duration of response was 18 months (range, 13 to 20+) for complete responses and 10 months (range, 7 to 10+) for partial responses. The four patients with a complete response had documented reversal of bone marrow fibrosis (two illustrative cases are shown in Fig. 1 and 2), and three of them also had molecular remissions (two illustrative cases are shown in Fig. 3); additional details on each of these four patients are provided in the Results section in the Supplementary Appendix.

At the data-cutoff date, a relapse had occurred in one patient who had had a complete response (Patient 4 in Table 2) and another patient who had had a partial response (Patient 7 in Table 2); an additional patient who had had a partial response (Patient 6 in Table 2) discontinued imetelstat therapy while still having a response, because of financial constraints. Three of the seven patients with a response who had been heavily dependent on red-cell transfusions at study entry became transfusion-independent and sustained a hemoglobin level of more than 10 g per deciliter for a minimum of 3 months during imetelstat therapy, with peak posttreatment hemoglobin levels of 17.4 g per deciliter (Patient 1), 13.9 g per deciliter (Patient 3), and 12.6 g per deciliter (Patient 5) (Table 2). At the data-cutoff date, these three patients were still transfusion-independent.

In addition to response, we measured other biologically relevant treatment effects and found that a high proportion of patients had such effects. Eight of the 10 patients (80%) with marked leukocytosis (defined as $>25\times10^9$ white cells per liter) had either complete resolution (3 patients) or more than a 50% reduction in the white-cell count (5 patients). All 11 patients (100%) with thrombocytosis had complete resolution (10 patients) or more than a 50% reduction in the platelet count (1 patient). A total of 22 of the 27 patients (81%) with leukoerythroblastosis had either complete resolution (13 patients) or more than a 50% reduction in the percentage of immature myeloid cells and nucleated red cells (9 patients). A total of 17 of the 21 patients (81%) with at least 1% circulating blasts had either complete disappearance of the circulating blasts (14 patients) or at least a 50% reduction in the blast count (3 patients). The proportion of patients who had a spleen response was 35% (8 of 23 patients in whom the spleen could be evaluated for response). Four of the 13 patients (31%) who had been dependent on red-cell transfusions became transfusion-independent for at least 3 months.

LABORATORY CORRELATES OF RESPONSE

Response rates were 27% among patients with a *JAK2* mutation (7 of 26 patients) versus 0% among those without a *JAK2* mutation (0 of 7 patients) ($P=0.30$) and 32% among patients without an *ASXL1* mutation (7 of 22 patients) versus 0% among those with an *ASXL1* mutation (0 of 11 patients) ($P=0.07$). Furthermore, 3 of the 4 patients who had a complete response harbored mutations in the spliceosome gene *U2AF1* or *SF3B1* (Table 2); the rate of complete response was 38% among patients with an *SF3B1* or *U2AF1* mutation (3 of 8 patients) versus 4% among those without a mutation in one of these genes (1 of 25 patients) ($P=0.04$). Furthermore, of the 5 patients with an *SF3B1* or *U2AF1* mutation who did not have a complete response, 1 (with an *SF3B1* K700E mutation) could not be evaluated for response because of early death from imetelstat-induced myelosuppression after weekly dosing, 1 (with a *U2AF1* Q157R mutation) also had an *IDH1* mutation, and 1 (with a *U2AF1* S34F mutation) also had an *ASXL1* mutation. In addition, the *U2AF1* or *SF3B1* mutation variants in patients who had a complete response (*U2AF1* Q157P, *U2AF1* Q157-Y158insYE, and *SF3B1* K666E)

were often different from those seen in patients who did not have a complete response (*U2AF1* Q157R, *U2AF1* S34F, and *SF3B1* K666N).

Exome sequencing was performed in Patient 4 (Table 2). A total of 421 genomic variations were identified in granulocytes at baseline, and after 3 months of imetelstat therapy, these variations were detected in 2% or fewer polymorphonuclear leukocytes. Included were changes in the percentage of polymorphonuclear leukocytes with *JAK2* V617F from 57% at baseline to 2% after 3 months of treatment, in the percentage with *SF3B1* K666E from 32% to 2%, in the percentage with *TET2* S354* from 5% to 0%, in the percentage with *PREX2* N1423D from 51% to 0%, in the percentage with *SHKBP1* H248L from 42% to 0%, in the percentage with *MFHAS1* A420V from 33% to 0%, in the percentage with *DNAH9* A2153T from 31% to 0%, and in the percentage with an insertion in *HTT* at position 3076672 A/ACCGCCGCCG from 47% to 0%. Figure 4 shows the validation of some of these mutations by Sanger sequencing and their concomitant disappearance during remission and reappearance at the time of relapse.

Telomere length was measured at baseline in 28 study patients and 16 normal controls. Analysis of telomere length in age-stratified normal controls indicated a clear effect of aging on relative telomere length ($P=0.003$ for the comparison of 50-to-65-year-old controls with controls <50 years of age and those >65 years of age) (Fig. S2A in the Supplementary Appendix). Study patients 50 to 65 years of age had significantly shorter telomere lengths than age-matched controls ($P=0.001$). However, the telomere lengths of older patients (>65 years of age) with myelofibrosis did not differ significantly from those of their age-matched controls. We found no significant difference in baseline telomere length between patients who had a response and those who did not ($P=0.34$) (Fig. S2B in the Supplementary Appendix) or between patients who had mutations in spliceosome component genes and those who did not ($P=0.30$) (Fig. S2C in the Supplementary Appendix). Similarly, in an evaluation of paired baseline and posttreatment blood samples among 5 patients who had a response, there was no consistent pattern of drug effect on telomere length (Fig. S3 in the Supplementary Appendix). Finally, screening for hTERT coding-region mutations was performed in all study patients, and no such mutations were found.

DISCUSSION

The current study suggests the potential value of telomerase-targeting treatment strategies in patients with myelofibrosis and identifies imetelstat as an active drug for this disease. The observed morphologic and molecular remissions confirm selective anticlonal activity, which has not previously been documented in drug treatment for myelofibrosis.[1] Moreover, the prospect of imetelstat-induced abrogation of dependency on red-cell transfusions is particularly noteworthy, because JAK inhibitor therapy, with some exceptions,[6] is more likely to cause rather than alleviate anemia in patients with myelofibrosis.[2] If the significant association between a complete response and spliceosome pathway mutations is validated in a larger group of informative cases, it would suggest a broader application of the drug in other hematologic cancers.[30] Equally important was the negative influence on treatment response of mutations that have previously been shown to be prognostically detrimental in myelofibrosis,[28] such as mutations in *ASXL1*. However, our observations regarding telomere length were inconclusive in terms of either prognostic relevance or mechanism of action.

Our preliminary correlative studies did not elucidate the precise mechanism by which imetelstat induces clinical responses in some patients but not others. However, it is reasonable to hypothesize that certain spliceosome mutations contribute to suboptimal telomerase upregulation through altered splicing, which in turn might have made affected patients vulnerable to further telomerase deprivation by imetelstat. This hypothesis is consistent with previous observations on the role of spliceosomal cleavage in the processing of telomerase RNA and the generation of functional telomerase.[31-33] A much larger clinical and laboratory study is needed to establish the most effective dosing schedules, clarify the mechanism of action, and address concerns about toxic effects of the drug, including the induction of genomic instability to telomerase-competent normal and clonal hematopoietic stem cells and progenitor cells.[34]

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