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Danazol Treatment for Telomere Diseases

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Abstract

BACKGROUND—Genetic defects in telomere maintenance and repair cause bone marrow failure, liver cirrhosis, and pulmonary fibrosis, and they increase susceptibility to cancer. Historically, androgens have been useful as treatment for marrow failure syndromes. In tissue culture and animal models, sex hormones regulate expression of the telomerase gene.

METHODS—In a phase 1–2 prospective study involving patients with telomere diseases, we administered the synthetic sex hormone danazol orally at a dose of 800 mg per day for a total of 24 months. The goal of treatment was the attenuation of accelerated telomere attrition, and the primary efficacy end point was a 20% reduction in the annual rate of telomere attrition measured at 24 months. The occurrence of toxic effects of treatment was the primary safety end point. Hematologic response to treatment at various time points was the secondary efficacy end point.

RESULTS—After 27 patients were enrolled, the study was halted early, because telomere attrition was reduced in all 12 patients who could be evaluated for the primary end point; in the intention-to-treat analysis, 12 of 27 patients (44%; 95% confidence interval [CI], 26 to 64) met the primary efficacy end point. Unexpectedly, almost all the patients (11 of 12, 92%) had a gain in telomere length at 24 months as compared with baseline (mean increase, 386 bp [95% CI, 178 to 593]); in exploratory analyses, similar increases were observed at 6 months (16 of 21 patients; mean increase, 175 bp [95% CI, 79 to 271]) and 12 months (16 of 18 patients; mean increase, 360 bp [95% CI, 209 to 512]). Hematologic responses occurred in 19 of 24 patients (79%) who could

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be evaluated at 3 months and in 10 of 12 patients (83%) who could be evaluated at 24 months. Known adverse effects of danazol — elevated liver-enzyme levels and muscle cramps — of grade 2 or less occurred in 41% and 33% of the patients, respectively.

CONCLUSIONS—In our study, treatment with danazol led to telomere elongation in patients with telomere diseases. (Funded by the National Institutes of Health; ClinicalTrials.gov number, NCT01441037.)

Telomeres are repeated hexanucleotides and associated proteins that are located at the ends of linear chromosomes; telomeres function to protect the chromosome ends from recognition as damaged or infectious DNA.[1] The repair of telomeres by the telomerase complex solves the "end replication problem" — the otherwise inevitable loss of genetic material with every cell division. Telomerase is active during embryogenesis and in proliferating adult tissue — for example, in hematopoietic stem cells and immune cells. In individual cells, critical shortening of telomeres leads to senescence or apoptosis and, in cells that continue to divide, to chromosome instability.[2,3]

In the telomere diseases, mutations in genes responsible for telomere maintenance and repair lead to organ dysfunction, including bone marrow failure, liver cirrhosis, and pulmonary fibrosis, as well as to an increased risk of cancer. The hematopoietic cells of patients with telomeropathy have very short telomeres, which cause a quantitative defect in stem-cell number and a qualitative deficiency in stem-cell regeneration. The term dyskeratosis congenita refers to the childhood syndrome of marrow failure with the dermatologic triad of leukoplakia, skin rashes, and dystrophic nails. Telomere diseases include not only dyskeratosis congenita but also aplastic anemia, pulmonary fibrosis, and liver cirrhosis, in isolation or in combination, all of which result from mutations in telomere repair and shelterin genes; these conditions have highly variable penetrance within affected pedigrees.[4–8]

Male hormones have been used to treat bone marrow failure since the mid-20th century.[9–12] Considerable evidence suggests that sex hormones directly regulate telomerase.[13,14] We have previously shown that human lymphocytes and CD34+ hematopoietic cells up-regulate both telomerase reverse transcriptase (*TERT*) gene expression and telomerase enzymatic activity in response to androgens in vitro.[15] Recently, treatment with male hormones was shown to lead to hematologic improvement and telomere elongation in a mouse model of telomere dysfunction.[16] In a large epidemiologic study, metabolites of testosterone and genetic polymorphisms affecting hormone exposure were each linked to leukocyte telomere length in almost 1000 healthy men.[17] Here, we report the results of a study that was designed to assess retardation of telomere loss with androgen therapy in patients with a variety of telomere diseases and the effect of androgen therapy in improving blood counts.

Methods

Study Design and Implementation

In our phase 1–2 study, the primary efficacy end point was amelioration of telomere attrition with long-term administration of danazol, a synthetic sex hormone with androgenic properties; the occurrence of toxic effects of danazol treatment was the primary safety end point. The protocol was approved by the institutional review board at the National Heart, Lung, and Blood Institute (NHLBI) and is available with the full text of this article at NEJM.org. There was no commercial support for this study. The authors vouch for the completeness and accuracy of the data and analysis and for adherence to the study protocol. All patients provided written informed consent.

Blood counts and the results of liver function tests were monitored monthly, and participants underwent comprehensive evaluations at the National Institutes of Health (NIH) at baseline and at 6, 12, and 24 months after the initiation of danazol treatment. Bone marrow biopsy and aspiration were performed before enrollment and at 12 and 24 months. Bone densitometry and ovarian, uterine, and testicular ultrasonography were performed at baseline and at 24 months. Pulmonary fibrosis was assessed by high-resolution computed tomography (CT) of the chest and by pulmonary function testing, which were performed at enrollment and at annual clinic visits.

Patients

Patients 2 years of age or older were eligible for enrollment at the NIH Mark O. Hatfield Clinical Research Center. The entry criteria included an age-adjusted telomere length at or below the first percentile, identified mutations in telomere maintenance and repair genes, or both, plus at least one low blood count (hemoglobin level, <9.5 g per deciliter; platelet count, <30,000 per cubic millimeter; or neutrophil count, <1000 per cubic millimeter), pulmonary fibrosis, or both.

Danazol

Oral administration of danazol (800 mg daily divided into two doses per day) was planned to continue for 2 years. The dose was reduced if a patient reported unacceptable side effects, and treatment was discontinued entirely if any grade 3 or 4 adverse events attributable to the drug occurred. Data on adverse events were collected in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Telomere Measurement and Gene Sequencing

Genomic DNA was purified from peripheral-blood leukocytes within 24 hours after collection with the use of the automated Maxwell 16 Instrument (AS2000, Promega). Telomere length was determined with a semiautomated, Clinical Laboratory Improvement Amendments (CLIA)–approved real-time quantitative PCR (qPCR) assay performed in triplicate and validated for human cells, as described previously.[18–20] In a subgroup of patients, telomere length was also measured by flow fluorescence in situ hybridization (flow-FISH) with the use of the Dako telomere PNA kit in accordance with the manufacturer's protocol. For further details, see the Supplementary Appendix, available at NEJM.org.

End Points

The primary aim of the study was to determine whether the attrition of telomeres could be slowed by completion of a 24-month course of danazol in patients with accelerated telomere loss of genetic origin. As compared with the normal rate of telomere loss of approximately

60 bp per year,[21–24] the telomere attrition rate in patients with telomerase gene mutations is conservatively estimated at approximately 120 bp per year[24] (Table S1 in the Supplementary Appendix). Biologic improvement was defined on the basis of the sensitivity of telomere length assays: we could reliably detect a 20% reduction in telomere attrition, to 96 bp per year or less, which was the primary biologic end point. An annual rate of change was calculated from telomere length measurements that were obtained at 24 months and compared with baseline pretreatment values. The primary safety end point was the occurrence of toxic effects over the 24 months of treatment with high-dose danazol. The secondary efficacy end point was a hematologic response at 3, 6, 12, and 24 months, which was defined as an increase in hemoglobin level of 1.5 g per deciliter or more (or no further need for transfusions or a reduction in the number of transfusions of >50%), an increase in platelet count of 20,000 per cubic millimeter or more, or an increase in neutrophil count of 500 per cubic millimeter or more, as compared with baseline. The other secondary end points were relapse, development of myelodysplastic syndrome or acute myeloid leukemia, progression of pulmonary fibrosis, and survival.

Statistical Analysis

The primary efficacy end point, biologic response at 24 months, was defined as a reduction in the telomere length attrition rate to 96 bp per year or less. The sample size was calculated for testing the null hypothesis that the 24-month rate of biologic response (the primary end point) would be 10% or less versus an alternative response rate of 30%. We calculated that with a sample size of 25, the study would have 80% power to test the null hypothesis, at a 5% significance level, with the use of a two-sided binomial test for proportions. The primary end point was analyzed with the use of the intention-to-treat principle by designating all patients who withdrew from the study before 24 months as not having had a response to treatment. To account for early withdrawals and allow for a sufficient number of patients who could be evaluated for secondary end points while maintaining statistical power for the primary end point, an upper sample-size limit of 35 patients was adopted. The rules regarding stopping the study for safety were based on an unacceptable frequency of severe adverse events.[18] There were no stopping rules for efficacy, but the NHLBI institutional review board required annual review of primary and secondary end-point data. Summary statistics, including proportions, means, standard deviations, and confidence intervals, were used to describe the primary and secondary end points. Changes in all the variables included in the secondary analyses that occurred between time points were calculated for patients for whom measurements were available. Statistical inferences with regard to the mean changes in the secondary end points were described with 95% confidence intervals and Student's t-test for the null hypothesis of zero means.

Results

Patients

All consecutive patients who were eligible for participation in the study were offered enrollment from August 2011 through May 2014 (Fig. S1 in the Supplementary Appendix). Of the 29 eligible patients who were evaluated at our center, 27 were enrolled in the study; 2 patients underwent immediate hematopoietic stem-cell transplantation. The median age of the patients was 41 years (range, 17 to 66), and 15 patients (56%) were female (Table 1). A total of 10 patients had mutations in *TERT*, 7 had mutations in *TERC* (the telomerase RNA component), 3 had mutations in *DKC1* (dyskeratosis congenita 1), and 1 had a mutation in *RTEL1* (the regulator of telomere elongation helicase 1) (Table S3 in the Supplementary Appendix). Six patients had leukocyte telomere lengths below the first percentile and a suggestive clinical phenotype, but they did not have an identifiable pathogenic mutation (Table S2 and Fig. S2 in the Supplementary Appendix). Eleven patients required regular transfusions of packed red cells, and 2 patients required regular transfusions of both red cells and platelets. The majority of patients (85%) had a family history suggestive of telomere disease,[3] and 6 patients had early graying of hair (Table 1, and Table S2 in the Supplementary Appendix).

Telomere Attrition

In April 2015, a total of 11 of the first 12 patients evaluated at 24 months were found to have consistent telomere elongation. In view of the unanticipated high level of efficacy that was achieved and the fact that there was sufficient information to reject the null hypothesis, the study was closed early by the NHLBI institutional review board.

All the patients who could be evaluated met the primary efficacy end point of reduction in the telomere attrition rate at 24 months as specified in the protocol (Fig. 1A); in the intention-to-treat analysis, the response rate was 12 of 27 (44%; 95% confidence interval [CI], 26 to 64). In exploratory analyses, the telomere length of peripheral-blood leukocytes at enrollment was compared with the telomere length after 6 months and 12 months of danazol administration; in addition, in a subgroup of 8 patients, measurements of telomere length at 30 months and 36 months (6 months and 12 months, respectively, after perprotocol discontinuation of danazol therapy) were compared with baseline measurements taken at enrollment (Fig. 1B and Table 2). Elongation of telomeres was found at all time points during danazol administration in patients who could be evaluated: 16 of 21 patients (76%) at 6 months, 16 of 18 (89%) at 12 months, and 11 of 12 (92%) at 24 months. The mean increase in telomere length as compared with baseline was 175 bp (95% CI, 79 to 271) at 6 months, 360 bp (95% CI, 209 to 512) at 12 months, and 386 bp (95% CI, 178 to 593) at 24 months (which was the time point used for the evaluation of the primary end point) (Fig. 1B and Table 2). A similar pattern of telomere elongation was confirmed by qPCR of flow-sorted lymphocytes and by flow-FISH (Fig. S4A and S4B and Table S4 in the Supplementary Appendix). Among the 8 patients who discontinued treatment per protocol at 24 months and had leukocyte telomere length measured at 6 months and 12 months after cessation of danazol treatment, the mean decrease in telomere length relative to the measurement obtained at 24 months of treatment was 135 bp at 6 months and 333 bp at 1 year after discontinuation of treatment (Fig. 1B and Table 2). Although we did not test the significance of the observation, telomere elongation was greater among patients with *TERT* mutations than in the group with unidentified mutations, and the smallest amount of elongation was found in the group with *TERC* and *DKC1* mutations (Table S3 in the Supplementary Appendix).

Hematologic Responses

Danazol therapy led to a hematologic response in 19 of 24 patients (79%) who could be evaluated at 3 months, in 17 of 21 patients (81%) at 6 months, in 14 of 18 patients (78%) at 12 months, and in 10 of 12 patients (83%) at 24 months (Fig. 2). Before danazol administration, 13 patients were transfusion-dependent; after treatment, all but 1 patient no longer required regular transfusions. Among the 14 patients who had hemoglobin levels lower than 9.5 g per deciliter at enrollment, we found a mean increase of 3.3 g per deciliter (95% CI, 2.1 to 4.4) and a mean increase in absolute reticulocyte count of 41,300 per cubic millimeter (95% CI, 25,320 to 57,280) at 1 year (Fig. 3, and Table S5 in the Supplementary Appendix). Neutrophil counts also increased, by a mean of 300 per cubic millimeter (95% CI, 124 to 476), and platelet counts increased by 14,250 per cubic millimeter (95% CI, 4880 to 23,620). To date, 10 of the 12 patients who could be evaluated after 2 years of danazol therapy have had a hematologic response (Fig. 2). Danazol treatment was discontinued in all patients at 2 years; 5 patients' blood counts then declined, but they improved with the reinstitution of danazol treatment "off protocol" by their treating physicians (Fig. 3).

Lung Fibrosis

Pulmonary fibrosis scores based on CT[25] were stable during the 2 years of treatment in all patients except Patient UPN9, who died of an acute exacerbation of pulmonary failure in association with viral pneumonia. The most prevalent abnormality was a defect in the diffusing capacity of the lungs for carbon monoxide (DLCO), which was present in 25 of 27 patients, with a mean DLCO (adjusted for hemoglobin) of 55% of the predicted value (range, 26 to 94%). In the 7 patients for whom results of pulmonary function tests before danazol administration were available, the adjusted DLCO measured at least 6 months before entry into the study had declined from a mean of 55% of the predicted value to a mean of 44% of the predicted value at the time of entry ($P = 0.01$ by paired t-test), whereas during danazol administration there was no significant decrease in lung function (Fig. S3 in the Supplementary Appendix).

Adverse Events and Missing Data

Data were missing for five patients at 24 months as a result of the study being halted early (Fig. S1 in the Supplementary Appendix). Data from earlier time points for these five patients were available for the evaluation of secondary end points.

Ten patients withdrew from the study before 2 years (Fig. S1 in the Supplementary Appendix): two patients discontinued treatment because of low-grade side effects, three discontinued after a grade 3 or grade 4 adverse event, two withdrew without a stated reason, two proceeded to receive alternative therapy, and one died from organ failure (pulmonary fibrosis). The most common adverse events were elevations in liverenzyme levels (in 41% of the patients), muscle cramps (in 33%), edema (in 26%), and lipid abnormalities (in 26%) (Table S7 in the Supplementary Appendix). Liver fibrosis measurements obtained by means of ultrasonic transient elastometry (FibroScan)[26] were available at baseline and at 24 months for four of six patients who had cirrhosis at baseline; fibrosis had been alleviated substantially in three patients and had worsened in one (UPN16) in association with continued alcohol abuse.

Three patients had progression of their disease during treatment with danazol: Patient UPN9 had severe pulmonary fibrosis at baseline and died at 10 months from acute respiratory failure, Patient UPN21 had moderate aplastic anemia that advanced to a severe form of the condition, and Patient UPN15 underwent portosystemic shunting with acute worsening of liver function. Marrow cytogenetic abnormalities appeared in two patients, without morphologic evidence of myelodysplastic syndrome: Patient UPN6, who had a hematologic response, had the cytogenetic abnormality trisomy 21 detected at 1 year of treatment; Patient UPN16, who also had a hematologic response, had duplication of chromosome arm 1q. Patient UPN7 had a diagnosis of myelodysplastic syndrome at enrollment, with a hypercellular marrow with trilineage dysplasia and normal karyotype, diagnostic of myelodysplastic syndrome (Table S2 in the Supplementary Appendix); at 1 year, deletion of chromosome arm 20q developed, occurring in 2 of 20 metaphases, without changes in bone marrow myeloblast percentage or dysplasia. All three patients continued to have a hematologic response to treatment.

Discussion

In this prospective clinical study involving patients with short telomeres, we found an increase in telomere length in response to a pharmacologic intervention. In patients with telomere disease, administration of male hormones resulted in telomere elongation in circulating leukocytes in association with hematologic improvement. Androgens have been a therapeutic option for marrow failure syndromes since the 1960s, without a clear mechanism for their action.[12,27] In retrospect, some patients with a response probably had telomere deficits. On the basis of our previous findings of increased telomerase activity in bone marrow hematopoietic progenitors cultured in the presence of sex hormones,[15] we designed this study to evaluate the effects of a synthetic androgen on telomere length and hematopoiesis in a cohort of patients with telomeropathy. Since enrollment began for our study, case reports[28,29] and an observational study[11] have described similar effects. The single patient carrying a *TERT* mutation described by Brummendorf and colleagues[28] had telomere length elongation as well as hematologic improvement in association with androgen therapy. Savage and colleagues described hematologic improvement in 11 of 16 patients with dyskeratosis congenita, mainly children, who received androgens.[11]

Our study was powered to detect a 30% improvement in telomere attrition after 2 years of danazol treatment. Not only was telomere loss prevented by treatment with danazol in our patients, but a mean increase of 386 bp telomeric repeats had occurred by study completion, with improvement usually observed early during the course of hormone therapy. Hematologic improvement in all blood counts accompanied telomere elongation.

Despite these robust results, our study has some limitations. First, mutations were not identified in some cases, despite the patients having very short leukocyte telomeres and a suggestive clinical phenotype. Heterogeneity in the genetic basis for telomere biologic deficiencies may have biased our estimation of telomere attrition. Second, telomere erosion can fluctuate with repeated measurements over time.[30] A longer period of observation before starting danazol would have been desirable to establish a firm baseline for the assessment of treatment effects. Third, we used the highest dose of danazol that is currently approved for use in humans, but a dose-finding strategy might have allowed identification of the minimum effective dose. Fourth, our study was not randomized and did not have a control group; this study design was adopted because telomere disease is not common, because such strong biologic and clinical effects were unanticipated, and because of ethical considerations.

Our in vitro data and studies in mice support a direct effect of hormone therapy on telomerase activity by up-regulation of *TERT* expression.[15] This effect is mediated through an estrogen-responsive element in the gene promoter and may also explain the longer telomeres found in postmenopausal women who have received hormone-replacement therapy.[31] We were unable to reliably measure telomerase activity, because relevant *TERT* regulation would occur in the hematopoietic stem cells, the numbers of which are severely reduced in these patients with marrow failure. Therefore, we cannot rule out other potential mechanisms, such as expansion of the hematopoietic stem and progenitor cell pool[32] or effects on bone marrow stroma.[33]

Male hormones are efficacious in the treatment of inherited bone marrow failure associated with telomere dysfunction, producing clinically meaningful hematologic improvement. The increase in telomere length seen in patients treated with hormones is consistent with hormone-mediated up-regulation of *TERT* and of telomerase enzymatic activity. Further studies are required to assess the effect of treatment on survival or progression to myelodysplastic syndrome or acute myeloid leukemia. Lower doses of danazol or other hormone formulations are likely to have better side-effect profiles. Sex hormones may be useful in the treatment of other types of accelerated telomere attrition, such as the attrition that occurs after chemotherapy[34] and hematopoietic stem cell transplantation,[35] and other drugs and small molecules could be screened in vitro for effects on telomerase. Our results may have broader relevance for the frequent use of androgens for blood diseases in the developing world and for testosterone replacement in aging men in developed countries. Longevity has been linked to telomere attrition rates in mammals[36]; the advantages and risks associated with the modification of telomere loss will need to be assessed in attempts to alter physiologic aging in humans. Telomere attrition and dysfunction have been implicated in the development of cancer in both mice[37] and humans.[2,38–40] Evolution to myelodysplastic syndrome or acute myeloid leukemia has been infrequent in historical studies of androgen treatment for bone marrow failure.[10] The mitigation of telomere erosion by sex hormones may abrogate early molecular steps in chromosome instability and oncogenesis and warrants investigation in clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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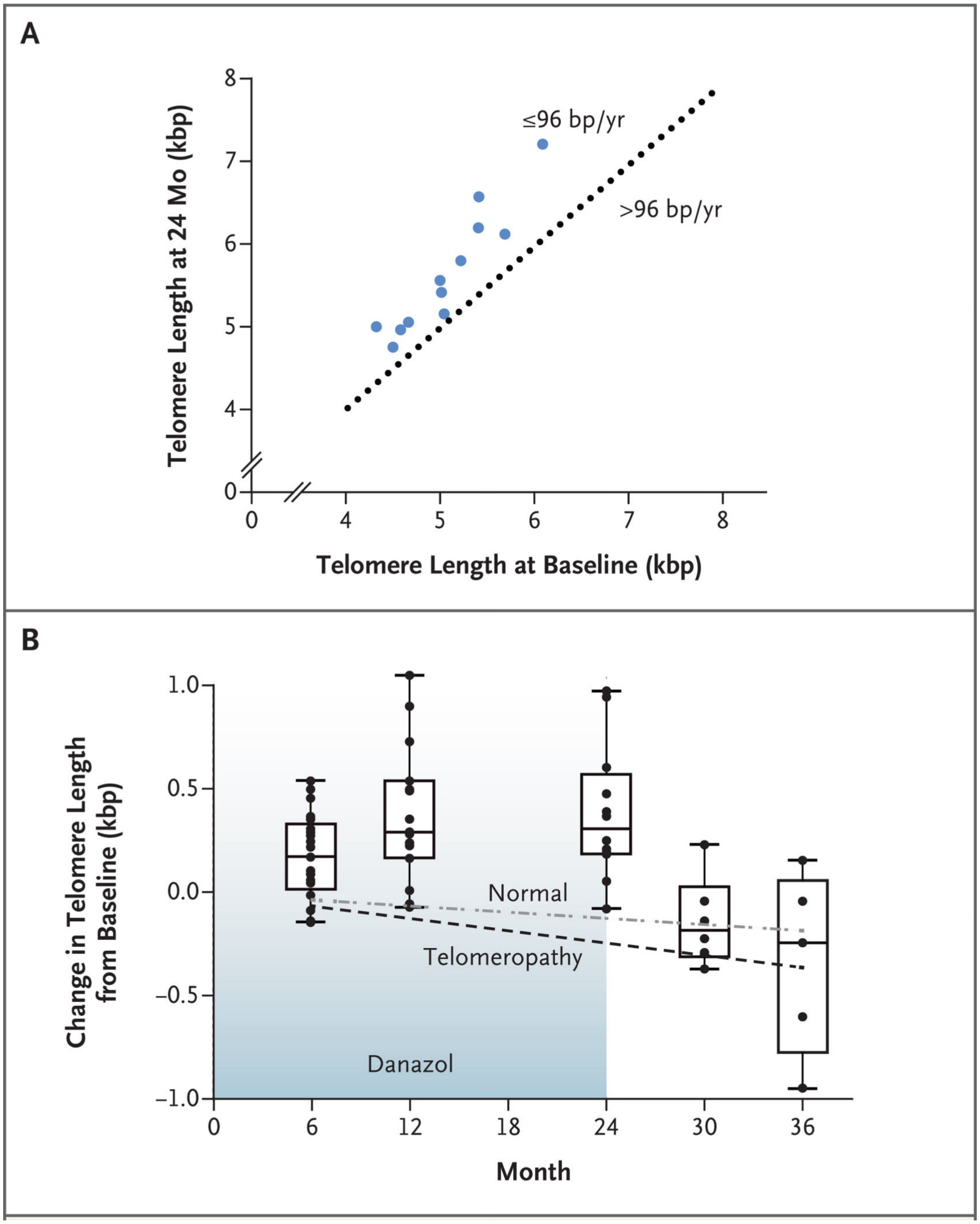

Figure 1. Changes in Telomere Length in Patients Receiving Danazol

Panel A shows the telomere length measured at 24 months (the time point used for evaluation of the primary end point) in 12 patients, plotted against each patient's baseline telomere measurement. The points above the dotted line represent patients who met the protocol-defined primary efficacy end point, a telomere attrition rate of 96 bp per year or less. Data for 15 patients were missing at 24 months; in the analysis of the primary end point, these patients were considered as not having had a response. In Panel B, summary statistics are shown in box plot format for changes in telomere length at landmark visits, as compared with baseline; the line within each box indicates the median, the top and bottom edges the 75th and 25th percentiles, respectively, and the I bars the range. The light dashed-and-dotted line represents the anticipated rate of telomere attrition with age in healthy persons (60 bp per year),[21–24] and the bold dashed line represents the anticipated rate of attrition in patients with telomere diseases (120 bp per year).[24] All patients who could be evaluated had improvements in telomere attrition while receiving danazol. Significant telomere elongation was found at 6, 12, and 24 months after the initiation of treatment with danazol, as compared with baseline. When treatment with danazol was stopped at 24 months, a decrease in telomere length was observed at 30 months and 36 months. All available data points were used in this analysis.

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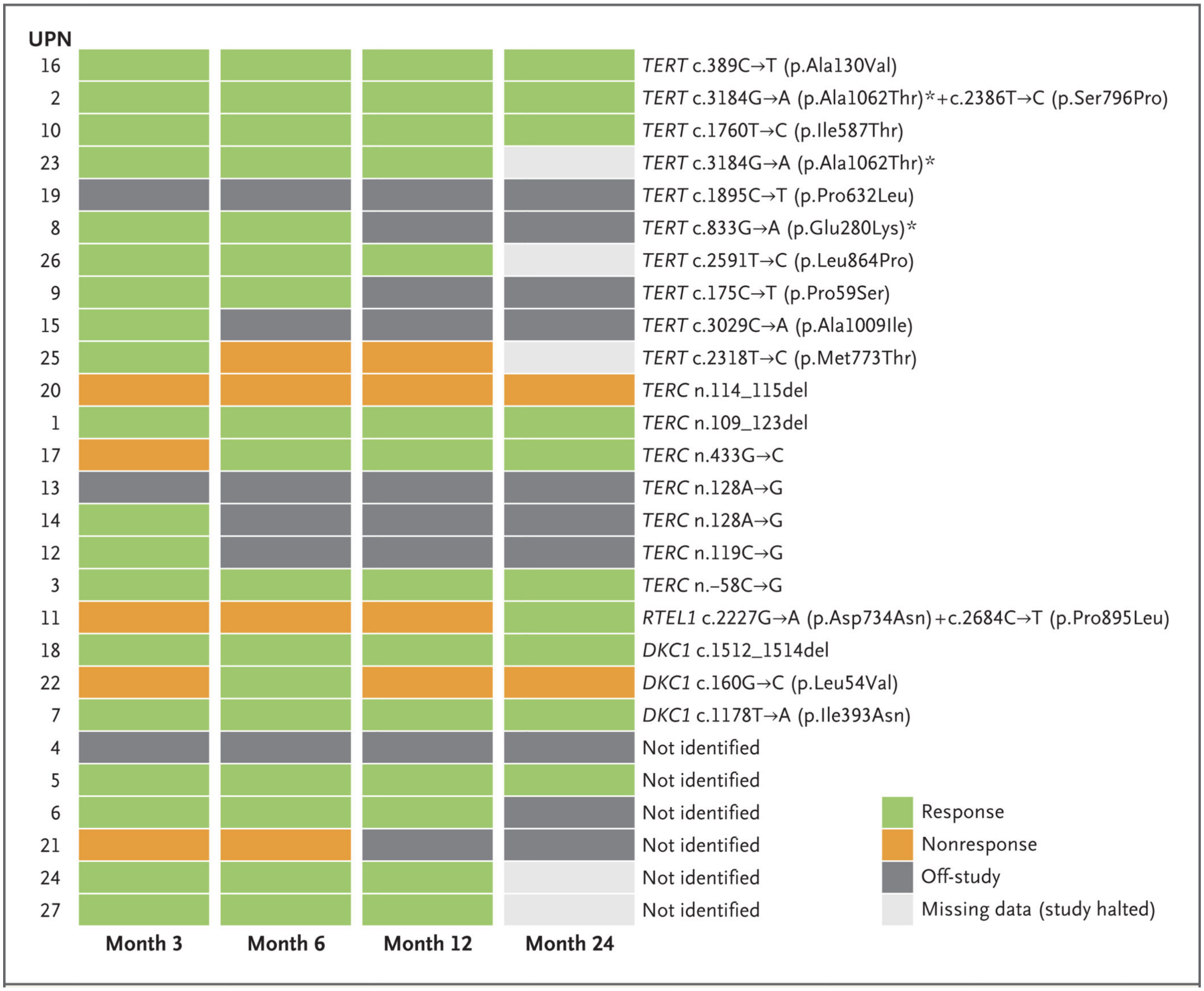

Figure 2. Hematologic Response in Patients Treated with Danazol, According to Mutation

For each landmark visit, the hematologic response to danazol is shown, with all patients listed according to patient number (UPN) on the left and genomic position of the heterozygous telomere gene mutation at right. Mutations were not detected in 6 patients despite screening for all known genes that have been reported to be mutated in telomere diseases (*CTC1, DKC1, NOP10, NHP2, RTEL1, TERC, TERT, WRAP53, TINF2*, and *USB1*). No data were available for 15 patients at 24 months: 10 withdrew from the study, and 5 had not reached the 2-year time point for evaluation of the primary end point because the study was halted early. An asterisk indicates that the pathogenicity was ambiguous; the p.Ala1062Thr and p.Glu280Lys variants have allele frequencies of 1.3% and 0.05%, respectively, in healthy controls (http://exac.broadinstitute.org/gene/ENSG00000164362).

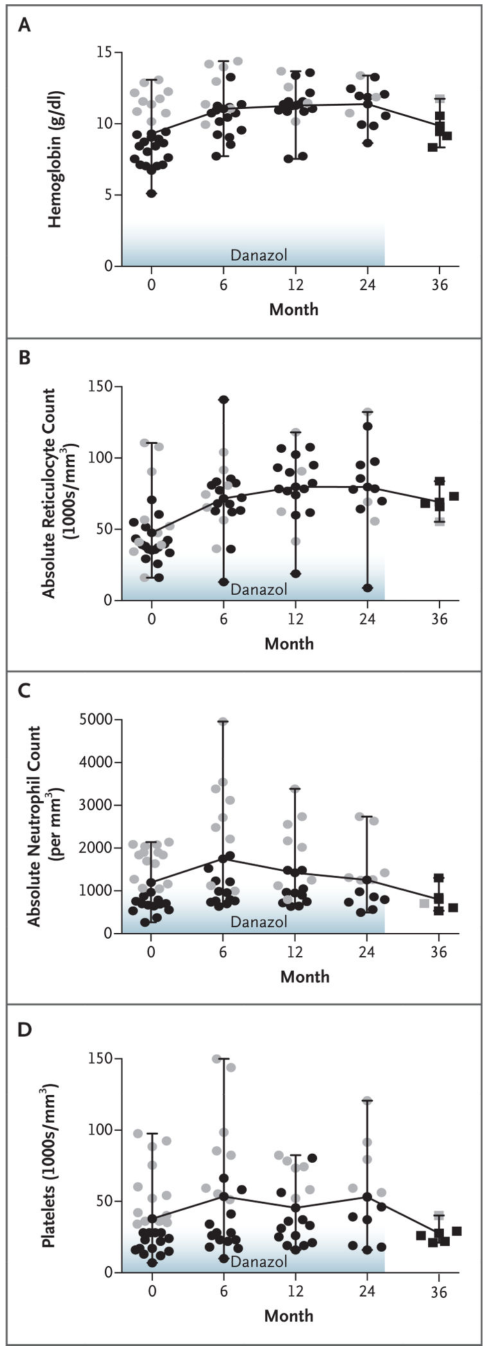

Figure 3. Blood Counts in Patients with Telomeropathy Treated with Danazol

Peripheral-blood counts at various time points during the study are shown. Each symbol denotes a blood count in one patient; circles denote counts assessed during the period in which danazol was administered, and squares denote counts assessed after treatment with danazol was discontinued, per protocol, at 24 months. Black symbols denote counts in patients with a preexisting abnormally low value in that cell lineage, which was used to satisfy the enrollment criterion, and paired t-test results were performed for only these patients; gray symbols denote counts in all other patients in the study. The enrollment criterion for protocol entry was anemia (hemoglobin level, <9.5 g per deciliter, or substantial requirements for red-cell transfusions), thrombocytopenia (platelet count, <30,000 per cubic millimeter, or <50,000 per cubic millimeter with bleeding), or neutropenia (absolute neutrophil count, <1000 per cubic millimeter) (summary statistics are provided in Tables S5 and S6 in the Supplementary Appendix).

Table 1

Baseline Characteristics of the Patients.*

Characteristic	All Patients (N = 27)	Patients with Mutation Identified				Patients with No Identified Mutation (N = 6)
		TERT (N = 10)	*TERC* (N = 7)	*DKC1* (N = 3)	*RTEL1* (N = 1)	
Median age (range) — yr	41 (17–66)	49 (23–66)	44 (18–59)	42 (30–49)	28	28 (17–40)
Female sex — no.	15	6	5	1	0	3
Bone marrow failure — no.						
MAA	19	7	4	2	1	5
SAA	4	1	2	0	0	1
MDS	2	1	0	1	0	0
Transfusion dependency — no.						
Red cells	11	4	4	2	0	1
Red cells and platelets	2	1	0	0	0	1
Pulmonary fibrosis — no.†						
Overt	10	3	4	2	0	1
Subclinical	15	6	3	1	1	4
Absent	2	1	0	0	0	1
Cirrhosis — no.†						
Overt	6	3	1	1	1	0
Subclinical	3	1	0	1	0	1
Absent	18	6	6	1	0	5
Early graying of hair — no.	6	2	1	2	1	0
Family history of telomeropathy — no.‡	23	9	7	3	1	3

*MAA denotes moderate aplastic anemia, MDS myelodysplastic syndrome, and SAA severe aplastic anemia.

†For pulmonary fibrosis and cirrhosis, the presentation was considered to be overt when patients had clinical manifestations or a previous diagnosis of the disease; the presentation was considered to be subclinical when the condition was diagnosed on screening.

‡Family history was defined as a history of any telomeropathy-associated disease (bone marrow failure, lung fibrosis, liver cirrhosis, early graying of hair) in a relative.

Table 2

Change in Telomere Length over Time.*

Time	Total No. of Patients	Mean Change in Telomere Length (95% CI)	P Value†	Patients with Increase in Telomere Length	
		kbp		*no.*	*% (95% CI)*
Receiving danazol					
0 to 6 months	21	0.175 (0.079–0.271)	0.001	16	76 (56–96)
0 to 12 months	18	0.360 (0.209–0.512)	<0.001	16	89 (73–100)
0 to 24 months‡	12	0.386 (0.178–0.593)	0.002	11	92 (73–100)
Not receiving danazol§					
24 to 30 months	6	−0.135	—	1	17
24 to 36 months	5	−0.333	—	1	20

*In the analysis of the primary end point, at 24 months, a rate of telomere loss of 96 bp per year or less was found in 12 of 27 patients (44%; 95% confidence interval [CI], 26 to 64; P<0.001 by one-sample proportions test with continuity correction for the null hypothesis of a 10% response rate). Data for 15 patients were missing at 24 months; in the analysis of the primary end point, these patients were considered as not having had a response.

†The P value is for testing the null hypothesis that the mean change in telomere content would be zero.

‡This time point was used for the primary end point.

§Confidence intervals and P values were not computed when fewer than 10 patients were in the sample.