Supplementary Material

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Methods

Patients

Male patients older than two years and female patients older than 16 years (to avoid virilization) were eligible for enrollment at Ribeirao Preto Medical School University Hospital (Ribeirão Preto, SP, Brazil). Entry criteria were age-adjusted mean TL under the first percentile and/or identified germline pathogenic variants in telomere-biology genes associated with at least one cytopenia and/or radiologic diagnosis of interstitial lung disease (ILD), varying from pulmonary fibrosis, in accordance with the American Thoracic Society criteria,(1) to other pulmonary phenotypes associated with telomere diseases.(2) Cytopenias were defined as hemoglobin level <9.5 g/dL or blood transfusion requirements more than two packs/month for at least two months or reticulocyte count <60,000/ µL; platelet count <30,000/µL or <50,000/µL associated with bleeding; neutrophil count <1,000/µL.

Patients with severe aplastic anemia and matching available donor were excluded, and patients lacking a germline pathogenic variant in a telomere-biology gene were screened for Fanconi anemia with diepoxybutane (DEB test) before enrollment and were also excluded if positive.

Exclusion criteria were concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease that would preclude the patient's ability to tolerate protocol therapy, or that death within 30 days was likely; ongoing treatment of cancer; current pregnancy, or unwillingness to avoid pregnancy if of childbearing potential; incapability to understand the study or give informed consent, or refusal to perform protocol procedures. Patients with severe aplastic anemia and matching available donor were also excluded. Patients lacking a germline pathogenic variant in a telomere-biology gene were screened for Fanconi anemia with diepoxybutane (DEB test) using peripheral blood before enrollment and were excluded if positive. The local Research Ethics Committee approved the protocol and all patients provided written

informed consent (CAAE: 19116913.4.0000.5440). This study is registered at clinicaltrials.gov (NCT02055456).

Treatment and monitoring

Patients were treated with 5 mg/kg of intramuscular nandrolone decanoate every 15 days for 2 years. This dose was chosen based on its previous effectiveness in aplastic anemia.(3) The dose was reduced to 3,5 mg/kg if any adverse event ≥ grade 3 related to the drug occurred, according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0, and to 2,5 mg/kg if symptoms related to the treatment persisted; treatment was discontinued if the latter dose was not tolerated.

Blood counts, liver and renal function tests, and blood chemistry were monitored biweekly in the first year, and then every four months. Bone marrow biopsy and aspirate for cytology and conventional karyotyping were performed at enrollment and at 24 months. For patients with hematopoietic failure at baseline, marrow evaluation was additionally performed at 12 months. Prostate-specific antigen, hepatic ultrasound, and lipid profile were performed yearly. Corticosteroids, immunosuppressants, growth factors, antifibrotic drugs, N-acetylcysteine, or other specific treatments for marrow failure or ILD were not allowed during study period.

Pulmonary evaluation

Pulmonary involvement was assessed by high-resolution computed tomography (HRCT) of the chest and pulmonary function testing for diffusing capacity for carbon monoxide (DLCO) at baseline and at 24 months. Patients with lung disease had an additional pulmonary evaluation at 12 months. Predicted forced vital capacity (FVC) and predicted DLCO values, corrected for anemia as previously described,(4) were used to evaluate lung disease progression.

HRCT scans were reviewed by a certified thoracic radiologist who was blinded to genetic status and TL data. To objectively compare HRCT evaluation, additional quantitative analyses of the HRCT scans were performed with a fully automated program (Yacta version 2.7, University of Heidelberg, Heidelberg, Germany).(5) This software provides quantitative measures of lung volumes and densities in HRCT scans. Pulmonary volume (PV, in mL), mean pulmonary density (MPD, in Hounsfield units - HU), and the 90th percentile of lung density (P90) were measured, as P90 was the strongest parameter that negatively correlated with functional FVC in a previous study.(6)

Telomere length measurement and massively parallel targeting sequencing panel

Peripheral blood leukocyte TL was measured by flow-FISH, as previously described(7) at landmark time points: enrollment, 12 and 24 months of nandrolone treatment. TLs below the first and tenth percentiles for age-matched controls were considered very short and short, respectively.

Massively parallel DNA sequencing

Patients were screened for germline variants in genes related to telomere biology, bone marrow failure, and hematologic malignancies by massively parallel targeted sequencing (**Supplementary Table 1**).(8-11) Enrichment-indexed libraries were prepared with genomic DNA from peripheral blood leukocytes using the SureSelect QXT Target Enrichment Multiplexed System (Agilent Technologies, CA). Libraries were further 150bp paired-end and sequenced on a NextSeq 500 instrument (Illumina, USA) with the NextSeq 500/550 Mid Output v2.5 Kit for 300 cycles (Illumina, USA) with a mean coverage of 400X. Reads were aligned to the human reference genome (GRCh37/hg19) using Burrows–Wheeler Aligner (BWA),(8) and data quality was

assessed using FastQC. GATK,(9) and VarDict(10) were used to call variants that were further annotated with ANNOVAR.(11) Variants were classified as germline or somatic based on their variant allele frequency (VAF); variants with VAF >35% were confirmed as germline by the screening of a germline control or family members using Sanger sequencing. Germline variants in telomere-biology genes were manually inspected and classified for pathogenicity using the Sherloc/American College of Medical Genetics and Genomics (ACMG) criteria.(12)

Variants were called if fulfilled the following criteria:

1. Mapping Quality score ≥ 25
2. Base Quality score ≥ 15
3. Number of SNVs on the same read < 5
4. Number of insertions and deletions on the same read < 2
5. Number of total reads ≥ 20
6. Number of variant reads ≥ 15
7. Variant allele frequency ≥ 0.4
8. Variants found in coding exons, affecting the amino acid composition of proteins and variants of non-coding RNA, were selected. Variants affecting splice sites of coding and non-coding genes were also selected.
9. Variants with a maximum frequency of 2% but > 0.1% in the overall population were manually inspected and included in the analysis if they were:
    a. Variants previously reported in ClinVar as uncertain significance or pathogenic (http://www.ncbi.nlm.nih.gov/clinvar), Human Gene Mutation Database (HGMD; http://www.hgmd.org) and/or telomerase database (http://telomerase.asu.edu/)
    b. Homozygous variants (variants associated with recessive phenotype may have high frequency in general population).
    c. Variants in genes linked to autosomal recessive phenotype, such as FANC genes, that have more than one variant within the same gene.

d. Stoploss or stopgain, frameshift deletion or insertion variants.

e. Predicted deleterious in at least 5 of the following tools: PolyPhen-2 based on HumDiv and based on HumVar (http://genetics.bwh.harvard.edu/pph2/), SIFT (Sorting Intolerant From Tolerant) (http://sift.jcvi.org/), likelihood ratio test, MutationTaster (http://www.mutationtaster.org), mutationassessor (http://mutationassessor.org), FATHMM (Functional Analysis through Hidden Markov Models) (http://fathmm.biocompute.org.uk/index.html), VEST3 (Variant Effect Scoring Tool) (http://karchinlab.org/apps/appVest.html), CADD (Combined Annotation Dependent Depletion) (http://cadd.gs.washington.edu) and GERP++ (Genomic Evolutionary Rate Profiling) (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html). RadialSVM score and LR_score were also used to have an integrated view of prediction scores.

Variants with VAF >35% were confirmed as germline by the screening of a germline control or family members using Sanger sequencing. PCR conditions and primers sequences are available upon request.

Supplementary table S1: Customized SureSelectXT Target Enrichment panel with 164 genes related to bone marrow failure and hematologic malignancies

Gene	**Gene function**	**Reported disease**	**Genomic position**
ABL1	Oncogene	AML	chr9:133589218-133763112
AEBP2	Epigenetic regulation	Secondary AML	chr12:19556929-19873785
ASXL1	Epigenetic regulation	MDS/AML	chr20:30946097-31027172
ATM	DNA damage and repair	MDS	chr11:108093161-108239879
ATRX	Epigenetic regulation	MDS/AML	chrX:76760306-77041805
BCOR	Transcription related	AA/AML	chrX:39909018-40036632
BCORL1	Transcription related	AA/AML	chrX:129115033-129192108
BRAF	Signal transduction	AML	chr7:140419077-140624614
BRCA2	DNA damage and repair	AML	chr13:32889561-32973859
BRCC3	Ubiquitination	MDS	chrX:154299645-154351399
BRIP1	DNA repair	Fanconi anemia	chr17:59756497-59940970
CALR	Transcription related	MPD	chr19:13049342-13055354
CBL	Ubiquitination	MDS	chr11:119076702-119178909
CBLB	Oncogene	neoplasms	chr3:105374255-105588446
CBLC	Oncogene	neoplasms	chr19:45281076-45303953
CDAN1	Chromatin changes	Congenital dyserythropoietic anemia	chr15:43015707-43029467
CDH23	Cell adhesion	AML	chr10:73156641-73575754
CDKN2A	Tumor suppressor	neoplasms	chr9:21967701-21995350
CEBPA	Transcription related	AML	chr19:33790790-33793520
CSF3R	Receptor	AML/ Congenital neutropenia	chr1:36931594-36948965
CSMD1	Signal transduction	AML	chr8:2792825-4852544
CTC1	Telomere biology gene	DC/AA/IPF	chr17:8128089-8151463
CTCF	Transcription related	MDS	chr16:67596260-67673138
CUX1	Transcription related	MDS	chr7:101458909-101927300

DAXX	Transcription related	AML	chr6:33286285-33297096
DCLRE1B (Apollo)	Telomere biology gene	DC/AA/IPF	chr1:114447713-114456758
DDX41	RNA splicing	MDS	chr5:176938528-176944520
DHX36	Telomerase maturation	None	chr3:153990285-154042336
DIDO1	Transcriptional factor	Familial MDS	chr20:61509040-61569354
DIS3	RNA processing	AML	chr13:73329490-73356316
DKC1	Telomere biology gene	DC/AA/IPF	chrX:153990967-154006014
DNMT3A	Epigenetic regulation	MDS/AML	chr2:25455446-25565509
EED	Epigenetic regulation	MDS/AML	chr11:85955376-85989905
ELANE	Elastases	Congenital neutropenia	chr19:850964-856296
ERCC4	DNA damage and repair	neoplasms	chr16:14013964-14046255
ETNK1	Phosphatidyletanolamine synthesis	MDS	chr12:22777959-22843658
ETV6	Transcription related	MDS	chr12:11802738-12048386
EZH1	Epigenetic regulation	MDS/AML	chr17:40852243-40897121
EZH2	Epigenetic regulation	MDS/AML	chr7:148504414-148581491
FANCA	DNA repair	Fanconi anemia	chr16:89803907-89883115
FANCB	DNA repair	Fanconi anemia	chrX:14861479-14891241
FANCC	DNA repair	Fanconi anemia	chr9:97861286-98080041
FANCD2	DNA repair	Fanconi anemia	chr3:10068048-10143664
FANCE	DNA repair	Fanconi anemia	chr6:35420088-35434931
FANCF	DNA repair	Fanconi anemia	chr11:22644029-22647437
FANCG	DNA repair	Fanconi anemia	chr9:35073782-35080063
FANCI	DNA repair	Fanconi anemia	chr15:89787130-89860542
FANCL	DNA repair	Fanconi anemia	chr2:58386328-58468565
FANCM	DNA repair	Fanconi anemia	chr14:45605092-45670143
FBXW7	Ubiquitination	MDS	chr4:153242360-153457303
FLT3	Receptor	AML	chr13:28577361-28674779
G6PC3	gluconeogenic and glycogenolytic pathways	Congenital neutropenia	chr17:42148048-42153762
GATA1	Transcription factor	Dysfunctional hematopoiesis	chrX:48644912-48652768

GATA2	Transcription factor	GATA2 deficiency	
GFI1	Transcription related	Congenital neutropenia	chr1:92940268-92952483
GNAS	Signal transduction	MDS	chr20:57414723-57486300
GPRC5A	Signal transduction	MDS	chr12:13043666-13070921
HAX1	Signal transduction	Congenital neutropenia	chr1:154244937-154248405
HRAS	Oncogene/DNA repair	neoplasms	chr11:532192-537337
HSP90AA1	Signal transduction	None	chr14:102547025-102606136
IDH1	Epigenetic regulation	MDS/AML	chr2:209100901-209130848
IDH2	Epigenetic regulation	MDS/AML	chr15:90626227-90645836
IKZF1	Regulation of telomere maintenance	ALL	chr7:50343629-50472849
IRF1	Transcription related	MDS	chr5:131817251-131826540
JAK1	Signal transduction	MPD	chr1:65298856-65432237
JAK2	Signal transduction	MPD	chr9:4984983-5128233
JAK3	Signal transduction	MPD	chr19:17935539-17958930
JARID2	Epigenetic regulation	MDS/AML	chr6:15246156-15522323
KDM6A	Epigenetic regulation	MDS/AML	chrX:44732371-44971907
KIT	Receptor	AML	chr4:55524035-55606931
KMT2A (MLL)	Transcription related	AML	chr11:118307155-118397589
KRAS	Oncogene	neoplasms	chr12:25357673-25403920
LAMB4	Cell adhesion	MDS	chr7:107663943-107770851
LUC7L2	RNA splicing	MDS	chr7:139025055-139108253
MAP3K4	Signal transduction	AML	chr6:161412709-161538467
MPL	Receptor	AA/MDS	chr1:43803425-43820185
MRE11A	DNA damage and repair	Ataxia-telangiectasia-like disorder	chr11:94150419-94227124
MYC	Oncogene	AML	chr8:128747630-128753730
MYD88	Signal transduction	Cancer differentiation	chr3:38179919-38184563
NAF1	Telomerase biogenesis	None	chr4:164031175-164088123
NBN (NBS1)	DNA damage and repair	AA	chr8:90945514-91015506
NCOR2	Transcription related	MDS	chr12:124808907-125052185

NF1	Signal transduction	MDS	chr17:29421895-29709184
NHP2	Telomere biology gene	DC	chr5:177576411-177581018
NOP10	Telomere biology gene	DC	chr15:34633864-34635428
NOTCH1	Signal transduction	CLL	chr9:139388846-139440364
NPM1	Transcription related	AML	chr5:170814070-170838191
NRAS	Oncogene	neoplasms	chr1:115247035-115259565
OBFC1	Telomere maintenance	None	chr10:105637268-105678095
PALB2 (FANCN)	DNA repair	Fanconi anemia	chr16:23614433-23652728
PARN	Telomere biology gene	DC/AA/IPF	chr16:14529507-14726635
PCNA	DNA damage and repair	None	chr20:5095549-5107322
PDGFRA	Signal transduction	CML	chr4:55095214-55164464
PEG3	Apoptosis	Acquired AA	chr19:57321395-57352146
PHF6	Transcription related	MDS	chrX:133507233-133562872
PIF1	Telomerase regulator	None	chr15:65107779-65117917
PIGA	GPI anchor	Acquired AA	chrX:15337523-15353726
PML	Tumor suppressor	AML	chr15:74286964-74340205
POT1	Telomere biology gene	DC/AA/IPF	chr7:124462390-124570087
PRF1	Perforin	Acquired AA	chr10:72357054-72362581
PRPF8	RNA splicing	MDS	chr17:1553873-1588226
PTEN	Tumor suppressor	neoplasms	chr10:89622820-89731737
PTGES3 (p23)	Telomerase related	None	chr12:57057075-57082242
PTPN11	Signal transduction	MDS	chr12:112856105-112947767
RAD21	Cohesin	AML	chr8:117858123-117887155
RAD51C	DNA repair	Fanconi anemia	chr17:56769884-56811753
RB1	Oncogene	neoplasms	chr13:48877833-49056172
RBBP4	Epigenetic regulation	MDS/AML	chr1:33116693-33151862
RBBP7	Epigenetic regulation	MDS/AML	chrX:16857356-16888587
RIT1	Signal transduction	MDS	chr1:155867549-155881245

RPL11	Ribosomal related gene	DBA	chr1:24018219-24022965
RPL15	Ribosomal related gene	DBA	chr3:23957986-23965237
RPL35A	Ribosomal related gene	DBA	chr3:197676808-197683531
RPL5	Ribosomal related gene	DBA	chr1:93297532-93307531
RPS10	Ribosomal related gene	DBA	chr6:34385181-34393952
RPS17	Ribosomal related gene	DBA	chr15:82821108-83209345
RPS19	Ribosomal related gene	DBA	chr19:42363938-42377044
RPS24	Ribosomal related gene	DBA	chr10:79793468-79816621
RPS26	Ribosomal related gene	DBA	chr12:56435587-56438166
RPS7	Ribosomal related gene	DBA	chr2:3622745-3628559
RTEL1	Telomere biology gene	DC/AA/IPF	chr20:62289113-62330087
RUNX1	Transcription related	MDS/AML	chr21:36160048-37377015
RUVBL1	TERC maturation	None	chr3:127783571-127872807
RUVBL2	TERC maturation	None	chr19:49496655-49519302
SBDS	Ribosomal related gene	SDS	chr7:66452614-66460685
SETBP1	Oncogene	MDS	chr18:42260088-42648525
SF3B1	RNA splicing	MDS	chr2:198254458-198299865
SH2B3	Signal transduction	AA/MDS	chr12:111843702-111889477
SHQ1	Telomerase biogenesis	None	chr3:72798378-72911115
SLX4 (FANCP)	DNA repair	Fanconi anemia	chr16:3631132-3661649
SMC1A	Cohesin	AML	chrX:53401020-53449727
SMC3	Cohesin	AML	chr10:112327399-112364444
SRP72	Signal transduction	AA/MDS	chr4:57333031-57369897
SRSF2	RNA splicing	MDS	chr17:74730147-74733543
STAG2	Cohesin	AML	chrX:123094012-123556564
STAT3	Signal transduction	AA/MDS	chr17:40465292-40540636
SUZ12	Epigenetic regulation	MDS/AML	chr17:30263987-30328114
TCP1	TriC related - telomeres	None	chr6:160199480-160210831
TEN1	Telomere maintenance	None	chr17:73975248-73996717
TERC	Telomere biology gene	DC/AA/IPF	chr3:169482258-169482898

TERF1	Telomere biology gene	DC/AA/IPF	chr8:73921047-73960407
TERF2	Telomere biology gene	DC/AA/IPF	chr16:69389414-69442524
TERT	Telomere biology gene	DC/AA/IPF	chr5:1253212-1295234
TET2	Epigenetic regulation	MDS/AML	chr4:106066982-106201023
TGS1	Telomerase maturation	None	chr8:56685651-56738057
THPO	Signal transduction	AA/Thrombocytopenia	chr3:184089673-184097526
TINF2	Telomere biology gene	DC/AA/IPF	chr14:24708799-24711930
TNKS	Signal transduction	neoplasms	chr8:9413374-9639906
TP53	Oncogene	MDS/AML	chr17:7565047-7590918
TPP1	Telomere biology gene	DC	chr11:6633947-6640742
U2AF1	RNA splicing	MDS	chr21:44513016-44527747
U2AF2	RNA splicing	MDS	chr19:56165366-56186132
UMODL1	Cell adhesion	AML	chr21:43483018-43563613
WAS	Signal transduction	Thrombocytopenia	chrX:48534935-48549868
WRAP53	Telomere biology gene	DC/AA/IPF	chr17:7589339-7606870
WT1	Transcription related	AML	chr11:32409271-32457226
ZRSR2	RNA splicing	MDS	chrX:15808524-15841433
ZSWIM4	Cell adhesion	AML	chr19:13906224-13943094

Error-corrected DNA sequencing

Patients were screened for somatic mutations in peripheral blood at enrollment, 12 and 24 months of nandrolone treatment. An error-correcting DNA sequencing was performed with 100ng of DNA using a customized panel covering 60 genes associated with myeloid malignancies and clonal hematopoiesis (VariantPlex ArcherDx, CO; **Supplementary Table 2**). Libraries were paired-end 150-bp sequenced on NovaSeq 6000 system (Illumina, CA) with average coverage of 6000x. Data were next analyzed using the Archer Analysis software Version 6.2.3, which used Bowtie2 to align de-duplicated reads to the reference genome and FreeBayes, Lofreq, and Vision for variant calling. Somatic variants were included in the analysis if at variant allele frequency (VAF) > 0.05%, with de-duplication ratio > 3:1, and at VAF above the minimum detectable allele fraction at which a variant could be distinguished from the background noise at statistical power of 0.95 (95 MDAF provided by the Archer analysis). Variants were next prioritized according to their predicted functional consequences and quality control statistics regarding unique molecular identifiers (UMI), strand bias, and outlier detection.

Supplementary table S2: Error-corrected DNA sequencing customized panel with genes related to telomere biology myeloid malignancies

Gene	Accession	Genomic Location	Target Exons
ACD	NM_022914	chr16:67691414-67694718	All coding region
ASXL1	NM_015338	chr20:30946146-31027122	11, 12, 13
BCOR	NM_001123385	chrX:39910498-39956719	All coding region
BCORL1	NM_021946	chrX:129139163-129192058	All coding region
BRAF	NM_004333	chr7:140433812-140624564	6, 10, 11, 12, 13, 15, 16, 17
CALR	NM_004343	chr19:13049413-13055304	8, 9
CBL	NM_005188	chr11:119076985-119178859	8, 9
CEBPA	NM_004364	chr19:33790839-33793430	All coding region
CSF3R	NM_172313	chr1:36931643-36948915	10, 14, 15, 16, 17, 18
CTC1	NM_025099	chr17:8128138-8151413	All coding region
CUX1	NM_001913	chr7:101459183-101927250	All coding region
DNMT3A	NM_022552	chr2:25455829-25564784	All coding region
DKC1	NM_001363	chrX:153991030-154005964	All coding region
ETV6	NM_001987	chr12:11802787-12048325	All coding region
EZH2	NM_004456	chr7:148504463-148581441	All coding region

FLT3	NM_004119	chr13:28577410-28674729	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21
GATA1	NM_002049	chrX:48644981-48652717	2, 4
GATA2	NM_032638	chr3:128198264-128212030	All coding region
GNAS	NM_000516	chr20:57466425-57486250	8, 9, 10, 11
IDH1	NM_005896	chr2:209100952-209118910	3, 4
IDH2	NM_002168	chr15:90627211-90645708	4, 6
JAK2	NM_004972	chr9:4985244-5128183	12, 13, 14, 15, 16, 19, 20, 21, 22
JAK3	NM_000215	chr19:17935592-17958841	3, 11, 15, 16
KIT	NM_000222	chr4:55524094-55606881	1, 2, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18
KRAS	NM_004985	chr12:25358179-25403854	All coding region
MDM4	NM_002393	chr1:204485506-204527248	All coding region
MECOM	NM_004991	chr3:168801286-169381563	All coding region
MPL	NM_005373	chr1:43803474-43820135	All coding region
NAF1	NM_138386	chr4:164049822-164088073	All coding region
NHP2	NM_017838	chr5:177576464-177580961	All coding region
NOP10	NM_018648	chr15:34633916-34635362	All coding region
NPM1	NM_002520	chr5:170814707-170837888	11
NRAS	NM_002524	chr1:115247084-115259515	All coding region
OBFC1	NM_024928	chr10:105637317-105678045	All coding region
PARN	NM_002582	chr16:14529556-14724128	All coding region
PHF6	NM_032335	chrX:133507341-133549321	All coding region
POT1	NM_015450	chr7:124462439-124570037	All coding region
PPM1D	NM_003620	chr17:58677543-58743640	6
PTPN11	NM_002834	chr12:112856535-112947717	3, 4, 7, 8,11, 12, 13
RPA1	NM_002945	chr17:1733272-1802848	All coding region
RTEL1	NM_032957	chr20:62289162-62328544	All coding region
RTEL1	NM_001283009	chr20:62326833-62327003	All coding region
RUNX1	NM_001754	chr21:36160097-36421462	All coding region
SAMD9	NM_017654	chr7:92728825-92747336	All coding region
SAMD9L	NM_152703	chr7:92759367-92777680	All coding region
SETBP1	NM_015559	chr18:42531699-42532155	4
SF3B1	NM_012433	chr2:198256697-198299771	13, 14, 15, 16
SRSF2	NM_003016	chr17:74730196-74733493	All coding region
STAG2	NM_006603	chrX:123095555-123236505	All coding region
TERC	NR_001566	chr3:169482397-169482848	All coding region
TERT	NM_198253	chr5:1253286-1295162	All coding region
TET2	NM_001127208	chr4:106067841-106200960	All coding region
TINF2	NM_001099274	chr14:24708850-24711880	All coding region
TP53	NM_001276696	chr17:7571719-7590868	All coding region
U2AF1	NM_006758	chr21:44513065-44527688	2, 6, 7
UBA1	NM_003334	chrX:47053200-47074527	3
WRAP53	NM_018081	chr17:7591666-7606820	All coding region
WT1	NM_000378	chr11:32409321-32457081	All coding region
ZCCHC8	NM_017612	chr12:122956145-122985543	All coding region
ZRSR2	NM_005089	chrX:15808573-15841382	All coding region

End points

Primary objectives of the study were safety and nandrolone decanoate activity in slowing telomere attrition in patients with telomeropathies. The expected telomere erosion rate in leukocytes of patients with telomerase disease is approximately 120 bp/year, considerably higher than the mean loss of 50 bp/year for the general population.(13, 14) The primary biologic end point was a reduction of ≥ 20% in the annual rate of telomere attrition in patients with telomere disease (to ≤ 96 bp/year) during nandrolone administration. The primary safety end point was the occurrence of adverse events throughout the treatment period.

Secondary end points were hematologic response, as defined by increase in hemoglobin > 1.5 g/dL or transfusion independence or increase in reticulocytes > 50% above baseline, and/or increase in platelets > 20,000/μL above baseline, and/or increase in neutrophils > 500/μL above baseline; and pulmonary response, as defined by 10% increase in FVC% or 15% increase in predicted DLCO. Other secondary end points were the incidence of clonal hematopoiesis bearing somatic variants related to myeloid malignancies determined by next-generation sequencing of whole blood leukocytes after treatment, and hematologic relapse, as defined by restart of blood transfusion requirement or any decrease in blood counts to levels required for study entry. Changes in chest HRCT scan quantitative measures were exploratory end points in patients with lung involvement.

Statistics

Summary statistics were used to describe the primary biologic and secondary end points, including means, medians, standard deviation, range, and confidence intervals. Linear regression models with mixed effects were used to obtain pairwise comparisons of the means of the variables of interest (TL, complete blood counts, CVF, and DLCO) between different periods. These models include a random effect that accounts for dependence between values from the same individual

(paired data). Assumption of normality of residuals was visually checked for the models using normal probability plots. The presence of outliers and influential observations were verified by graphs of the studentized residuals and by the Cook's D statistics. The SAS version 9 (proc mixed) was used to fit the data to each model, considering a level of significance of 0.05.(15)

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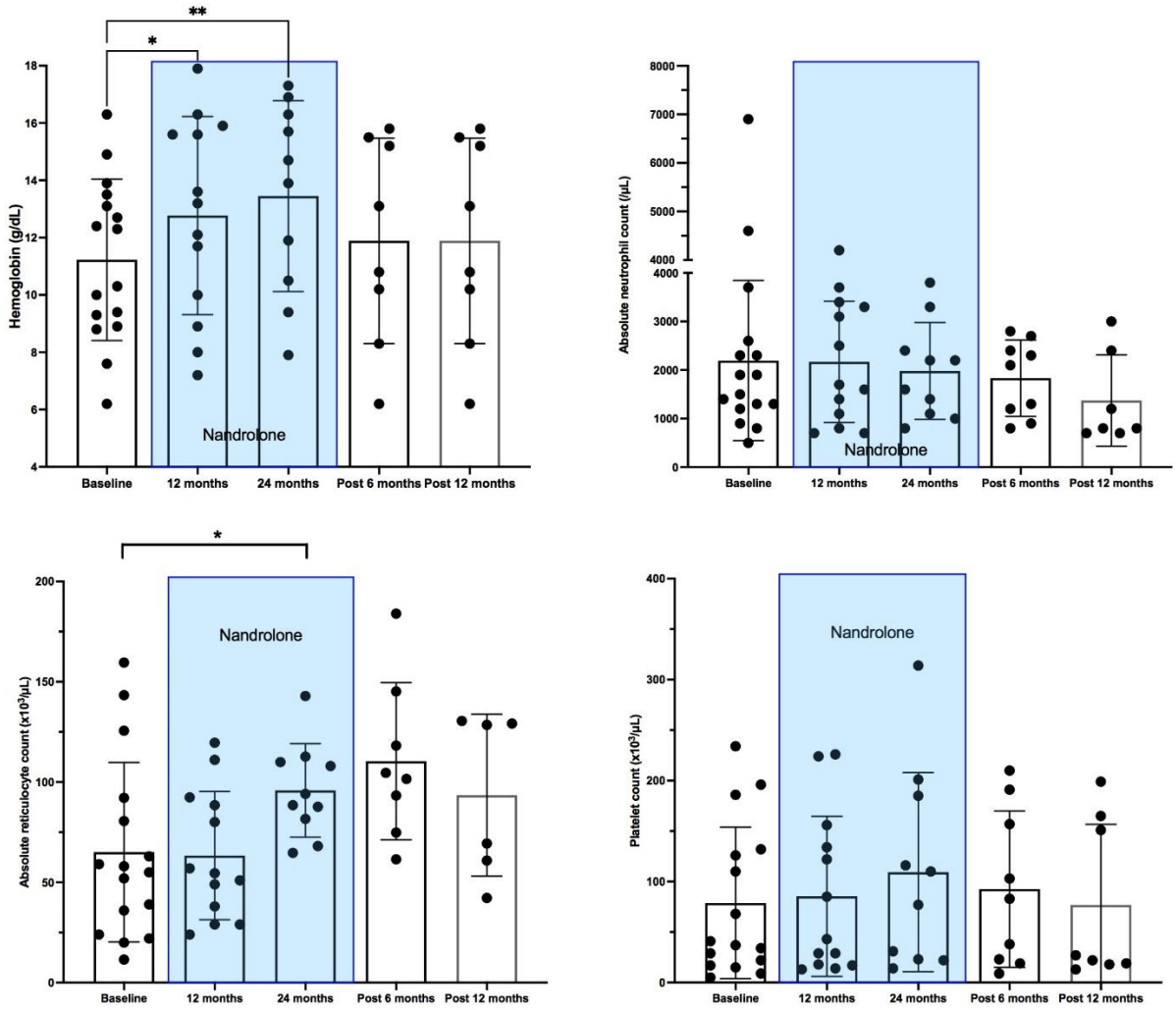
Supplementary Figure S1: Blood counts in patients with marrow failure treated with nandrolone



A: Hemoglobin (g/dl); **B**: Absolute neutrophil count (cell/µL); **C**: Absolute reticulocyte count (x10^3/µL) and **D**: Platelet count (x10^3/µL) at baseline, during nandrolone treatment and after drug discontinuation (6 and 12 months) in patients with marrow failure. * $p<0.05$

Supplementary Figure S2: Baseline telomere length of patients measured by flow-FISH with the age-adjusted percentile curves.