Protocol with Statistical Analysis Plan Cover Page:

Official Title:	Low Dose Danazol for the Treatment of Telomere Related Diseases
NCT number:	NCT03312400
Document Type:	Protocol with Statistical Analysis Plan (SAP)
Document Date:	September 10, 2026

CLINICAL RESEARCH PROJECT **PROTOCOL #: 18-H-0004**

Title: Low Dose Danazol for the Treatment of Telomere Related Diseases

Other Identifying Words: Telomeres, Aplastic Anemia, Pulmonary Fibrosis, Androgens,Cytopenia, Hepatic Fibrosis

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Subjects of Study:	Number	Sex	Age-Range
	40	Either	≥3 years and weight ≥ 12kg

Project Involves Ionizing Radiation?	Yes
Off-Site Project?	No
Multi-Center Trial?	No
DSMB Involvement?	Yes
Tech Transfer?	No

PRECIS

Telomere disease is caused by accelerated telomere attrition and results in multi-organ dysfunction. Telomeres are nucleotide repeats of non-coding DNA at the end of the chromosomes which function as protective caps to prevent erosion of genomic DNA during cell division and to protect chromosomes from recognition as single stranded DNA. Telomeric DNA is elongated by the telomerase complex, which is comprised of a reverse transcriptase catalytic subunit (encoded by TERT), an RNA template (encoded by *TERC*), and associated proteins.

Telomerase activity is crucial in maintaining telomere length in cells with a high proliferative capacity, such as hematopoietic stem cells (HSCs) and lymphocytes. Presentation of telomeropathies can vary from severe aplastic anemia (SAA) and dyskeratosis congenital (DKC)early in childhood, to pulmonary or hepatic fibrosis later in life. There is no standard of care for the treatment of telomere disease.

Considerable evidence suggests that sex hormones regulate telomerase. Calado et al. demonstrated that human lymphocytes and CD34+ hematopoietic cells up regulate both *TERT* gene expression and telomerase enzymatic activity in response to androgens in vitro. A recent observational cohort study demonstrated hematologic response in 14 of 16 pediatric patients withDKC treated with androgens. In a prospective trial from our Branch, Townsley et al demonstrated that patients with telomere diseases who were treated with the synthetic sex hormone danazol showed telomere elongation, and hematologic response was seen in 79% of patients after only three months of treatment. This study used the highest dose of danazol, 800 mg daily, and known adverse effects, such as elevated liver enzyme levels and muscle cramps, occurred in 41% and 33% of patients, respectively. Overall, the treatment was well tolerated, but some patients did require dose reduction. 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 endpoint. Because of the limited power, we were unable to draw definitive conclusions regarding further clinical effect of danazol, but stabilization or improvement was observed in a few cases in other organ function, measured by DLCO for pulmonary fibrosis and Fibroscan for cirrhosis.

We now propose a phase II study designed to determine the efficacy of low dose danazol in decreasing the rate of telomere attrition in subjects with a short age-adjusted telomere length. The secondary aim is to determine the clinical effect of this therapy in conditions that are related to short telomeres, to include cytopenia(s), pulmonary fibrosis, and/or hepatic fibrosis.

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1 OBJECTIVES

a. Primary Objective

- The primary objective of the study is to evaluate the safety and activity of danazol 400 mg versus 200 mg in slowing telomere attrition rates after 6 months on therapy in subjects with telomere related diseases.

b. Secondary Objective

- Assess the efficacy of danazol in ameliorating cytopenia(s) related to telomere disorders
- Assess the efficacy of danazol in slowing the progression of pulmonary fibrosis related to telomere disorders
- Assess the efficacy of danazol in slowing the progression of hepatic fibrosis related to telomere disorders
- Assess the toxicity profile during 12 months of treatment

c. Ancillary Studies

- Assess the accuracy of qPCR, Prime Flow-FISH, and conventional flow-FISHin measuring leukocyte telomere length
- In patients with very short leukocyte telomere length, evaluate hepatocyte telomere length using qPCR, flow-FISH, and terminal restriction fragment(TRF) analysis by Southern blot
- Evaluate hormone metabolite levels in relation to danazol dose and rate of telomere attrition

2 BACKGROUND AND SCIENTIFIC JUSTIFICATION

TELOMERES

Telomeres are structural elements that cap the ends of chromosomes, protecting them from recombination, end-to-end fusion, and recognition as damaged DNA. In human somatic cells, telomeres typically consist of more than 1000 tandem repeats of nucleotides and associated proteins. These repeats are gradually lost with cellular replication. Attrition eventually shortens telomeres critically; the result is usually arrested proliferation and senescence, shortened cellular life span, apoptosis, and occasionally genomic instability of the cell. Maintenance of the integrity of telomeres requires the telomerase ribonucleoproteincomplex, which consists of telomerase reverse transcriptase (encoded by *TERT*) and its integral RNA template (encoded by *TERC*), in addition to other proteins. Telomerase enzyme copies the RNA template into telomeric DNA to extend the 3' end of the chromosome. The normal attrition rate is about 60 base pairs per year.

Telomeres have been implicated in physiologic aging, certain fibrotic disorders, marrow failure, and in the development of cancer.[1] The direct link between telomeres, especially telomere repair mechanisms, and human disease comes from family studies of patients with X-linked dyskeratosis congenita (DKC).

Analysis of large pedigrees identified a location containing the *DKC1* gene, which encodes dyskerin, a small nucleolar ribonucleoprotein that is physically associated with the telomerase complex.[2] Subsequently, telomere length was measured as extremely short in patients with dyskeratosis congenita.[3] This initial discovery led to the purposeful investigation of another gene product, the RNA template encoded by the gene *TERC*, which was found to be mutated in patients with autosomal dominant dyskeratosis congenita.[4] The telomere is protected by the shelterin complex, and genes encoding for these protein components, such as *TINF2,* also may be mutated, producing a dyskeratosis phenotype. To date, eight different genes have been implicated in dyskeratosis congenita. These gene products are integral components of telomerase or telomere maintenance.[5] Patients with these defects in telomere repair have an increased attrition rate of about 120-170 bp/year. CLIA certified genomic panels are available to identify mutations in telomere maintenance genes.

Accelerated telomere loss also can occur physiologically, without a genetic basis. It is important to distinguish between short telomeres due to constitutional mutations and short telomeres due to stem cell depletion. The accelerated rate of telomere attrition in patients who develop a malignant clone may be evidence of restricted clonal hematopoiesis and proliferative stress leading to chromosomal instability.[6] This was illustrated by Dumitriu et al., who showed that SAA patients who progress to monosomy 7 myelodysplastic syndrome (MDS) have oligoclonal hematopoiesis and increase telomere attrition caused by accumulation of very short telomeres before development of aneuploidy.[6] In this population, rapid telomere attrition did not appear to be genetic; patients had normal telomere length at time of diagnosis with SAA and lacked mutations in *TERT* and *TERC*.[6]

Telomeres and Aplastic Anemia

Over the past decade, a significant body of research has been dedicated to the relationship between bone marrow failure and shortened telomeres. When we measured telomere length by a flow cytometric method, about 1/3 of patients with aplastic anemia had short telomeres for age.[7]

Dyskeratosis congenita was the first inherited bone marrow failure syndrome to be linked to accelerated telomere attrition. *DKC1* mutations cause x-linked dyskeratotsis. This mutation in DKC1 results in the loss of dyskerin, functionally destabilizing the telomerase repair complex.[5] In this syndrome, features almost always are present early in childhood, and boys demonstrate the classic triad of dystrophic nails, leukoplakia, and hypopigmented skin.

We later identified mutations in *TERC* and also in *TERT*, the gene encoding the reverse transcriptase telomerase, in some patients with apparently acquired anemia.[5] Affected patients and their family members carrying the mutation have short telomeres and evidence of diminished hematopoiesis.[8] For example, *TERC* and *TERT* mutations correlate with decreased bone marrow cellularity, low CD34 cell numbers, diminished functional hematopoietic progenitor cells in vitro, and increased levels of hematopoietic growth factors. Genetic defects in the telomere maintenance mechanisms include the shelterin genes, such as *TINF2*, and helicase genes, such as *RTEL1,* are also etiologic in dyskeratosis congenita.[9,10]

The clinical consequences of cytopenia(s) derive from low blood counts. Anemia leads to fatigue, weakness, headaches, and in older patients dyspnea and chest pain which are commonly responsible for the clinical presentation. Thrombocytopenia produces mucosal bleeding; petechiae of the skin and mucous membranes, epistaxis, and gum bleeding are frequent and early complaints. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage. Bacterial and fungal infections in

the setting of neutropenia are a major cause of morbidity and mortality, most often the cause of death in refractory cytopenias.

In patients with severe aplastic anemia (SAA), the current standard of care is transplant or immunosuppressive therapy (IST), regardless of telomere length. In a recent analysis, a short for age-adjusted telomere length was not sufficient to preclude hematologic recovery; however, higher rates of relapse, clonal evolution, and mortality were observed in this group.[11]

Telomeres and Pulmonary Fibrosis

Pulmonary fibrosis (PF) is a chronic progressive fibrotic lung disorder usually affecting adults over the age of 40 years. Common symptoms are cough and dyspnea, and pulmonary function defects include impaired gas exchange and reduced lung volume. Histopathology reveals patchy fibrosis of the lungs, interstitial inflammation, and disorganized deposition of collagen, and extracellular matrix with distortion of pulmonary architecture. In about 20% of patients with dyskeratosis congenita, lung complications ultimately diagnosed as pulmonary fibrosis develop.[12] This association led to the discovery of telomerase mutations in about 15% of patients with familial PF.[13]

In a recent cohort analysis of patients evaluated at a transplant center, idiopathic lung disease associated with a gene mutation in telomere related gene is universally progressive with mean rate of decline of forced vital capacity (FVC) of 300 mL/year with the median time to death or transplant as 2.87 years.[14]

The initial conception of the pathogenic process in idiopathic PF as partially inflammatory resulted in consideration of broad immunosuppression as a potential therapy. However, trials using this approach resulted in increased mortality when compared with placebo.[15] Recent clinical trials have focused on therapies that attenuate fibrosis, such as pirfenidone, a cytokine inhibitor, and nintedanib, a tyrosine kinase inhibitor, and these studies demonstrated that these drugs may slow the rate of progression.[15] However, drugs which completely halt or reverse disease progression are generally unavailable, and eventually patients will require supplemental oxygen and, in selected cases, will undergo lung transplantation.[15]

Telomeres and Hepatic Fibrosis

We have observed that many relatives of patients with aplastic anemia and a mutation in telomere maintenance have liver disease.[8] The two most common pathologies associated with *TERT* and *TERC* mutations are fibrosis with inflammation and nodular regenerative hyperplasia, a leading cause of noncirrhotic portal hypertension.[8] A recent cohort analysis of patients with idiopathic lung disease and short telomere length referred for lung transplant, found that 6 of 7 patients showed liver biopsies with abnormal findings, further supporting the association between shortened telomeres and hepatic fibrosis.[16] Some patients with dyskeratosis congenita have liver abnormalities after bone marrow transplantation.[17] Patients with pulmonary fibrosis and short telomeres can also have cryptogenic hepatic cirrhosis; this implicates telomere loss in both fibrotic processes.[1]

Transplants and Telomere Disease

There is no standard of care for the treatment of telomere disease. For patients presenting early in life with bone marrow failure, the typical recommendation is to pursue bone marrow transplant. However, there is a significant amount of late mortality after bone marrow transplant, primarily from pulmonary complications. In a systematic review of 109 patients,the overall survival was 70% at 5 years and 25% at 10 years.[18] This report confirmed improvement in early survival after the year 2000, when transplantation was performed prior to the age of 20, and when the donor was a matched sibling, but long-term survival

remains poor. Prognosis after the development of pulmonary fibrosis post-transplant was very poor. Reduced-intensity conditioning was not found to be associated with improved long-term survival.[18] Patients who develop progressive lung fibrosis or liver fibrosis are often not eligible for transplant because of their cytopenias and multi-organ disease.

Androgens and Telomere Length

There is a significant body of evidence that supports the role of sex steroid hormones and the molecular regulation of telomerase activity.[19] In men, higher serum dihydrotestosterone (DHT) and estradiol correlated with longer leukocyte telomere length independent of age.[20] Androgens have been used to treat bone marrow failure syndromes without a clear understanding of their mechanism of action. In vitro studies of peripheral blood and bone marrow samples suggest that telomere length can be modulated by sex hormones. Exposure of normal peripheral blood lymphocytes and human bone marrow–derived CD34+ cells to androgens increased telomerase activity in vitro, and androgens increased low baseline telomerase activity in individuals carrying a loss-of-function *TERT* mutation to normal levels.[21] Our recent study demonstrated this effect in vivo; treatment with danazol, a synthetic sex hormone, led to telomere elongation in patients with telomere disease.[22] Danazol is a synthetic androgen derivative, 17-alpha-ethinyl testosterone. It primarily inhibits the luteinizing hormone surge and increases free testosterone and decreases estrogenlevels. The exact mechanism that reverses telomere attrition remains unknown.

Scientific and Clinical Justification of The Protocol

Currently, there is no standard of care for the treatment of patients with telomere diseases. Organ dysfunctions secondary to telomere disorders are typically treated symptomatically, as outlined above.

Recently, our group published a prospective clinical study that demonstrated telomere elongation in circulating leukocytes in association with hematologic improvement in patients with telomere disease treated with danazol.[22] 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 endpoint. In exploratory analysis, increases in telomere length were observed at 6 months (16 of 21 patients), 12 months (16 of 18 patients) and 24 months (11 of 12 patients). Hematologic responses occurred in 19 of 24 patients (79%) evaluated at 3 months and in 10 of 12 patients (83%) evaluated at 24 months.[22]

Androgens have been used to treat bone marrow failure since the mid-20th century, with hematologic improvement rates of 50% or better in a few case series.[5] In a prospective analysis of 16 patients on androgen therapy (oxymetholone, fluoxymesterone, and nandrolone), which included mostly children with dyskeratosis congenita, 11 achieved clinically significant hematologic responses.[23] Additionally, a recent case report described, for the first time, marked improvement in the clinical and laboratory parameters of a child with dyskeratosis congenita-associated pulmonary fibrosis after initiating danazol.[24]

This follow-on study will evaluate the rate of telomere attrition with low dose danazol. The prior study used oral administration of danazol 800 mg daily divided into two doses per day. This route and dose were extrapolated from that used to treat other conditions such as endometriosis and fibrocystic breast disease, as approved by the FDA, and is the highest dose of danazol that is currently approved for use in humans. Ten patients withdrew from the study before 2 years. Five of the 10 patients withdrew due to side effects, as follows: two patients discontinued treatment because of low grade side effects; three discontinued after grade 3 or grade 4 adverse events. Two withdrew without a stated reason, two proceeded to receive alternative therapy, and one died from organ failure related to pulmonary fibrosis.

The most common adverse events were elevations in liver enzyme levels (in 41% of patients), muscle cramps (in 33%), edema (in 26%), and lipid abnormalities (in 26%).[22] Anecdotal evidence from the prior study found similar benefits with fewer side effects whenthe dose of danazol was decreased.

We propose the use of a two arm study design. The goal of this study is to identify the minimal effective dose of danazol. The primary endpoint is the rate of telomere attrition after 6 months on therapy. The study is powered to support this finding. The secondary endpoints are evaluating the clinical effect after 6 months and 12 months on therapy. After the initial 6 months of therapy, patients will switch to the other dosing arm, allowing us to evaluate the different genotypes on each dosing arm. In telomere disease, there is significant variability in the clinical severity, even within a pedigree. In this protocol, we will include all patients with very short telomeres and a mutation in any of the telomere maintenance genes. In the prior danazol study, we found variability in the clinical response. For example,some of the patients with DKC1and TERC mutations did not respond as briskly as the patients with TERT mutation. The current trial design may provide insight into the question about how the different genotypes respond to treatment. Toxicity is generally mild and reversible; therefore, we do not need a washout period for toxicity. In the prior study, dosage effect was typically seen in the first 3 months on drug. Given the prior experience with dosing danazol at much higher doses, 800 mg/day for 2 years, we do not expect harm from the current dosage strategy.

We propose a dose comparison approach to identify a low effective dose of danazol. Additionally, our study will further define the clinical benefit of danazol in treating pulmonary fibrosis, hepatic fibrosis, and cytopenias associated with telomere diseases.

3 STUDY DESIGN

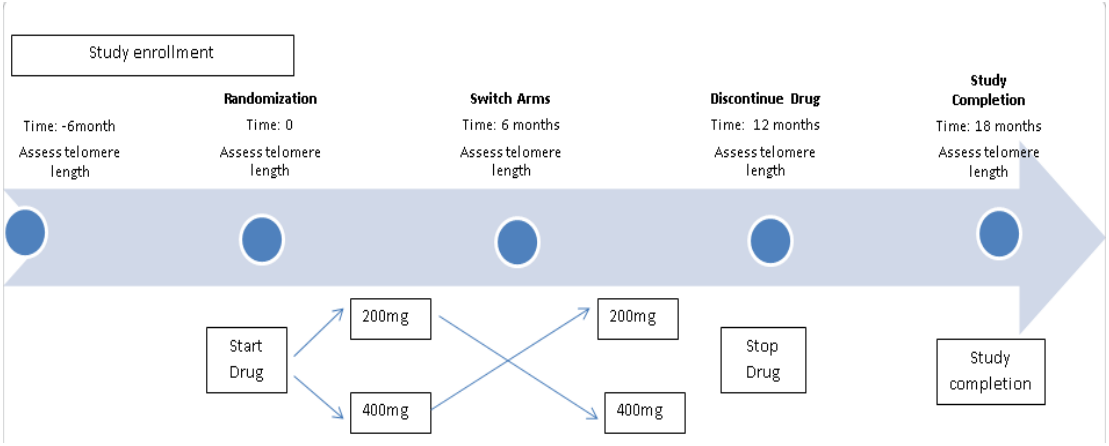
This is a phase II study designed to determine the efficacy of low dose androgens in decreasing the rate of telomere attrition in subjects with a short age-adjusted telomere length (<1st-10th percentile). This will be a superiority study evaluating the efficacy of danazol 200 mg daily versus 400 mg daily after 6 months of treatment at each dose. The secondary aim is to determine the clinical effect of danazol therapy in conditions that are related to telomere dysfunction. Prior to starting danazol, we will determine the baseline rate of telomere attrition over at least a 6-month period. This period of baseline evaluation may occur on other NHLBI screening protocols or based on testing from external institutions.Telomere length measurements by flow-FISH obtained at commercial labs outside of the NIH prior to evaluation at NIH will also be accepted. Telomere length measurements must be at least 6 months prior to starting danazol at the randomization visit to allow accurate determination of the telomere rate of attrition per year t. Subjects may sign consent any time during this baseline period prior to starting danazol.

After determining baseline rate of telomere attrition, subjects will be randomized to either receive danazol 200 mg/day for 6 months or danazol 400 mg/day for 6 months. After 6 months, the patients will switch to the other dosing arm for an additional 6 months of treatment. Telomere length and clinical evaluation will be monitored at each time point. The primary end point will be evaluated after the first 6 months on danazol, the second 6 month period when the subjects are switched to the other dose will evaluate secondary aims.

Patients will be monitored for an additional 6 months after completing a total of 12 months of danazol treatment.

Pediatric patients will be randomized to either receive danazol 4 mg/kg/day divided in twice daily dose (max 400 mg daily) for 6 months or danazol 2 mg/kg/day divided in twice daily dose (max 200 mg daily)

for 6 months. The dose will be rounded to the nearest 50-mg dose.



Telomere length will be measured by flow-FISH in a CLIA certified laboratory.

4 ELIGIBILITY ASSESSMENT

Eligibility will be determined either on the NHLBI screening protocol (97-H-0041), by using any existing subject's test result from other active Hematology Branch protocol, or from external medical records (see below). The time between eligibility determination and signing consent to participate on this protocol may not exceed 300 days.

The following testing may be used from external records to satisfy inclusion criteria (provided they are performed within the 180-300-day eligibility window): CBC, genetic testing in a CLIA(or international equivalent) certified laboratory, telomere length by flow-FISH in a CLIA certified laboratory, PFTs, CT chest, liver ultrasound, liver fibroscan, and lung or liver histology reports. Genetic testing in a CLIA (or international equivalent) certified laboratory performed at any time demonstrating a mutation in a telomere related gene may be used to determine eligibility.

4.1 Inclusion Criteria

4.1.1 Age-adjusted telomere length less than or equal to the first percentile by flow-FISH method. In patients with a known pathogenic or likely pathogenic mutation in a telomere maintenance gene, age adjusted telomerelength less than or equal to the 10^{th} percentile is sufficient.

4.1.2 A mutation in telomere maintenance genes (TERT, TERC, DKC1, TINF2, NHP2, NOP10, WRAP53, TERF2, PARN, RTEL1, ACD, CTC1, USB1) as tested in a CLIA (or international equivalent) certified laboratory

4.1.3 Age ≥ 3 years

4.1.4 Weight ≥ 12 Kg

AND

4.1.5 At least one of the following criteria:

a. Anemia with a hemoglobin ≤ 10 g/dL without red blood cell transfusion
b. Thrombocytopenia with a platelet count ≤50,000/μL without transfusion
c. Neutropenia with an absolute neutrophil count ≤ 1,000/μL

OR

Pulmonary fibrosis diagnosed by either a lung biopsy or computed tomography scan of the chest according to guidelines from the American Thoracic Society and European Respiratory Society.

OR

4.1.6 Hepatic fibrosis diagnosed by Transient Elastography by Fibroscan® value greater than 10 kpa or US evidence of cirrhotic liver or splenomegaly, or transjugular liver biopsy demonstrating fibrosis.

4.2 Exclusion Criteria

4.2.1 Patients on androgen hormones to include testosterone or high dose estrogen (estradiol 0.5 mg/day or greater) for the12 months prior to enrollment

4.2.2 Patients with active thrombosis or thromboembolic disease and history of such events, undiagnosed abnormal genital bleeding, porphyria, androgen-dependent tumor, or prostatic hypertrophy

4.2.3 Patients with pulmonary fibrosis who are receiving anti-fibrotic drug treatment, such as pirfenidone or nintedanib unless stable on anti-fibrotic drug for at least 6 months prior to starting on danazol as demonstrated by PFTs.

4.2.4 Patients with active hepatitis B or C

4.2.5 Patients who have received a bone marrow transplant

4.2.6 Patient with other hereditary bone marrow failure syndromes such as Fanconi anemia or Diamond Blackfan anemia

4.2.7 Patients with infections not adequately responding to appropriate therapy

4.2.8 Current pregnancy, or unwillingness to take oral contraceptives or use the barrier methods of birth control or practice abstinence to refrain from pregnancy if of childbearing potential during the course of the study

4.2.9 Lactating women, due to the potentially harmful effects on the nursing child

4.2.10 Patients with cancer who are actively receiving systemic chemotherapeutic treatment or who take drugs with hematological effects

4.2.11 Patients with decompensated liver disease to include persistent ascites, encephalopathy, variceal hemorrhage, or MELD score of 10 or greater

4.2.12 Inability to understand the investigational nature of the study or to give informed consent or without a legally authorized representative or

surrogate that can provide informed consent

4.2.13 Inability to swallow a capsule

5 TREATMENT PLAN

5.1 Danazol Administration

Once the 6-month baseline telomere measurements are determined, patients will be randomized to one of two treatment arms, and after 6 months of treatment they will switch to the other treatment arm. One arm will be danazol 100 mg twice a day, the other arm will be danazol 200 mg twice a day. Every patient will receive 6 months danazol 200 mg/day and 6 months 400 mg/day, thereby completing 12 months of danazol treatment. The danazol dose was chosen based on our experience with patients observed in our earlier protocol: after termination of study treatment, some individuals showed hematologic benefit when lower doses of danazol were administered by their local physicians. The drug will be administered in divided doses. Children less than 50 kg will be dosed at 4 or 8 mg/kg/day not to exceed 200 or 400 mg/day, respectively. Danazol is supplied in capsule form. The smallest capsule available is 50 mg, therefore pediatric danazol doses will be rounded to the nearest 50 mg.

Patients who do not tolerate 400mg/day of danazol will be moved to the other treatment arm and dose reduced to 200mg/day of danazol. Because the goal of the trial is to define the lowest effective dose of danazol, patients who do not tolerate 200mg/day of danazol will be removed from the study, rather than attempting further dose reduction. Danazol will be discontinued for any grade IV toxicity considered to be probably or definitely related to danazol.

Subjects may also require dose interruptions for other clinically indicated reasons, such as toxicity from a different medication. Dose interruptions will typically not be for more than 14 days (either interrupted or continuous) and will be monitored. The treatment period is 6 months, so the rare need to interrupt dosing will not affect the study objectives or compromise subject safety. If tolerated, danazol will be continued for a total of 12 months.

Treatment compliance will be monitored by surveying patients at the 6 month and 12 month visit regarding compliance and frequency of missed doses. We will monitor requests for refills. Additionally, we will check sex hormones to include LH and FSHwhich may give some insight into patient compliance.

There is no standard of care for patients with telomeropathy. Organ dysfunctions secondary to telomere disorders are treated symptomatically. For marrow failure, immunosuppression is usually ineffective. For pulmonary fibrosis, therapies are only effective in slowing disease progression. For hepatic fibrosis, there are no effective therapies.

5.2 Drug Interactions

The use of danazol may be associated with increased serum cholesterol, because of the short duration of the study, patients will likely not require treatment. Under the discretion of the primary investigator, lipid lowering agents should be started if clinically indicated. The risk of myopathy and rhabdomyolysis is increased by concomitant administration of danazol with statins such as simvastatin, atorvastatin and lovastatin.

Therapy with danazol may cause an increase in carbamazepine levels in patients taking both drugs.

6 CLINICAL MONITORING

All patients will be evaluated by hematology, hepatology, and endocrinology services at enrollment, and then at the 6 months, and 12-month clinic visits as needed. At PI discretion patients with abnormal pulmonary function testing or chest CT will be evaluated by NHLBI Pulmonary Branch. Once eligibility is determined, the consent process may be conducted either at the NIH Clinical Center (CC) or via telephone or an approved telehealth platform. In the case a patient is unable to travel to the NIH clinical center, enrollment can take place remotely via telephone or an approved telehealth platform. Patients enrolled remotely will not need formal evaluation by a hepatologist or endocrinologist unless clinically required at PI discretion.

The baseline is defined as the time period prior to starting danazol.

6.1 Baseline Evaluation (Prior to Randomization)

The following studies will also be considered baseline if available under other NIH protocols or from home medical provider and reviewed at NIH within the past 300 days. For patients enrolled remotely, studies can be performed at commercial labs or outside hospitals within 300 days prior to enrollment and records reviewed by the NIH clinical team.

Baseline status will be evaluated as follows:

- Medical history and physical examination
- Baseline clinical laboratory studies
    - complete blood count with differential
    - reticulocyte count
    - acute care (Na, K, Cl, CO2, creatinine, glucose, urea nitrogen)
    - mineral (phosphorus, magnesium, albumin, and calcium)
    - hepatic (ALK phosphatase, ALT, AST, total and direct bilirubin, GGT)
    - Telomere length assay by flow-FISH from a CLIA certified laboratory
- If there is a cytopenia on prior labs (at NIH or outside clinics) within the past 300 days (hemoglobin ≤10, platelets ≤50,000 or absolute neutrophilcount ≤1,000):
    - obtain bone marrow aspirate and biopsy with cytogenetic analysis as appropriate to stage and classify underlying disease within 6 months of study enrollment
    - flow cytometry of the peripheral blood for glycosylphosphatidylinisotol anchored proteins
- Pulmonary Evaluation
    - pulmonary function testing
    - 6-minute walk test (once prior to starting danazol)
    - echocardiogram with bubble study if PFTs concerning for pulmonaryAVM (per PI or pulmonary consultant; may be omitted)
    - chest CT can be performed any time in the screening period prior to starting

danazol. Scan can be omitted if patient has normal PFTs or due to participant choice.

- Liver Evaluation

    - complete ultrasound of abdomen with doppler flow (once prior to starting danazol)
    - liver elastography by Fibroscan and/or Acoustic radiation force impulse (ARFI) (once prior to starting danazol)
        - If on Fibroscan there is evidence of significant fibrosis including Fibroscan greater than 10 kpa or ultrasound evidence of cirrhotic liver or splenomegaly, then patient may have transjugular liver biopsy and portal pressure measurements if required for diagnosis of fibrosis/cirrhosis per PI or hepatology consultant

Either liver ultrasound or fibroscan can be performed for baseline liver evaluation provided both are performed at least once prior to the commencement of danazol. If liver fibrosis is the eligibility criterion used for the study, both liver ultrasound and fibroscan should be performed at both baseline and randomization.

- The following labs are part of the Screening Evaluation and need only be obtained once prior to starting danazol. They can be obtained at screening evaluation or at the randomization visit, prior to starting danazol.

    - fasting lipid profile, apolipoprotein panel
    - lipoprotein particle
    - pregnancy test (urine HCG in women of child-bearing potential)
    - other (total protein, CK, LDH, uric acid) panels
    - hepatitis B surface antigen (HBsAg)
    - antibody to hepatitis B core antigen (anti-HBc)
    - hepatitis B surface antibody (anti-HBs)
    - antibody to hepatitis A virus
    - antibody to hepatitis C virus (anti-HCV)
    - Estradiol
    - Estrone
    - Free and total testosterone
    - DHEA
    - Dihydrotestosterone
    - HIV (anti-HIV 1 and 2 antibody)
    - Peripheral blood immunophenotyping (TBNK)

Endocrine Evaluation: For children, endocrine evaluation will be performed twice over at least a 6-month period to obtain a baseline growth rate and bone maturation prior to starting danazol. These evaluations can occur at screening/baseline and then at time of randomization, for example. For patients over age 18 years testing need only occur

once prior to starting danazol.

- height and weight
- pubertal staging (children 3-18 yo only)
- testicular size (males only). This may be omitted in the case of telehealth or telephone visits.
- Ferriman Gallwey score (measure of hirsutism) (females only)
- menstrual history (females only)
- bone age X-ray of left hand (children 3-18 yo)

If blood volumes permit, the following baseline examinations may be performed. May be omitted per PI discretion.

Prioritize as follows.:

1. thyroid binding globulin, free T4, TSH
2. fasting insulin, hemoglobin A1C
3. anti-liver-kidney-microsomal antibody (Anti-LKM)
4. alpha-1-antitrypsin levels (A1AT)
5. alpha-fetoprotein (AFP)
6. ceruloplasmin
7. cryoglobulins
8. pro-BNP
9. sex hormone binding globulin, LH and FSH
10. antinuclear antibody (ANA)
11. antimitochondrial antibody (AMA)
12. rheumatoid factor (RF)

6.2 Study Time: at Randomization, 6-Months on danazol, And 12-Months on danazol

After initiation of drug, subjects will be followed by their home physician or at the Clinical Center and have blood testing as detailed below. Subjects will be seen by their home physician as clinically indicated and the Clinical Center and home physician will continue to communicate clinically significant updates as needed. Progress notes and laboratory results from home physicians will be faxed to the Research nurse, at 301- 402-3088. Subjects must be evaluated at the NIH Clinical Center at randomization, 6 months (+/-14 days) and 12 months (+/-14 days). Subjects may undergo additional in person or telehealth visits to follow-up on study related laboratory results or evaluations per PI discretion.

In the case of a Public Health Emergency preventing travel to the NIH, the Clinical Center visit testing at randomization, 6 months and 12 months can be performed at a local healthcare center with results sent to the study team to be used both as research and clinical follow up. A telehealth or telephone visit will be performed in lieu of an in person visit to the clinical center in these cases.

- Local care providers will obtain renal and hepatic panel weekly (+/- 6 days) for the first month after starting a new danazol dose and then every 2-4 weeks

(+/- 7 days). These labs will first be obtained one week after starting danazol. CBC should be monitored every 3 months/12 weeks (+/- 7 days). More frequent monitoring of specific disease related issues may be recommended per PI discretion.

- If first randomized to 400 mg, when switched to the lower dose arm, patients do not need to do weekly labs unless clinically indicated and will continue with monthly labs as described above. Patients who switch from the 200 mg to the 400 mg dosing arm will need to resume weekly labs as described above.
- After discontinuation of danazol, should a research subject require clinical labs, we will request results of these labs for our study.

Evaluation at randomization, 6 and 12-month landmark visits (+/-14 days):

- complete blood counts with differential
- reticulocyte count
- Bone marrow biopsy
    - Bone marrow biopsy, immunophenotyping, cytogenetics and may be performed at the discretion of the PI if clinical deterioration in blood counts or concerns regarding evolution to myelodysplastic syndrome.
- acute care (Na, K, Cl, CO2, creatinine, glucose, and urea nitrogen)
- mineral (phosphorus, magnesium, albumin, and calcium)
- hepatic (Alk phosphatase, ALT, AST, total and direct bilirubin, GGT)
- other (total protein, CK, LDH, uric acid) panels
- Telomere length assay using flow-FISH in a CLIA certified laboratory
- fasting lipid profile, apolipoprotein panel, lipoprotein particle
- fasting insulin, hemoglobin A1C (Hemoglobin A1c will not be performed on patients receiving PRBC transfusions)
- thyroid binding globulin, free T4, TSH
- sex hormone binding globulin, LH and FSH
- estrone, estradiol, free and total testosterone, DHEA, dihydrotestosterone
- women of childbearing potential will have a urine pregnancy test
- pro-BNP
- Peripheral blood immunophenotyping (TBNK)
- chest CT (can be omitted per PI discretion if participant is deemed to have normal or unchanged PFTs or due to participant choice)
- echocardiogram with bubble study if PFTs concerning for pulmonary AVM (per PI or pulmonary consultant)
- 6-minute walk test
- pulmonary function test
- complete ultrasound of abdomen with doppler flow
- liver elastography by Fibroscan or Acoustic radiation force impulse (ARFI)
    - If on fibroscan there is evidence of significant fibrosis including fibroscan greater than 10 kpa or US evidence of cirrhotic liver or

splenomegaly, or if there is unexpected progression of disease, then patient may have transjugular liver biopsy and portal pressure measurements.

- Endocrine Evaluation
  - height and weight
  - pubertal staging (children 3-18 yo)
  - testicular size (males only). This may be omitted in the case of telehealth or telephone visits.
  - Ferriman Gallwey score (measure of hirsutism) (females only)
  - menstrual history (females only)
  - bone age X-ray of left hand (only patients with prior bone age not yet fused)

6.3 Follow-Up Evaluation (6 Months off danazol)

Subjects will be evaluated at the Clinical Center after being off danazol for 6 months (+/- 30 days) and have blood work done as detailed below. Subjects will be seen by their home physician as clinically indicated and the Clinical Center and home physician will continue to communicate clinically significant updates as needed. Subjects may undergo additional in person or telehealth visits to follow-up on study related laboratory results or evaluations per PI discretion. Progress notes and laboratory results from home physicians will be faxed to the Research nurse, at 301- 402-3088:

In the case of a public health emergency preventing travel to the NIH, the Clinical Center visit testing at 6 months off danazol can be performed by a local healthcare provider with results sent to the study team. A telehealth or telephone visit will be performed in lieu of an in person visit to the clinical center in these cases.

- complete blood counts with differential
- reticulocyte counts
- Bone marrow biopsy
  - Bone marrow biopsy, cytogenetics, and immunophenotyping may be performed at the discretion of the PI if the clinical deterioration in blood counts or concerns regarding evolution to myelodysplastic syndrome.
- acute care, mineral, hepatic panels, GGT
- Telomere length assay using flow-FISH in a CLIA certified laboratory
- fasting lipid profile, apolipoprotein panel, lipoprotein particle
- thyroid binding globulin, free T4, TSH
- fasting insulin, hemoglobin A1C
- sex hormone binding globulin, LH and FSH
- pro-BNP
- women of childbearing potential will have a urine pregnancy test
- estradiol, estrone, free and total testosterone, DHEA, dihydrotestosterone
- Peripheral blood immunophenotyping (TBNK)
- Echocardiogram with bubble study if PFTs concerning for pulmonary AVM (per PI or pulmonary consultant)

- 6-minute walk test
- pulmonary function test
- chest CT (can be omitted per PI discretion if participant is deemed to have normal or unchanged PFTs or due to participant choice).
- complete ultrasound of abdomen with doppler flow
- liver elastography by Fibroscan or Acoustic radiation force impulse (ARFI)
- Endocrine evaluation
    - height and weight
    - pubertal staging (children 3 -18yo)
    - testicular size (males) This may be omitted in the case of telehealth or telephone visits.
    - Ferriman Gallwey score (measure of hirsutism) (females)
    - menstrual history (females)
    - bone age X-ray of left hand (only patients with prior bone age not yet fused)

7 CRITERIA FOR RESPONSE

7.1 Criteria for Telomere Response

Response will be determined as decreased rate of telomere attrition by 50%, as compared to the baseline rate, after 6 months of treatment.

7.2 Criteria for Clinical Response

7.2.1 Hematologic response will be determined as improvement in one or more of the listed parameters:

Subjects with severe pancytopenia, defined as having 2 of the following criteria prior to starting danazol: ANC <500 /μL, platelet count <20,000 /μL, and reticulocyte count <20, 000, will be considered to have a response when hematologic criteria for severe aplastic anemia is no longer met defined as meeting at least 2 of the following criteria:

- Absolute neutrophil count >500 /μL
- Platelet count > 20,000 /μL
- Reticulocyte count > 60,000 /μL

In subjects with moderate pancytopenia, or patients with cytopenia not qualified as severe, peripheral blood parameters (improvement in one or more of the listedparameters) will be recorded as a response:

a) Absolute neutrophil count (ANC)

- If baseline in ANC is below 500 /μL, increase in ANC ≥ 300 /μL
- If baseline in ANC is above 500 /μL, increase in ANC ≥ 500 /μL

b) Platelets

- Response is an increase in platelet count by ≥20,000 /μL

- Hemoglobin

Any increase in hemoglobin by 1.5 g/dL of blood in transfusion independent patients and in absolute reticulocyte count to ≥ 60,000 /μL of blood in transfusion-dependent patients

Subjects will be considered responders if their blood counts meet these criteria on at least a minimum of 2 serial measurements at least one week apart. Subjects that are dependent upon exogenously administered growth factors will not be considered as fulfilling response criteria.

7.2.2 For patients with pulmonary fibrosis, a positive response would be seen if the rate of FVC decline on drug is less than baseline rate of decline over a 6-month interval. This will be defined as 5% predicted difference between baseline rate of decline and rate of decline in FVC on drug. This is consistent with a difference of at least 10% predicted FVC in 52 weeks thatwas used in other landmark IPF clinical trials, such as the ASCEND trial.[15]

7.2.3 Hepatic response will be determined as improvement in one or more of the listed parameters:

a) Reduction in transient elastography by Fibroscan® of ≥ 20% after 6 and 12 months of treatment when compared to start of therapy.

OR

b) Reduction in hepatic venous portal gradient by ≥ 20% after 12 months of treatment when compared to start of therapy in subjects who had liver biopsy for significant liver disease.

OR

c) Reduction in transient elastography by ARFI ≥ 20% after 6 and 12 months of treatment when compared to start of therapy.

8. ANCILLARY LABORATORY RESEARCH STUDIES

8.1 Sample Collection, Storage, Biospecimen and Data Management Plan

Intended use: During the course of participating on this study, 60 cc of blood and 5 cc of bone marrow may be requested for the following correlative laboratory research studies. Baseline samples may also be obtained under protocol 04-H-0012.

These studies are undertaken for descriptive or ancillary studies which may or may not be done and if done, may be correlated with response. In pediatric subjects, 3 ml/kg of blood not to exceed 60 ml may be requested for laboratory research studies. These specimens will not be examined by a pathologist.

- Telomere length assays at baseline over a 6-month period prior to starting danazol, then after being on danazol for 6-months and 12-months, then again 6-months after

stopping danazol
- Evaluation of hepatocyte telomere length by flow-FISH, qPCR, Southern blot
- Evaluate hormone metabolite levels in relation to danazol dose and rate of telomere attrition
- Serum alpha-2-antiplasmin
- Telomere length measurement in peripheral blood / bone marrow by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA
- Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: DKC1, TERC, TERT, SBDS, NOP10, NHP2, NAF1, PARN, RTEL1.
- Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.
- Quantification of gene expression with RNA-seq
- Characterization of chromatin and promoter/enhancer landscapes with ATAC-seq
- Next Generation Sequencing (NGS) of blood and bone marrow for myeloid somaticmutations
- NGS for gene related to other (non-telomere disease) inherited bone marrow failure syndromes

Management of Genetic Testing Results

In this study, targeted sequence will be performed on a panel of genes primarily focused on bone marrow failure. However, there is a small possibility that we may find a variant on a small number of clinically actionable genes included in the panels we are testing. Should we identify a clinically relevant (actionable) gene during NGS testing, which is done in a CLIA-certified lab, we will discuss the findings with the research subject and refer them to a genetic counselor as appropriate.

Biospecimen Management: Specimens and their derivatives (e.g., genomic material, cell lines) will be coded and stored in conformity with DIR Policy (e.g., BSI). Coded biospecimens may be sent to collaborators outside of the NIH with IRB approval in accordance with applicable NIH and DIR Policy for sharing research resources, including an executed material transfer agreement. Biospecimens with subject personal identifiers may be sent to associate investigators and collaborators outside of the NIH only after approvals of all applicable IRBs, an executed reliance agreement with the NIH IRB, or an extension of the NIH's FWA through an Individual Investigator Agreement.

Data Management: The principal investigator, associate investigators, research nurses and/or a data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as intake forms and the case report forms. Laboratory data from NIH will be reviewed using CRIS. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from the subjects' home physician.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. Laboratory values from referring home physicians will be entered into the system. The principal investigator, associate investigators/research

nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable.

Research data will be prospectively collected by authorized Investigator personnel and entered into an NHLBI-approved database that is 21 CFR 11 compliant, database which will consist of the study specific set of electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.

The database will maintain complete data records on each research subject. Subjective and objective patient experiences during the duration of the study will be documented in the patient medical record notes. These protocol notes will serve as the primary source material from which data will be collected in the database. Any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records will be used as additional source for data collection.

We will maintain the confidentiality of identifiable private information collected in this Clinical Trial and protect the privacy of the individual human subjects. Primary data containing individually identifiable information obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH information security standards. Neither individual personal identifiers nor the key linking coded data to individuals will be released without prior IRB approval and an executed CDA or MTA. Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed CDA or MTA.

Storage: All samples will be stored in the laboratory of Dr. Young. Collected samples will be de-identified prior to storage in the laboratory of the principal investigator following current NHLBI DIR BSI Policy. Efforts to ensure protection of patient information include:

- Each sample is assigned a unique number
- Vials holding patient samples are labeled with the sequential laboratory accession ID number that does not contain any personal identifier information
- An electronic database is used to store patient information related to the coded samples
- The laboratory is located in a controlled access building and laboratory doors are kept locked. Visitors to the laboratory are required to be accompanied by laboratory staff at all times
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.

Tracking: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

End of Study Procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. At the completion of the protocol(termination), samples and data will be maintained in a repository for future research.

Loss or Destruction of Data: Should we become aware that a major breach in our plan to protect patient

confidentiality and trial data has occurred, the IRB will be notified.

Privacy and Confidentiality: All efforts, within reason, will be made to keep subjects' private identifiable information (PII) private. Using or sharing ("disclosure") such data must follow federal privacy rules. Under certain circumstances, the United States Office of Human Research Protections (OHRP), The US Food and Drug Administration (FDA), and the Institutional Review Board (IRB), will be able to inspect and copy confidential study-related records which identify participants by name. Therefore, absolute confidentiality cannot be guaranteed.

Future Use of Stored Specimens and Data:

We may share specimens and data with other researchers for future use.
Following analyses of biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a research biospecimen repository. Biospecimens may be destroyed only when permitted by the Clinical Director. Any future research use of identifiable biospecimens not defined in the research protocol will occur only after IRB review and approval.

8.2 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to OHSRP office of Compliance and Training and investigated withing 7 calendar days. Minor protocol deviations will be reported in aggregate at the time of continuing review. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

8.2.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

8.3 Publication and Data Sharing Policy

Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for

publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers up until 10 years after having been posted in clinicaltrials.gov by contacting the Principal Investigator, Bhavisha Patel M.D., Ph 301-402-3477, bhavisha.patel@nih.gov.

Genomic Data Sharing Plan

This study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

Per GDS policy, sequence data will be deposited in dbGap.

8.4 Collaborations

Rebecca Ortolano, M.D. is the protocol author, and will continue to collaborate on trial with protocol questions. Dr. Ortolano will not have access to PII and will no longer participate in direct patient care.

Joseph Parambil, M.D. is a physician with expertise in pulmonary fibrosis. Dr. Parambil will provide patient referral and expert consultation. Dr. Parambil will not have access to PII outside of his own patient referrals.

Carrie Diamond, M.D. will continue to collaborate on trial with protocol questions. Dr. Diamond will not have access to PII and will no longer participate in direct patient care.

9 BIOSTATISTICAL CONSIDERATIONS

9.1 Primary Endpoints

The primary objective of the study is to evaluate the superiority of 400 mg danazol versus 200 mg in reduction of telomere attrition rate over 6 months. Reduction of telomere attrition rate is defined as a 50% reduction compared to baseline.

9.2 Secondary Endpoints

Secondary endpoints to be evaluated for the study include:

(a) hematologic response after 6 and 12 months of treatment
(b) progression of pulmonary function testing after 6 and 12 months of treatment
(c) progression of fibroscan and transient elastography by ARFI after 6 and 12 months of

treatment
(d) Assess the toxicities associated with low dose danazol use.

9.3 Sample Sizes

The study will enroll up to n=20 patients per arm, so that the total sample size is N=40(20 x 2) patients in both the 200 mg arm and the 400 mg arm.

9.4 Statistical Methods

Primary Analysis and Sample Size Justification:

The primary endpoint is response rate and will be analyzed using the t-test (or equivalently the Chi-square test) for proportions between two samples. The proposed sample size of 20 patients per arm is calculated based on testing the rates of telomere response (see definition in Section 7.1) between the danazol 400-mg arm and the danazol 200-mg arm. Let p_1 and p_2 be the response rates for the danazol 200 mg arm and the danazol 400mg arm, respectively. The statistical objective is to test the null hypothesis H_0: $p_1=p_2$ versus the two-sided alternative that H_0: $p_1 \neq p_2$. To demonstrate a meaningful clinical reduction in telomere attrition for the danazol 400 mg arm under the alternative, we would like the response rate p_2 to be at least 50% decrease in telomere attrition rate by 6 months. Assuming that the danazol 200 mg arm is almost equivalent to the telomere attrition rate without danazol, the response rate p_1 is a 10% decrease in telomere attrition rate by 6 months. Using the normally approximated binomial test for proportions under the alternative that $p_1=0.10$ and $p_2=0.50$, the sample size of 20 patients per arm (total 40 patients for both arms) has 80% power at 0.05 significance level. The primary endpoint of response rate at 6 months after the treatment will be analyzed using the "intention-to- treat" (ITT) principal in the sense that all patients who randomized will be included in the analysis and patients who do not have telomere measurements will be treated as non- responders.

Interim Analysis:

Because the sample size is small, we expect that almost all the patients will be successfully enrolled before the first 5 or 6 patients complete their evaluations after 6 months of treatment. We do not plan any statistical stopping boundaries for either efficacy or futility from an interim analysis. The study will only be stopped because of safety reasons (see Section 9.5 below).

Statistical Analysis for Secondary Endpoints:

The planned analyses will include descriptive statistics on the clinical efficacy of danazolin treating the cytopenia(s), pulmonary fibrosis, and hepatic fibrosis associated with telomere disease. It will also include descriptive statistics on the incidence and severity of adverse events, such as the percentage of subjects with toxicities. Sample proportion and its standard deviations and 95% confidence intervals will be used to estimate the response probability of the primary endpoint and evaluate its statistical inferences. When it is deemed appropriate, sample proportions and their inferences, such as standard deviations, confidence intervals, and t-tests, for the response probabilities under subgroups of patients will also be evaluated. Such subgroup analysis will be useful to evaluate the similarities and differences between different types of patients. Potential subject subgroups may be stratified by age, sex or ethnicity, among others. The proportions of adverse events, serious adverse events (SAE) and treatment related SAEs would be

estimated using the sample proportions, and 95% confidence intervals given.

The time to adverse events will be analyzed using appropriate survival analysis methods such as Kaplan-Meier estimates.

9.5 Stopping Rules for Safety

In subjects experiencing side effects from low dose danazol, the drug treatment may be discontinued. In these cases, the subject can pursue alternative approaches which will likely involve other experimental therapies (likely elsewhere) as there are no standard therapies for telomeropathies. The study will be monitored to ensure that the occurrence of a specified set of treatment related serious adverse events (TRSAEs) within 6 months of treatment in the trial does not substantially exceed an anticipated rate. The following two types of TRSAEs will be considered for early stopping of the study:

- Death considered to be probably or definitely related to danazol
- Any grade IV toxicity considered to be probably or definitely related to danazol

Each arm of the study will be monitored separately using the stopping rules as outlined below and will be seriously considered for early stopping if the number of subjects in the corresponding arm who have developed one or more of the above specified TRSAEs is over a pre-specified threshold value. TRSAEs are those attributed as definitely or probably related to the research. From our experience using this agent in higher doses, we anticipate the rate of developing at least one of the above specified TRSAEs for this subject population to be 10% or less. Following Geller et al. (2004, "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach"), our stopping rule is determined by a Bayesian approach. The stopping boundary for the study is reached if the Bayesian posterior probability that the true probability of developing one or more of the above specified TRSAEs exceeds this benchmark rate of 10% is at least 90%. We take our prior distribution to be a beta distribution with the sumof the two beta parameters to be 3, i.e. the parameters of the beta prior distribution are

0.30 and 2.70. Since we have seen in the past that the first few subjects to be accrued are possibly sicker than the rest of the subjects in the sample, we will start safety monitoring the study when 2 or more subjects have developed specified TRSAEs. The following tables summarize the threshold numbers for stopping the study:

Number of Subjects in the trial	Stop if the number of subjects who develop any of the specified TRSAEs reaches:
5	2
11	3
18	4

20	5

We conducted a simulation study to evaluate the statistical property of the stopping rule. In each simulation run, we generated a study with 20 independent Bernoulli trials, each had a probability p for having the above TRSAE and q=1-p for not having such TRSAE and compared the TRSAE outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computedthe proportion of stopped studies (i.e., "number of stopped studies"/100,000), which were stopped using the above stopping rule. The following table summarizes the proportions of stopped studies under a number of scenarios for p:

Probability of "Monitored" TRSAE= p	5%	10%	15%	20%	25%	30%
Proportion of Stopped Studies	3.55%	16.88%	36.87%	58.27%	75.85%	87.38%
Average Number of Subjects	19.53	18.04	15.85	13.36	10.95	8.93
Average Number of Subjects Suffering a specified TRSAE	0.98	1.81	2.37	2.67	2.74	2.68

These results suggest that the above stopping rules have a low probability of stopping a study when the proportion of the corresponding specified TRSAE is below the benchmark value of 10%, and the probability of stopping a study is high when the true proportion of the above-specified TRSAE exceeds this benchmark value. Based on these results, we believe that our Bayesian stopping rules have satisfactory statistical properties.

9.6 Off Study/Off Treatment Criteria

Subject choice: Subjects may withdraw from study at any time. The risks of withdrawing will be discussed, as will alternative treatment options.

Principal investigator decision: Should any of the following events occur, study drug administration will be discontinued. The subject will be followed until resolution of the event and laboratory values monitored through 6 months or until initiation of alternative therapy:

- Intractable intolerance to danazol
- Pregnancy or unwillingness to refrain from pregnancy
- Initiation of the following specific therapy: bone, lung, or liver transplant, immune suppressive therapy with anti-thymocyte globulin or alemtuzumab, treatment with eltrombopag or systemic chemotherapy.

Completion of protocol participation: After 2 years, protocol participation will be deemed complete (6 months of baseline evaluation, 12 months on danazol, 6 months aftercompleting danazol).

Lost to Follow *up:* Research team was unable to contact the subject on at least three occasions.

Non Compliance*:* If subject does not comply with medication and/or visit schedule or protocol restrictions.

Death: Death of a subject.

10 DATA AND SAFETY MONITORING

10.1 Safety Monitoring

Principal Investigator: The protocol oversight monitoring, including accrual, efficacy and safety data, will be monitored by the Principal Investigator, Bhavisha Patel M.D.

***NIH Intramural IRB*.** Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to Title 45 CFR 46.
This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual, follow-up of subjects or data analysis.

NHLBI Hematology DSMB*:* The NHLBI Hematology Data Safety and Monitoring Board will review the protocol regularly and the interval will be determined by DSMB. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

10.2 Event Characterization and Reporting

Events include adverse events (AE), serious adverse events (SAE), protocol deviations (PD), unanticipated problems (UP), and non-compliance. The principal investigator will review all events (AEs, protocol deviations, UPs, SAEs) to determine the seriousness, expectedness, and reportability of the event. As required and/or needed, the principal investigator will review the events with the Sponsor to make the final determination of seriousness and reportability.

10.2.1 Definitions

Please refer to Policy 801 for current definitions.

10.2.2 Adverse Event Management

Thromboembolism, thrombotic and thrombophlebitic events including sagittal sinus thrombosis and life-threatening or fatal strokes have been reported. Peliosis hepatis and benign hepatic adenoma have been observed with long-term use.
Danazol has been associated with several cases of benign intracranial hypertension also known as pseudotumor cerebri.

Because danazol is FDA approved with known toxicity profiles, any observed or volunteered adverse events that are as listed on the label will not be reported unless (1) the adverse event is previously unknown (not on the label); (2) the adverse event is more severe than on the label; or (3) meets the criteria for a serious adverse event. Toxicities will be monitored weekly after initiation of androgens for about 1 month. Less frequently monitoring every 2-4 weeks will be done depending on tolerability.

Adverse events to evaluate the safety of this protocol regimen will be captured during subject participation starting on the first day of initiation of the study drug.All AEs will be captured in the medical record for up to 6 months following danazol therapy or until the subjects starts a different treatment for their disease.

This will include any unlabeled unfavorable and unintended signs, symptoms or diseases which either occurs during the study, having been absent at baseline or if present at baseline appear to worsen. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and AEs will be graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be downloaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Abnormal laboratory findings used to evaluate the safety of this protocol regimen will be collected to include any unlabeled change from baseline laboratory assessments (done prior to first dose of study medication) that result ina progression to a grade 3 or 4 laboratory toxicity or are characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

The laboratory toxicities will be attributed (unrelated, unlikely, possibly, probably or definitely related) to study medication and/or disease and graded by severity utilizing CTCAE version 4.0.

10.2.3 Serious Adverse Event Management

Serious adverse events will be attributed as definitely (clearly related to the research), probably (likely related to the research), possibly (may be related to the research), unlikely (doubtfully related to the research) and unrelated (clear not related to the research).

TRSAEs are those attributed as definitely or probably. As detailed in section 9.5 stopping rules, TRSAE that will be monitored and considered for early stopping the study according to statistically determined criteria include Death and any grade IV toxicity considered to be probably or definitely related to study medication.
Hospitalizations for administrative issues (e.g., to receive a transfusion) or upgrading to ICU for routine monitoring will not be reported as an SAE.

10.2.4 Reporting of Events

Principal Investigator.
All serious adverse events will be reported to:

Bhavisha Patel, M.D., HB, NHLBI (E)
Hematology Branch (HB)
National Heart, Lung and Blood Institute (NHLBI)
Building 10, Rm 3-5132
9000 Rockville Pike
Bethesda, MD 20892

Phone: 301-402-3477
E-mail: bhavisha.patel@nih.gov

NIH Intramural IRB and NHLBI Clinical Director (CD) Reporting

Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per HRPP Policy 801 "Reporting Research Events".

Reports to the IRB at the time of Continuing Review (CR)*:*

The PI or designeewill refer to HRPP Policy 801 "Reporting Research Events" to determine IRBreporting requirements and timelines.

Reports to the CD*:*

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

NHLBI DSMB Reporting

Reports of serious adverse events that are unexpected and thought to be related (possible, probably or definitely) to the experimental drug will also be forwarded no later than seven days in the case of death or life-threatening serious adverse events or within fifteen days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). All SAEs will be included in the DSMB reports for review by the DSMB at the time of scheduled DSMB meetings (DSMB meetings held 2 times a calendar year and DSMB determines the frequency of protocol review per DSMBminutes).

10.3 Unanticipated Problems

10.3.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

10.3.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

10.4 Protocol Monitoring

As per ICH-GCP 5.18 and FDA 21 CFR 312.5 clinical protocols are required to be adequately monitored by the study sponsor.

The objectives of a monitoring visit will be:
1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject;
2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;
3) to compare abstracted information with individual subjects' records and source documents (subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and
4) to help ensure investigators are incompliance with the protocol.
The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections- OHRP) and applicable guidelines (ICH-GCP) are being followed. During the monitoringvisits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit. The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records) readily available for inspection by the local IRB, thesite monitors, and the NHLBI staff for confirmation of the study data.

11 HUMAN SUBJECT PROTECTION

11.1 Rationale for Subject Selection

The study will be open to all subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. No subjects will be excluded from participation based on sex, race or ethnicity.

Pregnant women will be excluded from participation in this study because the use of Danazol in pregnancy is contraindicated. Exposure to Danazol in utero may result inandrogenic effects on the female fetus; reports of clitoral hypertrophy, labial fusion, urogenital sinus defect, vaginal atresia, and ambiguous genitalia have been reported.

Recruitment efforts:
The study will be listed on the clinicaltrials.gov website, Clinical Center (CC) Search the Studies, the CC Recruitment website with dedicated webpage, and Craigslist. A recruitment plan will be developed by the NHLBI Patient Recruitment Office (PRO) that includes IRB-approved recruitment materials and tools, developed in partnership with CC Office of Patient Recruitment (OPR), to include a recruitment flyer, iStock photos for marketing the study, and a Public Service Announcement (PSA) including recruitment language for use with social media (Facebook and Twitter), OPR and NIH Listservs, Craigslist postings, NIH Record and CC News internal publications and/or other potentialofficial NIH external publications, as available. Informational letters to researchers and physicians internal and external to NIH will be available for electronic (and hard copy as appropriate) distribution to local and national contacts. All

options mentioned in IRB approved PSA (i.e., Craigslist, NIH publications, etc.) may be used for recruitment: Social media language may be used by the accounts of the Clinical Center, NHLBI, and any collaborating patient advocacy groups, including Aplastic Anemia and MDS Foundation, Dyskeratosis Congenita Outreach.

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation: $0. The study participants will not be reimbursed for their time and inconvenience.

Competition between Branch Protocols: There are no competing Branch protocols for this patient population.

11.2 Participation of Children

Danazol Use in The Pediatric Population

Danazol has been used, off label, in the pediatric population for more than 50 years. It is employed as long-term prophylaxis for the treatment of hereditary angioedema (HAE) and there are numerous case reports and case series of its use in pediatric patients with bone marrow failure. In a retrospective review of 8 Fanconi anemia patients treated with danazol (dosing 1.7-7.2 mg/kg/day) none of the patients developed severe or unacceptable side effects from the danazol treatment that led to the withdrawal of therapy.[25] Khincha et al. published a study on the use of androgen therapy in pediatric patients with dyskeratosis congenita. Patients were as young as 1.5 years of age.[23] Fourteen of the 16 patients were treated with oxymetholone, a more potent androgen.

The side effects reported were similar to those seen in adults, including hypercholesterolemia and transaminitis.[23] One of the potential side effects of androgentherapy on the pediatric population is its effect on linear growth. The European HAE community recommends waiting until age 12 years to start long term danazol treatment because of the insufficient clinical data regarding danazol's effect on growth.[26] The study by Khincha et al. did demonstrate growth acceleration in 6 pre-pubertal male dyskeratosis congenital patients on androgen therapy.

Congenital telomere diseases can be severe in the pediatric population. For example, Hoyeraal-Hreidarsson syndrome, considered a variant of dyskeratosis congenital, presents in early infancy with low birth weight, immunodeficiencies, cerebellar hypoplasia, developmental delay, and bone marrow failure. Currently, supportive care or bone marrow transplant in childhood is the only treatment option. However, even when the marrow failure is cured by transplant, these patients then can go on to develop pulmonary or hepatic fibrosis as young adults.

We are limiting the protocol to subjects who are age 3 years and older because our clinic does not have the expertise to care for infants. In addition, per clinical center guidelines,we are limiting participation to

those children who weigh ≥ 12 kg. An endocrinology consult will be done at baseline with follow-up to monitor growth and development of children while on androgen therapy. An endocrine consult will be done with yearly follow-up in cases where female subjects experience the endocrine effects of danazol such as severe reproductive related problems including menstrual cycle disturbances or amenorrhea.

This treatment regimen will be entirely outpatient. We do not anticipate the need to care for critically ill pediatric patients.

11.3 Risks and Discomforts

11.3.1 Related to Danazol

Androgenic effects include weight gain, acne and seborrhea. Mild hirsutism, edema, hair loss, voice change, which may take the form of hoarseness, sore throat or of instability or deepening of pitch, may occur and may persist after cessation of therapy. Hypertrophy of the clitoris is rare. Other possible endocrine effects are menstrual disturbances including spotting, alteration of the timing of the cycle and amenorrhea. Although cyclical bleeding and ovulation usually return within 60-90 days after discontinuation of therapy with danazol, persistent amenorrhea has occasionally been reported. Flushing, sweating, vaginal dryness and irritation and reduction in breast size, may reflect lowering of estrogen. Nervousness and emotional lability have been reported. In the male, a modest reduction in spermatogenesis may be evident during treatment. Abnormalities in semen volume, viscosity, sperm count, and motility may occurin patients receiving long-term therapy.

Hepatic dysfunction, as evidenced by reversible elevated serum enzymes and/or jaundice, has been reported in patients receiving a daily dosage of danazol of 400 mg or more. Serious hepatic toxicity including cholestatic jaundice, peliosis hepatis, and hepatic adenoma have been reported. Abnormalities in laboratory tests may occur during therapy with danazol including CPK, glucose tolerance, glucagon, thyroid binding globulin, sex hormone binding globulin, other plasma proteins, lipids and lipoproteins.

The following reactions have been reported, for which a causal relationship to the administration of danazol has neither been confirmed nor refuted allergic: urticaria, pruritus and rarely, nasal congestion; CNS effects: headache, nervousness and emotional lability, dizziness and fainting, depression, fatigue, sleep disorders, tremor, paresthesias, weakness, visual disturbances, and rarely, benign intracranial hypertension, anxiety, changes in appetite, chills, and rarely convulsions, Guillain-Barre syndrome; gastrointestinal: gastroenteritis, nausea, vomiting, constipation, and rarely, pancreatitis and splenic peliosis; musculoskeletal: muscle cramps or spasms, or pains, joint pain, joint lockup, joint swelling, pain in back, neck, or extremities, and rarely, carpal tunnel syndrome which may be secondary to fluid retention; genitourinary: hematuria, prolonged post-therapy amenorrhea; hematologic: an increase in red cell and platelet count. Skin: rashes (maculopapular, vesicular, papular, purpuric, petechial), and rarely, sun sensitivity, Stevens-Johnson syndrome and erythema multiforme; other: increased insulin requirements in diabetic patients, change in libido, myocardial infarction, palpitation, tachycardia, elevation in blood pressure, interstitial pneumonitis, and rarely, cataracts, bleeding gums, fever, pelvic pain, nipple discharge. Malignant liver tumors have been reported in rare instances, after long-term use.

11.3.2 Related to Bone Marrow Aspirate and Biopsy

No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

11.3.3 Related to Phlebotomy

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

11.3.4 Related to Bone X-Ray and Chest CT
The amount of radiation that pediatric subjects will receive in this study from a bone X-ray and Chest CT is 0.48 rem.

11.3.5 Related to Liver Biopsy
The major side effects of liver biopsy are pain, bacteremia, puncture of another organ and bleeding. Local pain and discomfort at the liver biopsy site occurs in about 20% of persons undergoing liver biopsy. This is transient (lasting one to twelve hours) and is usually mild, rarely requiring analgesics. Bacteremia occurs in 1-2% of persons undergoing liver biopsy. In the absence of bile duct obstruction, this is almost always self-limited and is rarely symptomatic.

Significant bleeding after liver biopsy is the most serious side effect of this procedure. In the absence of a blood coagulation defect or hepatic malignancy, significant bleeding is rare, occurring in less than one in a thousand cases of liverbiopsy. Death due to bleeding after liver biopsy has been reported in less than 1/10,000 cases.

11.3.6 Related to Genetic Testing
There are risks associated with information that can be obtained from doing genetic testing. Patient privacy and confidentiality will be protected. With regard to genetic testing and patient safety and protection, we will take the following precautions: First, all information collected or discovered will be confidential; blood or bone marrow samples will be coded to ensure patient confidentiality and identifier data will be kept locked by the research team; and no genetic information with patient identifiers will be placed in the medical chart or in public record. Second, data obtained will be used only for research relevant to the study of bone marrow failure syndromes.

11.4 Risks in Relation to Benefit

For Adult Subjects:

The benefits to the subjects could be reduction or even abolition of transfusion requirements and/or stabilization or improvement of cytopenias, pulmonary fibrosis, or liver fibrosis resulting in improved quality of life and decreased morbidity and mortality. Potentially, treatment with other more toxic therapies, to include bone marrowor lung transplant, could also be avoided or postponed.

For Pediatric Subjects:

Pediatric subjects are included because:

(a) *the risk is justified by the anticipated benefit to the subjects*: We are offering pediatric subjects an alternative to symptomatic therapy.

(b) *the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches*. The benefits to the patients could be reduction or even abolition of transfusion requirements and/or stabilization or improvement of cytopenias, pulmonary or liver fibrosis resulting in improved quality of

life and also decreased morbidity and mortality. Potentially, treatment with other more toxic therapies, to include bone marrow or lung transplant, could also be avoided or postponed.

There may be difficulties with regards to children complying with taking pills daily. In such cases, the research team will ensure that parents or caregivers are given clear instructions about drug accountability procedures (recording dates of missed dosing) as well as the importance of not doubling-up the dose to make up for the missed dose.

Follow-up phone calls will be done at regular intervals for identified potential non- compliant subjects and protocol dosing violations will be tracked and evaluated by the PI.

For Cognitive Impaired Subjects:

The benefits to the patients could be reduction or even abolition of transfusion requirements and/or stabilization or improvement of cytopenias, pulmonary or liver fibrosis resulting in improved quality of life and also decreased morbidity and mortality. Potentially, treatment with other more toxic therapies, to include bone marrow or lung transplant, could also be avoided or postponed.

11.5 Informed Consent Processes and Procedures

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.

An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting. The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic, both the investigator and the participant will sign the document with a

hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic).

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed consent document.

Following the enrolment of each subject, the research coordinator will be informed of the enrolment. The research coordinator will conduct a Quality Control (QC) review of the consent process and documentation periodically to confirm compliance".

Procedures to Obtain Assent and Documentation of Assent or Dissent:

Where deemed appropriate by the clinician and the parents or guardian, the child will also be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts, and benefits of participation and a minor assent will be obtained.
If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

Written assent will be obtained as appropriate from children ages 7 and older. The consent/assent process will be documented in the child's medical record, including the assessment of the child's abilityto provide assent as applicable. Children between the ages of 13 and above will provide written assent by signing either the assent form or the assent line of the consent form depending on their level of comprehension.
In cases where parents share joint legal custody in making medical decisions of their child (e.g., by a custody agreement or court order) both parents must give their parental permission regardless of the level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent, or is not reasonably available (e.g., in prison).

We will inform potential adult and minor female participants during the consent and/or assent process that for safety, we need to do a pregnancy test. She will also be told that if it is positive, we will counsel her. If the patient does not want to proceed, she will beadvised not to sign the assent and her enrollment on this protocol will end.

If at any time during participation in the protocol, new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to each enrolled or prospective patient. Documentation will be provided to theIRB and if necessary, the informed consent amended to reflect relevant information.

Consent for Minors when They Reach the Age of Majority: When a pediatric subject reaches age 18, continued participation will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis

continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit.

If reconsent is not feasible, we request a waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority

Requirements for Waiver of Consent consistent with 45 CFR 46.116(f)(3):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.
    a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.
(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
(5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

Informed consent for adult research participants unable to provide consent:

If there is an unexpected enrollment of a research participant unable to provide informed consent, the PI will ensure that study investigators will identify an appropriate LAR consistent with requirements of Policy 403 and will obtain consent from the LAR as outlined in the consent process in Section 11.5 before initiating research interventions.

Consent and Assent:

Procedures to Determine Capacity: If documentation of decision-making capacity is not present in the medical record or the investigator questions the decision-making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429) will be contacted to make the determination.

11.6 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the in a password protected database on the NIH secure network drive. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

11.7 Conflict of Interest

The Principal Investigator assures that each associate investigator on the protocol received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation will be provided a copy of the document when they are added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director.

This protocol has no associated patents, CRADAs or CTAs.

12 PHARMACEUTICALS

12.1 Danazol

Generic name: danazol

Brand Name: Danocrine

Supply: Commercially available.

Pharmacology: Danazol is a derivative of the synthetic steroid ethisterone and is a weak androgen. It suppresses the pituitary-ovarian axis and is approved for the treatment of endometriosis and fibrocystic breast disease.

Product description: Danazol is available in 50, 100, and 200 mg tablets.

Storage and Stability: Controlled room temperature, 15° C to 30° C (59° F to 86° F)

Route of administration: Oral

Administration: Danazol may be taken with or without food. Danazol is metabolized by the liver and has two major metabolites: ethisterone and 17-hydroxymethylethisterone. Danazol is excreted by the kidney.

12.2 FDA Regulatory Considerations

An IND application with the FDA is not required for this project. The proposed research with danazol meets the exemption requirements noted in 21 CRF 312.2, specifically:

1. The drug product is lawfully marketed in the United States.
2. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
3. In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
4. The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).
5. The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).
6. The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

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APPENDIX A. Adverse Events Related to Danazol (800 mg/day) Administration. (Townsley et al. NEJM 2016)

	<Grade 3	≥Grade 3
Alanine aminotransferase increase	11 (41%)	
Muscle spasm or cramps	9 (33%)	
Aspartate aminotransferase increase	8 (30%)	
Edema	7 (26%)	
High cholesterol	7 (26%)	
Headaches	4 (15%)	
Weight gain	4 (15%)	
Arthralgia	3 (11%)	
Hair thinning	3 (11%)	
Dizziness	2 (7%)	
Hirsutism	2 (7%)	
Nasal congestion	2 (7%)	
Rash	2 (7%)	
Testicular atrophy	5 (19%)	
Alkaline phosphatase increase	1 (4%)	
Acne	1 (4%)	
Agitation*	1 (4%)	
Cognitive disturbance	1 (4%)	
Depression	1 (4%)	
Nausea	1 (4%)	
Reduced appetite	1 (4%)	
Vomiting	1 (4%)	
Voice alteration	1 (4%)	
Hot flashes	1 (4%)	
Thromboembolic cerebrovascular accident*		1 (4%)
Hemangioma*		1 (4%)

Suspected treatment-related adverse events in all 27 patients. Alterations in serum lipid profiles occurred in all patients, namely increase in serum low-density lipoprotein and decrease in high-density lipoprotein, and changes met criteria for an adverse event in seven cases (grade 1 or 2 hypercholesterolemia). Testicular size decreased in allmale subjects at 14 months (n=5). Bone densitometry and uterine/ovarian ultrasounds were unchanged while on danazol. Three patients chose to discontinue drug due to adverse events.

*Severe Adverse Event that prompted discontinuation of danazol by investigator due to possible relationship to danazol. In one subject, UPN#8, with preexisting cardiovascular risk factors a thromboembolic cerebrovascular accident causing dysphasia and extremity weakness prompted discontinuation of the drug; the subject had a prolonged hospital course but fully recovered. A hemangioma was diagnosed in another subject, UPN#6, who developed swelling and pain in the lower extremity while on danazol; symptoms resolved with discontinuation of danazol, but the hemangioma persisted on repeat imaging.

[+]Resolved with dose-reduction of danazol.

APPENDIX B. Estimated Telomere Attrition Rates in Healthy and Telomeropathy Patients.

Institution	Population	N	Method	Study design	Attrition rate, Bp loss / year (error)
NHLBI	Healthy	175	qPCR	Cross-Sectional	60 (95% CI, 45-71)
NHLBI	Healthy	298	qPCR	Cross-Sectional	54 (95% CI, 47-60)
Univ British Columbia	Healthy	400	Flow-FISH	Cross-Sectional	51 (NR)
Univ of Sao Paulo	Healthy	261	qPCR	Cross-Sectional	40 (95% CI, 48-33)
Univ of Sao Paulo	Healthy	180	Flow-FISH	Cross-Sectional	47 (95% CI, 53-41)
NCI	DKC	9	Flow-FISH	Longitudinal	170 (±100 SD)
NHLBI	Telomere disease	25	qPCR	Longitudinal	147 (±281 SD)

Townsley et al. NEJM 2016

APPENDIX C: Schedule of Events (SOE)

Activity	Screening	At Randomization	Every 1-4 weeks after starting	Every 3 months (12 weeks) after starting danazol, more frequent prn	After 6 months on danazol	Every 1-4 weeks after switching doses	Every 3 months after switching doses, more frequent prn	After 12 months on danazol	Follow-up 6 months off danazol
			Obtained by home provider			Obtained by home provider			
Informed Consent	x								
Medical History	x	x			x			x	x
Physical Exam	x	x			x			x	x
Telomere Length	x	x			x			x	x
Genetic Testing	x								
Endocrine Consult	Pediatric only	x			x			x	x
Hepatology Consult	x	x			x			x	x
6 Minute Walk Test & PFT	x	x			x			x	x
Pro BNP	x				x			x	x
Ultrasound of Abdomen with Doppler Flow	x	x			x			x	x
Liver Elastography	x	x			x			x	x
Mineral Panel	x				x			x	x
Acute Care Panel	x		x		x	x		x	x
Hepatic Panel	x		x		x	x		x	x
GGT	x				x			x	x
CBC with Diff	x	x		x	x		x	x	x
Reticulocyte Count	x	x			x			x	x
Total Protein	x				x			x	x

Activity	Screening	At Randomization	Every 1-4 weeks after starting	Every 3 months (12 weeks) after starting danazol, more frequent prn	After 6 months on danazol	Every 1-4 weeks after switching doses	Every 3 months after switching doses, more frequent prn	After 12 months on danazol	Follow-up 6 months off danazol
			Obtained by home provider			Obtained by home provider			
CK, LDH, Uric Acid	x				x			x	x
Fasting Lipid panel, Apolipoprotein Panel,Lipoprotein Particle	x				x			x	x
Sex Hormone Binding Globulin, LH, FSH, DHEA, Estrone, Free & Total Testosterone,Dihydrotestosterone	x				x			x	x
Thyroid Binding Globulin, FT4, TSH	x				x			x	x
Fasting Insulin, Hgb A1C	x				x			x	x
Anti-LKM. A1AT, AFP, ANA, AMA, RF	x								
Ceruloplasm & Cryoblobulins	x								
Serum alpha-2-antiplasmin	x								
HBsAg, anti HBc, anti HBs, anti- HCV, and HAV	x								
Anti-HIV 1/2 Antibody	x								
Female patients									
Urine Pregnancy Test	x				x			x	x
Ferriman Gallwey Score	x				x			x	x
Menstrual History	x				x			x	x
Male Patients									
Testicular Size Measurement	X[1]				X[1]			X[1]	X[1]
Pediatrics patients									
Height and Weight	x	x			x			x	x
Pubertal Staging	x	x			x			x	x

Activity	Screening	At Randomization	Every 1-4 weeks after starting	Every 3 months (12 weeks) after starting danazol, more frequent prn	After 6 months on danazol	Every 1-4 weeks after switching doses	Every 3 months after switching doses, more frequent prn	After 12 months on danazol	Follow-up 6 months off danazol
			Obtained by home provider			Obtained by home provider			
Bone Age X Rays	x	x			x			x	x
Only if indicated:									
Pulmonology Consult	x	x			x			x	x
Echocardiogram with Bubble Study	x	x			x			x	x
Bone Marrow Biopsy & PB Flow for GP Protein	x	x			x			x	x
Liver Biopsy and Portal Pressure Measurements	x	x			x			x	x
Chest CT	x	x			x			x	x

[1] May be omitted in the case of telehealth or telephone visits.