PROTOCOL NUMBER:

TITLE: Danazol for Treatment of Cytopenias in Patients with Cirrhosis

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STUDY ARMS: Single Arm Pilot Study

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1.0 Background and Hypothesis

1.1 Cirrhosis and Cytopenias

Most studies estimate that between 6 and 77% of all patients with cirrhosis have abnormal hematologic indices (AHI), including anemia, thrombocytopenia and leukopenia. In a homogenous population of patients with compensated Child-Pugh Class (CPC) A/B cirrhosis, as many as 84% have AHI.[18,19] The presence of AHI contributes to increased morbidity and mortality in a large proportion of cirrhotic patients. For example, thrombocytopenia can be a limiting factor when considering invasive surgical procedures due to the increased risk for bleeding. Leukopenia increases the risk for infections and chronic anemia contributes to worse outcomes after hemorrhagic episodes.[18] Thrombocytopenia and leukopenia have been shown to be associated with death, transplant, clinical decompensation and hepatocellular carcinoma (HCC).[19]

The pathogenesis of AHI in patients with cirrhosis is often multifactorial, with splenic sequestration, portal hypertension, bone marrow suppression, and changes in hematopoietic stimulating factors contributing to the etiology.[18] The severity of cytopenias does not consistently correlate with the degree of cirrhosis and may not correct after liver transplant. Current therapies have variable efficacy in improving cytopenias and management focuses primarily on supportive care with transfusions and growth factors.

1.2 Cirrhosis and Telomeres

Telomeres are repetitive DNA sequences located at the natural ends of linear chromosomes. They function to cap and protect chromosome ends from being recognized as damaged or infected DNA.[3] During cell division, the "end-replication problem" arises as telomeres continually shorten because DNA polymerase cannot fully replicate the 3' end of chromosomes. The telomerase complex counters telomere attrition by elongating the telomere DNA after each cell division. Germline genetic defects in telomere maintenance and repair can cause dyskeratosis congenita, bone marrow failure, liver cirrhosis, pulmonary fibrosis, as well as increased susceptibility to various cancers. [4-5, 24-25]

As a major complication of liver disease, cirrhosis is the main risk factor for progressive liver failure and HCC. To better understand the pathogenesis of cirrhosis, the connection between telomere attrition and cirrhosis has been examined in preclinical studies. Rudolph et al. found that telomerase reverse transcriptase (TERT)-deficient mice displayed reduced liver regeneration after partial hepatectomy and increased hepatic fibrosis after carbon tetrachloride exposure. After restoration of telomerase activity, there was improved liver function and cirrhosis reduction.[21] Similarly, studies in humans found significantly accelerated telomere shortening and more telomere mutations in livers with cirrhosis and chronic hepatitis compared to normal livers.[15] Patients with dyskeratosis congenita, pulmonary fibrosis, aplastic anemia, and short telomeres also showed an increased frequency of liver fibrosis and cirrhosis.

In an analysis of the Surveillance, Epidemiology and End Results (SEER)-Medicare database from 1992 through 2009, the development of liver disease was compared between 82,938 men with prostate cancer who did and did not undergo androgen deprivation therapy (ADT).[12] Exposure to ADT was significantly associated with an increased subsequent risk of non-alcoholic fatty liver disease (54%), cirrhosis (35%) and

any liver disease 47%). These data support a relationship between androgens and liver health, the mechanism of which is likely multifactorial.

1.3 Summary of Danazol Efficacy Data

Androgens have historically been used to treat bone marrow failure syndromes, including aplastic anemia and myelodysplastic syndrome.[8, 17, 14, 22] Evidence in both tissue culture and animal models suggest sex hormones play a role in regulating telomerase. *Calado et al.* showed that the addition of sex hormones resulted in the up regulation of TERT gene expression and telomerase enzymatic activity in hematopoietic stem cells *in vitro*.[6] In a mouse model of telomere dysfunction, male hormone therapy also resulted in telomere elongation and hematological improvement.[2] Danazol is a derivative of the synthetic steroid ethisterone, which is a modified testosterone. Although the drug's mechanism of action is unclear, it is speculated that danazol is potentially able to modify antiplatelet levels (in immune-mediated thrombocytopenia), inhibit the mononuclear phagocyte system, and increase telomerase activity.

In 2016, *Townsley et al.* conducted a phase I/II prospective study in which 27 patients with congenital telomere disorders and bone marrow failure were treated with danazol 800 milligrams daily for a total of 24 months.[23] The primary efficacy endpoint was a 20% reduction in the annual rate of telomere attrition. The primary endpoint was achieved in 92% of patients and 83% had a hematologic response (HR) at 24 months. Of four patients with cirrhosis liver disease who had baseline Fibroscan data available for comparison, three had significant alleviation of liver fibrosis at 24 months. The majority of patients in this study had *TERT* or *TERC* gene mutations and one patient had a *RTEL1* mutation. While 79% of patients were noted to have HR as early as 3 months, the *RTEL1* patient did not achieve HR until 24 months.

Danazol has been evaluated for its effect on cytopenias in patients with cirrhosis. In a study of 49 patients with chronic hepatitis C virus (HCV), 90% of whom had cirrhosis, danazol was administered to mitigate thrombocytopenia in patients undergoing HCV treatment with peginterferon (PEG-INF) and ribavirin.[1] Danazol was used concurrently with HCV therapy over a duration of 2 to 11 months. Patients received danazol with doses ranging from 300 to 600 mg daily, which was titrated based on platelet counts. Patients showed a tendency for an average platelet loss of 5,217/mm^3/month prior to the study which trend reversed upon treatment with danazol. Platelet counts increased to above 100,000/mm^3 in 10.6 % of patients, by 10,000-100,000 in 53% of patients and were maintained at baseline in 20.4% of patients. Fourteen percent (14.2%) of patients were considered unresponsive after 11 months of treatment. While platelet response was delayed in patients without sustained virological response (SVR), their platelet levels were similar to those with SVR at the end of the study period.

1.4 Summary of Danazol Safety Data

In the study by *Townsley et al*, which included nine patients with cirrhosis (6 overt and 3 subclinical), the most common side effects were elevation in liver-enzyme levels (41% of patients), muscle cramps (33%), edema (26%), and lipid abnormalities (26%). Two patients (4%) discontinued danazol due to grade 3/4 adverse events (AE). One patient with preexisting cardiovascular risk factors had a thromboembolic cerebrovascular accident prompting discontinuation of the drug. One patient developed a hemangioma in

the lower extremity during danazol treatment causing pain and swelling. Symptoms resolved with discontinuation of danazol although the hemangioma persisted on imaging.

In the study by *Alvarez et al*, all patients had at least one AE. Five patients withdrew prematurely from therapy: 3 discontinued due to intolerance of Peg-INF and ribavirin, 1 required splenectomy, and 1 died (cause of death not stated). Most treatment-related AEs were mild to moderate and consistent with AEs associated with PEG-INF and ribavirin treatment. The most common AEs included anemia (40%), headache (38%), arthralgia (31%), myalgia (31%) and malaise (29%). No patients discontinued therapy due to AEs. There were no reports of liver toxicity or withdrawal due to decompensation of liver disease.

The most frequent adverse events seen in patients treated with danazol for other conditions include modest elevation in liver enzymes and weight gain. One study compared side effects of danazol in treated and untreated hereditary angioedema patients.[11] The most common AEs included menstrual abnormalities, weight gain, arterial hypertension and acne. In the setting of myelofibrosis, WHO grade 1-2 transaminitis occurred in 8/50 (16%) patients during the first two months of therapy and improved following reduction of danazol from 600mg to 400mg daily.[9] Two additional patients developed cholestasis that resolved after discontinuation of danazol and 4 patients stopped therapy, 1 developed prostate adenocarcinoma, 1 developed liver peliosis, 1 died due to splenic rupture (a known complication of myelofibrosis), and 1 withdrew despite anemia response.

There have been case reports of HCC occurring in patients with and without cirrhosis who are treated with danazol although this is rare. In one survey study of physicians treating patients with hereditary angioedema, 1/886 patients (0.1%) developed HCC while on danazol.[22]

1.5 Study Rationale

In our previous study, approximately 20% of patients with cirrhosis presenting for evaluation for liver transplantation were found to have a telomere gene variant. Patients with these gene variants tended to have lower blood cell counts and longer post-transplant hospital stays compared to patients without mutations. It is possible that cirrhotic patients with low blood cell counts may have bone marrow failure related to telomere dysfunction as fibrosis and bone marrow failure are both found in patients with other inherited telomeropathies. Given that danazol has been effective in decreasing telomere attrition and improving blood cell counts in patients with inherited telomere disorders, danazol may have similar benefit in cirrhotic patients found to have telomere mutations or accelerated telomere shortening. There is a suggestion, from one small study, that danazol may also improve liver fibrosis. As leukopenia and thrombocytopenia are associated with increased morbidity and mortality in cirrhotic patients, treatment of these cytopenias may improve clinical outcomes.

1.6 Hypothesis

We hypothesize that danazol treatment may improve cytopenias in patients with compensated Child-Pugh Class (CPC) A/B cirrhosis. We also hypothesize that danazol may reduce progression or decompensation of cirrhosis among patients with telomere dysfunction.

2.0 Objectives

2.1 Primary Objectives

- Determine the prevalence of telomere gene mutations and/or shortened telomere lengths in patients with compensated CPC A/B cirrhosis and leukopenia and/or thrombocytopenia.
- Evaluate the safety of danazol in patients with compensated CPC A/B cirrhosis and leukopenia and/or thrombocytopenia, with or without telomere gene mutations and/or shortened telomere lengths.
- Evaluate the efficacy of danazol in improving cytopenias in patients with compensated CPC A/B cirrhosis, with or without telomere gene mutations and/or shortened telomere lengths.

2.2 Secondary Objectives

- Measure the change in peripheral blood telomere lengths after treatment with danazol.
- Measure the change in liver fibrosis and liver function parameters after treatment with danazol.
- Evaluate the outcomes of decompensation, death and transplant in patients treated with danazol.

3.0 Study Design

This is a phase II pilot study designed to assess the safety and efficacy of danazol for treatment of cytopenias in patients with CPC A/B cirrhosis. Subjects with telomere mutations and/or shortened telomeres will be treated with danazol 600 mg per day by mouth for a duration of 24 months. The goal will be to treat a total of 10 patients with telomere gene mutations and/or shortened telomere lengths.

4.0 Drug Information

Danazol is a synthetic steroid derived from ethisterone, which is a modified testosterone that has antigonadotropic and anti-estrogenic activities. Danazol suppresses the pituitary-ovarian axis by inhibiting the pituitary output of gonadotropins. This suppression is thought to be through a combination of depressed hypothalamic-pituitary response to lowered estrogen production, the alteration of sex steroid metabolism, and interaction of danazol with sex hormone receptors. The only other demonstrable hormonal effect is weak androgenic activity. The pituitary-suppressive action of danazol is reversible. Danazol has been approved in treating endometriosis, fibrocystic breast disease, hereditary angioedema, thrombocytopenic purpura, and other conditions.

Danazol is lipophilic and can partition into cell membranes, indicating the likelihood of distribution into deep tissue compartments. Danazol appears to be metabolized and eliminated by renal and fecal pathways. The reported elimination half-life of danazol is variable across studies. The mean half-life of danazol in healthy males is 9.7 hours. After 6 months of 200 mg three times a day dosing in endometriosis patients, the half-life of danazol was reported as 23.7 hours.

Adverse reactions from danazol include androgen like effects (i.e. weight gain, acne, mild hirsutism, edema, hair loss, voice change) and menstrual disturbances. The use of danazol in

pregnancy is contraindicated. Other common side effects also include elevations in liver-enzyme levels and lipid abnormalities.

5.0 Eligibility Assessment

5.1 Patient Eligibility

Eligibility waivers are not permitted. Subjects must meet all of the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

Accrual time frame will be 18 months.

5.2 Inclusion Criteria

- Age 18 years or older and able to provide informed consent
- ECOG 0-2
- Compensated Child-Pugh class A of any etiology with the exception of chronic hepatitis B with one or more of the following cytopenias
    1. Leukopenia defined as white blood cell count $\leq$2000/mm^3 or absolute neutrophil count $\leq$1000/mm3 along with thrombocytopenia <150,000/mm^3 measured on two separate occasions at least 3 months apart within 6 months of enrollment
    2. Thrombocytopenia defined as platelet count <50,000/mm^3 along with white blood cell count $\leq$4000/mm^3 measured on two separate occasions at least 3 months apart within 6 months of enrollment

- Compensated Child-Pugh class B cirrhosis of any etiology with the exception of chronic hepatitis B with one or more of the following cytopenias:
    1. Leukopenia defined as white blood cell count $\leq$ 3500/mm^3 measured on two separate occasions at least 3 months apart within 6 months of enrollment
    3. Thrombocytopenia defined as platelet count $\leq$ 100,000/mm^3 measured on two separate occasions at least 3 months apart within 6 months of enrollment

- Enrolled patients must have one or more of the following:
    - Presence of a genetic variant (defined as a known mutation, variant likely to be pathogenic or variant of undetermined significance with likely deleterious effect on transcription or translation) in at least one of the following genes: *TERT, TERC, RTEL1, DKC, NOP10, NHP2, TINF2*, *WRAP53*
    - Shortened telomere length in peripheral blood mononuclear cells (defined as age-adjusted telomere length at or below the 5th percentile)
    - Of note, patient's found to have telomere mutations know to confer a gain of function will be excluded

- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year during the treatment period

    - A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (>= 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus)
    - Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices
    - The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient; periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception
- Women of childbearing potential (WOCBP) must have a negative serum test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]) within 72 hours prior to the start of treatment

5.3 **Exclusion Criteria**

- Cirrhosis secondary to chronic hepatitis B or any history of hepatitis B
- Patients with telomere related mutations know to confer gain of function will be excluded
- Patients known to be infected with HIV
- History of any hormone sensitive malignancy, including breast cancer, prostate cancer, hepatocellular carcinoma or liver adenoma as well as any patient considered high risk for developing malignancy (i.e. history of familial cancers including a first degree relative)
- Patients who are actively receiving anti-cancer therapy
- Liver decompensation event within the last 6 months (i.e. variceal bleed, ascites requiring paracentesis, hepatic encephalopathy)
- Active thrombosis or history of unprovoked thromboembolic disease, including cardiovascular events. If a patient has received and completed adequate anticoagulation for a provoked thrombosis, they can be included in the study.
- Pregnant or planning to become pregnant
- Females patients who are breast feeding
- Any contraindication to danazol use
- Uncontrolled co-morbid condition which would make the administration of danazol unsafe, including decompensated heart failure or known EF less than 40%, unstable angina pectoris, uncontrolled cardiac arrhythmia, decompensated liver failure, renal failure defined as creatinine greater than >1.6 or psychiatric illness that would limit compliance with study requirements
- Alanine aminotransferase and/or aspartate aminotransferase >3x upper limit of normal
- Alkaline phosphatase >2.5 x upper limit of normal
- Total bilirubin or direct bilirubin >2.5 x upper limit of normal
- Patients with known alcohol or drug abuse within the last year
- Concomitant use of hormone stimulants or hormone blocking agents.
- Concomitant use of other bone marrow stimulating agents that may affect white blood cell and platelet counts (i.e. G-CSF, romiplostim, eltrombopag, corticosteroids). Short term use of growth factors per standard of care in preparation for procedure or for other medical indications is acceptable. Patients taking corticosteroids above 5 mg of

prednisone or the equivalent who are on a stable dose for at least 8 weeks prior to enrollment can be included.

- Concomitant treatment with systemic immunosuppressive medications (including but not limited to cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [anti-TNF] agents)

5.4 **Withdrawal Criteria**

Patients will be removed from therapy when any of the criteria listed below apply. Notify the Principal Investigator, and document the reason for study removal and the date the patient was removed from treatment in the Case Report Form. The patient should be followed-up per protocol.

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation of treatment will be documented and may include:

- Any decompensation event (i.e variceal bleed, ascites requiring paracentesis, hepatic encephalopathy)
- Any grade 4 toxicity that is considered likely related to study drug
- Development of liver enzyme or bilirubin elevation to grade 3 that does not resolve to baseline grade with cessation or reduction of study drug
- Any new diagnosis of malignancy
- Any new thrombotic event
- Patient becomes pregnant
- Worsening of cardiac, hepatic or renal function from baseline defined as, known EF less than 40% or creatinine greater than >1.6
- Patient withdraws consent (follow up)
- Patient is unable to comply with protocol requirements
- Treating physician determines continuation on the study would not be in the patient's best interest
- Lost to follow-up: If a research subject cannot be located to document survival after a period of 2 years, the subject may be considered "lost to follow-up." All attempts to contact the subject during the two years must be documented in CRF's.

5.5 **Replacement Criteria**

- Any patient who discontinues danazol therapy prior to receiving at least 3 months duration
- Any patient who cannot tolerate danazol at a minimum dose of 200 mg/day

6.0 **Study Agent Administration**

6.1 **Treatment Dosages and Administration**

AGENT	DOSE	ROUTE	FREQUENCY	DURATION
Danazol	600 mg	Oral	Daily	24 months

Danazol will be administered orally in a dose of 600 milligrams daily. Danazol treatment will be for a total duration of 24 months. The treatment duration is chosen based on the same study showing that patients with *RTEL1* mutations, which we found to have a high prevalence in cirrhotic patients, may require as long as 24 months to demonstrate a hematologic response to danazol.[23]

Danazol will be discontinued for treatment-related serious adverse events or if the patient decides to withdraw from the study. As response will be assessed at the conclusion of the study, response will not be used as a criterion to determine if danazol should be held during the study period.

6.2 Monitoring

Patients will have routine blood draws every 2 weeks for the first month, then every 4 weeks for the following 3 months, then every 3 months thereafter for toxicity monitoring including assessment of renal and liver function.

Patients will be asked about symptoms at each study visit with particular attention to symptoms known to be associated with androgen therapy including but not limited to fatigue, arthralgias, acne, weight gain, hirsutism and mood changes.

Physical exam will be performed at each 3-month study visit to assess for signs of liver decompensation (i.e. ascites, edema, encephalopathy) or treatment-related effects.

During the study period, patients will continue to follow with their primary care physician for standard of care evaluations typical for patients with cirrhosis and low blood cell counts. This should include abdominal ultrasound every 6 months or per local standard of care (no less frequently than yearly) for HCC surveillance.

After the study is completed, patients will continued to be followed for an additional 24 months with abdominal ultrasounds as a part of the standard of care surveillance by chart review and phone calls made to the patient.

Safety will be evaluated in this study through the monitoring of all serious and non-serious AEs, defined and graded according to NCI CTCAE v4.0. Patients will be assessed for safety (including laboratory values) according to the schedule in *Appendix I*.SEP General safety assessments will include serial interval histories, physical examinations, and specific laboratory studies, including serum chemistries and blood counts (see study calendar for the list and timing of study assessments). All serious adverse events (SAEs) will be reported in an expedited fashion. In addition, the investigators will review and evaluate observed AEs on a regular basis. Patients who have an ongoing study treatment−related AE upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anticancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or until it has been determined that study treatment or participation is not the cause of the AE.

Some people may find it upsetting to learn that they have certain mutations or errors in genes that could lead to future health problems for themselves or their children. A referral to a genetic counselor will be made available to the participant for the purpose of answering questions about the implications of the genetic testing results. If a genetic counselor is not available, hematology is accustomed to discussing the implications of testing results.

6.3 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Study Calendar (Appendix 2). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity (Appendix 1).

6.4 Concomitant Medications/Treatments

Concomitant therapy includes any prescription medications or over-the-counter preparations used by a patient between the 7 days preceding the screening evaluation and the treatment discontinuation visit. These are recorded during each study visit as dictated by study calendar (Section 7.0). Males and females of reproductive potential should use highly effective means of contraception.

It is strongly recommended that: Traditional or herbal medicines not be administered because the ingredients of many herbal medicines are not fully studied and their use may result in unanticipated drug-drug interactions that may cause, or confound assessment of, toxicity.

6.5 General Plan to Manage Safety Concerns

Measures will be taken to ensure the safety of patients participating in this trial, including the use of stringent inclusion and exclusion criteria (see *Section 5.0*) and close monitoring (as indicated above). See *Section 8.4* for complete details regarding safety reporting for this study.

7.0 Study Assessments and Calendar

7.1 Response Criteria

Hematologic Response (HR) defined as:

- Normalization of WBC to ≥ 4000/μL or doubling of WBC from baseline AND
- Normalization of platelet count to ≥150,000/μL or doubling of platelet count from baseline

Patients dependent on exogenous hematopoietic growth factors or requiring transfusions will not be considered as fulfilling response criteria.

7.2 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining written informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained. All screening procedures must be performed within 14 days prior to registration unless otherwise stated.

7.3 Medical History

Complete medical and surgical history, history of infections, prior cancer history and treatment, transfusion requirements will be performed.

Medical history includes clinically significant diseases within the previous 5 years, smoking history, cancer history (including tumor characteristics such as hormone receptor status), prior cancer therapies and procedures, and all medications used by the patient within 7 days before the screening visit (including prescription, over-the-counter, and herbal/homeopathic remedies and therapies).

7.4 Physical Examination

A complete physical examination will be performed at screening and at the treatment discontinuation visit and should include the evaluation of head, eye, ear, nose, and throat and cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, and neurologic systems.

A limited physical examination will be performed at other visits to assess changes from baseline abnormalities and any new abnormalities and to evaluate patient-reported symptoms. New or worsened abnormalities should be recorded as AEs if appropriate.

7.5 Telomere gene mutation analysis

All eligible patients who have signed informed consent will be screened at baseline for mutations in known telomere genes using a CLIA-certified panel, which includes the following genes: *TERT, TERC, RTEL1, DKC, NOP10, NHP2, TINF2*, and *WRAP53*. Three milliliters of whole blood from each patient will be sent to Fulgent Diagnostics Laboratory (Temple City, CA). Each sample will be tested for the presence of telomere complex variants. Genomic DNA is first isolated from peripheral blood mononuclear cells, then enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries are then sequenced using next-generation sequencing technology. Following alignment, variants are detected in regions of at least 10x coverage.

7.6 Telomere length measurement

Telomere lengths of peripheral blood mononuclear cells will be measured at baseline and 24 months using a CLIA-certified test performed by Repeat Dx.

7.7 Blood draws

Blood draws will be performed at baseline and every 2 weeks for the first 4 weeks, then every 4 weeks for 3 months, then every 3 months thereafter as part of the standard of care for monitoring patients with low blood cell counts.

7.8 Transient Elastography

FibroScan is a non-invasive tool to measure the fibrosis of the liver. FibroScan or transient elastography creates vibration of the skin that passes through the liver. The tool uses ultrasound to track the vibration and measure the stiffness of the liver which is then reported. The method can indirectly measure the degree of fibrosis in the liver tissue

without means of an invasive procedure like liver biopsy. The entire procedure is performed through a hand-held probe that is placed on the upper right lateral wall of the abdomen. On average, the procedure takes 5 minutes. The technique has no risks associated with the procedure.

This will be performed at baseline and 24 months by Dr. Brian Kim at Keck Hospital of the University of Southern California. Fibroscan is not necessarily required as part of standard of care work up for patients with cirrhosis and cytopenias, but it will be a more sensitive test to measure changes in degree of fibrosis for the purposes of this study.

7.9 Bone Marrow Biopsy

A bone marrow biopsy will be performed at baseline (per standard of care for evaluation of unexplained low blood cell counts). If indicated to reassess cytopenias, a bone marrow biopsy will be performed again at 24 months. Additional biopsy will be done only if there are changes in clinical condition and it is required for standard of care assessment.

7.10 Study Calendar - See Appendix 2

The flowchart of scheduled study assessments is provided in *Appendix 2*. Patients will be closely monitored for safety and tolerability throughout the study. All assessments must be performed and documented for each patient.

Patients should be assessed for toxicity prior to each dose; dosing will occur only if the clinical assessment and local laboratory test values are acceptable.

If the timing of a protocol-mandated study visit coincides with a holiday and/or weekend that precludes the visit, the visit should be scheduled on the nearest following feasible date, with subsequent visits rescheduled accordingly. A +/- 4 day window is allowed for scheduling of study visits that must be moved for other reasons.

7.11 Duration of Follow Up

After the study is completed, patients will continued to be followed for an additional 24 months with abdominal ultrasounds as a part of the standard of care surveillance by chart review and phone calls made to the patient.

8.0 Assessment of Safety and Efficacy

Baseline adverse events will be assessed. See Section 8.4 for Adverse Event monitoring and reporting.

Analyses of safety/ toxicity will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 4 (See Appendix 1) for reporting of adverse events (http://ctep.cancer.gov/reporting/ctc.html).

Safety assessments will consist of monitoring and reporting AEs and SAEs that are considered related to Danazol, all events of death, and any study-specific issue of concern.

8.1 Side effects/toxicities to be monitored at each visit:

- Fatigue
- Weight gain
- Acne or oily skin
- Hair changes (hirsutism or hair loss)
- Edema or fluid retention
- Voice changes
- Menstrual disturbances
- Breast atrophy or decreased breast size
- Flushing or sweating
- Mood changes
- Elevation of liver enzymes
- Muscle cramps
- Hypertension
- Headache
- Thrombotic events

8.2 **Long-term toxicities to be monitored after completion of therapy:**

- Development of ovarian cancer
- Development of liver tumors (i.e. hepatic adenoma, hepatocellular carcinoma)
- Development of peliosis hepatitis
- Development of other hepatic toxicity

8.3 **Dosage change based on toxicity:**

- Danazol will be initiated at a dose of 600 mg/d.
- Danazol will be dose reduced to 400 mg/d if not tolerating based on side effects and toxicity assessment in 8.1 – see Table 1.
- If patient achieves hematologic response, danazol dose may be reduced to 200mg/day; patients who are unable to tolerate Danazol dose of 400 mg/d prior to achieving hematologic response will be removed from study
- Patients who develop grade 4 toxicity thought to be possibly or definitely related to danazol, will be removed from the study.
- Danazol will be held for imaging findings suspicious for tumors until work up has been completed. If negative, patient can resume danazol at previous dose.
- Danazol will be discontinued for any acute decompensation event; new diagnosis of arterial or venous thrombosis; new malignancy; pregnancy.
- Toxicity grading will be determined per CTAE version 5.0 guidelines.

Table 1. Management of Danazol Toxicities

NCI CTAE Grade	Management
Any Grade 1 Toxicity	Continue Danazol at current dose with continued monitoring

Grade 2 Toxicity at 600 mg/d dose	Danazol should be dose reduced to 400 mg/d
Grade 2 Toxicity at 400 mg/d dose	Discontinue Danazol and patient will be removed from the study
Grade 3 Toxicity at 600 mg/d dose	Danazol should be held for at least 2 weeks then resumed at reduced dose of 400 mg/d if labs have returned to baseline
Grade 3 Toxicity at 400 mg/d dose	Discontinue Danazol and patient will be removed from the study
Any Grade 4 Toxicity	Discontinue Danazol and patient will be removed from the study

Table 2. Edema Management

NCI CTAE Grade	Management	Danazol Dosage
Grade 1 Edema	Consider starting low dose diuretics or increasing current diuretic dose	Continue Danazol at current dose
Grade 2 Edema	Start low dose diuretic or increase current diuretic dose	Resume Danazol at dose reduced to 400 mg/d
Grade 3 Edema	Start low dose diuretic or increase current diuretic dose	Danazol should be held for at least 2 weeks then resumed at reduced dose of 400 mg/d if edema improves

8.4 **Adverse Event Reporting:**

8.4.1 **Adverse Events**

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention. An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms that were not present prior to the AE reporting period
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations)
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated interventions

- Pre-existing medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

The following events meet the definition of unanticipated problem (UPR):

- Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
- Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
- Any new information (e.g., publication, safety monitoring report, updated safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
- Any breach in confidentiality that may involve risk to the subject or others.
- Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

8.4.2 **Serious Adverse Events**

A "serious" adverse event (SAE) is defined in regulatory terminology as any untoward medical occurrence that:

- Results in death (If death results from progression of the disease, the disease should be reported as event SAE itself);

- Is life-threatening (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe);

- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours;

- Results in persistent or significant disability or incapacity;

- Is an important medical event; or

- Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event" (for example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency).

The Principal Investigator must be notified within 24 hours of learning of any serious adverse events (SAE), regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.

SAE occurring after consent but before first dose will not require expedited reporting and for which there will be no protocol-mandated intervention; management should be per investigator or treating physician and in accordance with standard of care.

8.4.3 **Reporting of Adverse Events and Serious Adverse Events**

The USC NCCC Data and Safety Monitoring Committee (DSMC) must be notified within 24 hours of submission of such reportable event to the IRB. The patient ID and the study number as well as identifier of the AE or SAE report should be submitted to the DSMC Coordinator via email or Fax to the attention of the DSMC Coordinator at 323-865-0089.

The USC NCCC Data and Safety Monitoring Committee (DSMC) must be notified within 24 hours of submission of such reportable event to the IRB. The patient ID and the study number as well as identifier of the SAE report should be submitted to the DSMC Coordinator via email or Fax to the attention of the DSMC Coordinator at 323-865-0089. The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 60 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

The Institutional IRB (US) must be notified of "any unanticipated problems involving risk to subjects or others" in accordance with the Institutional policy. Such policies will be provided to the USC Clinical Investigation Support Office (CISO) QA prior to enrolling 1st patient. (for USC refer to HSPP Policies and Procedures chapter 14 available at https://oprs.usc.edu/files/2017/04/2017-Policies-and-Procedures-PDF-1JUN2017.pdf).

8.5 **Adverse Event Monitoring**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be monitored until the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline; any abnormal laboratory values have returned to baseline; or death.

All AEs and SAEs, whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means, will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the study drug (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes There is a plausible temporal relationship between the onset of the AE and administration of Danazol, and the AE cannot be readily explained by the patient's clinical state, inter-current illness, or concomitant therapies; and/or the AE follows a known pattern of response to Danazol; and/or the AE abates or resolves upon discontinuation of Danazol or dose reduction and, if applicable, reappears upon re-challenge.

No Evidence exists that the AE has an etiology other than Danazol (e.g., pre-existing medical condition, underlying disease, inter-current illness, or concomitant medication); and/or the AE has no plausible temporal relationship to Danazol administration (e.g., cancer diagnosed 2 days after first dose of study drug). Expected AEs are those AEs that are listed or characterized in the Package Insert (PI) or current Investigator's Brochure. Unexpected AEs are those not listed in the PI or current Investigator's Brochure or not identified. This includes AEs for which the specificity or severity is not consistent with the description in the PI or Investigator's Brochure. For example, under this definition, hepatic necrosis would be unexpected if the PI or Investigator's Brochure only referred to elevated hepatic enzymes or hepatitis.

8.6 **Procedures for Eliciting, Recording and Reporting Adverse Events**

8.6.1 **Eliciting Adverse Events**

A consistent methodology for eliciting AEs at all patient evaluation time-points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

8.6.2 **Routine Reporting**

All other adverse events - such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission. This report will be forwarded to the USC DSMC Coordinator. All toxicities will be included in the IND annual report.

8.6.3 **Monitoring Rules for Safety**

A USC Data Safety Monitoring Board will be notified of all SAEs reported during the course of this trial.

8.6.4 **Deaths**

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death."

8.6.5 **Pre-existing Medical Conditions**

A pre-existing medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A pre-existing medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept

that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

8.6.6 **Hospitalizations for Medical or Surgical Procedures** Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a patient is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a patient is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE. Hospitalizations for the following reasons do not require reporting: 1. Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for pre-existing conditions; 2. Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study; or 3. Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

8.6.7 **Pregnancies in Female Patients** Female patients of childbearing potential should utilize contraception and take active measures to avoid pregnancy while undergoing Danazol treatment and for at least 90 days after the last dose of Danazol. Female patients of childbearing potential will be instructed to immediately inform the investigator if they become pregnant during the study or within 90 days after the last dose of study drug. A Pregnancy Report CRF should be completed by the investigator immediately (i.e., no more than 24 hours after learning of the pregnancy) and submitted via fax. Pregnancy should not be recorded on the Adverse Event CRF. The investigator should discontinue study drug and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any SAEs associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event CRF.

9.0 Criteria for Evaluation and Endpoint Definitions

The outcome status (in terms of toxicity, response, reason off study, progression, and survival) of all eligible patients will be reported. All eligible patients who begin treatment will be included in the analysis of survival and time-to-failure.

Evaluable for toxicity: All patients who receive one dose of danazol are evaluable for safety and toxicity.

Evaluable for response: All patients who receive danazol for at least 3 months will be evaluable for response. In instances of drug interruptions, patients who receive danazol for at least 3 months with no more than 4 weeks of interruption will be assessed for hematologic response. Liver and telomere response will be assessed in patients who receive at least 12 months of danazol with no more than 8 weeks of interruption. Patients who do not receive danazol for at least 3 months will be replaced.

Endpoint Definitions

Primary endpoints:

1) Hematologic response, defined as normalization of WBC to ≥ 4000/μL or doubling of WBC from baseline, AND/OR normalization of platelet count to ≥150,000/μL or doubling of platelet count from baseline, from study entry to three months.
2) Occurrence of grade 3+ CTCAE 5 adverse events.

Secondary endpoints include:

1) Change in blood cell counts baseline to three months.
2) Change in peripheral blood telomere length from baseline to 24 months.
3) Change in liver fibrosis as measured by Fibroscan from baseline to 24 months
4) Change in liver function parameters from baseline to 24 months;
5) Transplant-free survival, defined as time from study entry until liver transplant. Patients who have not undergone transplant will be censored at the time of last contact.
6) Overall survival defined as the time from study entry until death. Patients who are alive at last follow-up will be censored.
7) Occurrence of clinical decompensation events (variceal hemorrhage, ascites requiring intervention, and hepatic encephalopathy).

10.0 Data Collection and Monitoring

10.1 Active Monitoring Program Details

10.1.1 **Adherence to Protocol/Per Patient:** It is the responsibility of the USC Principal Investigator (PI) to ensure that patient recruitment and enrollment, treatment, follow-up for toxicities and response, and documentation and reporting at USC are all performed as specified in the protocol.

10.1.2 **Day-to-Day Monitoring – Eligibility**: At USC, the Study Coordinator will assist the Investigator in reviewing eligibility and will assemble the required source documents, and do a final review by completing an Eligibility Registration Worksheet.

10.1.3 **Day-to-Day Monitoring – Informed Consent**: Prior to registering the patient on study, the Study Coordinator will review the informed consent, to ensure that the patient has signed and dated the most current IRB-approved form, and that the form has been signed and dated by the person obtaining the consent as well as appropriate witnesses. A copy of the ICF will also be provided to CISO QA for review. CISO SOP 3.3 will be followed.

10.1.4 **Day-to-Day Monitoring – Treatment**: The PI and co-investigators are responsible for ensuring that treatment is given per protocol. The Study Coordinator will review the treatment orders and calendar-specified diagnostic/monitoring items with the treating investigator.

10.1.5 **Data Management – Patient Charts**: At USC, all written source documents not associated with the study research are maintained in the patient chart, which is stored in the Department of Medical Records at the appropriate hospital. At the Norris Hospital, the official medical record is on Cerner. Radiographical images are stored in the Department of Radiology and in an electronic system called Synapse. At Los Angeles County General Hospital the official medical record is on Orchid. These are the permanent, official documents for each patient on-study. A copy of the signed informed consent, physician's notes, orders, test results and pathology notes are maintained in the patients' hospital charts. It is the responsibility of the research staff to ensure that the patient chart contains the required documents and work closely with treating investigators to ensure all protocol-related assessments are carefully documented.

10.1.6 **Data Management – Research Charts**: At USC, to facilitate adherence to the protocol schedule and data management, research charts are created to collect copies of the relevant notes, orders and results that are in the Patient Chart. In addition, all source documents related to the research, such as original informed consent forms, HIPAA Forms, AE assessment worksheets and disease response worksheets are maintained in the Research Charts. Protocol calendars, worksheets, and checklists, are also kept in the research chart. These are maintained in the locked offices of the clinical trial research unit until the study is completed and the results are published and no further need is anticipated. These are then stored off-

site. It is the responsibility of the Data Manager to ensure that the research chart contains all the required documents.

10.1.7 **Data Management – Case Report Forms:** It is the responsibility of the Data Manager to complete the required case report forms. For in-house trials, case report forms are developed for each trial; these are used to finalize the data entry screens in the Cancer Center clinical trials database. It is the responsibility of the PI to review the Off-Study Summary form which summarizes pertinent toxicity, response and adherence information, once the patient has completed treatment.

10.2 **Quality Assurance Monitoring Committee (QAMC) Oversight:** The QAMC will be provided by CTSI at the University of Southern California Keck School of Medicine.

10.2.1 **Data and Safety Monitoring Committee (DSMC) Oversight:** The data safety and monitoring group for this study will be comprised of at least 3 individuals from different specialties (at least 1 MD and 1 representative of CTSI) who will be asked by the principal investigator to review toxicity data at 6 months and then every 12 months until study completion.

10.2.2 **Adherence to the Protocol:** Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

10.2.3 **Emergency Modifications:** Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval. For any such emergency modification implemented, an IRB modification form must be completed within five (5) business days of making the change.

10.2.4 **Non-Emergency departures from protocol:** A protocol deviation is any variance from an IRB approved protocol. If the deviation meets all of the following criteria, it is considered a minor protocol deviation that:

- Is generally noted or recognized only after it occurs;
- Has no substantive effect on the risks to research participants;
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected; or
- Did not result from willful or knowing misconduct on the part of the investigator(s).

If the deviation meets any of the following criteria, it is considered a protocol violation:

- Has harmed or increased the risk of harm to one or more research participants;
- Has damaged the scientific integrity of the data collected for the study;
- Results from willful or knowing misconduct on the part of the investigator(s); or
- Demonstrates serious noncompliance with federal regulations, State laws, or University policies.

Protocol Deviations: personnel will report to the Study team data and safety monitoring committee in accordance with their policies.

Protocol Violations: All protocol violations will be entered in the clinical trial database by the Research Coordinator. In addition, Research Coordinator and Investigator should report all protocol violations within one (1) week of the knowledge of the event using iStar.

10.2.8 **Amendments to the Protocol:** Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB as well as to all the sponsoring agencies (FDA, NCI, etc.) for review and for approval prior to implementation. It is the responsibility of the study PI to ensure that the appropriate agencies have been informed of the proposed amendments and that these have been reviewed and approved.

10.2.9 **Retention of Records:** Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Study team correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.0 **Statistical Considerations**

11.1 **Sample Size**

In this pilot study, 10 patients meeting trial inclusion and exclusion criteria will be enrolled to receive danazol 600 mg per day for 24 months. Based on the previous study by *Chiu et al,* we expect to screen around 50 patients to find 10 patients for our study [10]. The sample size of 10 was based on feasibility and available study resources, including study product.

For estimated proportions of telomere shortening/mutation ranging from 0.2-0.8, a sample size of 50 will provide 95% binomial confidence intervals with a width (from estimate to upper or lower confidence limit) of 0.237-0.289.

The sample size of 10 who will be treated with danazol will allow estimation of 95% binomial (specifically, exact Clopper-Pearson) confidence intervals with a width (from estimated proportion to upper or lower limit) of ±0.53-0.63 for estimated proportions ranging from 0.20-0.80. For the secondary continuous outcomes of change from baseline, the sample will also allow estimation of 95% confidence intervals on a one-sample mean with a width (from estimated mean to upper or lower limit) of ±0.71 of a standard deviation unit

11.2 **Statistical Analysis**

Primary Aim 1: Determine the prevalence of telomere gene mutations and/or shortened telomere lengths in the study population. Among consenting patients evaluated for telomere mutations and shortening, the proportion (with binomial 95% CI) of each telomere mutation, and any telomere mutation will be computed.

Primary Aim 2: Evaluate the efficacy of danazol in improving cytopenias in the study population. The proportion (with binomial 95% CI) of patients showing a Hematologic Response will be computed.

Primary Aim 3: Evaluate the safety of danazol in the study population. Proportions (with 95% CI) of patients experiencing danazol-targeted AEs/SAEs, other AEs/SAEs, and toxicity-based danazol dose reduction will be computed.

Secondary Aims: Continuous measures assessed at baseline and at 24 months include peripheral blood telomere length and liver fibrosis. Mean (SD) (median, IQR) change will be computed; difference of the mean change from a null value of 0 will be evaluated with a paired t-test (or non-parametric Wilcoxon signed rank test). Blood counts (WBC, ANC, platelets) and liver function tests will be completed throughout the trial (every 2 weeks for the first month, every 4 weeks for the next 3 months, every 3 months thereafter). Mean (95% CI; median, IQR) of values at each time point and changes from the baseline pre-treatment values will be summarized; changes from baseline will be tested with a linear mixed effects model for longitudinal data, specifying a patient-level random effect. Survival distributions (median, 25th and 75th percentiles) for death and transplant will be computed with 95% confidence intervals.

12.0 Registration Guideline

12.1 **Patient Informed Consent Process** Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing a dated IRB approved consent form. In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki. Patients enrolling at USC will receive the *California Experimental Research Subject's Bill of Rights*. At the time of registration, signed and dated copies of the patient Informed Consent document with the *California Experimental Research Subject's Bill of Rights (California patients only)* and the HIPAA authorization must be given to the patient. Institutional policy regarding distribution and location of original consent documents should be followed. When a study is opened at two or more institutions, a copy of the signed consent and HIPAA should be sent to USC Clinical Investigation Support Office (CISO) QA team as soon as possible, and not later than within 5 business days of obtaining consent. For patients consented at USC/LAC, institutional policy should be followed: a copy of ICF and HIPAA should be uploaded through True to USC CRO and to CISO QA Team. The original will be kept in the patient research chart maintained by the study assigned Data Manager. The informed consent document must be signed by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent document must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language. Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at any time.

12.2 **Registration Eligibility Worksheet** At the time of registration, the completed Eligibility Worksheet will be submitted to the QA Monitor at CISO for review of eligibility compliance.

12.3 **Institutional Review Board (IRB) Approval** It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. The study will be conducted in accordance with FDA, applicable national and local health authorities, and IRB requirements. The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, the IRB must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant AEs. Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to SAEs that are not already identified in the Investigator's Brochure and that are considered possibly or probably related to the molecule or study drug by the investigator. Some IRBs may have other specific AE requirements to which investigators are expected to adhere.

12.4 **USC Registration:** For patients enrolled at USC, the Research Coordinator must complete the protocol eligibility form to ensure that the patient is eligible. The PI will review the patient eligibility (with assistance from the Research Coordinator - who will assemble the required source documents, and do an initial review) prior to registering the patient on study. The Research Coordinator or data manager will then register the patient into the Cancer Center database, Café, by accessing the Registration forms. Likewise, after the patient has completed the study, the Off Study forms in cafe will need to be completed, for Off Treatment and Off Study.

12.5 **Confidentiality of Records:** The original data collection forms will be kept in secure file cabinets, for USC patient's forms will be kept in the Clinical Investigations Support Office (CISO). Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the ICF (or separate authorization to use and disclose personal health information) signed by the patient or unless permitted or required by law. Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare for treatment purposes. Data generated by this study must be available for inspection upon request by representatives of the FDA and other regulatory agencies, national and local health authorities, and the IRB/Ethics Committee (EC) for each study site, if appropriate.

13.0 Biohazard Containment

All blood samples drawn for study testing will be obtained handled and discarded through CLIA-certified laboratory according their standard operating protocol. Bone marrow aspiration and biopsies obtained for the study will be performed per protocol in the LAC+USC hematology outpatient clinic (A4A) by medical personnel and sent to the LAC+USC pathology department for processing and reporting. Any biohazard material obtained as a part of this study will be handled according to the guidelines outlines in the USC Biosafety Manual (https://ehs.usc.edu/files/BSM-2020.pdf).

14.0 Ethical and Regulatory Considerations

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice. The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of

Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub- investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion. This clinical research study will be monitored both internally by the PI and externally by the USC IRB. In terms of internal review, the PI will continuously monitor and tabulate AEs. Appropriate reporting to the USC IRB will be made. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled,
- Stopping rules for toxicity and/or response are met,
- Risk/benefit ratio is not altered to the detriment of the subjects,
- Appropriate internal monitoring of AEs and outcomes is done,
- Over-accrual does not occur,
- Under-accrual is addressed with appropriate amendments or actions, and
- Data are being appropriately collected in a reasonably timely manner.

Routine monitoring will be carried out via a periodic team conference among investigators during which toxicity data, including all SAEs, will be reviewed and other issues relevant to the study such as interim assessment of accrual, outcome, and compliance with study guidelines, will be discussed. Monitoring will be carried out on an ongoing basis. The severity, relatedness, and whether or not the event is expected will be reviewed.

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APPENDICES

APPENDIX 1 – CURRENT NATIONAL CANCER INSTITUTE COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (NCI CTCAE)

Please use the following link to the NCI CTCAE website:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

	Grade 1	Grade 2	Grade 3	Grade 4
ALT	> ULN - 3.0 x ULN if baseline was normal or 1.5 - 3.0 x baseline if baseline was abnormal	>3.0 - 5.0 x ULN if baseline was normal or 3.0 - 5.0 x baseline if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal or 5.0 - 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal or >20.0 x baseline if baseline was abnormal
AST	> ULN - 3.0 x ULN if baseline was normal or 1.5 - 3.0 x baseline if baseline was abnormal	>3.0 - 5.0 x ULN if baseline was normal or 3.0 - 5.0 x baseline if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal or 5.0 - 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal or >20.0 x baseline if baseline was abnormal
Alkaline phosphatase	> ULN - 2.5 x ULN if baseline was normal or 2.0 - 2.5 x baseline if baseline was abnormal	>2.5 - 5.0 x ULN if baseline was normal or 2.5 - 5.0 x baseline if baseline was abnormal	>5.0 - 20.0 x ULN if baseline was normal or 5.0 - 20.0 x baseline if baseline was abnormal	>20.0 x ULN if baseline was normal or >20.0 x baseline if baseline was abnormal
Bilirubin	> ULN - 1.5 x ULN if baseline was normal or 1.0 - 1.5 x baseline if baseline was abnormal	> 1.5 - 3.0 x ULN if baseline was normal or 1.5 - 3.0 x baseline if baseline was abnormal	>3.0 - 10.0 x ULN if baseline was normal or 3.0 - 10.0 x baseline if baseline was abnormal	>10.0 x ULN if baseline was normal or >10.0 x baseline if baseline was abnormal
Creatinine	> ULN - 1.5 x ULN	>1.5 - 3.0 x baseline or >1.5 - 3.0 x ULN	>3.0 x baseline or >3.0 - 6.0 x ULN	>6.0 x ULN
Edema	5 - 10% inter-limb discrepancy in volume or circumference at point of greatest visible difference; swelling or obscuration of anatomic architecture on close inspection	>10 - 30% inter-limb discrepancy in volume or circumference at point of greatest visible difference; readily apparent obscuration of anatomic architecture; obliteration of skin folds; readily apparent deviation from normal anatomic contour; limiting instrumental ADL	>30% inter-limb discrepancy - - in volume; gross deviation from normal anatomic contour; limiting self care ADL	

APPENDIX 2 – Study Calendar

Event/Visit	**Pre-Treatment**	**Week 0 (Start of Treatment)** (	**Week 2**	**Week 4**	**Week 8**
Written informed consent	X				
Eligibility assessment	X	X			
Demographics	X				
Comprehensive History & Physical Examination	X				
Targeted History & Physical Examination		X	X	X	X
Complete Blood Count	X	X	X	X	X
Complete Metabolic Panel	X	X	X	X	X
Reticulocyte Count	X	X	X	X	X
PT/INR	X				
Alpha fetoprotein	X				
Abdominal Ultrasound	X				
Fibroscan	X				
Telomere Gene Panel Testing	X				
Telomere Length Measurement	X				
Bone Marrow Biopsy*	X				
Danazol administration		X	X	X	X
Side effect and toxicity Assessment			X	X	X
Current medication list Review	X	X	X	X	X

APPENDIX 3 – ECOG Performance Scale

ECOG	Description
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on selfcare. Totally confined to bed or chair
5	Dead