


# TELO-SCOPE study: a randomised, double-blind, placebo-controlled, phase 2 trial of danazol for short telomere related pulmonary fibrosis

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## ABSTRACT

**Introduction** Recent discoveries have identified shortened telomeres and related mutations in people with pulmonary fibrosis (PF). There is evidence to suggest that androgens, including danazol, may be effective in lengthening telomeres in peripheral blood cells. This study aims to assess the safety and efficacy of danazol in adults and children with PF associated with telomere shortening.

**Methods and analysis** A multi-centre, double-blind, placebo-controlled, randomised trial of danazol will be conducted in subjects aged >5 years with PF associated with age-adjusted telomere length ≤10th centile measured by flow fluorescence in situ hybridisation; or in children, a diagnosis of dyskeratosis congenita. Adult participants will receive danazol 800 mg daily in two divided doses or identical placebo capsules orally for 12 months, in addition to standard of care (including pirfenidone or nintedanib). Paediatric participants will receive danazol 2 mg/kg/day orally in two divided doses or identical placebo for 6 months. If no side effects are encountered, the dose will be escalated to 4 mg/kg/day (maximum 800 mg daily) orally in two divided doses for a further 6 months. The primary outcome is change in absolute telomere length in base pairs, measured using the telomere shortest length assay (TeSLA), at 12 months in the intention to treat population.

**Ethics and dissemination** Ethics approval has been granted in Australia by the Metro South Human Research Ethics Committee (HREC/2020/QMS/66385). The study will be conducted and reported according to Standard Protocol Items: Recommendations for Interventional Trials guidelines. Results will be published in peer-reviewed journals and presented at international and national conferences.

**Trial registration numbers** NCT04638517; Australian New Zealand Clinical Trials Registry (ACTRN12620001363976p).

## INTRODUCTION

Progressive pulmonary fibrosis (PF) is a relatively rare condition that leads to substantial morbidity and mortality. The treatment of progressive PF, particularly in its idiopathic

form (IPF), has undergone dramatic change over the last decade. In 2012, a major shift in the management of IPF occurred, with the publication of the Prednisone, Azathioprine, and N-Acetylcysteine: A Study That Evaluates Response in Idiopathic Pulmonary Fibrosis (PANTHER-IPF) trial which demonstrated harm from this combination, which had been the standard of care until that point.<sup>1</sup> Subsequently two agents, nintedanib and pirfenidone, jointly coined antifibrotics, have proved efficacious to slow disease progression, reduce the frequency of acute exacerbation and improve survival.<sup>2–4</sup> However, these medications are not a cure, and do not completely halt disease progression. While they both have demonstrated broad activity across the spectrum of fibrosing interstitial lung diseases,<sup>5–7</sup> their activity targets down-stream pathways of lung fibrosis. Disease modifying therapies in their truest sense, require activity at the very origins of disease pathogenesis.

Recent discoveries have begun to unravel fundamental genetic abnormalities in a significant proportion of patients with PF. The most frequent genetic abnormalities are found in genes involved in telomere maintenance. Telomeres are nucleoprotein complexes consisting of long, TTAGGG repeat segments, which protect chromosomes from loss of genomic material during cell replication. Telomere length is regulated by the enzyme telomerase and shortening occurs naturally with age. Mutations in telomere-maintenance genes cause extreme shortening, and were first recognised to lead to fatal PF in children with dyskeratosis congenita (DC).<sup>8</sup> Subsequently, similar mutations were identified in adults with PF.<sup>9</sup> Telomere shortening is frequently identified in adult



PF and defines disease behaviour.<sup>10,11</sup> In IPF, telomere length is directly proportionate to survival—those with the shortest telomere lengths have the worst survival.<sup>12–15</sup> Additionally, post-hoc evaluation of the pivotal PANTHER trial suggests that a proportion of the adverse outcomes with immunosuppression was attributable to the treatment's impact in patients with short telomeres.<sup>16</sup> In chronic hypersensitivity pneumonitis, the presence of shortened telomeres predicts a survival identical to IPF.<sup>17</sup> Shortened telomeres and their associated genetic mutations (TERT, TERC, RTEL1 and PARN) predict adverse outcomes among families with inherited interstitial lung diseases (ILDs), despite heterogeneous radiology and histology.<sup>12</sup> Finally, the presence of telomere shortening impacts lung transplantation complications and survival, and data are emerging to suggest that such patients should be managed differently in the post-transplantation period.<sup>18</sup>

The evidence above suggests that the telomere apparatus is a potential treatment target in PF. Preserving, and potentially elongating telomere length may help to prevent progressive PF. Danazol, a synthetic androgen, has demonstrated potential efficacy in this regard.<sup>19</sup> In 27 subjects with a variety of short telomere related blood disorders, telomeres were lengthened with danazol.<sup>19</sup> Ten participants with PF had stable lung function over 2 years of treatment. A case report from our group demonstrated similar benefit with danazol.<sup>20</sup> Moreover, in 17 patients, including 6 with PF, a similar androgen (intramuscular nandrolone) resulted in telomere elongation by 1119 base pairs (bp) at 12 months, and resolution of respiratory failure in 1 patient.<sup>21</sup> Further support for a potential role of androgens in PF stems from data demonstrating lower testosterone levels in males with IPF, which correlated with shorter telomere length.<sup>22</sup> However, previous trials have significant limitations, including missing data, no comparator group, and the majority of participants had extremely short telomeres (less than first centile). Additionally a study in 10 haematology patients failed to confirm an effect of danazol on telomere length.<sup>23</sup> Ongoing studies of androgens highlight the worldwide interest in this therapy for short telomere syndromes. However, these studies either have a haematological (USA, NCT03312400; France, EudraCT 2018-001686-17, South America, NCT02055456) or paediatric (Boston, NCT01001598) focus.

The TELO-SCOPE Study: A Randomised, Double-Blind, Placebo-Controlled, Phase 2 Trial of Danazol for Short Telomere Related Pulmonary Fibrosis, will be the first study to specifically study danazol in PF associated with short telomeres and has been deliberately designed to align with international protocols so that subsequent meta-analyses are facilitated. The primary endpoint of the study is change in telomere length over 12 months of treatment, allowing the study to be adequately powered. We hypothesise that danazol will result in reduced telomere attrition in patients with PF associated with short telomeres.

## METHODS AND ANALYSIS

### Design

TELO-SCOPE is a national, multi-centre, double-blind, placebo-controlled, randomised trial which will be conducted in subjects aged over 5 years with a multidisciplinary diagnosis of PF and with age-adjusted telomere length less than or equal to the 10th centile in adults; and for children (aged less than 16 years), a confirmed diagnosis of DC. The study is designed according to Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines, will be conducted in accordance with the principles of Good Clinical Practice and reported according to Consolidated Standards of Reporting Trials standards. The study has been notified to the Therapeutic Goods Administration Australia (CT-2020-CTN-04 759-1).

### Participants

Adults and children (aged older than 5 years) with a fibrosing ILD on high-resolution CT (HRCT) who meet all other inclusions with no exclusions are eligible to participate in this trial (box 1). A key inclusion will be the presence of shortened telomeres, defined as age adjusted peripheral blood leucocyte telomere length less than or equal to the 10th centile measured by flow fluorescence in situ hybridisation (flow-FISH). Flow-FISH was chosen for screening purposes as it is the clinically accepted diagnostic test for telomere length.<sup>24,25</sup> Flow-FISH measures median telomere length in a cell population and allows for a patient's telomere length to be rapidly referenced to a control population, with telomere lengths below the 10th centile being indicative of underlying telomere related gene mutations.<sup>26</sup> Other inclusion criteria are as follows: (1) males and females aged over 5 years, able to take capsules orally; (2) fibrosing interstitial pneumonia (including, but not limited to: IPF, non-specific interstitial pneumonia, chronic hypersensitivity pneumonitis, pleuroparenchymal fibroelastosis, unclassifiable ILD) diagnosed according to current international guidelines<sup>27,28</sup>; (3) forced vital capacity >40% predicted; (4) diffusion capacity for carbon monoxide >25% predicted; (5) if receiving background pirfenidone or nintedanib, stable dose for 28 days prior to screening; (6) able to understand and sign a written informed consent form (or legally authorised representative); (7) agreement to use a medically approved form of non-hormonal contraception (if of childbearing potential) and noting that oral contraceptives are advised not to be used concurrently with danazol.

Subjects will be excluded if any of the following criteria are present: actively or imminently listed for lung transplantation; undergone, awaiting, or likely to require bone marrow transplantation within 12 months; concurrent enrolment in another study; females with a positive pregnancy test at screening or currently breast feeding; pelvic infection; past jaundice with oral contraceptives; undiagnosed abnormal genital bleeding; undiagnosed

**Box 1** Inclusion and exclusion criteria

**Inclusion criteria**

- ▶ Males and females aged >5 years, able to take capsules orally.
- ▶ Fibrosing interstitial pneumonia (including, but not limited to, idiopathic pulmonary fibrosis (PF), non-specific interstitial pneumonia, chronic hypersensitivity pneumonitis, pleuroparenchymal fibroelastosis, unclassifiable interstitial lung disease).
- ▶ Age-adjusted peripheral blood leucocyte telomere length ≤10th centile on flow fluorescence in situ hybridisation.
- ▶ Forced vital capacity >40% predicted.
- ▶ Diffusion capacity for carbon monoxide >25% predicted.
- ▶ If receiving background pirfenidone/nintedanib, stable dose for 28 days prior to screening.
- ▶ Able to understand and sign a written informed consent form (or legally authorised representative).
- ▶ Agreement to use a medically approved form of non-hormonal contraception (if of childbearing potential) (noting that oral contraceptives are advised not to be used concurrently with danazol).

**Exclusion criteria**

- ▶ Actively or imminently listed for lung transplantation.
- ▶ Undergone, awaiting or likely to require bone marrow transplantation within 12 months.
- ▶ Concurrent enrolment in another study.
- ▶ Females with a positive pregnancy test at screening or currently breast feeding.
- ▶ Pelvic infection.
- ▶ Past jaundice with oral contraceptives.
- ▶ Undiagnosed abnormal genital bleeding.
- ▶ Undiagnosed ovarian/uterine masses.
- ▶ Any history of malignancy likely to result in significant disability or likely to require significant medical or surgical intervention within the next 12 months.
- ▶ History of androgen-dependent tumour.
- ▶ Any condition other than PF that, in the opinion of the investigator, is likely to result in the death of the participant within the next 12 months.
- ▶ History of end-stage liver disease or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3 times the upper limit of normal.
- ▶ History of end-stage kidney disease requiring dialysis.
- ▶ Markedly impaired cardiac function.
- ▶ Known increased risk of or history of thromboembolism (eg, Factor V Leiden, protein C or S deficiency).
- ▶ Uncontrolled hypertension.
- ▶ Uncontrolled lipoprotein disorder.
- ▶ Poorly controlled diabetes mellitus.
- ▶ History of marked or persistent androgenic reaction to previous gonadal steroid therapy.
- ▶ History of epilepsy induced or worsened by previous gonadal steroid therapy.
- ▶ History of raised intracranial pressure.
- ▶ Known intolerance to danazol.
- ▶ Porphyria.
- ▶ Use of any of the following agents within 28 days before screening: danazol or other androgen therapy, warfarin or other anticoagulant, carbamazepine, phenytoin, investigational therapy, cytotoxic therapy, tacrolimus, cyclosporine, simvastatin.
- ▶ Professional singer due to potential for voice change.
- ▶ Competitive athletes.

Continued

**Box 1** Continued

- ▶ Lactose intolerance.
- ▶ Prostate specific antigen above the upper limit of normal (adult males only).

ovarian/uterine masses; any history of malignancy likely to result in significant disability or likely to require significant medical or surgical intervention within the next 12 months; history of androgen-dependent tumour; any condition other than PF that, in the opinion of the investigator, is likely to result in the death of the participant within the next 12 months; history of end-stage liver disease or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3 times the upper limit of normal; history of end-stage kidney disease requiring dialysis; markedly impaired cardiac function; known increased risk of or history of thromboembolism (eg, factor V Leiden, protein C or S deficiency); uncontrolled hypertension; uncontrolled lipoprotein disorder; poorly controlled diabetes mellitus; history of marked or persistent androgenic reaction to previous gonadal steroid therapy; history of epilepsy induced or worsened by previous gonadal steroid therapy; history of raised intracranial pressure; known intolerance to danazol; porphyria; use of any of the following agents within 28 days before screening: danazol or other androgen therapy, warfarin or other anticoagulant, carbamazepine, phenytoin, investigational therapy, cytotoxic therapy, tacrolimus, cyclosporine, simvastatin; professional singer due to potential for voice change; competitive athletes; lactose intolerance; and prostate specific antigen above the upper limit of normal (adult males only).

The inclusion criteria have been developed in line with those for previously reported studies of antifibrotics in PF and the Townsley *et al* danazol study.<sup>2 3 19</sup> The Townsley *et al* study used a threshold of age-adjusted telomere length less than the first centile.<sup>19</sup> A slightly longer, but still abnormal, telomere length threshold (less than or equal to the 10th centile) has been chosen in this study as it has been demonstrated in multiple studies to be associated with mutations in the telomere-maintenance genes and outcomes in PF,<sup>9–11 14</sup> and since the treatment effect of danazol was found to be independent of baseline telomere length in the study by Townsley *et al*.<sup>19</sup>

**Recruitment**

Participants will be recruited from an existing IPF Registry, the investigator's ILD clinics, and the ILD multi-disciplinary meetings held at all sites. Patients with PF known to be associated with short telomeres or DC will be approached initially. Patients with clinical features suggestive of a short telomere syndrome (eg, young age of onset, a family history of PF, early greying of the hair, haematological abnormalities) will be targeted for recruitment.<sup>8</sup> If not already enrolled, subjects with age-adjusted





telomere length above the 10th centile will be offered enrolment in the Australian ILD Registry so that the impact of telomere length on the natural history of PF can be ascertained.<sup>29</sup> Participants will be advised of their telomere length result alongside genetic counselling. All participants with a telomere length less than or equal to the 10th centile will be offered a clinical genetics consultation. Whole genome sequencing will be performed for those individuals with telomere length  $\leq$ 10th percentile. Known telomere related genes will be screened for pathogenic variants that meet the American College of Medical Genetics and Genomics (ACMG) guidelines for interpretation of sequence variants. Clinically significant findings will be returned to participants following best practice guidelines. Additionally, subjects with positive family histories for ILD, will be offered enrolment in the Genetic Research in Idiopathic Pulmonary Fibrosis study.

### Flow-FISH for telomere length screening

For screening, relative (percentile) telomere length will be assessed with flow-FISH, using the peptide nucleic acid (PNA) Kit/FITC for Flow Cytometry (DAKO) according to the manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) will be isolated by density gradient separation, within 40 hours of collection, before freezing for further use. Peripheral blood leucocyte telomere length has been used by multiple investigators as a surrogate for telomere related pathology in solid organs, including the lung, in observational<sup>10 11 13–17</sup> and previous interventional studies.<sup>19 21</sup> PBMCs washed with phosphate-buffered saline (PBS) will be mixed with the control cell line (1301) and their DNA denatured in a heating block adjusted to 82°C for 10 min in hybridisation solution with or without a fluorescently labelled telomere specific probe. After an overnight incubation at room temperature, samples will be washed and the DNA stained. Finally, samples will be run on a flow cytometer and telomere length estimated by comparison to the control cell line.

### Intervention

Adult participants will receive danazol at a dose of 800mg daily in two divided doses or identical placebo capsules orally for 12 months, in addition to standard of care (eg, pirfenidone or nintedanib). This dose was chosen as it was studied by Townsley *et al* and was tolerated well over 24 months.<sup>19</sup> In subjects who have difficulty tolerating danazol/placebo, the dose will be reduced by 200mg/day and side effects will be reassessed. If symptoms related to the study drug persist, subsequent 200mg/day dose reductions will be allowed until a tolerated dose is achieved. If the subject cannot tolerate danazol/placebo 200mg/day, the drug will be discontinued. Background antifibrotic therapy is allowed as drug pharmacokinetics do not predict interactions or additive hepatotoxicity, but these will be a key focus of the safety assessments.

Paediatric participants will receive danazol 2mg/kg/day orally in two divided doses rounded to the nearest 100mg (maximum 800mg daily) or identical placebo for the first 6 months of the trial. If no side effects are encountered, the dose will be escalated to 4mg/kg/day (max 800mg daily) orally in two divided doses rounded to the nearest 100mg for a further 6 months.

### Randomisation and blinding

Concealed random allocation will be employed using an online randomisation system in a 2:1 fashion generated with permuted blocks of various sizes. Blinding will be achieved by an over-encapsulation method using gelatin capsules. Placebo capsules will be packed with microcrystalline cellulose. Individual participant treatment assignments will not be unblinded during the study unless there is a regulatory obligation, or a patient safety issue arises in which unblinding is necessary to ensure optimal patient management. While the study is ongoing, individual patient unblinding not related to regulatory requirements will only occur after approval from the medical monitor or designee.

### Outcome measures

The primary outcome is change in absolute telomere length in base pairs (bp) at 12 months in the intention to treat population. Absolute telomere length (bp) will be measured at baseline, 3, 6, 9 months and end-of-study visits using the

#### Box 2 Secondary and safety outcome measures

##### Secondary outcome measures

1. Treatment-emergent adverse events.
2. Treatment-emergent changes in clinical laboratory parameters.
3. Treatment-emergent deaths.
4. Change in telomere length (bp) at 3, 6 and 9 months.
5. Change in forced vital capacity (FVC, mL) at 6 and 12 months.
6. Change in FVC (% predicted) at 6 and 12 months.
7. Change in diffusing capacity for carbon monoxide (% predicted) at 6 and 12 months.
8. Change in 6-minute walk distance (m) at 12 months.
9. Change in participant reported outcomes (Leicester Cough, Parent Cough-Specific Quality of Life and King's Brief Interstitial Lung Disease questionnaires).
10. Change in haematological parameters (haemoglobin, platelet count, white cell count).
11. Change in telomere length (bp) at 12 months stratified by telomere-maintenance mutation.
12. Change in high-resolution CT.

##### Safety outcome measures

1. Treatment-emergent adverse events (AEs).
2. Treatment-emergent serious adverse events (SAEs).
3. Treatment-emergent treatment-related AEs.
4. Treatment-emergent treatment-related SAEs.
5. AEs leading to early discontinuation of study treatment.
6. Treatment-emergent deaths.
7. Cause of death.
8. Treatment-emergent changes in clinical laboratory findings.



telomere shortest length assay (TeSLA). TeSLA was chosen to measure primary outcome because it measures individual telomere lengths, and provides more precise information regarding the abundance, distribution and length of the shortest telomeres.<sup>25 30</sup> In brief, genomic DNA will be ligated with overhang adaptors, digested with a restriction enzyme panel, and ligated with double-stranded adaptors.<sup>25</sup> Ligated telomeric DNA will be amplified using PCR and products resolved by agarose gel electrophoresis. Gels will be dried, denatured, neutralised and subject to in-gel hybridisation with a  $\gamma$ -[<sup>32</sup>P]-ATP-labelled (CCCTAA)<sub>3</sub> probe in Church buffer. Gels will be washed and exposed to a PhosphorImager screen. Secondary and safety outcome measures, including changes in lung function parameters, are listed in box 2.

### Genetic analysis

Whole genome sequencing will be performed using the Illumina Novaseq6000 platform. Genomes will be processed using an established sequencing pipeline (JLD). Briefly, sequences will be aligned to human genome reference GRCh38/hg38 and variant calling will be undertaken using GATK Haplotype caller.<sup>31</sup> Variant annotation will be performed using ANNOVAR<sup>32</sup> and standard quality control (QC) performed to remove sequence artefacts/low quality calls. Annotated telomere-associated gene sequences including promoter and 3' regulatory sequences will be extracted. A curated gene list will include genes previously shown to harbour mutations associated with short telomeres and pulmonary

fibrotic conditions in the first instance (table 1) and will be expanded to include genes associated other telomere disorders and lung fibrotic conditions.<sup>33 34</sup> Single nucleotide polymorphisms (SNPs) meeting QC will be filtered as follows: population frequency <0.01% as determined using publicly available databases gnomAD (<http://gnomad.broadinstitute.org/>), 1000Genomes (<https://www.internationalgenome.org/>) and ExAC (<http://exac.broadinstitute.org/>), predicted pathogenicity using functional prediction tools including CADD,<sup>35</sup> MutationTaster<sup>36</sup> and other prediction tools as appropriate; ensuring inclusion of previously reported disease associated SNPs. Structural variation will also be examined.<sup>37</sup> All potentially pathogenic SNPs will be curated and evaluated using currently available databases such as VarSome<sup>38</sup> and ClinVar<sup>39</sup> and, in addition to published literature. Curated variant lists will be discussed with the TELO-SCOPE gene review panel and considered in line with current standards and guidelines for the interpretation of sequence variants (the joint consensus recommendations of the ACMG and the Association for Molecular Pathology and the ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020).<sup>40</sup> Agreed clinically significant genetic findings will be returned following best practice guidelines for the return of genetic results guided by CI's clinical genetics expertise.

### Exploratory end-points

A pharmacogenomic study will follow the trial. After unblinding, subjects with identifiable mutations will be triaged by their response to danazol. Mutations from the top and bottom two 'responders' (bp/year) will be introduced into the Kucg2 iPS cell line using CRISPR-Cas9-mediated genome editing. Mutant cell lines will be assessed for telomere length by flow-FISH and terminal restriction fragment length analysis, telomerase activity by the immunoprecipitation-telomere repeat amplification protocol and human telomerase reverse transcriptase (hTERT) expression by quantitative reverse transcription PCR, prior to and during treatment (8 days) with 100  $\mu$ M danazol (Sigma-Aldrich). This approach will determine whether the specific underlying mutation can explain a differential response to danazol.

### Schedule of assessments

The assessment schedule is detailed in table 2. Two screening visits will occur, the first being to facilitate screening of the participant's telomere length following informed consent. Participants meeting the inclusion criteria with no exclusions will return to complete the final screening assessments at a second visit. Trial visits will incorporate a combination of telephone and face-to-face consultations. Monitoring for known side effects of danazol will occur at all visits, including, physical examination for changes in physical features, laboratory tests for changes in liver function, coagulation profile, cholesterol and hormonal levels. A liver ultrasound is scheduled at 6 months to exclude the development of peliosis

**Table 1** Genes harbouring mutations associated with telomere disorders<sup>33 34</sup>

Genes	Disease phenotype
<i>TERT</i>	DC, IPF/FIP, AA/MDS, familial liver cirrhosis, HHS
<i>TERC</i>	DC, IPF/FIP, AA/MDS, familial liver cirrhosis
<i>DKC1</i>	DC, HHS, IPF/FIP
<i>RTEL1</i>	DC, HHS, IPF/FIP
<i>TINF2</i>	DC, HHS, RS, IPF/FIP
<i>PARN</i>	IPF/FIP, DC, HHS
<i>ZCCHC8</i>	FIP
<i>NAF1</i>	IPF/FIP, CPFE
<i>NOP10, NHP2, WRAP53, CTC1</i>	DC
<i>ACD</i>	AA, DC, HHA
<i>POT1, TPP1</i>	BMF, CP, IPF and CLL
Genes in the Shelterin CST complexes	–
Genes: MUC5B, surfactant genes, vesicular trafficking genes	IPF

AA, aplastic anaemia; CP, coats plus; CPFE, combined pulmonary fibrosis and emphysema; DC, dyskeratosis congenita; FIP, familial interstitial pneumonia; HHS, Hoyeraal-Hreidarsson syndrome; IPF, idiopathic pulmonary fibrosis; MD, myelodysplastic syndrome; RS, Revesz syndrome.



Table 2 Assessment schedule

Visit	Screening			Baseline			Treatment			Follow-up									
	1	2	3a	3b	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Day	-42	-42	1	2	7*	28	56	84	112*	140*	168	196*	224*	252	280*	308*	336*	364	28 days after last doset
		to																	
Telephone visit			X	X					X		X	X	X	X	X	X	X		
Window (days)	NA	NA	NA	+1	±1	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±7
Informed consent	X																		X
Eligibility assessments	X	X	X																
Demographics, medical history	X																		
Physical examination, vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height, weight	X							X			X		X						X
ECG	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Telomere length measurement	X†		X§				X§				X§		X§						X§
Laboratory tests	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PFTs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Six-minute walk test	X									X	X	X	X	X	X	X	X	X	X
Questionnaires¶	X									X	X	X	X	X	X	X	X	X	X
Liver ultrasound										X									
HRCT	X																		X
Randomisation																			
Study drug administration			X				X			X	X	X	X	X	X	X	X	X	X
Review adherence					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
End of treatment/study form																			X

\*Phone visit.

†Follow-up visit will occur for all participants, including those terminating early; telomere length will be measured using the following notes.

‡Flow fluorescence in situ hybridisation (screening).

§Telomere shortest length assay (other visits).

¶Questionnaires include King's Brief Interstitial Lung Disease (adults only), Leicester Cough Questionnaire (adults only) and Parent Cough-Specific Quality of Life (children only). HRCT, high-resolution CT; NA, not applicable; PFT, pulmonary function test.

hepatitis. Blood will be collected for the measurement of telomere length at baseline, 3, 6, 9 and 12 months. Questionnaires will be completed at baseline, 6 months and end of treatment. Adult participants will complete the King's Brief Interstitial Lung Disease<sup>41</sup> and Leicester Cough Questionnaire.<sup>42</sup> In children, the parent/guardian will complete the Parent Cough Specific Quality of Life questionnaire.<sup>43</sup>

### Sample size

The normal rate of telomere attrition is 60bp/year,<sup>19</sup><sup>44</sup> but doubles to roughly 120bp/year in people with telomere-maintenance gene mutations. Assuming a conservative effect size (normalisation of telomere attrition) of 10% in the control group and 50% in the treated group, and assuming a power of 80% and two-sided significance level of 5%, using a 2:1 randomisation a sample size of 15 is required in the control group and 30 in the treated group, giving a total sample size of 45, with 50 enrolled to allow for a 10% drop-out rate. Analysis will be by intention-to-treat. It is estimated that the prevalence of PF associated with short telomeres is approximately 25% among undifferentiated PF patients, requiring screening of up to 200 to enrol 50 participants.

### Data analysis plan

Cohort descriptive statistics, incidence of short telomeres, telomere-maintenance gene mutations and adverse events will be reported. Data will be analysed on an intention-to-treat basis using two-sided tests with *p* values <0.05 considered statistically significant. The primary endpoint will be assessed using two-sided test for proportions. Additional tests for secondary endpoints and rate of telomere attrition will be performed using standard parametric or non-parametric tests, controlling for confounding variables, as appropriate.

### Patient and public involvement

Feedback on PF research priorities and TELO-SCOPE was obtained from the Lung Foundation Australia Pulmonary Fibrosis Consumer Advisory Group. Feedback themes were (1) lack of effective treatments; (2) access to clinical trials for regional and rural patients; (3) access to genetic counselling; (4) lack of research into genetic causes of PF; (5) impact on family members after identification of potentially heritable mutations; (6) exposure to placebo and (7) access to danazol beyond TELO-SCOPE. This feedback informed the design of TELO-SCOPE. The study protocol and participant information and consent forms underwent review by the Pulmonary Fibrosis Australasian Clinical Trials Network Consumer Advisory Group. Modifications to these documents were made following this review to address all feedback.

### Data monitoring

The Data Safety and Monitoring Board (DSMB) will comprise an independent group of international experts

in ILD and a statistician. A review of safety data will occur after 20 participants have completed the week 4 visit. The DSMB will report the findings to the trial steering committee, consisting of chief investigators.

### ETHICS AND DISSEMINATION

Ethics approval has been granted in Australia by the Metro South Human Research Ethics Committee (HREC/2020/QMS/66385) and ratified by the University of Queensland (2020/HE002889). The study will be conducted and reported according to SPIRIT guidelines. Results will be published in peer-reviewed journals and presented at international and national conferences. The results will also be disseminated to patients and the public through lay publications and seminars.

### Trial status

Recruitment commenced September 2021.

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