Telomere biology disorders are a heterogeneous group of diseases arising from germline mutations affecting genes involved in telomere maintenance. Telomeres are DNA-protein structures at chromosome ends that maintain chromosome stability; their length affects cell replicative potential and senescence. A constellation of bone marrow failure, pulmonary fibrosis, liver cirrhosis and premature greying is suggestive, however incomplete penetrance results in highly variable manifestations, with idiopathic pulmonary fibrosis as the most common presentation. Currently, the true extent of TBD burden is unknown as there is no established diagnostic criteria and the disorder often is unrecognised and underdiagnosed. There is no gold standard for measuring telomere length and not all TBD-related mutations have been identified. There is no specific cure and the only treatment is organ transplantation, which has poor outcomes. This review summarises the current literature and discusses gaps in understanding and areas of need in managing TBD.

**MECHANISMS/PATHOPHYSIOLOGY**

**Telomere biology**

Telomeres are crucial for chromosomal stability and protect them from deterioration during mitosis or aberrant fusion with neighbouring chromosomes. They address the “end-replication” problem that describes DNA loss and progressive chromosomal shortening with each cell division. Linear DNA and broken chromosomes are unstable and have a propensity to fuse. Critical telomere shortening or broken chromosomes, thus, result in aberrant recombination with end-to-end fusions and “breakage-fusion-bridge” cycles, which are termed “crisis”. Crisis is characterised by replicative senescence, chromosome end-to-end fusions and extensive apoptosis, causing unevolve derivative chromosomess and genomic instability.

The telomere structure may result in DNA damage responses recognising their ends as double-stranded DNA breaks and various mechanisms help to maintain its integrity. Telomeres have a stabilising “D-loop-T-loop” configuration; the T-loop is formed by insertion of the single-stranded G-strand terminus into the double-stranded telomere sequence, it displaces the sequence strand of the duplex telomeric DNA and forms the D-loop at the point of insertion. Telomeres are protected by shelterin, which is a protein complex that generates and stabilises the “T-loop” structure, and facilitates and regulates telomere elongation. It comprises six subunits, namely, telomere repeat-binding factor 1 (TRF1), telomere repeat-binding factor 2 (TRF2), repressor/activator protein 1 (RAP1), TRF1-interacting nuclear protein 2 (TIN2), TIN2-interacting protein 1 (TPP1) and protection of telomeres 1 (POT1). TRF1, TRF2 and POT1 localise the telomeric sequence, while TIN2, TPP1 and RAP1 form a complex that distinguishes telomeres from sites of DNA damage. Ku is a DNA-end-binding heterodimer involved in DNA repair, but also associates with telomeres and protects them from recombination and degradation, regulates telomere addition and maintains telomere length.

Telomerase is a ribonucleaseprotein complex that adds telomere repeats to chromosomal ends and maintains TL. Its essential components are telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC).

**INTRODUCTION**

Telomeres are noncoding repeats of the TTAGGG nucleotide sequence at chromosomal ends that provide genomic stability. They shorten with each cellular division, triggering senescence and apoptosis when critically short. Genetic mutations affecting telomere homoeostasis, thus, result in disorders of premature aging, with various manifestations like bone marrow failure, pulmonary fibrosis, liver cirrhosis, premature greying and increased cancer risk. Various terms such as short telomere syndrome, telomeropathies and telomere biology disorders (TBD) have been employed to describe these diseases; in this review, we use the term TBD as it reflects the spectrum of the disorders and pathophysiology.

The archetypal TBD is dyskeratosis congenita (DC), which was first described by Zinsser in the early twentieth century as a mucocutaneous triad of skin pigmentation, nail dystrophy and oral leukoplakia, with other systemic features identified later. DC accounts for less than 5% of all TBD and has an estimated incidence of 1 in 1,000,000. It is estimated that 41% of familial haematologic and interstitial lung disease (ILD) and 3% of familial haematologic disorders are due to TBD, however the overall incidence is unknown. There is no specific cure and the only treatment is organ transplantation, which has poor outcomes. This review summarises the current literature and discusses gaps in understanding and areas of need in managing TBD.

**REFERENCES**

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(TERT) and an RNA component (TERC), which provides the template for nucleotide addition\(^1\). Immature TERC is non-functional with short extended oligoadenylated forms\(^{25}\). Its maturation involves PARN, which removes oligoadenylated tails that mark nuclear RNAs for exosome-dependent degradation\(^{25}\). In contrast, PAPDS oligoadenylates immature TERC and thus targets it for exosome degradation\(^{26}\). The nuclear exome targeting (NEXT) complex directs a subset of noncoding RNAs that mark nuclear RNAs for exosome-dependent degradation\(^{25}\). Further genetic mutations have yet to be identified.

Telomere length influences and effects

The Hayflck limit describes the fixed number of cell replications prior to senescence and apoptosis, which is triggered by short telomeres\(^{1,3}\). TL thus reflects somatic cell replicative history and life span, with shorter TL correlating with increasing age\(^{36,37}\). At birth, the average leucocyte TL (LTL) is estimated to be 10 kb and attrition rate ranges from 27 to 41 base pairs per year\(^{37,38}\). The factors affecting TL and attrition are complex, but genetics has significant influence. Telomeres are longer in individuals with African ancestry and LTL attrition in Down’s syndrome is thrice that of age-matched controls\(^{36,37}\). Although TL and variants in non-telomeric regions related to telomere maintenance are hereditable, variable local gene expression across different cell lines results in varying TL within an individual\(^{26,36,37,39,40}\). For example, tests have longer TL than other cell types and higher TERT expression, while cells with a higher rate of turnover like blood and lung epithelial cells, have a shorter TL\(^{36}\).

Variation in TL is also demonstrated between twins and family members, suggesting that extrinsic factors also affect TL and attrition\(^{36,39,40}\) (Fig. 3). Some of these include obesity, cigarette smoking and environmental exposures\(^{36,38,40}\). Exposures and chronic inflammatory states like obesity and recurrent infection, result in reactive oxygen species generation and DNA damage, leading to shorter telomeres and accelerated cellular aging\(^{38,40,41}\). Short telomeres are implicated in the degenerative defects of aging and carcinogenesis; however the exact mechanisms have not been fully delineated yet\(^{4,8}\).

**CLINICAL PRESENTATION**

**Childhood onset syndromes**

DC is the best described childhood TBD, it is a syndrome of inherited bone marrow failure with 80–90% developing aplastic
anaemia (AA) or bone marrow failure by age 30. Inheritance may be X-linked recessive, AD, or AR, but sporadic cases also occur. Although patients classically develop mucocutaneous manifestations and anaemia in adolescence, the severe X-linked form may present within the first decade of life, and presentation may be at any age (Fig. 4). There may be multisystem involvement; up to 20% develop pulmonary fibrosis, and other traits include liver disease, premature greying of hair, oropharyngeal candidiasis, and haematological malignancies. The most common cause of death is bone marrow failure, followed by pulmonary complications and malignancy.

The diagnostic criteria for DC are summarised in Table 2. The severe forms of DC, Hoyeraal–Hreidarsson Syndrome (HHS) and Revesz Syndrome (RS) often present in infancy, and patients often do not survive long enough to develop all the syndromic features or mucocutaneous manifestations. HHS presents with cerebellar hypoplasia, immunodeficiency, progressive bone marrow failure and intrauterine growth retardation. RS has the additional traits of Coats-like retinal vasculopathy, although cerebral calcifications are often present. More recently, Coats plus, which overlaps with RS, has been described and is characterised by exudative retinopathy, gastrointestinal vascular ectasia and leukoencephalopathy.

**Pulmonary manifestations**

Idiopathic pulmonary fibrosis (IPF) is the most common TBD manifestation. It is a disease of progressive lung fibrosis with a deteriorating disease course and median survival of 3 years. It has a prevalence of 1.25–23.4 per 100,000 in Europe. IPF commonly affects male smokers over 60 and typically has a usual interstitial pneumonia (UIP) pattern on chest high-resolution computed tomography (HRCT) or lung biopsy in the appropriate clinical context.

At least 30% of patients with sporadic or familial pulmonary fibrosis have genetic predisposing factors that are known to increase the risk of pulmonary fibrosis. TERT and TERC mutations account for 8–15% of familial and 1–3% of sporadic IPF, while other telomere-related mutations like NAF1, PARN, RTEL1, DKC1, TIN2 and ZCCHC8 account for 1–3% of familial cases. FIP presents earlier with a more aggressive disease course than sporadic cases, and radiological and histological heterogeneity exists even within a pedigree. Non-specific interstitial pneumonia (NSIP), cryptogenic organising pneumonia (COP), respiratory bronchiolitis-interstitial lung disease (RB-ILD), unclassifiable ILD and CPFE have also been described in FIP.

TL below the 10th percentile of age-matched controls are found in sporadic and familial IPF with no telomere-related mutations. Other sporadic forms of ILD have also been found to have shorter TL below the 10th percentile of age-matched controls are found in sporadic and familial IPF with no telomere-related mutations. Other sporadic forms of ILD have also been found to have shorter

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**Fig. 2 Telomere and telomerase complex components and their associated diseases.** Telomeres are noncoding tandem repeats of the sequence TTAGGG, found at the ends of chromosomes in a duplex “D-loop-T-loop” configuration. RTEL1 is a helicase that disrupts T-loops for telomere replication and repair. Shelterin protects telomeres from DNA damage surveillance and comprises of six polypeptide components: TRF1, TRF2, RAP1, TIN2, TPP1 and POT1. Telomerase comprises of the essential components TERT and TERC, which synthesise telomeres and maintain telomere length. Dyskerin forms a complex with NHP2, NOP10 and GAR1. It binds to TERC to stabilise the telomerase complex. TCAB1 regulates the recruitment of telomerase to telomeres. Mutations in any of these components results telomere dysfunction, manifesting as telomere biology disorders. DC, dyskeratosis congenita; GAR1, H/ACA ribonucleoprotein complex subunit 1; HHS, Hoyeraal–Hreidarsson Syndrome; IPF, idiopathic pulmonary fibrosis; NHP2, H/ACA ribonucleoprotein complex subunit 2; NOP10, H/ACA ribonucleoprotein complex subunit 3; POT1, protection of telomeres 1; RAP1, repressor/activator protein 1; RTEL1, regulator of telomere length 1; TCAB1, Telomerase Cajal body protein 1; TERT, telomerase reverse transcriptase; TERC, telomerase RNA component; TIN2, TRF1-interacting nuclear protein 2; TPP1, TIN2-interacting protein 1; TRF1, telomere repeat-binding factor 1; TRF2, telomere repeat-binding factor 2.
More recently, pulmonary arteriovenous malformations (AVM) have been described in patients with DC and TBD even in the absence of hepatopulmonary syndrome (HPS), another known manifestation of TBD53.

### Haematological manifestations

Bone marrow failure from AA or myelodysplastic syndrome (MDS) may occur as an isolated manifestation of TBD, most commonly due to TERT or TERC mutations5,6,20,37,54. TBD cases who first present with AA are younger than those who first present with IPF, suggesting that AA is a more severe phenotype5,7,54. Telomerase-related mutations account for 3–5% of AA and TERC mutations have more severe disease; they also have significantly shorter LTL compared to idiopathic and secondary cases of AA7,54. A short LTL correlates with poorer outcomes in AA and MDS, including higher rates of transformation to acute leukaemia, poorer survival and higher rates of non-relapse mortality after transplantation55,56.

Other haematological conditions described in TBD include macrocytosis, isolated cytopenias, paroxysmal nocturnal haemoglobinuria and essential thrombocythaemia5,6,8,15,20,54 (Fig. 5). Immunodeficiencies like hypogammaglobulinemia, lymphopenia

| Gene mutation | Product | Inheritance | Disease and phenotype | Frequency | Comment |
|---------------|---------|-------------|-----------------------|-----------|---------|
| TERT5,7,17,47,50,54,59 | TERT | Autosomal dominant, Autosomal recessive | DC, IPF FIP, AA, MDS, Familial Liver Cirrhosis, DC, HHS | 15–25% | TERT and TERC mutations are the most common TBD mutations and present later in life. |
| TERC6,6,20,47,50,54,59 | Telomerase RNA component | Autosomal dominant | DC, IPF FIP, AA, MDS, Familial Liver Cirrhosis | Rare | TERT and TERC mutations are the most common TBD mutations and present later in life. |
| DKC110,48 | Dyskerin | X-linked recessive | DC, HHS, IPF FIP | 15–25% | Described in severe X-linked DC, which presents in the first decade of life |
| RTEL19,29,50 | RTEL1 | Autosomal recessive, Autosomal dominant | DC, HHS, IPF FIP | Rare, Rare | – |
| TINF216,49 | TIN2 | Autosomal dominant | DC, HHS, RS FIP | Rare, Rare | Results in very short telomeres. Presentation ranges from milder disease (e.g., FIP) to early-onset severe diseases (e.g., HHS, DC). Only mutation identified with RS |
| PARN11,29 | PARN | Autosomal dominant, Autosomal recessive | IPF FIP, DC, HHS | 5% | Biallelic and monoallelic variants are associated with DC, HHS, and IPF, respectively |
| ZCCHC828 | ZCCHC8 | Autosomal dominant | FIP | Rare | – |
| NAF1 | NAF1 | Autosomal dominant | IPF FIP, CPFE | <1% | – |
| NOP113 | NOP10, NHP218 | Autosomal recessive | DC | Rare | CTC1 has been described in Coats’ plus |
| WRAP5322 | TCA81, CTC112,35 | Autosomal dominant | DC | Rare | |
| ACD14 | TPP1 | Autosomal dominant, Autosomal recessive | AA, DC, HHA | Rare | – |

References are in numerical superscript.
5Dyskeratosis congenita.
Idiopathic pulmonary fibrosis.
Familial interstitial pneumonia.
Aplastic anaemia.
Myelodysplastic syndrome.
Hoyeraal–Hreidarsson syndrome.
Revesz syndrome.
Combined pulmonary fibrosis and emphysema.
Acute myeloid leukaemia-myelodysplastic syndrome.
Increased cancer risk due to failure of underlying immune dysfunction and accelerated immunosenescence increased cancer risk due to failure of cancer surveillance and contribute to poor tolerance of immunosuppression.

**Hepatobiliary manifestations**

Up to 40% of patients with TBD have liver involvement. Manifestations include transaminitis, non-alcoholic steatohepatitis, nodular regenerative hyperplasia, cryptogenic liver cirrhosis, noncirrhotic portal hypertension and HPS (Fig. 5).
findings include inflammatory and fibrotic components, hepatic nodular regeneration, cirrhosis and idiopathic hemosiderosis. HPS in TBD typically presents during the first four decades of life, which is earlier than that for pulmonary fibrosis: thus when younger TBD patients present with breathlessness, HPS should be evaluated for, particularly in the absence of pulmonary fibrosis.

**Table 2. Summary of dyskeratosis congenita diagnostic criteria.**

| Diagnosis | Criteria |
|-----------|----------|
| DC* | 1. All three mucocutaneous features:  
| | a. Abnormal skin pigmentation  
| | b. Nail dystrophy  
| | c. Leukoplakia  
| | 2. At least 1 out of 3 mucocutaneous feature,  
| | combined with bone marrow failure and 2 other somatic features of DC  
| | 3. Aplastic anaemia, myelodysplastic syndrome  
| | or pulmonary fibrosis associated with a pathogenic telomerase variant  
| | 4. 2 or more features seen in DC associated  
| | with very short telomeres that are below 1st centile. |
| Hoyeraal–Hreidarsson Syndrome | Four or more features of:  
| | 1. Growth retardation  
| | 2. Developmental delay  
| | 3. Microcephaly  
| | 4. Bone marrow failure  
| | 5. Immune deficiency  
| | 6. Cerebellar hypoplasia |

*Dyskeratosis congenita. References are in numerical superscript.

**Shorter TL have been found in non-alcoholic steatohepatitis, which is postulated to make them vulnerable to a “second hit”**. Besides telomere-related germline mutations, chronic cell injury and exposures to hepatotoxic drugs and alcohol may accelerate telomere attrition, contributing to progression to liver fibrosis. Similarly, shorter telomeres, TERC and TERT promoter mutations are linked to the pathogenesis of various hepatobiliary tumours including hepatocellular carcinoma.

**DIAGNOSIS**

**Measuring telomere length**

Most methods that are currently employed measure the average TL. Currently, there is no common reference or gold standard method to measure and trend TL. Furthermore, TL measured using different methods do not correlate well. These factors limit objective comparisons between different cohorts and changes in TL within an individual over time.

The earliest method used to measure TL is terminal restriction fragment (TRF) analysis by Southern blot, which measures the average TRF. TL is calculated by comparing the electrophoresis-separated TRF against known molecular weight markers. This is time consuming and requires substantial amounts of DNA. Furthermore, it overestimates TL as subtelomeric DNA is included in the measurement.

Quantitative polymerase chain reaction (qPCR) is most commonly used. It involves using two primer pairs, one targeting telomere repeats (T) and another targeting a known single-copy gene (S). The ratio between the T and S amplification products (T/S ratio) is calculated and this correlates with TL; the relative difference in T/S ratio between samples is proportional to the...
relative difference in TL. Although it does not require a large amount of starting DNA, it does not give an absolute TL value and there may be substantial variation between cohorts due to different single-copy loci used.

Quantification fluorescence in-situ hybridisation (Q-FISH) has several variations that have been used. Q-FISH quantifies the fluorescence intensity after hybridisation with a nucleic acid telomeric repeat, which is then compared against a reference population for a comparative assessment of TL. Flow-FISH is a commonly used variation that can be used for clinical purposes. It combines FISH with flow cytometry, using labelled peptide nucleic acid probes, which hybridise to telomeric repeats in cells and allows measurement of TL in specific cell subpopulations. Q-FISH results are reproducible but it is labour intensive.

Some methods used in research settings include Single-Telomere Length Analysis (STELA), which uses a single-molecule PCR to generate highly accurate TL measurements on individual chromosomes, and Telomere Shortest Length Assay (TeSLA), which uses a single-molecule assay. These methods are labour intensive, and low throughput. The methods available to measure TL are summarised in Table 3; the selection of the test would also depend on the purpose as large-scale population-based studies would have different requirements from clinical or mechanistic ones.

Diagnosing TBD

Recognising and diagnosing TBD can be challenging due to the spectrum of presentations (Fig. 4). Heterogeneity within a pedigree exists due to incomplete penetration, anticipation, and the influence of TL on disease severity. Environmental insults like smoking may result in genetically predisposed individuals developing IPF earlier in life than nonsmokers, or the use of immunosuppression may result in increased susceptibility to bone marrow failure. In addition, TERC and TERT mutations may present as isolated AA or IPF and be regarded as sporadic. Furthermore, not all mutations have been identified and some cases thus remain genetically uncharacterised.

Diagnostic criteria based on the various systems such as pulmonary, haematological and liver should be defined to help facilitate recognition of TBD. A thorough family history and evaluation of other involved organ systems should be considered and we propose a possible schema to guide clinicians (Fig. 6).

MANAGEMENT

Transplantation and immunosuppression

There is no specific treatment for TBD and only transplant is curative. However, immunosuppression is often poorly tolerated and outcomes are worse in TBD. Shorter TL correlate with reduced survival and a faster onset of severe chronic lung allograft dysfunction in pulmonary fibrosis patients who undergo lung transplantation. TBD lung transplant recipients also have a shorter median survival ranging 214 days to 1.9 years compared to non-TBD recipients. Solid organ transplant recipient with TBD are at higher risk of complications such as severe cytopenias, higher infection rates, bone marrow failure, renal complications and calcineurin inhibitor toxicity.

Furthermore, myeloablative regimens used in bone marrow transplantation for TBD are associated with poorer survival and high rates of pulmonary, liver and endothelial related complications, including unusual complications like veno-occlusive disease. Although non-myeloablative fludarabine-based regimens have fewer complications and allow for successful engraftments, TBD recipients may still develop complications in organs that were unaffected prior to transplantation.

To date, seven liver transplants in TBD are reported with decompensated liver cirrhosis as the most common indication. A combined liver and lung transplant has also been performed for noncirrhotic hepatopulmonary syndrome in end-stage pulmonary fibrosis. Although there is now more experience in performing transplantation in TBD, it remains high risk and poses challenges. Further experience is needed to draw conclusions longer term outcomes of transplant in TBD, particularly with regards to liver and combined organ transplantations.

Therapeutics

Androgen hormonal therapy. Androgens have been shown to improve blood counts, transfusion dependence and liver fibrosis, while also stabilising lung function in TBD patients. Although androgens directly increase TERT transcription and normalise telomerase activity in mouse models, effects on TL in clinical studies is mixed, with some demonstrating improvement while others have attrition rates similar to placebo group.

Further studies are still required before danazol can be routinely used in TBD and potential adverse effects should be considered. Common complications include transaminitis, muscle cramps, lipid abnormalities and virilisation, while rare complications such as splenic peliosis and rupture have been described with concomitant granulocyte colony-stimulating factor (GCSF) use.

Non-transplant treatment in IPF. Treatment options are very limited for patients with IPF and currently only the antibiotics pirenidone and nintedanib are approved. They retard the rate of forced vital capacity deterioration by 50% a year and pooled analysis suggests mortality benefit, but they are non-curative and do not improve symptoms nor quality of life. They are used to treat FIP; however patients have a persistently more aggressive trajectory compared to sporadic IPF and lung transplantation should be explored early in suitable patients.

Non-transplant treatment for bone marrow failure. In acquired severe aplastic anaemia where transplant is not suitable, immunosuppression with the use of growth factors like GCSF, erythropoietin and eltrombopag can improve treatment response and accelerate count recovery. GCSF does not improve trilineage response or overall survival, and potentially increases the risk of secondary clonal disorders like AML and MDS. Concomitant use of GCSF with danazol should be used cautiously or avoided if possible due to the risk of splenic peliosis and rupture.

Eltrombopag has been shown to restore haematopoiesis even after discontinuation, however further study is needed before this can be used as monotherapy in severe aplastic anaemia.

MDS should be managed according to risk stratification, lower risk patients may be considered for immunosuppression, thrombopoiesis-stimulating agents or DNA-hypomethylation agents such as azacitidine. In higher risk non-transplant candidates, DNA-hypomethylation agents may be used as tolerated. Supportive treatments like blood transfusions alleviate symptoms such as fatigue, and antimicrobial prophylaxis reduces the risk of opportunistic infections.

Non-transplant treatment of liver cirrhosis. Advanced liver cirrhosis is often complicated by portal hypertension with sequelae like ascites, oesophageal varices, and HPS. This may lead to fatal gastrointestinal tract bleeding and multi-organ failure. Medications like non-selective beta-blockers to reduce portal venous pressures and endoscopic interventions such as endoscopic band ligation to reduce variceal bleeding risk are often required. Ascites is the most common cause of decompensated liver cirrhosis and a moderate sodium-restrictive diet with diuretics should be prescribed; large volume paracentesis may be required.
Table 3. Summary of methods to measure telomere length.

| Method          | Principle                                                                 | Analyte            | Results                        | Strengths                                                                 | Limitations                                                                 |
|-----------------|---------------------------------------------------------------------------|--------------------|--------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| TRF<sup>61,63</sup> | DNA fragments are visualised by Southern blot following digestion, and then compared against DNA ladder. | DNA (0.5–10 µg)    | Average TL<sup>k</sup>         | ■ Reproducible<br■ Gives direct measurement of TL<br■ Large amount of DNA required.<br■ Subtelomeric polymorphisms impact data.<br■ Low hybridisation efficiency for very short telomeres.<br■ Risk of DNA degradation.<br■ Variation between labs (different restriction enzymes used). | ■ Large amount of DNA required.<br■ Subtelomeric polymorphisms impact data.<br■ Low hybridisation efficiency for very short telomeres.<br■ Risk of DNA degradation.<br■ Variation between labs (different restriction enzymes used). |
| qPCR<sup>b</sup> | qPCR: measures ratio between the telomere (T) and single-copy gene (S) amplification products. mmqPCR: Telomere DNA and single-copy DNA amplified in the same tube. aTLqPCR: Standard curve from known TL | DNA (<100 ng)       | T/S ratio<sup>i</sup> (relative quantification) | qPCR:<br■ Small amount of DNA required<br■ High-throughput<br■ Reduced human error related inaccuracy in qPCR<braTLqPCR:<br■ Standard single-copy gene reference | mmqPCR:<br■ Reduced human error related inaccuracy in qPCR<braTLqPCR:<br■ Standard single-copy gene reference |
| STELA<sup>c</sup> | STELA: Telomeric DNA amplified using subtelomeric specific primers. U-STELA: DNA digestion followed by specific amplification for short telomeric fragments | Cells (1–1 × 10<sup>5</sup>) | Chromosome-specific TL | Both:<br■ Small amount of material required.<br■ Does not require viable cells<br■ STELA: Detects shortest telomeres on specific chromosomes.<br■ U-STELA: Detects shortest telomeres from every chromosome | Both:<br■ Small amount of material required.<br■ Does not require viable cells<br■ STELA: Detects shortest telomeres on specific chromosomes.<br■ U-STELA: Detects shortest telomeres from every chromosome |
| TeSLA<sup>67</sup> | Southern blot with hypersensitive digoxigenin-labelled probes | DNA (<1 mcg)       | Chromosome-specific TL | Both:<br■ Measures very short TL<br■ Detects longitudinal changes in TL | Both:<br■ Less suitable for cells with long heterogeneous TL.<br■ Labour intensive.<br■ Low throughput. |
| Q-FISH<sup>66</sup> (Interphase Q-FISH; Metaphase Q-FISH) | Telomere fluorescent intensity visualised after hybridisation with (CCCTAA)<sub>3</sub> probe. | Interphase Q-FISH: Interphase cells (can be done on fixed tissues and cells)<brMetaphase Q-FISH: Actively dividing cells (15–20 metaphase chromosomes) | Interphase Q-FISH: Average TL measured as relative fluorescence unit<brMetaphase Q-FISH: Average TL, chromosome-specific TL (both measured as relative fluorescence unit) | Interphase Q-FISH:<br■ Provides telomere length and histological information.<br■ Higher resolution.<br■ Less labour intensive than Metaphase Q-FISH.<br■ High-throughput Q-FISH available.<br■ Does not require mitotically active cells<brMetaphase Q-FISH:<br■ Suitable for telomeres of various lengths.<br■ Recognises ‘telomere-free’ ends.<br■ Higher accuracy than Interphase Q-FISH. | Interphase Q-FISH:<br■ Unable to detect ‘telomere-free’ ends.<brMetaphase Q-FISH:<br■ Requires mitotically active cells.<br■ Does not detect telomeres that are very short and do not hybridise with the probe.<br■ Skilled expertise needed for analysis |
| Method        | Principle                                                                 | Analyte                        | Results                                                                 | Strengths                                                                 | Limitations                                                                                           |
|--------------|---------------------------------------------------------------------------|--------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| Flow FISH    | Combination of flow cytometry with hybridisation of pantelomeric (CCCTAA)$_3$ probe to cells in suspension | White blood cells (0.5–2 × 10$^6$) | Cell-specific average TL measured as relative fluorescent unit         | • Cells can be sorted into subpopulations. <br>• Provides 3D telomeric signals within cells. <br>• May be adapted for a higher throughput. | • Sensitive to cell types (mostly done on peripheral blood mononuclear cells). <br>• Challenging to process suspension cells. <br>• Non-specific binding of telomeric probe. <br>• Does not detect chromosome-specific individual TL or telomere-free ends. <br>• Labour intensive. <br>• Costly. |
| PRINS        | Labels telomeric sequences in-situ on metaphase chromosomes/interphase nuclei | 15–20 metaphase chromosomes    | Average TL and chromosome-specific TL (both are measured as relative fluorescence unit) | • Higher resolution <br>• Measures TL in specific cell types <br>• Can detect individual telomeres, telomere-free ends and average TL when used on metaphase chromosomes. | • Labour intensive. <br>• Relative quantification. <br>• Mitotically active cells required for metaphase chromosomes. |
| HPA          | Compares telomeric repeats and Alu repeats                                | 10–3000 ng DNA                 | Average TL                                                              | • Quick <br>• Small amount of DNA required                                                                 | Measures mean telomere length only <br>• Alu repeats in sample can vary                                 |

References are in numerical superscript.  
*Terminal restriction fragment.  
*Quantitative polymerase chain reaction.  
*Monochrome multiplex polymerase chain reaction.  
*Absolute telomere length quantitative polymerase chain reaction.  
*Single-telomere length analysis.  
*Universal single-telomere length analysis.  
*Telomere shortest length assay.  
*Quantitative fluorescence in-situ hybridisation.  
*Primed in-situ subtype of Q-FISH.  
*Hybridisation protection assay.  
*Telomere length.  
*Telomere repeat to single-copy gene ratio.
in symptomatic or massive ascites\textsuperscript{90}. Hepatotoxic drugs and alcohol should be avoided, and laxatives prescribed to prevent constipation, which may precipitate hepatic encephalopathy. Patients with recurrent hepatic encephalopathy may require rifaximin prophylaxis\textsuperscript{90}.

Potential new therapies and trials

Imetelstat is a 13-mer lipid-conjugated oligonucleotide that competitively inhibits telomerase and has been shown to inhibit cellular proliferation in tumour xenografts and cancer\textsuperscript{91}. A pilot study in myelofibrosis demonstrated that 21% achieved partial or complete remission, while another trial in advanced non-small cell lung cancer showed a trend towards improvement in progression-free and overall survival in patients with short telomeres\textsuperscript{92,93}. The main adverse effects were myelosuppression and transaminases\textsuperscript{92,93}. Further trials are underway to evaluate safety profile and efficacy.

Other investigational treatments include small molecule PAPD5 inhibitors, TA-65 and telomerase gene therapy\textsuperscript{94–96}. PAPD5 inhibitors like BCH001 and RG7834, demonstrated restoration of telomerase activity and TL in DC induced pluripotent stem cells and mouse models\textsuperscript{94}. TA-65 is a small molecule activator of telomerase that extended TL compared to placebo in healthy subjects\textsuperscript{95}. Telomerase gene therapy using adeno-virus-associated virus (AAV)9 gene therapy vectors has been shown to lengthen telomeres, improve blood counts and survival in mice with aplastic anaemia\textsuperscript{96}. However, further studies are needed to evaluate safety and outcomes before these can be translated to further trials.

Quality of life

Early palliation should be commenced in TBD patients not suitable for transplant. Common areas of need identified in patients with bone marrow failure, IPF and advanced liver disease include improving quality of life through management of symptoms such as fatigue, dyspnoea and pain, psychological support, advanced care planning, and access to resources like specialised care and hospice services\textsuperscript{97–99}. The disease burden in patients with single organ disease is high and may compounded in those with multisystem involvement.

Genetic counselling and testing

TBD is rare and complex with limited treatment options. A certain level of genetic literacy is thus needed for patients to make challenging informed decisions and share information with relatives\textsuperscript{100}. Furthermore, inaccurate informal sources may further hinder understanding\textsuperscript{100}. Although genetic counselling and patient education initiatives improve knowledge, surveyed patients still answer less than 60% of disease-specific and general genetic knowledge questions correctly when assessed, suggesting patient education has further room for improvement\textsuperscript{100}.

In suspected cases of inherited bone marrow failure syndrome, other conditions to consider besides TBD would include Diamond-Blackfan anaemia, Fanconi anaemia and Shwachman–Diamond syndrome\textsuperscript{15}. IPF cases are not routinely offered genetic counselling unless they present young and have features suggestive of FIP, due to a low pretest probability\textsuperscript{83,87}. Patients with short TL should be offered screening for TBD, while those with a personal or family history of early-onset lung malignancy or disease, should be counselled for surfactant-related mutation screening\textsuperscript{85} (Fig. 6). A thorough assessment should be done prior to offering genetic testing with appropriate counselling on the risks and benefits, as a confirmatory diagnosis has far-reaching implications.

OUTLOOK

Since DC was first described, there have been significant developments in telomere biology and its implications on senescence and disease pathogenesis. TBD is a syndrome of premature aging and the prototype for degenerative diseases, as telomeres shorten with age. It is likely the most common monogenic premature aging disorder and identifying its syndromic nature has important clinical implications as seemingly sporadic diseases share a common pathways of telomere dysfunction. Its role in more complex pathogenesis such as carcinogenesis, suggests that genotype–phenotype correlations extend beyond what is currently understood. Other mechanisms...
of telomere dysfunction and mutations will likely be identified with further genotype-phenotype correlations.

Future research would include standardisation of TL measurement methods and benchmarking TL across different cell types and ages. This would allow for comparison of different study results and establishing a gold standard for clinical testing, where TL potentially could have utility as a biomarker of disease predisposition, prognosis and treatment response.

Establishing clinical and genetic criteria for TBD diagnosis would be invaluable for risk stratification and genetic counselling. Increased awareness and education of medical practitioners can help early recognition and facilitate diagnosis. Multidisciplinary genetics clinics with genetic counselling and support services would also help improve care. Early advanced care planning should be initiated with open discussions to explore patients’ ideas, concerns, and expectations so that appropriate supportive care is received in a timely manner.

Regarding therapeutics, androgen treatment and novel therapies targeting telomere homoeostasis pathways are areas for development. These will require further trials before they are suitable for clinical use. If successful, given the involvement of telomere dysfunction in various disease pathways, such treatments could potentially be used to treat other diseases beyond TBD that are currently incurable.

Although various aspects of care discussed in this review are regarded as standard of care in TBD, in many parts of the world, these services and enrolment into trials may not be available or easily accessible to patients. Greater awareness of TBD is needed to improve overall care of patients with this disease.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

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AUTHOR CONTRIBUTIONS
M.L.W.K. and J.Y.Y.N. conceptualised the idea. M.L.W.K. prepared the manuscript. All authors contributed to the design and wrote the paper. M.L.W.K. and T.T.T.N. conceptualised and created the figures and tables. J.Y.Y.N. provided critical review of the manuscript. All authors approved the manuscript for this submission and agree to be accountable for all aspects of the work.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION

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