

## Review

## Telomere biology disorders: time for moving towards the clinic?

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**Telomere biology disorders (TBDs) are a group of rare diseases caused by mutations that impair telomere maintenance. Mutations that cause reduced levels of TERC/hTR, the telomerase RNA component, are found in most TBD patients and include loss-of-function mutations in hTR itself, in hTR-binding proteins [NOP10, NHP2, NAF1, ZCCHC8, and dyskerin (DKC1)], and in proteins required for hTR processing (PARN). These patients show diverse clinical presentations that most commonly include bone marrow failure (BMF)/aplastic anemia (AA), pulmonary fibrosis, and liver cirrhosis. There are no curative therapies for TBD patients. An understanding of hTR biogenesis, maturation, and degradation has identified pathways and pharmacological agents targeting the poly(A) polymerase PAPD5, which adds 3'-oligoadenosine tails to hTR to promote hTR degradation, and TGS1, which modifies the 5'-cap structure of hTR to enhance degradation, as possible therapeutic approaches. Critical next steps will be clinical trials to establish the effectiveness and potential side effects of these compounds in TBD patients.**

**Telomeres and telomerase are essential for tissue homeostasis**

Telomeres are repetitive DNA sequences that cap chromosomal ends and are composed of an array of double-stranded TTAGGG repeats followed by a single-stranded 3' overhang of 40–500 nt in length [1–3]. Telomeres are protected by shelterin, a six-protein complex that prevents telomeres from being recognized as DNA double-stranded breaks, through a conformational change in telomeric DNA where the 3' overhang folds back to form a telomere (t)-loop structure (Figure 1A).

Although this complex biochemical structure is essential to prevent erroneous activation of DNA damage responses at chromosomal ends [4], it also makes the replication of DNA ends a challenging process (recently reviewed [5]). In fact, telomeres shorten by up to 200 bp at each cell division, due to the inability of replicative DNA polymerases to fully extend the lagging strand of DNA replication at telomeres [6]. Telomeres therefore become progressively shorter over time, and, once a critical length is reached, cellular senescence is triggered [7,8].

In cells with high proliferative potential, such as germline and somatic stem cells, this DNA 'end-replication' problem is solved by **telomerase** (see **Glossary**), a ribonucleoprotein complex composed at its core by TERT (the telomerase reverse transcriptase component) and hTR (the telomerase RNA component) that serves as the template for the addition of telomeric repeats to the ends of linear chromosomes [6,9]. Although these core components are sufficient for telomerase activity *in vitro*, the assembled telomerase complex *in vivo* is far larger, and includes the RNA-binding proteins DKC1, NOP10, NHP2, GAR1, and TCAB1 (Figure 1B). These components are assembled through direct or indirect binding to hTR, thereby increasing the stability of the hTR RNA and increasing telomerase activity *in vivo*. Several other proteins interact transiently with

**Highlights**

Mutations in genes associated with telomere maintenance occur in telomere biology disorders (TBDs). These poorly treated disorders are characterized by multisystemic phenotypes including bone marrow failure/aplastic anemia, pulmonary fibrosis, liver cirrhosis, and increased cancer incidence.

Mutations that cause reduced levels of TERC/hTR, the telomerase RNA component, are found in most TBD patients and include loss-of-function mutations in hTR itself, in several hTR-binding proteins, and in proteins required for post-transcriptional hTR processing. These mutations culminate in increased hTR 3'-end oligo-adenylation by the non-canonical poly(A) polymerase PAPD5, which triggers exonucleolytic decay of the telomerase RNA component.

Recent evidence shows that genetic or chemical inhibition of PAPD5 can rescue telomere homeostasis and cellular functionality in both murine and human models of TBDs, indicating this could serve as a possible therapeutic approach for several TBDs.

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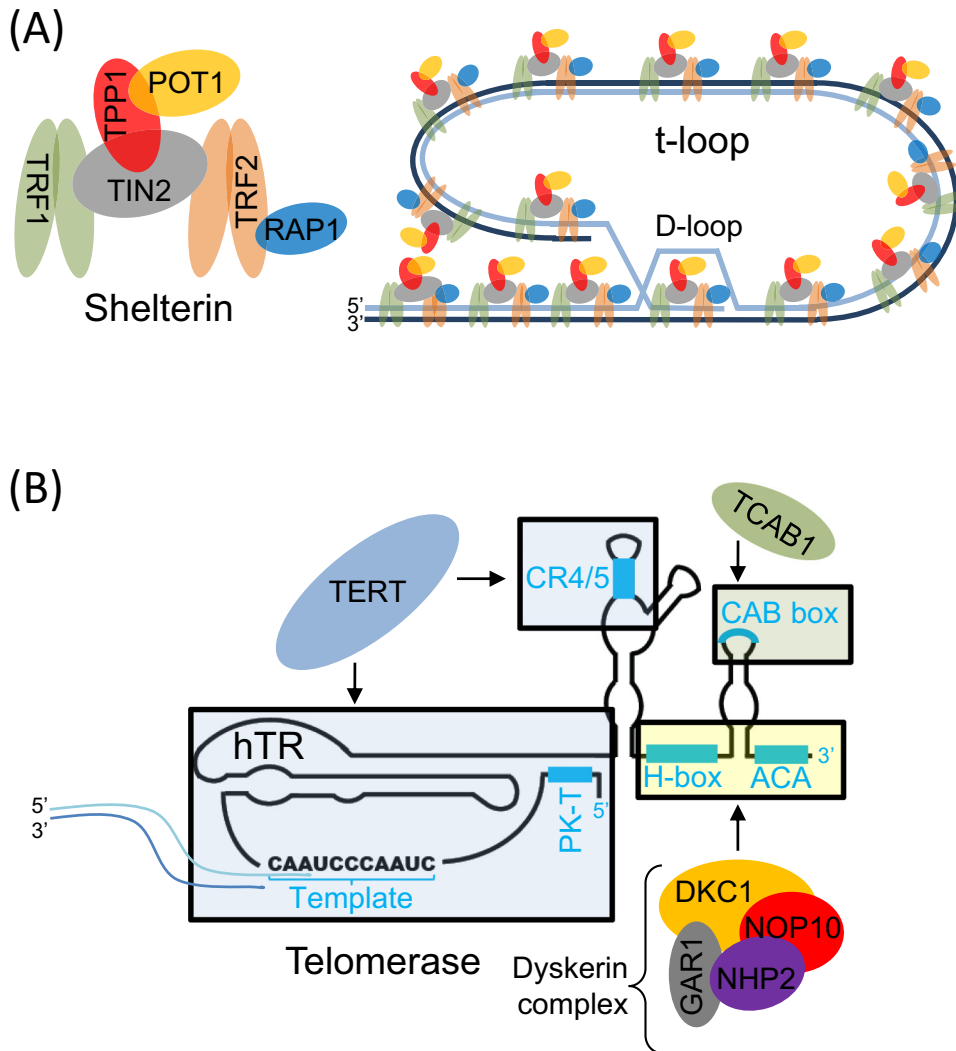
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**Figure 1. Shelterin and telomerase bind to telomeres.** (A) Depiction of the six-subunit shelterin complex (left) and its association with telomeric DNA (right). Shelterin components TRF1 and TRF2 (depicted as dimers) bind to double-stranded telomere DNA. RAP1 binds directly to TRF2, while TIN2 binds to both TRF1 and TRF2. TPP1 binds to TIN2 and to POT1, which also binds to the single-stranded 3' overhang. TPP1 also binds to TERT (telomerase reverse transcriptase component) and recruits telomerase to telomeres (not depicted in the figure). The formation of the telomere (t)-loop and displacement (D)-loop structures are shown, which help to repress activation of DNA damage responses at telomeres. (B) Depiction of the telomerase RNA component (hTR, black line) and its many binding partners that constitute the telomerase complex. Different domains of hTR are highlighted. TERT binds to hTR through the CR4/5 and template/pseudoknot domains. The dyskerin complex binds to hTR through the H/ACA domain, which contains a single-strand hinge (H-box), followed by a loop and the ACA sequence. The H/ACA domain contains the CAB box region to which TCAB1 binds. Finally, the template region of hTR also binds to the 3' end of telomeres. In (A) and (B), shelterin and telomerase components are not depicted to scale or abundance.

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telomerase and are necessary for its assembly, trafficking, and binding to telomeres (a review on telomerase assembly and function can be found in [10]). In metazoans, telomerase expression is primarily regulated by the expression of TERT, which in humans is mostly restricted to germline and somatic stem and progenitor cells [11]. As these cells are responsible for tissue repopulation and long-term viability [12], it is not surprising that telomerase activity is essential for continued

stem cell function and tissue homeostasis [13,14]. While the regulation of telomerase activity remains a complex issue, recent advances in our understanding of TERT expression [15], hTR stability [10], and the recruitment of telomerase to telomeres [3] could finally allow targeted modulation of telomere length in different cell types. Such knowledge could have broad implications for the regulation of physiological aging, or, as we will discuss in more detail here, in the clinical management of disorders where telomeres are exceptionally short.

### Telomere biology disorders

Given the importance of telomerase in somatic stem cells, germline mutations that impair telomere structure, replication, and telomerase function are linked to severe, widespread phenotypes and a shortened lifespan in patients [16–18]. First genetically characterized in 1998 in pediatric dyskeratosis congenita (DC) patients suffering from BMF [19], mutations that cause exacerbated telomere shortening are currently identified in different age groups and in patients suffering from different conditions. Collectively, these conditions are referred to as telomeropathies or TBDs [20].

TBDs commonly involve multiple organ systems and are associated with the premature loss of tissue-repopulating cells, leading to a reduction in the regenerative capacity of the organs affected [17]. This loss of tissue homeostasis is directly linked to activation of DNA damage responses from dysfunctional telomeres [21–26], and *in vitro* and *in vivo* experiments show that reactivation of telomerase activity efficiently restores tissue functionality [23,27]. In some families affected with TBDs, disease manifestations occur at an earlier age and with more severe phenotypes in successive generations, a phenomenon referred to as genetic anticipation [28]. Although a common phenotype associated with TBDs is BMF/AA, multisystemic defects are also usually found in patients. In this regard, DC is a pediatric BMF syndrome that is associated with a 'triad' of nail dysplasia, oral leukoplakia, and lacy reticular pigmentation [29].

Clinical presentations in DC can vary depending on age of diagnosis, mode of inheritance (autosomal dominant, AD; autosomal recessive, AR; X-linked; or *de novo*), and the specific genes mutated [30], and associated features typically include premature greying of hair, pulmonary fibrosis, emphysema, and liver fibrosis [29]. More severe pediatric manifestations of telomere shortening can be found in Hoyeraal–Hreidarsson syndrome (HHS), where patients also present with cerebellar hypoplasia, in Revesz syndrome, where patients typically present with associated retinal pathology, and in Coats plus, where patients show an array of neurological abnormalities that lead to slow growth, seizures, movement disorders, and decreasing intellectual function [17]. In cases of adult onset, the most common phenotype of TBDs is pulmonary fibrosis, but these can also present with AA and liver disease [31]. Patients with DC are also at an increased risk of developing cancer compared with the general population. The types of malignancies most commonly associated with TBDs are head and neck squamous cell carcinoma, myelodysplastic syndromes, and acute myeloid leukemia [32,33]. Several other severe complications can be found in these patients, ranging from gastrointestinal to immunologic abnormalities [33].

While enormous progress has been made into the genetics and the characterization of TBDs, these syndromes remain without a general cure [34]. Management of hematopoietic failure in TBDs is challenging. The use of **erythropoietin** and **granulocyte colony-stimulating factor (GCSF)** does not produce durable improvements in red cell and neutrophil counts [35]. Similarly, immunosuppressive therapy with **cyclosporin** and **anti-thymocyte globulin** does not yield a good response in DC patients [36], and is not a recommended approach. While **eltrombopag** has recently been given approval for therapy in patients with severe AA, the use of this small molecule is not recommended for patients with DC as case reports show no response to

### Glossary

**Anti-thymocyte globulin:** antibodies prepared from animals (horse or rabbit) immunized with human thymocytes, and which are used as an immunosuppressive therapy

**Cajal body:** a nuclear organelle that is enriched in small RNAs, including the telomerase RNA component hTR and its RNA-binding proteins.

**Cyclosporin:** a calcineurin inhibitor used clinically as an immunosuppressant.

**Eltrombopag:** a thrombopoietin receptor agonist used to promote platelet production.

**Erythropoietin:** a glycoprotein hormone that stimulates red blood cell production.

**Fludarabine:** an inhibitor of DNA polymerase  $\alpha$  that is used to treat chronic lymphocytic leukemia as well as part of bone marrow transplant conditioning regimes.

**Granulocyte colony-stimulating factor (GCSF):** a hormone that stimulates neutrophil production.

**Nuclear exosome targeting complex (NEXT):** recruits nuclear exosomes to RNAs for processing or degradation; nuclear exosomes are a 3' to 5' exonuclease degradative complex that can be recruited to noncoding RNAs by 3' oligo(A) tails.

**Poly(A) polymerases PAPD5/7:** polymerases that add oligo(A) tails to hTR and other noncoding RNAs to promote their degradation.

**Poly(A)-specific ribonuclease (PARN):** removes oligo(A) tails from hTR and other noncoding RNAs to protect them from degradation.

**RG7834:** a pharmacological inhibitor of PAPD5/7.

**Telomerase:** an enzyme complex that extends telomeres on chromosomes consisting of catalytic TERT protein and a template RNA (hTR).

**Trimethylguanosine synthase 1 (TGS1):** an enzyme that modifies the cap structure of mRNAs and hTR, which promotes RNA export from nucleus.

**ZCCHC8:** a component of the NEXT complex that when mutated can give rise to telomere biology disorder (TBD).

treatment [37]. Androgen therapy leads to multiple lineage rescue in a variety of BMF patients, and it has been described to elicit a good response in many DC patients. Danazol is now the preferred androgen in the treatment of DC [38]. It can produce very good clinical responses in ~70% of patients. The precise mechanisms of danazol action and its effects on telomere elongation remain unclear, but may be related to increased expression of TERT [33]. However, in a subgroup of patients the drug has to be discontinued because of toxicity [39].

Hematopoietic stem cell transplantation (HSCT) represents the only curative therapy for BMF/AA in TBDs, but the outcome of this procedure remains highly variable [40]. High morbidity and mortality were observed with conventional myeloablative HSCT approaches, and the use of reduced-intensity **fludarabine**-based conditioning regimens has led to significant progress in HSCT in DC patients. A comprehensive description of HSCT in TBDs is outside the scope of this article and can be found in [35]. Despite progress with current HSCT approaches, therapy for the extra-hematopoietic defects remains a challenge. A fundamental limitation is that TBDs often present as multisystem syndromes and HSCT does not correct the extra-hematopoietic abnormalities. Indeed, HSCT is usually only recommended in TBD cases with severe and progressive BMF who have minimal comorbidities [33]. Therefore, alternatives to HSCT are desirable; ideally, therapies capable of targeting the disease specific defect need to be developed and brought to the clinic.

### Telomerase RNA component: a central element in telomere biology disorders

Current understanding of the biogenesis and regulation of the telomerase complex, including the telomerase RNA component, identifies multiple perturbations that can cause TBDs and points to possible targets for therapeutic intervention. Mutations in several genes that regulate different aspects of telomere homeostasis are found in TBD patients [16]. These include mutations in shelterin, mutations in proteins involved in telomere replication, and mutations in the telomerase complex itself. Table 1 presents a summary of mutations in TBDs, and a comprehensive analysis of how these different mutations lead to patient phenotypes can be found in [20]. We focus here on mutations that impair telomerase function through a reduction in mature hTR levels caused by

Table 1. Genes associated with telomere dysfunction in human disease

Gene/product	Biological role	Associated diseases <sup>a</sup>
<i>ACD/TPP1</i>	Component of the shelterin complex	AA, DC
<i>CTC1/CTC1</i>	Component of the CST complex	DC, CP
<i>DKC1/Dyskeratin<sup>b</sup></i>	hTR stability; component of telomerase	AA, DC, PF
<i>NAF1/NAF1<sup>b</sup></i>	Component of the dyskerin complex	PF
<i>NOLA2/NHP2<sup>b</sup></i>	Component of the dyskerin complex	DC
<i>NOLA3/NOP10<sup>b</sup></i>	Component of the dyskerin complex	DC
<i>PARN/PARN<sup>b</sup></i>	hTR processing	DC, PF
<i>RTEL1/RTEL1</i>	Telomere replication	DC, PF
<i>RPA1/RPA1</i>	DNA replication/repair	DC, PF
<i>TERC/TR *</i>	Component of telomerase	AA, DC, PF
<i>TERT/TERT</i>	Component of telomerase	AA, DC, PF
<i>TINF2/TIN2</i>	Component of the shelterin complex	AA, DC
<i>WRAP53/TCAB1<sup>b</sup></i>	Component of telomerase	DC
<i>ZCCHC8/ZCCHC8<sup>b</sup></i>	hTR processing	PF

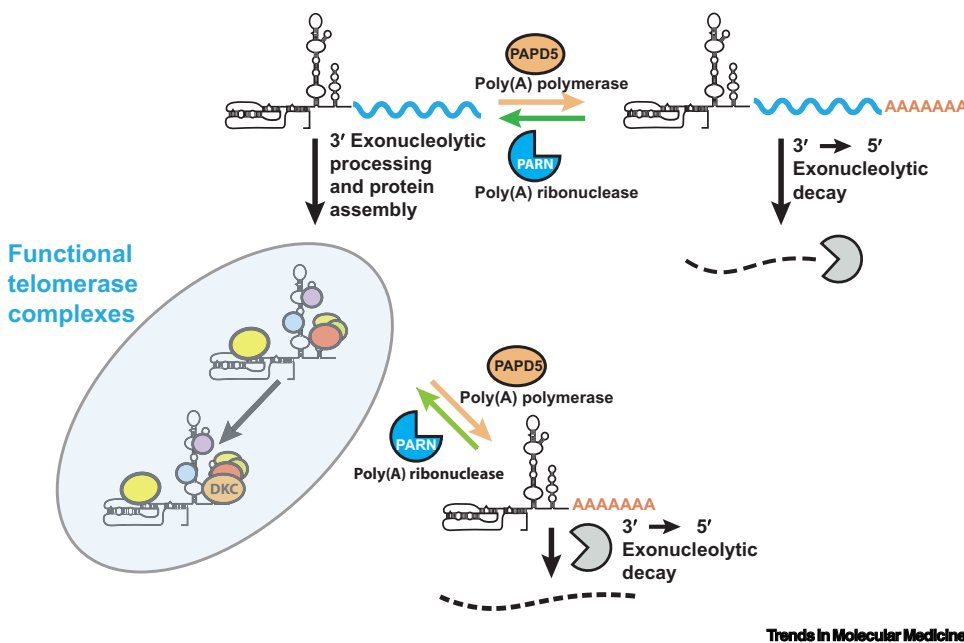
<sup>a</sup>Abbreviations: AA, aplastic anemia; CP, Coats plus; DC, dyskeratosis congenita; PF, pulmonary fibrosis.

<sup>b</sup>Mutations that impair hTR levels/function.

unbalanced maturation and degradation of these RNA molecules *in vivo*. These are the types of mutations where targeted pharmacological intervention now appears to be possible.

hTR is a small **Cajal body** RNA (scaRNA) and small nucleolar RNA (snoRNA) that contains an H/ACA domain [41]. This H/ACA domain guides the pseudouridylation of both ribosomal (rRNA) and small nuclear (snRNA) RNAs by the dyskerin complex (composed of DKC1, NOP10, NHP2, and NAF1) [42,43]. The interaction of hTR with the dyskerin complex is crucial for hTR stability and processing, and is thus essential for telomerase function [44,45]. Mutations in either hTR or members of the dyskerin complex that disrupt the hTR–dyskerin interaction are found in TBD patients (Table 1), and cause low telomerase activity and progressive telomere shortening in TERT-positive cells [20]. A key point is that these mutations destabilize hTR and that this is the pathogenic mechanism in patients harboring such mutations. This is demonstrated by the fact that overexpression of hTR in cells harboring mutations that prevent the stable assembly of hTR with the dyskerin pseudouridylation complex rescues telomerase activity, promotes telomere elongation, and improves cellular viability [46,47]. Mutations in other sites of hTR have also been found in TBD patients, including mutations in the pseudoknot domain (which contains the template region) and mutations in the CR4/5 domain which also disrupt the catalytic function of telomerase.

An understanding of hTR transcription, processing, and degradation has been crucial for the development of possible therapeutic interventions (Figure 2). hTR is transcribed by RNA polymerase II from its own promoter [48]. Although most mature hTR transcripts end at position 451 [49], hTR is initially transcribed as longer precursors (461–1500 nt in length) which are post-transcriptionally processed into the mature form by 3' to 5' exonuclease



**Figure 2. Telomerase RNA component (hTR) biogenesis and processing.** Nascent hTR contains 3' extensions (depicted in blue) that are polyadenylated by PAPD5, leading to their exonucleolytic decay by the exosome. Alternatively, 3' exonucleases can trim these 3' extensions, leading to the formation of mature hTR molecules. PAPD5 can also adenylate fully trimmed hTR molecules to promote their 3' to 5' degradation, which is increased in patient cells lacking dyskerin (DKC). Binding of hTR to the dyskerin complex (through its H/ACA domain) increases hTR stability. Binding to TCAB1 (through its CAB box domain) is necessary for telomerase trafficking to Cajal bodies (not shown), which aids in the formation of functional telomerase complexes.

trimming, as well as by oligo-adenylation of the 3' end of nascent transcripts by the noncanonical **poly(A) polymerase PAPD5** [50–52]. PAPD5-mediated polyadenylation of hTR 3' ends can also target hTR for degradation by the RNA exosome complex [50,51]. In turn, the recruitment of the RNA exosome complex is performed by the **nuclear exosome targeting complex (NEXT)** and the cap-binding complex (CBC) [53]. Working in opposition to PAPD5, **poly(A)-specific ribonuclease (PARN)** deadenylates hTR transcripts, preventing their degradation and leading to accumulation of mature, functional hTR transcripts [49–51].

This balance between hTR maturation and degradation is disturbed in TBDs patients harboring loss-of-function mutations in PARN. These patients show low levels of mature hTR, resulting in reduced telomerase activity and telomere shortening [51,54–56]. Similarly, patients harboring mutations in the NEXT component **ZCCHC8** were recently identified [57], where levels between immature and mature forms of hTR were also imbalanced. Mutations in ZCCHC8 cause significantly reduced levels of mature forms of hTR, leading to reduced telomerase activity and exacerbated telomere shortening [57].

Collectively, it becomes clear that correct hTR processing and stability is essential for telomerase function, and is impaired in a significant number of patients suffering from TBDs. A common consequence of defective RNA processing, or ribonucleoprotein (RNP) assembly, is degradation of the telomerase RNA, leading to TBDs. Thus, strategies to restore mature hTR levels represent an attractive target to restore telomere instability in these patients, which could lead to significant clinical improvement.

### Restoring hTR levels through PAPD5 inhibition as a novel approach against TBDs

The role of PAPD5 in adenylating hTR to promote its degradation by the exosome suggests that inhibition of PAPD5 might be an approach to restoring telomerase activity in TBDs where hTR levels are reduced. Importantly, genetic inhibition of PAPD5 by short hairpin (sh)RNAs in human cells harboring different mutations that affect hTR biogenesis (i.e., in DKC1 and PARN) leads to a reduction of 3' extended hTR molecules, as well as to an increase in the number of deadenylated, functional hTR molecules [49,50,58]. Moreover, inhibition of PAPD5 is followed by an increase in telomerase assembly and recruitment, as well as an increase in telomerase activity, in cells harboring pathogenic mutations that impair hTR levels [50,58]. This causes significant elongation of telomeres in these cells and a reduction in the activation of DNA damage responses (DDR), opening the possibility that inhibition of PAPD5 could mitigate the molecular trigger leading to tissue failure in TBDs.

As a major phenotype associated with hTR deficiency is impaired hematopoietic development that culminates in BMF, experiments were performed to determine whether PAPD5 inhibition could alleviate the hematopoietic deficit caused by telomerase dysfunction. Experiments with DKC1 mutant human embryonic stem cells (hESCs) determined that silencing of PAPD5 in these cells restores their *in vitro* hematopoietic differentiation capacity, through direct regulation of the 3'-end maturation of hTR [59]. Silencing of PAPD5 restored trilineage hematopoietic differentiation and increased myeloid, erythroid, and T cell cellularity. Of note, unlike the silencing of the exosome component 3 (EXOSC3), silencing of PAPD5 was not toxic to human hematopoietic cells [59], suggesting that pharmacological inhibition of PAPD5 could be a possible therapy for TBDs caused by reduced hTR levels. However, it remains to be determined whether the broad and persistent inhibition of PAPD5 could be toxic to different cell types, and, if so, to what extent this could impair tissue functionality in clinical settings.



PAPD5 is a good target for drug discovery. Since PAPD5 and its paralog **poly(A) polymerase PAPP7** are required for hepatitis B virus (HBV) infections [60], some biotech companies have developed inhibitors of PAPD5/7 which have proved effective in restoring hTR levels in relevant TBDs. Treatment with **RG7834**, a dihydroquinolinone that acts as a PAPD5 and PAPP7 inhibitor [61], culminated in a reduction of 3'-end oligoadenylation in hTR molecules in PARN and DKC1 mutant cells [62]. This was followed by increased mature levels of hTR in these cells, increased telomerase assembly, and higher levels of telomerase activity and telomere lengthening. Moreover, treatment with low concentrations of RG7834 rescued hematopoietic development of DKC1 mutant cells, leading to higher formation of erythroid and myeloid colonies [62]. These improvements were achieved with a modest increase in hTR levels (approximately twofold), suggesting a reasonable therapeutic window. In addition, pharmacological inhibition of PAPD5 (achieved with either RG8734 or BCH001) significantly reduced 3'-end oligoadenylation in PARN-mutant primary hematopoietic stem and progenitor cells (HSPCs), without detectable toxicity [63]. Finally, RG7834 rescued hTR 3'-end maturation over time in xenotransplantation experiments of PARN-mutant human CD34<sup>+</sup> cells into immunocompromised mice [63]. In these experiments, RG7834 was supplied orally over months through drinking water, and no adverse effects were reported [63].

These recent observations indicate that pharmacological inhibition of PAPD5 represents an exciting new therapeutic approach in TBDs, which currently remain without a cure. As TBDs are characterized by multisystemic defects, it will be important to determine the extent to which PAPD5 inhibition can also rescue functionality in other tissues that are frequently impaired in patients (i.e., it remains to be determined to what extent restoration of hTR levels is effective in slowly proliferative or postmitotic tissues). Similarly, deeper studies are necessary to identify other possible targets of PAPD5/7 in different cell types, and how these could influence tissue functionality or treatment. These are necessary studies that will also help to determine the safety and breadth of this approach before future clinical trials.

### TGS1 as alternative target for restoring hTR levels

A second approach to restoring hTR levels in relevant TBDs would be to target the enzyme trimethylguanosine synthase 1 which modifies the hTR RNA during biogenesis. Specifically, during the early stages of transcription, hTR molecules acquire a 5'-end m<sup>7</sup>G trimethylguanosine (MMG) cap, which is then further methylated to a N<sup>2</sup>,2,7-trimethylguanosine (TMG) cap by **trimethylguanosine synthase 1 (TGS1)** [64]. The formation of the TMG cap controls telomerase trafficking and accumulation, and inhibition of TGS1 increases hTR levels, possibly by restricting its localization to Cajal bodies and thereby preventing its degradation in the cytoplasm [10]. Ablation of TGS1 results in increased telomerase activity and telomere extension [65]. Interestingly, inhibition of TGS1 has also been shown to trigger different molecular markers of alternative lengthening of telomeres (ALT) in human cells, pointing to a possible dual role of TGS1 in telomere maintenance [66]. In fact, treatment of different human cell types with sinefungin, a compound that inhibits the methyltransferase activity of TGS1, leads to telomerase elongation in wild type and PARN-mutant settings [67]. Therefore, the chemical inhibition of TGS1 could represent another route for rescue of telomerase activity in a subset of TBD patients where hTR levels are disrupted, although how well inhibition of TGS1 can rescue the hematopoietic deficits remains to be determined. One possibility is that TGS1 inhibition could be combined with concomitant inhibition of PAPD5 for more robust restoration of telomerase RNA levels. Such strategies should be further tested in different *in vitro* and *in vivo* models.

### Clinician's corner

Dyskeratosis congenita and related disorders of telomere biology are very diverse. This makes clinical diagnosis difficult. Clinicians in all specialties need to be aware of the multisystem phenotypic presentations (dermatologic, hematologic, hepatic, pulmonary, etc.) that can affect different parts of the body.

Telomere length measurement and genetic analysis of the known disease genes has facilitated diagnosis. Improving access to these diagnostic tests for all patients is important for effective treatment.

In a significant subset of patients it is not possible to substantiate the diagnosis at the genetic level at present. Further research will be necessary to characterize their genetic basis and pathophysiology. It is also important to recognize that some of the pathology is not telomere-related since many of the disease genes have multiple biological functions.

Danazol, which can upregulate TERT expression, and reduced intensity fludarabine-based hematopoietic stem cell transplantation (HSCT) have had an important role in improving clinical outcomes in these patients. However, some patients do not respond to danazol or have significant side effects, and HSCT is not feasible in all patients.

### Concluding remarks

The progressive shortening of telomere length observed in the human population is intrinsically associated with physiological aging, and regulates multiple biological traits, including life-expectancy [68]. Germline mutations in genes involved in telomere elongation, replication, and repair exacerbate these phenotypes and lead to severe tissue failure in patients with TBDs. Although these Mendelian diseases are rare and present with a multitude of different phenotypes, our ability to identify these patients has significantly improved over the past few years. However, treatment options remain unsatisfactory, and novel approaches are needed to improve patients' quality of life.

A possible therapeutic approach is to modulate the pathways that regulate the levels of hTR in human tissue stem and progenitor cells. Collectively, mutations that impair hTR processing and lead to increased hTR degradation represent the majority of cases identified in patients suffering from TBDs. Critically, our knowledge of the mechanisms that control the biogenesis and degradation of hTR has significantly increased in recent years, allowing targeted modulation of the pathways that regulate hTR levels in tissue progenitor cells.

Pharmaceutical inhibition of PAPD5 seems to be the most promising route to be pursued. Inhibition of PAPD5 restores hTR levels and function, leading to rescue of telomerase activity and improvement in hematopoietic potential. Compounds that specifically inhibit PAPD5/PAPD7 can effectively rescue hematopoietic phenotypes associated with telomere dysfunction in culture and in animal models.

There are several unanswered questions (see [Outstanding questions](#)). One critical aspect is the specificity and potential toxicity of PAPD5 inhibitors in the diversity of different cell types in a patient. On the optimistic side, chemical inhibition of PAPD5 leads to only small changes in the transcriptome of hESCs or induced pluripotent stem cells (iPSCs) [62,63], and was relatively non-toxic in mice [63]. However, it remains possible that PAPD5 has important functions in some yet to be identified cell type(s). If there are unanticipated toxicities due to PAPD5 inhibition, one possibility is that patients could be treated in cycles that would allow an increase in telomerase activity and telomere length, followed by an off-treatment period to mitigate possible toxicities.

Taken together, the promising biochemical and cellular data collected so far strongly suggest that regulation of hTR levels by PAPD5 inhibition could prove beneficial for TBD patients. This possibility should be pursued further because it represents an exciting opportunity for transition into the clinic as a possible therapy for some TBDs if future studies demonstrate that they have a favorable efficacy/toxicity profile.

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### Declaration of interests

R.P. is a founder and consultant for Faze Medicines. The other authors declare no conflicts of interest.

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### Outstanding questions

How effective is the inhibition of PAPD5 in rescuing tissue functionality in different human somatic cell populations outside the hematopoietic system?

How specific is inhibition of PAPD5 in modulating hTR levels in different stem cell populations?

Are there other targets of PAPD5 in these cellular populations? What are the potential side effects of PAPD5 inhibition in the long-term functionality of these cells?



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