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Late-Onset Telomere Biology Disorders: Clinical Insights and Treatment Outcomes from a Retrospective Registry Cohort

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Abstract:
Pathogenic germline variants affecting proper telomere maintenance result in premature telomere shortening and cause telomere biology disorders (TBDs). While classical dyskeratosis congenita in children is rather well defined, late-onset (“cryptic”) TBDs remain underrecognized, resulting in underdiagnosis and inadequate treatment in affected adults. Here, we present a series of adult TBD cases collected through the German TBD reference center between 2014 and 2024. Patients ≥18 years with an age-matched telomere length (TL) < 10th percentile in lymphocytes and detection of either a variant of uncertain significance, a pathogenic or a likely pathogenic variant in TBD-associated genes, and available clinical data were included in this analysis. On this basis, a novel point-based algorithm for categorization into proven, probable and suspected-only TBD cases, respectively, was developed. Out of a total of 1,537 TL analyses, 42 patients with proven (n=29) or probable (n=13) TBD were identified. Median age at first clinical manifestation and at diagnosis was 20.0 years and 34.1 years, respectively. Bone marrow failure (BMF) was the most frequent manifestation observed in our cohort (73.8%), followed by liver or interstitial lung diseases (50.0% and 41.5%, respectively). Immunosuppressive therapy was carried out in six patients with BMF, none of them responded. In comparison, eight of eight evaluable patients treated with androgen derivatives showed hematologic response. Our data provide novel real-world insight into the clinical manifestation spectrum, diagnosis as well as clinical course and treatment of TBD in adult, late-onset cases of this hereditary disease.

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Late-Onset Telomere Biology Disorders in Adults: Clinical Insights and Treatment Outcomes from a Retrospective Registry Cohort

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Running title: Outcome in adult TBD

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Abstract

Pathogenic germline variants affecting proper telomere maintenance result in premature telomere shortening and cause telomere biology disorders (TBDs). While classical dyskeratosis congenita in children is rather well defined, late-onset ("cryptic") TBDs remain underrecognized, resulting in underdiagnosis and inadequate treatment in affected adults. Here, we present a series of adult TBD cases collected through the German TBD reference center between 2014 and 2024. Patients ≥18 years with an age-matched telomere length (TL) < 10th percentile in lymphocytes and detection of either a variant of uncertain significance, a pathogenic or a likely pathogenic variant in TBD-associated genes, and available clinical data were included in this analysis. On this basis, a novel point-based algorithm for categorization into proven, probable and suspected-only TBD cases, respectively, was developed. Out of a total of 1,537 TL analyses, 42 patients with proven (n=29) or probable (n=13) TBD were identified. Median age at first clinical manifestation and at diagnosis was 20.0 years and 34.1 years, respectively. Bone marrow failure (BMF) was the most frequent manifestation observed in our cohort (73.8%), followed by liver or interstitial lung diseases (50.0% and 41.5%, respectively). Immunosuppressive therapy was carried out in six patients with BMF, none of them responded. In comparison, eight of eight evaluable patients treated with androgen derivatives showed hematologic response. Our data provide novel real-world insight into the clinical manifestation spectrum, diagnosis as well as clinical course and treatment of TBD in adult, late-onset cases of this hereditary disease. *(word count: 250)*

Key points

- Clinical manifestation patterns of TBDs in adults differ significantly from children and adolescents.

- TBDs are still severely underdiagnosed in adults, and proper diagnosis is often substantially delayed following first clinical manifestations.

Introduction

Telomeres shorten with each cell division but can be re-elongated by the enzyme telomerase [1, 2]. Telomere biology disorders (TBDs) are characterized by premature telomere shortening leading to organ failure due to replicative senescence/apoptosis. Pathogenic (P) variants in genes responsible for proper telomere maintenance have been identified as the cause of TBDs. Until now, 20 TBD-associated genes have been discovered (*ACD, DCLRE1B, CTC1, DKC1, NAF1, NHP2, NOP10, NPM1, MDM4, PARN, POT1, RPA1, RTEL1, STN1, TERC, TERT, TINF2, TYMS-ENOSF1, WRAP53, ZCCHC8*). *TERT* and *TERC*, the main functional components of human telomerase are among the most frequently affected genes [3-8]. While TBDs overall follow different modes of inheritance, autosomal dominant (AD) inheritance is the predominant pattern in adults. The gold-standard and functional screening for underlying TBDs is the determination of telomere length (TL) deviation in peripheral blood (PB) lymphocytes using flow cytometry and fluorescent in situ hybridization (flow-FISH) [9-12].

The best-characterized clinical disorder within the group of TBD is dyskeratosis congenita (DC) [5]. Classical DC is characterized by the mucocutaneous triad of oral leukoplakia, nail dysplasia and abnormal skin pigmentation. Frequently, and increasingly over time, multiple organ systems are involved in DC, eventually resulting in e.g. bone marrow failure (BMF), interstitial lung disease (ILD) or liver disease (LD) [3, 5, 13-15]. In addition, prognosis of affected individuals is significantly impaired by a substantially increased risk for the development of hematologic or solid malignancies [16]. Particularly in adults, growing evidence shows that TBDs initially often present mono- or oligosymptomatically. However, organ systems affected, age of onset, as well as clinical significance of organ dysfunction are highly

heterogeneous between affected individuals, somewhat linked to the underlying genetic variant [16]. This heterogeneity, in combination with its ultra-rarity, results in adult-onset, cryptic TBDs both being severely underdiagnosed and often remaining hidden behind so-called “idiopathic” BMF syndromes, predominantly aplastic anemia (AA), idiopathic pulmonary fibrosis or liver cirrhosis, immune defects or other disease states [17, 18]. For the affected patients and their families, this is particularly unfortunate, since TL, measured by flow-FISH - a well-established and even functional screening parameter - is available to identify the vast majority of at-risk cases and trigger complementary genetic work-up for defects in genes linked to altered telomere maintenance. Even more importantly, a missed diagnosis of an underlying inherited TBD may have dramatic consequences for both, the correct treatment of the affected patient (including best selection of potential allogeneic family donors), as well as for potentially co-affected family members themselves. Due to the rarity and limited recognition of adult-onset TBDs, recommendations for diagnostic algorithms for these patients remain limited, and more data are needed to develop better diagnostic and management strategies for TBD, ultimately improving patient outcomes and quality of life [3, 19]. In our study, we provide a comprehensive registry analysis on adult TBD patients collected over a decade within the German TBD reference center. The focus is on the clinical characterization of disease manifestation patterns in adult TBDs with the aim to increase clinical awareness for these disorders.

Materials and methods

Patient recruitment and history

Patients of the AA-BMF-Registry und Aachen Telomeropathy Registry, included from October 2014 to April 2024, were considered for this analysis if the following criteria were met: 1.) TL < 10th percentile for age in PB lymphocyte subsets analyzed by flow-FISH or 2.) genetic data with variant of uncertain significance (VUS) or P/likely pathogenic (LP) variant in a TBD-associated gene detected via panel based next generation sequencing (NGS) or whole exome sequencing (WES) and 3.) age at last follow-up ≥18 years and 4.) sufficient clinical data allowing TBD diagnosis.

Medical and family history as well as patient follow-up were obtained by patient interview and/or medical records and reviewed by June 30, 2024. All patients signed written informed consent for registry inclusion and genetic analyses (*EK332/20*, *EK225/14;* RWTH Aachen University). Recruitment and work-up of the registry were carried out as published previously [9]. All variants, except those in *TERC*, were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines for the interpretation of sequence variants: class 3 (VUS), class 4 (LP), and class 5 (P) [20]. Since *TERC* encodes a non-coding RNA, many of the ACMG criteria supporting pathogenicity are not applicable. Therefore, for the classification of *TERC* variants, we added shortened TL as a moderate pathogenicity criterion as published before [9]. Time of confirmed TBD diagnosis was defined as either the time point of detection of significantly shortened TL or the genetic result of a TBD-associated variant.

TBD classification

The diagnosis of TBD in this study was established as follows: patients with P/LP variants in a TBD-associated gene were classified as proven TBD. Patients with VUS in TBD-associated genes were categorized into probable TBD (≥ 4 points, maximum score 9 points) or suspected-only TBD (< 4 points) using refined diagnostic criteria including parameters such as the degree of telomere shortening observed, positive family history of a TBD as well as presence of TBD related clinical features. We adapted these diagnostic criteria from those proposed by Niewisch et al. [16] (see Supplemental Methods and Supplemental Figure S1).

Statistical analysis

Survival probabilities for overall survival were calculated using Kaplan Meier estimates. Survival probabilities were compared by the log-rank test. P-values lower than 0.05 were considered significant. Data were collected via Microsoft Excel 2007 and analysed using GraphPad Prism (GraphPad Software version 9.0.0, La Jolla, CA, USA).

Results

Out of 1,537 patients undergoing TL screening during the designated ten year study period from 2014 to 2024 at the German TBD reference center in Aachen, 253 patients underwent further genetic work-up. Among them, 57 patients were identified to fulfil the inclusion criteria for the study mentioned above (Supplemental Figure S1). Of those, 29 patients had a P/LP variant in a TBD-associated gene, and, accordingly, were considered as proven TBD. Of the 28 remaining patients with VUS in TBD-related genes, 13 patients were classified as probable TBD (≥ 4 points), and 15 patients (< 4 points) were considered as suspected-only TBD (Supplemental Figure S1). Patients classified as suspected-only TBD exhibited significantly better survival outcomes compared to those with probable and proven TBD (median survival: 80.2 and 60.1 years, respectively; p=0.01; Supplemental Figure S2A). Given this significant prognostic difference, we defined the TBD cohort for further analysis as the 42 adult patients with probable and proven TBD.

Clinical and molecular characteristics of the adult TBD cohort

Clinical and molecular characteristics of the patient population are depicted in Figure 1. Twenty-two out of 42 adult TBD patients were female (52.4%). TL measured in PB lymphocytes by flow-FISH [9, 11, 12] was below the 1st percentile in 31 patients (73.8%), between the 1st and 10th percentile in nine (21.4%) and above the 10th percentile in two patients (4.8%), respectively. The most common underlying pattern of inheritance was AD, with variants detected in *TERC* (n=17), *TERT* (n=12), *RTEL1* (n=5), and *CTC1* (n=1), respectively. One female patient had a heterozygous X-chromosomal *DKC1* variant and was included in the AD analysis, one patient with

shortened TL was heterozygous carrier of a NHP2 variant and was also included in the AD analysis. Three patients carried autosomal recessive variants (AR; *CTC1*, n=2 and *WRAP53*, n=1). One patient had a homozygous variant in *TERT* (categorized for analysis as AR) and one male patient an X-linked (XL) *DKC1* variant (detailed list see Supplemental Table 1). In this combined prospective/retrospective analysis, the median prospective follow-up after inclusion into the registry was 2.0 years (range: 0 to 8.0 years).

Overall, of 36 patients evaluable for the TBD related clinical features, only 16 patients (44.4%) expressed typical DC skin stigmata and surprisingly, in none of these (adult) patients detection of typical DC skin manifestations was reported as first manifestation (FM) or led to the diagnosis of the underlying TBD.

In comparison, 31 patients developed suspected BMF (73.8%), most frequently presenting with leukopenia (73.8%, n=31) followed by thrombocytopenia (71.4%, n=30) and anemia (66.7%, n=28), and in 31 of 33 evaluable patients (93.9 %) in conjunction with bone marrow hypoplasia or aplasia. Retrospective classification of cytopenias according to the Camitta criteria for AA [21] was not possible for most of the patients due to insufficient data on the exact bone marrow cellularity or distribution of cellular subtypes (e.g. missing reticulocyte counts). Despite of these limitations, which were mainly related to the retrospective nature of this part of the analysis, BMF was considered the FM of the TBD in 25/42 (59.5%) patients.

Nineteen of 38 patients (50.0%) evaluable for BMF manifestation of an adult TBD developed LD; in four patients (10.5%), LD even represented the FM of the TBD. Similarly, 17 of 41 patients (41.5%) evaluable for this manifestation developed ILD and again, in four of those patients (9.8%), this represented the FM of the TBD.

Malignancies were reported in eight out of 41 patients with information being available (19.5%), two of which (4.8%) were head and neck squamous cell carcinoma (HNSCC, also representing FM of a yet unknown TBD) and myelodysplastic syndrome (MDS), respectively. The remaining cases were endometrial cancer, breast cancer, spindle cell carcinoma and diffuse large B-cell lymphoma (n=1 each).

In six cases, patients were presumably asymptomatic (#2, #12, #31, #38, #39, #41), i.e. without a clinical features suggestive of TBD (three patients had mild skin abnormalities that did not trigger diagnostic work-up [#2, #12, #41]) and detected accidentally, e.g. during screening for related allogeneic stem cell donorship. In 20 out of 35 patients (57.1%), early hair greying (described qualitatively) was reported, however, in none of the cases this clinical feature was either the FM of the TBD or triggered the diagnostic work-up.

Notable selected additional clinical features reported, albeit with less stringently established causal relation to TBDs, were enteropathies (n=5) [22], psychiatric disorders (n=9), osteonecrosis (n=4), combined variable immunodeficiencies (CVID, n=2) [17] or growth retardation (n=1).

Twenty-seven of 38 patients with this information being available (i.e. 71.1%) reported a positive family history for a TBD-related disease.

Treatment

Based on the records and received treatment, six patients (14.3%) of our cohort were initially diagnosed as acquired AA and treated with anti-thymocyte globulin (ATG) and/or cyclosporine-A (CSA)-based immunosuppressive therapy (IST). Expectedly,

none of these patients had a sustained response to treatment, confirming the genetic rather than immunologic cause of these TBDs. Four patients (9.5%) received eltrombopag without response, five (11.9%) received tyrosine kinase inhibitor nintedanib for ILD. Androgen treatment with danazol or oxymetholone was given in 12 patients (28.6%) of whom all eight patients with available follow-up showed a response in at least one hematological lineage (no follow-up data were available on response in four patients). For patients with ILD, adequate follow-up data were not available to allow a proper assessment of the response to androgen or nintedanib treatment. Four patients (9.5%) received allogeneic transplantation, one of them was reported to have died during follow-up (16 years after allogeneic transplantation). No patient underwent lung transplantation for ILD, only one patient received liver transplantation for hepatic disease.

Survival of the TBD cohort, age at FM, and time to diagnosis of TBD

Previous data reported a substantially reduced life expectancy in patient cohorts with TBDs comprised mostly of pediatric patients with classical DC. Therefore, we were particularly interested in the analysis of the disease course and overall survival of this adult cohort of TBDs. The median survival according to Kaplan Meier analysis was found to be 60.1 years (Figure 2A). Given that the underlying genetic defect can influence outcomes, we next examined the impact of genotype on survival. We observed a trend towards reduced median survival in patients with AR/XL inheritance compared to those with AD inheritance (p=0.07, Figure 2B).

Despite the lack of data on the exact causes of death in our patients, we hypothesized that the severity of BMF or the presence of ILD or LD might contribute to adverse outcomes. We found significantly better survival for patients without BMF (n=11, according to our definition, median survival not reached) compared to those with BMF (n=31, 51.2 years, p=0.02; Figure 2C). Similarly, a trend towards impaired median survival was also found in patients with (n=17) compared to patients without (n=24) evidence of ILD (p=0.07, Figure 2D) and in patients with (n=19) compared to patients without (n=19) evidence of LD (p=0.07, Supplemental Figure 2B).

Given the substantial concern of underdiagnosis of adult TBDs, we were particularly interested in investigating the age at FM and the time from FM to diagnosis of TBD (DOT) in affected patients. Median age at FM was 20.0 years, 16 of 36 patients with clinical manifestations developed their individual FM already before their 18th birthday (Figure 3A). Twenty-five percent of the patients developed their FM of TBD only after the age of 36.4 years. Median age at DOT by Kaplan Meier analysis was 34.1 years (Figure 3B), 25% of the patients included here were only properly diagnosed for an underlying TBD above the age of 47.5 years (Figure 3B). Among the 36 patients that had already developed clinical manifestations of TBD at the time of this analysis, the median duration from FM to DOT was 6.4 years (range: 0 to 47.3 years). Median time from FM to death or last follow-up by Kaplan Meier analysis was 25 years (Figure 3C).

The underlying genotype might influence the age at FM. We observed that affected individuals with AR/XL inheritance tended to develop first clinical features earlier (median age: 13.4 years), compared to those with AD inheritance (median age: 21.6 years, p=0.13; Supplemental Figure 2C). Notably, median time from FM to death or

287 last follow-up did not seem to differ between these two groups of inheritance patterns

288 (Supplemental Figure 2D).

Discussion

TBDs are defined by a common pathophysiology based on impaired telomerase activity leading to altered telomere maintenance [23, 24]. Consequently, the clinical manifestations usually result from telomere-mediated replicative cellular exhaustion in organs or tissues characterized by high cellular turnover such as the hematopoietic compartment. Pediatric patients with classical DC typically first develop clinical signs at the age of 5-13 years [25]. However, a significantly more heterogeneous, multi-organ clinical manifestation pattern beyond childhood, the ultra-rarity of the disease as well as the lack of stringent diagnostic criteria triggering telomere screening and eventually genetic work-up for an underlying TBD result in a significant risk of underdiagnosis of TBDs in adults.

In this study, we aimed to propose refined diagnostic criteria for the diagnosis of adult TBDs in the subcohort of patients screened by flow-FISH and follow-up genetic work-up in whom a VUS had been detected in a TBD-associated gene. Based on the degree of telomere shortening detected, weighed clinical parameters and family history, these VUS could then either be classified as probable TBDs and added to the cases with proven TBDs or categorized as suspected-only cases. Our results showed that patients with proven and probable TBDs exhibited significantly poorer survival compared to suspected-only cases. Notably, the survival outcomes of our proven and probable TBD group closely aligned with those reported by Niewisch et al. for confirmed TBD cases [28]. Further and again consistent with the findings reported by Niewisch et al., we found that patients with AR or XL TBD tended to have worse overall survival compared to those with AD inheritance pattern.

In line with previous studies aimed at the incidence of TBDs within clinically defined cohorts such as BMF syndromes [9, 26], immunodeficiencies [17] or other underlying

diseases [27], we can confirm that TL once being measured by flow-FISH [10, 11, 28-30] provides a highly efficient screening tool for patients with TBD even beyond childhood [9, 16], reviewed in [3].

We demonstrate that more than half of our TBD patients do not exhibit first clinical features until adulthood, and in 50% of the patients, diagnosis of TBD was only established after the age of 34 years. Moreover, approximately 70% show only one or two major clinical features, consistent with a mostly mono-/oligosymptomatic course of the disease in adult patients [31, 32]. Similarly, no signs of vascular disease - commonly observed in children - were reported in this cohort. Corresponding to this observation, signs of the classical DC triad were not regularly identified in adult patients and did not trigger further diagnostic work-up for an underlying TBD. While TBD is typically considered as a potential differential diagnosis in pediatric patients with related clinical features, most physicians treating adult patients may not consider TBD as a possible underlying cause, given that a FM of a hereditary disease in adults is a rare (and therefore underappreciated) event. This underscores the importance of raising awareness among healthcare professionals about TBDs, particularly in older adult patients with cryptic or subtle clinical presentations affecting a highly heterogeneous pattern of organ systems.

Ten patients within our cohort were initially misdiagnosed and treated as acquired AA with IST or a thrombopoietin agonist without any response. In comparison, twelve patients were reported to be treated with androgen derivatives of whom eight had sufficient follow-up data available. All these eight patients exhibited at least a single-lineage response.

The dramatic difference in probability of response to IST versus androgen therapy in patients with acquired (mostly immune-mediated) AA compared to hereditary AA based on an underlying TBD further underscores the importance of considering TBD

as a differential diagnosis also in adult patients with newly diagnosed AA [26, 33, 34]. This is even more urgent once patients with AA have shown no or unsatisfactory response to first line IST and are considered to be treated with a second round of alternative ATG while an underlying TBD had not yet been ruled out in the first place.

Although primarily retrospective in nature, our data obtained from real-world clinical registries on the screening of patients with AA or suspected TBD provide valuable insight into the natural clinical course of adult TBDs as well as their initial manifestation patterns. Median overall survival (or data of last follow-up) was 60 years, and half of the patients survived 25 years after the onset of first clinical features, for the most part, changes in blood counts. Consistent with previous case studies in adult TBD patients, BMF, ILD and LD are the predominant clinical manifestations observed in our cohort, two patients were diagnosed on the basis of a malignancy as FM of TBD [13, 35-38]. Overall, 24% of our cohort were diagnosed with solid or hematological malignancies, a proportion similar to previously published data [39, 40].

The high prevalence of BMF with bi- or pancytopenia as most frequent clinical feature in this cohort can be attributed to the clinical scope and the inclusion criteria of the registries, which primarily focusses on individuals with hematological abnormalities [9]. No patient in our cohort exhibited a variant in the *PARN* gene, which is in line with the relative predominance of pathogenic variants in *PARN* being detected in a subgroup of patients with pulmonary fibrosis that is typically not associated with relevant hematological phenotypes [31]. Regarding DKC1, we identified a male patient exhibiting the characteristic X-linked mode of inheritance. Notably, we also identified a female patient with DKC1 who presented with TBD manifestations as reported in previous studies [41] . While we lack definitive causal data regarding

patient mortality, patients with BMF showed a higher mortality compared to those without BMF. Previous data suggest that upon the onset of symptomatic lung disease, individuals with TBD typically experience rapid decline, possibly contributing to the observed increased mortality associated with lung disease [42]. In line with this observation, patients both with ILD and LD in our cohort tended to have shortened overall survival.

Our multicenter pro- and retrospective registry analysis has obvious limitations. We present an analysis of mostly retrospective clinical registry data, which may lead to underreporting of clinical features of TBD. Furthermore, we cannot ascertain the definitive causes of death in most patients included. And, although continuously collected via a German reference center over a period of ten years, due to the ultra-rarity of the disease, our sample size is still limited. Further validation of our refined diagnostic criteria is beyond the scope of this study scope and warrants future research.

In summary, our study provides novel insights into the initial manifestation and natural time course and manifestation pattern of telomere diseases with particular focus on adult, late-onset TBD. Our data show that adult TBD is often mono-/oligosymptomatic, rarely characterized by the classical mucocutaneous triad and likely underdiagnosed, despite a highly convenient and robust blood-based assay available for both screening and facilitating genetic work-up in suspected individuals and their families. Too many patients still undergo several lines of unspecific and often ineffective treatments before an accurate diagnosis is established. This can take years up to decades despite early signs and manifestations of clinical features which should prompt TBD directed diagnostics. Increased awareness and systematic

392 inclusion of TBD patients in specific registries are urgently needed to allow for
393 progress in diagnosis, management and counseling of patients and their families.

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Authorship Contributions

Conceptualization: FB, MT, THB. Methodology: FB, RM, MV. Validation: MT, FB, RM, IK, ME. Formal analysis: FB, MT, RM. Data interpretation: MT, MK, YS, JW, MV, KK, AR, UP, MR, PS, SC, BH, SB, CH, JC, MH, MK, MWW, SK, JP, SI, ME, IK, RM,THB, FB. Investigation: THB, FB. Resources: THB. Data curation: MT, FB. Writing - original draft preparation: MT, FB, THB. Writing - review and editing: MT, MK, YS, JW, MV, KK, AR, UP, MR, PS, SC, BH, SB, CH, JC, MH, MK, MWW, SK, JP, SI, ME, IK, RM, FB, THB. Visualization: MT, THB, FB. Supervision: THB, FB. Project administration: THB, FB. All authors have read and agreed to the published version of the manuscript.

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Disclosure of Conflicts of Interests

THB and FB have a long-standing scientific collaboration with Repeat Dx., Vancouver, Canada. The other co-authors have no conflict of interest related to this manuscript.

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Figure legends

Figure 1. Clinical characteristics of all patients with a probable and proven telomere biology disorder (TBD, n=42).

patient identification number, bold numbers: presumably asymptomatic patients; f: female; m: male; red: Telomere length (TL) < 1st percentile; yellow: TL ≥ 1st but < 10th percentile; green: normal TL. Inheritance pattern is depicted in blue for autosomal dominant and brown for autosomal-recessive/X-linked inheritance. Presence of TBD manifestations are shown in grey. Dark grey represents first manifestations of clinical TBD features. Sufficient clinical data were defined as the documentation of a reported physical examination and family history, accompanied by a detailed list of pre-existing conditions or diagnostics addressing typical TBD symptoms, such as liver disease (LD) or interstitial lung disease (ILD). Where no relevant conditions were reported and/or specific diagnostics were not conducted, conditions were marked as not available (n.a.) and not done (n.d.), respectively. Family members are marked with asterisks (§, ʘ and ■). One patient was compound heterozygous (/), one patient was a heterozygous NHP2 variant carrier (//), and one patient was a heterozygous X-chromosomal carrier (///). Patients with CTC1 typically carry two pathogenic variants and follow an autosomal recessive inheritance pattern. In case #36, a variant in the other allele was not identified by WES (¶). One patient One patient received liver transplantation (x).

BMF, bone marrow failure; BM, bone marrow; DC, dyskeratosis congenita; CVID, common variable immune deficiency; ATG, anti-thymocyte globulin; CSA, cyclosporine-A; BMT, bone marrow transplant

Figure 2. Survival curves of probable and proven adult telomere biology disease (TBD) patients by Kaplan Meier analysis. A: Probability of survival of the cohort (median survival 60.1 years (y), n=42). **B:** Probability of survival dependent on mode of inheritance with a trend to better outcome for patients with autosomal dominant (AD) inheritance pattern (60.1 y) compared to autosomal recessive/X-linked (AR/XL; 36.4 y). **C.** Comparison of patients with bone marrow failure (BMF) and

without BMF shows significantly better survival for patients without BMF (median survival not reached) versus those with BMF (51.2 y). **D.** Probability of survival shows a trend to better survival for patients without evidence of interstitial lung disease (ILD) compared to those with evidence of ILD.

FU, follow-up

Figure 3. Course and follow-up of probable and proven adult telomere biology disease (TBD) patients. A: Swimmer plot showing the course of clinical TBD manifestations in years (y) without first manifestation (FM; blue), y after FM (orange) and y after diagnosis of TBD (DOT; green) for each patient. a.) patients with FM <18 y, b) patients with FM ≥18 y and c) patients without FM. Vertical red line marks the age of 18 y. (*), patient deceased; (#), autosomal recessive/X-linked inheritance pattern, (/) compound heterozygous, (//) heterozygous NHP2 variant carrier, (///) heterozygous X-chromosomal carrier, all others had autosomal dominant inheritance pattern. **B:** Kaplan Meier curve showing the age at DOT: Median age was 34.1 y, 25% of the patients were above the age of 47.5 y. **C:** Median time from FM to death or last follow-up (FU) by Kaplan Meier analysis was 25 y.

Figure 1

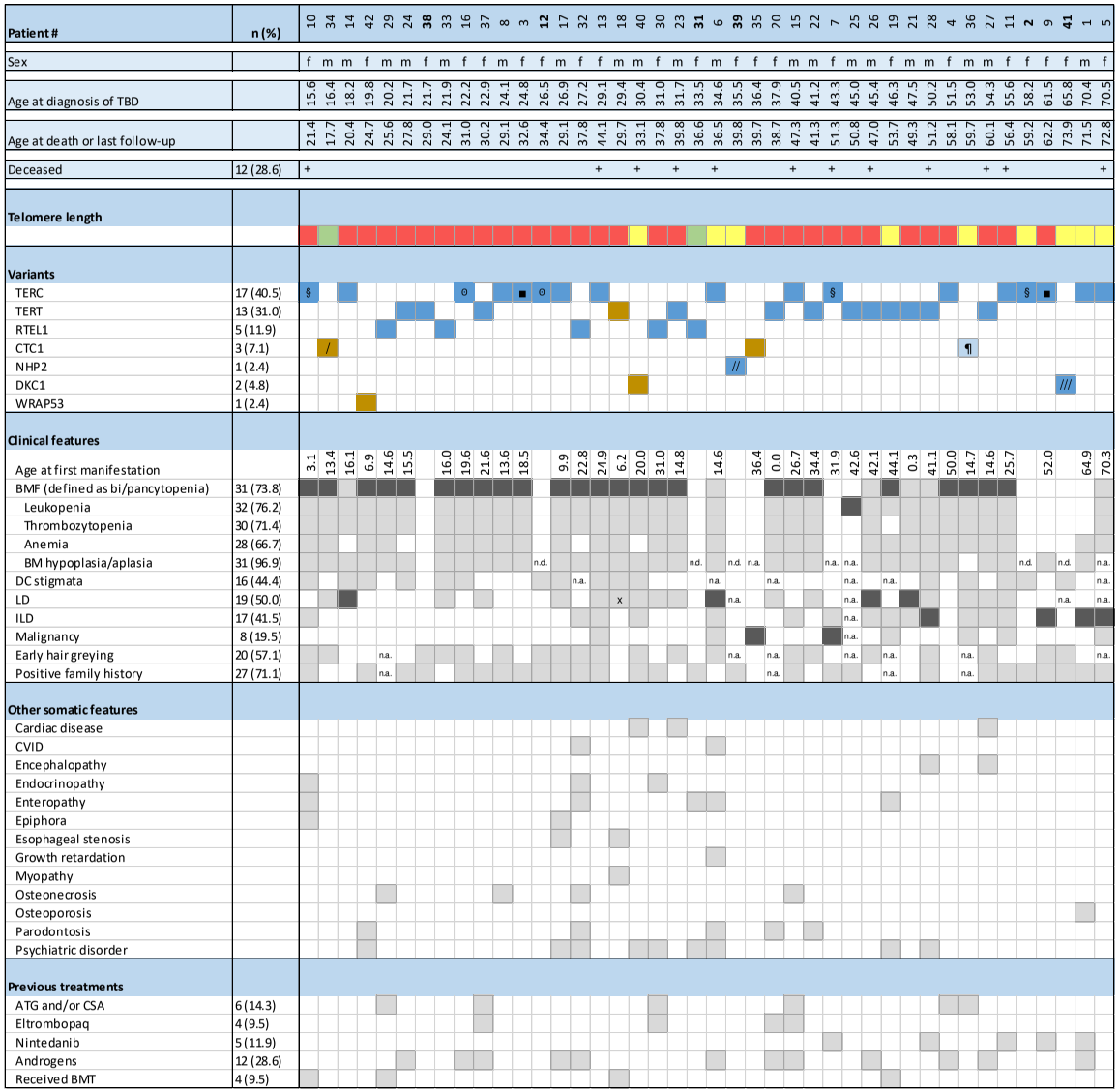

Patient #
n (%)
Sex
Age at diagnosis of TBD
Age at death or last follow-up
Deceased
Telomere length
Variants
TERC
TERT
RTEL1
CTC1
NHP2
DKC1
WRAP53
Clinical features
Age at first manifestation
BMF (defined as bi/pancytopenia)
Leukopenia
Thrombozytopenia
Anemia
BM hypoplasia/aplasia
DC stigmata
LD
ILD
Malignancy
Early hair greying
Positive family history
Other somatic features
Cardiac disease
CVID
Encephalopathy
Endocrinopathy
Enteropathy
Epiphora
Esophageal stenosis
Growth retardation
Myopathy
Osteonecrosis
Osteoporosis
Parodontosis
Psychiatric disorder
Previous treatments
ATG and/or CSA
Eltrombopaq
Nintedanib
Androgens
Received BMT

Figure 2

Figure 2

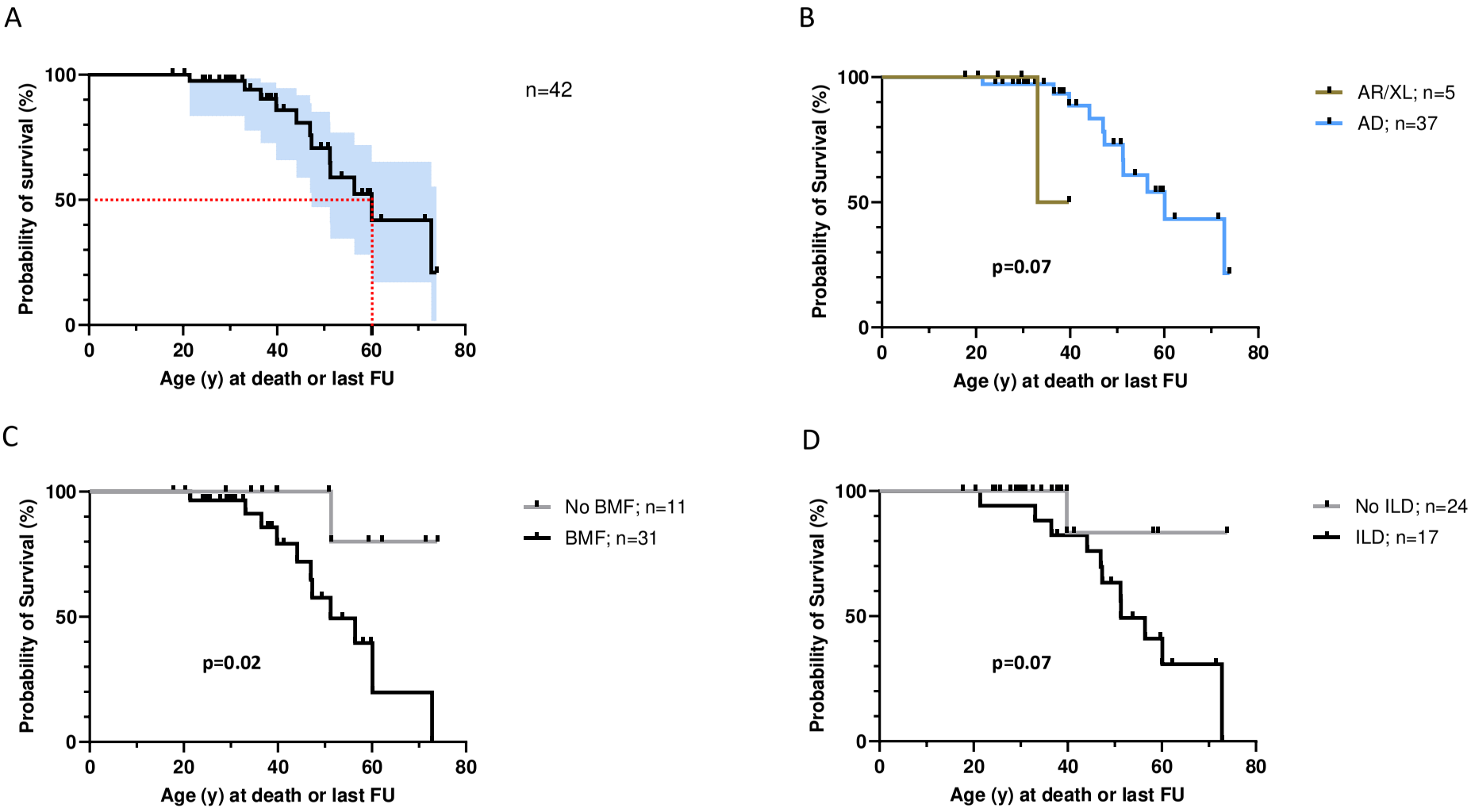
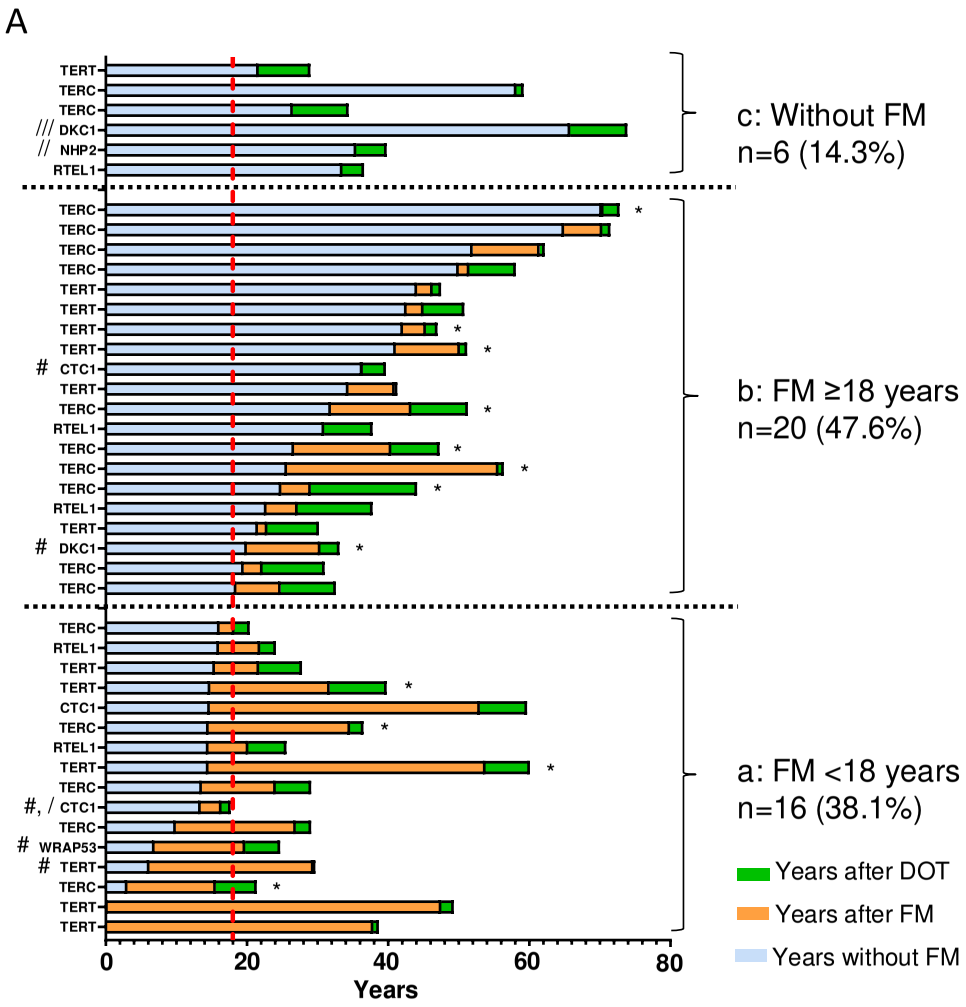
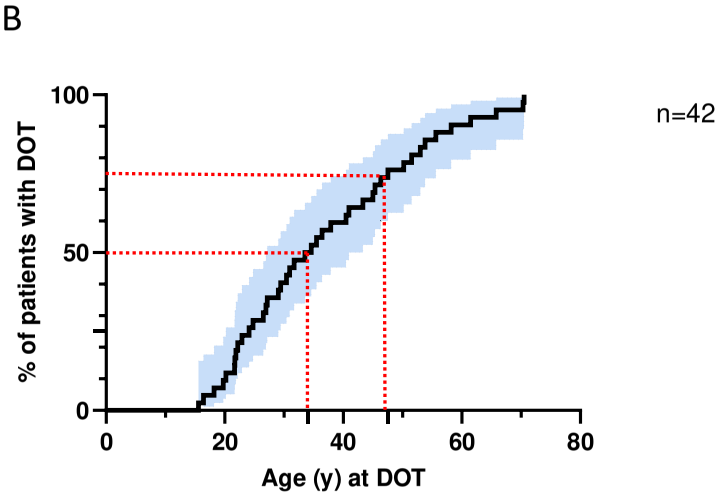
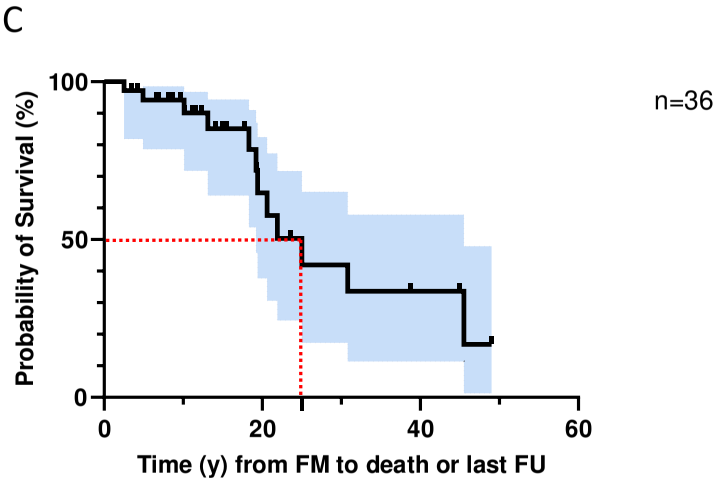


Figure 3


A
TERT
TERC
TERC
/// DKC1
// NHP2
RTEL1
c: Without FM
n=6 (14.3%)
TERC
TERC
TERC
TERC
TERT
TERT
TERT
TERT
CTC1
TERT
TERC
RTEL1
TERC
TERC
TERC
RTEL1
TERT
DKC1
TERC
TERC
b: FM ≥18 years
n=20 (47.6%)
TERC
RTEL1
TERT
TERT
CTC1
TERC
RTEL1
TERT
TERC
#, / CTC1
TERC
WRAP53
TERT
TERC
TERT
TERT
a: FM <18 years
n=16 (38.1%)
Years after DOT
Years after FM
Years without FM
0
20
40
60
80
Years


B
100
50
0
% of patients with DOT
0
20
40
60
80
Age (y) at DOT
n=42


C
100
50
0
Probability of Survival (%)
0
20
40
60
Time (y) from FM to death or last FU
n=36