bjh short report

Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita

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Summary

Dyskeratosis Congenita (DKC) is a systemic disorder caused by mutations resulting in impaired telomere maintenance. Clinical features include bone marrow failure and an increased risk of developing hematological malignancies. There are conflicting data whether androgen derivatives (AD) can elongate telomeres in vivo and whether AD treatment enhances the risk of gaining myelodysplastic syndrome-related mutations. Seven *TERC* or *TERT*-mutated DKC patients underwent AD treatment. All patients revealed hematological response. Telomere length of lymphocytes and granulocytes increased significantly and no MDS-related mutations were detected. Pending longer follow-up, treatment with AD seems to represent an efficient and safe therapy for DKC patients.

Keywords: telomere, androgens, dyskeratosis congenita, bone marrow failure, MDS.

Key Points

- Androgen derivatives significantly improve blood counts and elongate telomeres in patients with dyskeratosis congenita with *TERT* or *TERC* mutations *in vivo*.
- No myelodysplastic syndrome-related somatic mutations were observed during telomerase activation with androgen derivatives.

Classical dyskeratosis congenita (DKC) is a hereditary disorder mainly characterized by mucocutaneous features and bone marrow failure.[1] In adults, so-called 'cryptic' DKC manifests itself mostly with progressive bone marrow failure (occurring in >90% of the cases) and pulmonary fibrosis or secondary cancers; primarily affecting tissues with high cellular turnover. DKC is caused by mutations suppressing proper telomere maintenance and leading to premature telomere shortening.[1] Most mutations affect subunits of telomerase itself, namely telomerase reverse transcriptase (*TERT*) or the telomerase RNA component (*TERC*). However, various other mutations in genes coding for helicases, e.g. regulator of telomere elongation helicase 1 (*RTEL1*), or the ribosomal core component H/

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ACA ribonucleoprotein complex subunit DKC1 (*DKC1*) have been described.[1] Flow-FISH as the method of choice is used for assessment of telomere length (TL) to screen for and diagnose DKC.[2] Preclinical *in vitro*[3] and *in vivo*[4] studies as well as clinical case reports providing proof-of-principle[5] paved the way for systematic clinical trials eventually confirming that androgen derivatives (AD) such as danazol or oxymetholone can not only improve blood counts and reduce transfusion frequency but in fact increase telomere length in patients with DKC.[5,6] AD are thought to elongate telomeres by increasing the expression of telomerase thereby at least partially reversing the mutation-related haploinsufficiency of the telomerase complex.[3,4] However, whether consistent and stable telomere elongation can be maintained long-term *in vivo* is still debated.[7] Unfortunately, patients with DKC have been shown to harbor an increased risk of developing solid tumors as well as myeloid neoplasms such as acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS).[8] Recent studies favor the hypothesis that malignant transformation is mostly mediated by chromosomal instability due to critically short telomeres and not primarily via clonal hematopoiesis of indeterminate potential (CHIP) and eventual selection for MDS-related somatic mutations.[9] Hence, the question arises whether increased telomerase activity mediated by AD further increases the risk for CHIP development in DKC patients treated with AD. Here, we aimed to sequentially investigate the effect of AD both on TL as well as on MDS-related somatic mutations in patients with adult-onset (cryptic) DKC.

Methods and patients

Seven patients with molecularly confirmed DKC were enrolled in our analysis (see Table I). All TL assessments of peripheral blood granulocytes and lymphocytes were carried out by Flow-FISH, the results are given in kilobases (kb) as previously described.[2] Before initiation of AD treatment, telomere attrition below the 1st percentile of normal controls was confirmed in all cases in lymphocytes and in 6 out of 7 in the granulocytes. Six patients received danazol treatment while patient #5 was treated with low dose oxymetholone (see Table I). All patients were treated with AD for the first time and none had concomitant medication with growth factors. Patients were at a median age of 40 years (range: 21–53 years) at start of therapy. Median follow-up during treatment was 14 months (range: 3–29 months, Table I). Blood cell counts were assessed by the local treating physicians. TL and next-generation

Table I. Main characteristics of analyzed cohort.

Pat No	Age (years)	Gender	Mutation	Treatment†	Dose	Duration (months)	Status before treatment and best response	Additional clinical DKC features
1	40	male	*TERC** n.18_31del	danazol	700 mg (abs.) qd	19	severe neutropenia: stable anemia: stable thrombopenia: PR	Nail dystrophy, positive family history (ILD, leukemia, HNSCC)
2	52	female	*TERC** n.73G>A	danazol	600 mg (abs.) qd	14	no neutropenia anemia: CR thrombopenia: PR	Early hair greying
3	43	female	*TERC** n.54_57del	danazol	600 mg (abs.) qd	3	no neutropenia anemia: stable thrombopenia: PR	ILD, early hair greying Positive family history (ILD, BMF)
4	54	male	*TERT*** c.2639C>T p.(Ala880Val)	danazol	600 mg (abs.) qd	3	no neutropenia anemia: stable thrombopenia: PR	Lung fibrosis, hepatopathy, early hair greying
5	22	male	*TERT*** c.2372T>C p.(Val791Ala)	oxy-metholone	0·22 mg/kg qd	29	no neutropenia no anemia thrombopenia: PR	Positive family history (BMF)
6	25	female	*TERT*** c.383C>G p.(Thr128Ser)	danazol	600 mg (abs.) qd	15	neutropenia: CR anemia: CR thrombopenia: PR	Positive family history (BMF)
7	28	female	*TERC** n.128A>G	danazol	600 mg (abs.) qd	11	neutropenia: CR anemia: CR thrombopenia: PR	Nail dystrophy, hepatopathy, early hair greying, positive family history (HNSCC, BMF)

The population analyzed comprises adult patients screened for late onset of hereditary telomeropathies. Parts of the genetic characteristics of the patients population are described in Ventura Ferreira *et al.*[2,13] abs., absolute; PR, partial remission; CR, complete remission; ILD, interstitial lung disease; HNSCC, Head and neck squamous cell cancer; BMF, bone marrow failure. All DKC-confirming mutations were heterozygous. Transcript used for variant annotation: *TERC: NR_001566; **TERT: NM_198253; †no concomitant treatment with growth factor (e.g granulocyte stimulating factor or thrombopoietin receptor agonists).

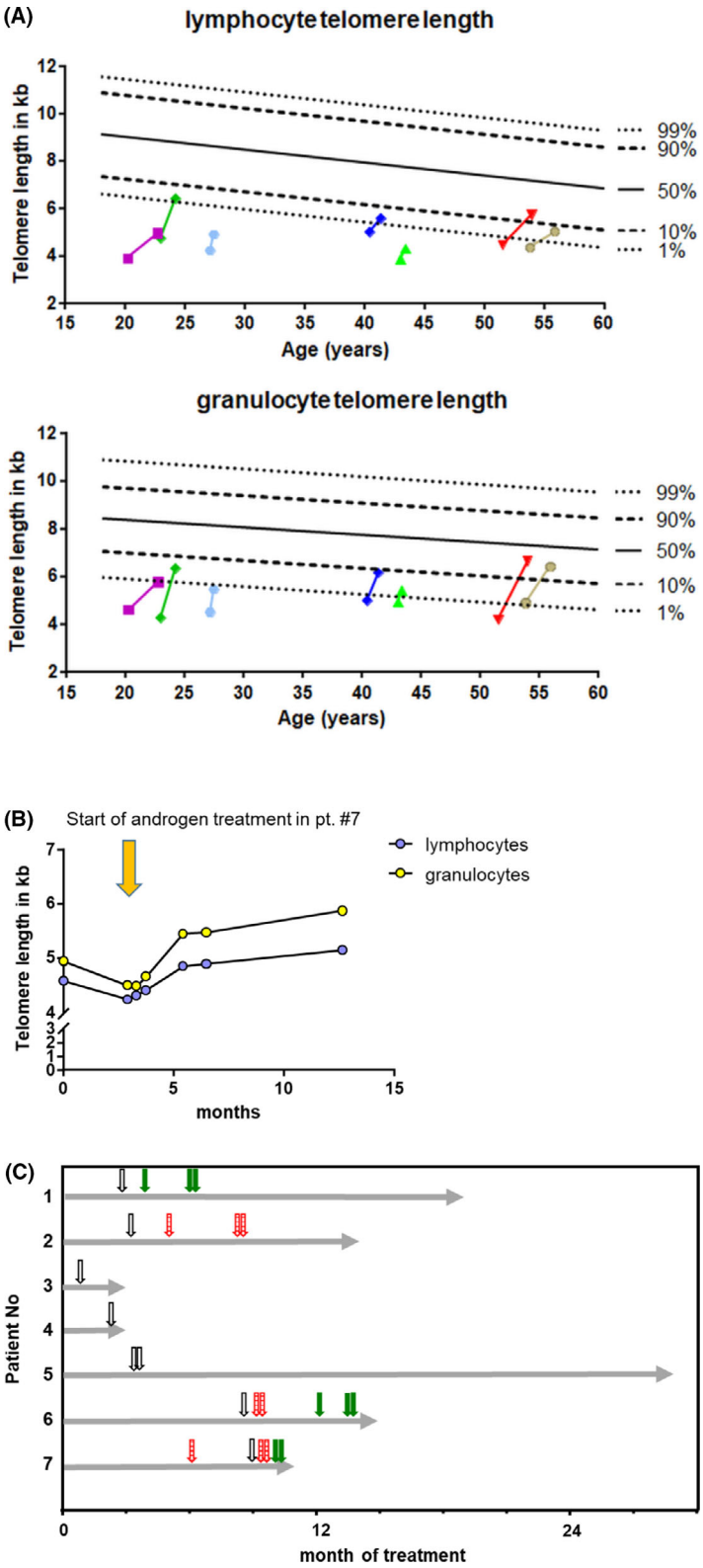

Fig 1. (A) Telomere length in lymphocytes (upper panel) and granulocytes (lower panel) at baseline and during androgen treatment. (B) Individual course of telomere length during treatment in case #7. (C) Improvement of hematological parameters during androgen treatment. The grey horizontal arrow represents the duration of the androgen treatment. One arrow indicates partial response (⇩), two arrows indicate complete response (⇩⇩). platelets/thrombopoiesis are shown in blank arrows (⇩), hemoglobin/erythropoiesis in red shaded arrows (⇩), neutrophils/granulopoiesis in green filled arrows (⇩). [Colour figure can be viewed at wileyonlinelibrary.com]

sequencing (NGS) analysis of patients were assessed before AD treatment and at the last time point available during treatment. Response assessment was carried out as described previously (for further detail see Methods S1).

Results and discussion

In all seven patients with mutations in the telomerase genes *TERC* and *TERT* and short TL at baseline, mean TL

increased significantly during AD treatment (Fig 1A). Lymphocyte TL increased significantly from a mean 4·45 ± 0·37 kb at baseline to 5·28 ± 0·68 kb ($P = 0·003$, Figure S2A). TL in the granulocyte subpopulation increased from 4·72 ± 0·40 kb before treatment start to 6·05 ± 0·49 kb under treatment ($P = 0·003$, Figure S2B). For patient #7, continuous TL measurement during treatment was available. TL increased quickly following treatment initiation with AD followed by a subsequent stabilization under continued treatment (Fig 1B). For all patients, median increase in TL per month for lymphocytes and granulocytes was 0·11 kb (0·019–0·223 kb) and 0·153 kb (0·019–0·513 kb).

All seven patients showed a hematological response during treatment with AD in at least one hematopoietic myeloid lineage (Figure S1). Peripheral blood counts revealed a significant increase in granulocyte counts from a mean of 1·4/µl ± 0·6 to 4·2/µl ± 2.1 ($P = 0·049$, Figure S1A), in hemoglobin levels from a mean of 9·2 g/dl ± 2·3 to 11·2 g/dl ± 2·9 ($P = 0·027$, Figure S1B), and in platelet counts increasing from a mean of 45/nl ± 45 before treatment to 69/nl ± 54 ($P = 0·036$, Figure S1C) during AD treatment. These data are in line with previous observations of patients harboring *TERC* or *TERT* mutations who clinically benefitted from AD derivatives.[6]

Here, in line with previous studies,[5,6] we were able to confirm that both blood counts and TL improve under treatment with AD in patients with DKC. However, a recent study by Khincha *et al*. failed to see a significant telomere elongation in ten DKC patients upon AD therapy after a median treatment duration of 3 years.[7] One potential explanation for this discordance could reside in the fact that the response to AD treatment might depend on the underlying mutational profile which can be observed in different cohorts of DKC patients. Our study includes a genetically rather homogenous population of patients with *TERC* and *TERT* mutations only, while Khincha *et al*.[7] analyzed a genetically much more heterogeneous cohort including only one *TERC*-mutated patient. In vitro data have shown that AD can lead to an increased expression of the telomerase protein TERT.[3] Consequently, in patients with mutation-related haploinsufficiency of TERC or TERT, AD treatment is expected to lead to an increase of telomerase activity resulting in telomere elongation. In contrast, mutations of other telomerase complex members can either cause substantial loss of function, such as in *DKC1*-mutated patients,[10] or - as in *RTEL1*-mutated patients[11] - indirectly impair telomerase activity while the telomerase complex itself still remains functional. In both cases, an AD-mediated increase in telomerase expression would not necessarily result in elongated telomeres since telomere maintenance is independent of the levels of telomerase activity. In line with this assumption, the *TERC*-mutated patient within the cohort reported by Khincha *et al*. did indeed experience a hematological response and mild telomere elongation.[7]

Finally, our NGS analysis looking for possible MDS-related mutations did not reveal any newly evolving mutations under AD treatment. We previously showed that MDS-related mutations are generally infrequent in patients with DKC.[9] This clearly differs from patients with myeloid diseases characterized by short telomeres but unimpaired functional telomerases such as patients with acquired aplastic anemia or elderly individuals with MDS.[12-15] Nonetheless, one major concern in regards to AD treatment of DKC patients is that reactivation of telomerase complex could stabilize genetically instable clones with short telomeres and eventually increase the frequency of MDS-related mutations.

In conclusion, AD treatment for patients with adult-onset, cryptic DKC represents an efficient and safe therapy for patients harboring *TERT* or *TERC* mutations and short TL by significantly improving peripheral blood counts with elongation of telomeres. Due to the rarity and genetic heterogeneity of the disease, valid conclusions regarding the persistence of the observed beneficial effects and long-term safety of AD treatment in DKC patients pends on further investigation.

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Authorship contributions

MK: Performed the experiments, analyzed and interpreted the data and wrote the manuscript. MV: Analyzed the data and wrote the manuscript. KK, MSVF, MWW, MS, SB, BR, SI, SK, BH and JP: Provided patient samples, clinical data and interpreted the data. THB: Analyzed and interpreted the data and wrote the manuscript. FB: Conceived and planned the study design, interpreted the data, and wrote the manuscript. All co-authors critically read and approved the final version of the manuscript.

Conflicts of interest

THB and FB receive scientific support from Repeat Dx, Vancouver, Canada. The authors declare that they have no conflict of interest.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig SI. Clinical parameters before and under treatment with AD. Improvement of clinical parameters, such as granulocytes (A), hemoglobin (B), and platelets (C) after treatment with AD. Statistical analysis was performed using students paired *t*-test.

Fig SII. Increase of telomere length measured by flow-FISH. The increase of telomere length in lymphocytes (A) and in granulocytes (B) after treatment with AD measured by flow-FISH is shown. Statistical analysis was performed using students paired *t*-test.

Methods S1. Supplementary methods.

References

1. Calado RT, Young NS. Telomere diseases. *N Engl J Med*. 2009;**361**:2353–65.
2. Ferreira MSV, Kirschner M, Halfmeyer I, Estrada N, Xicoy B, Isfort S, et al. Comparison of flow-FISH and MM-qPCR telomere length assessment techniques for the screening of telomeropathies. *Ann N Y Acad Sci*. 2020;**1466**(1):93–103.
3. Calado RT, Yewdell WT, Wilkerson KL, Regal JA, Kajigaya S, Stratakis CA, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood*. 2009;**114**:2236–43.
4. Bar C, Huber N, Beier F, Blasco MA. Therapeutic effect of androgen therapy in a mouse model of aplastic anemia produced by short telomeres. *Haematologica*. 2015;**100**:1267–74.
5. Ziegler P, Schrezenmeier H, Akkad J, Brassat U, Vankann L, Panse J, et al. Telomere elongation and clinical response to androgen treatment in a patient with aplastic anemia and a heterozygous hTERT gene mutation. *Ann Hematol*. 2012;**91**:1115–20.
6. Townsley DM, Dumitriu B, Liu D, Biancotto A, Weinstein B, Chen C, et al. Danazol treatment for telomere diseases. *N Engl J Med*. 2016;**374**:1922–31.
7. Khincha PP, Bertuch AA, Gadalla SM, Giri N, Alter BP, Savage SA. Similar telomere attrition rates in androgen-treated and untreated patients with dyskeratosis congenita. *Blood Adv*. 2018;**2**:1243–9.
8. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up. *Haematologica*. 2018;**103**:30–9.
9. Kirschner M, Maurer A, Wlodarski MW, Ventura Ferreira MS, Bouillon A-S, Halfmeyer I, et al. Recurrent somatic mutations are rare in patients with cryptic dyskeratosis congenita. *Leukemia*. 2018;**32**:1762–7.
10. Wong JM, Kyasa MJ, Hutchins L, Collins K. Telomerase RNA deficiency in peripheral blood mononuclear cells in X-linked dyskeratosis congenita. *Hum Genet*. 2004;**115**:448–55.
11. Walne AJ, Vulliamy T, Kirwan M, Plagnol V, Dokal I. Constitutional mutations in RTEL1 cause severe dyskeratosis congenita. *Am J Hum Genet*. 2013;**92**:448–53.
12. Brummendorf TH, Maciejewski JP, Mak J, Young NS, Lansdorp PM. Telomere length in leukocyte subpopulations of patients with aplastic anemia. *Blood*. 2001;**97**:895–900.
13. Brummendorf TH, Balabanov S. Telomere length dynamics in normal hematopoiesis and in disease states characterized by increased stem cell turnover. *Leukemia*. 2006;**20**:1706–16.
14. Stanley N, Olson TS, Babushok DV. Recent advances in understanding clonal haematopoiesis in aplastic anaemia. *Br J Haematol*. 2017;**177**:509–25.
15. Kulasekararaj AG, Jiang J, Smith AE, Mohamedali AM, Mian S, Gandhi S, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood*. 2014;**124**:2698–704.

