Supplementary Material

No effect of danazol treatment in patients with advanced idiopathic pulmonary fibrosis

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Supplementary Methods

Blood samples were taken around time of diagnosis. The samples were stored at -80 degrees Celsius until use. DNA was isolated from white blood cells using a magnetic beads-based method (chemagic DNA blood 10k kit; PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany). Telomere length was determined using a previously described quantitative polymerase chain reaction (PCR) method.[1] Briefly, telomere length was estimated for each sample from the ratio of telomere repeat copy number to a single gene (human β-globin gene) copy number (T/S ratio). Measurements were performed on the Bio-Rad CFX96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) in duplicate, with additional measurements if the duplicates differed more than 0.05; the mean value of the measured samples was used. Furthermore, quality control samples were included on each PCR-run, with a <0.05 margin of variance to reference values allowed. The control cohort included 164 healthy adults (71 male) with an age ranging between 20-70 years. Age-adjusted normal values for the T/S-ratio were calculated by determining the best-fitting linear regression line through the data, and percentiles were derived from the regression line.

After enrichment of the exome with the Agilent SureSelect CREV2 kit (Agilent Technologies, Santa Clara, California, USA), whole exome sequencing was performed on an Illumina Novaseq 6000 sequencer. The Illumina sequencing data was processed with the in-house pipeline, IAP v2.6.1, including GATK v3.4-46, according to the best practices guidelines.[2] Results were filtered for exonic variants with a population frequency below 0.5% in 36 genes related to telomere syndromes or pulmonary fibrosis (*ABCA3*, *ACD*, *AP3B1*, *CSF2RA*, *CSF2RB*, *CTC1*, *DKC1*, *FAM111B*, *HPS1*, *HPS4*, *ITGA4*, *LIG4*, *MARS*, *NAF1*, *NKX2-1*, *NOP10*, *PARN*, *POT1*, *RNF168*, *RTEL1*, *SAMD9L*, *SFTPA1*, *SFTPA2*, *SFTPB*, *SFTPC*, *SFTPD*, *STN1*, *TEN1*, *TERC*, *TERF1*, *TERF2*, *TERT*, *TINF2*, *TMEM173*, *USB1*, and *WRAP53*).

Supplementary Table 1: Outcomes stratified by leukocyte telomere length below or above the 10th percentile for age

	Leukocyte telomere length <10th percentile for age (N=22)	**Leukocyte telomere length >10th percentile for age (N=21)**	***p*-value**
Any side effects (%)	14 (64)	13 (62)	1.00
Stopped because of side effects (%)	10 (45)	11 (52)	0.76
Any dose adjustments (%)	7 (32)	7 (33)	1.00
Still using danazol >1 year after start (%)	7 (32)	3 (14)	0.28
Mean FVC-decline after one year, mL (95% confidence interval)	546 (326 - 766)	468 (-30 - 967)	0.77

Supplementary Table 2: Outcomes stratified by presence or absence of any clinical suggestion of a telomere syndrome

	Any clinical suggestion of a telomere syndrome (N=18)	**No clinical suggestion of a telomere syndrome (N=32)**	***p*-value**
Any side effects (%)	12 (67)	17 (53)	0.39
Stopped because of side effects (%)	10 (56)	13 (41)	0.38
Any dose adjustments (%)	5 (28)	9 (28)	1.00
Still using danazol >1 year after start (%)	3 (17)	8 (25)	0.72
Mean FVC-decline after one year, mL (95% confidence interval)	459 (216 - 702)	500 (163 - 837)	0.86

Supplementary Table 3: presumed side effects of danazol stratified by concurrent use of antibrotic medication

	Concurrent use of pirfenidone (n=9)	**Concurrent use of nintedanib (n=28)**	**Concurrent use of any antibrotic (n=37)**	**No concurrent antifibrotic (n=13)**	***p*-value**
Any side effects (%)	7 (78)	13 (46)	20 (54)	9 (69)	0.52
Elevated liver enzymes (%)	2 (22)	5 (18)	7 (19)	3 (23)	0.71
Stopped because of side effects (%)	6 (67)	11 (39)	17 (46)	7 (54)	0.75
Any dose adjustments (%)	3 (33)	6 (21)	9 (24)	5 (38)	0.47
Still using danazol >1 year after start (%)	2 (22)	7 (26)	9 (24)	2 (15)	0.70

p-values are calculated based on the difference between patients that concurrently used any antifibrotic and patients that did not use any antifibrotic.

References

1. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;30(10):e47.

2. Van der Auwera GA, Carneiro MO, Hartl C, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinforma*. 2013;43:11.10.1-33. doi:10.1002/0471250953.bi1110s43 [doi]