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Safety and efficacy of pirfenidone and nintedanib in patients with Idiopathic Pulmonary Fibrosis and carrying a telomere related gene mutation

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To the Editor

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and deadly interstitial lung disease (ILD). Over the past decade, familial occurrence of IPF led to the identification of genetic susceptibility traits [1]. Germline pathogenic variations in telomeres related genes (TRG) such as *TERT*, *TERC TINF2*, *DKC1*, *RTEL1*, *PARN*, *NAF1*, *ZCCHC8*, *NHP2*, and *NOP10* have been detected in 20-30% of patients with familial pulmonary fibrosis (FPF) and in 1-5% of sporadic IPF [2] [3], [4]. In comparison with IPF patients, carriers of a TRG mutation are significantly younger and show an accelerated decline of FVC [5][6][7]. Two drugs, pirfenidone and nintedanib, have been shown to reduce the decline of forced vital capacity (FVC) in IPF patients [8],[9]. So far, two studies reported on the safety and effectiveness of pirfenidone in patients with a TRG mutation [7],[6] whereas no study investigated nintedanib in this specific population. Thus, the aim of this retrospective study was to assess safety and efficacy of nintedanib and pirfenidone in IPF patients with a TRG mutation.

Patients from specialized European interstitial lung disease (ILD) centers from France (OrphaLung network), Netherlands, Spain, Greece, Belgium, and Switzerland were included in the study if they fulfilled the following criteria:1) a multidisciplinary team diagnosis of IPF, 2) carrier of a TRG variant interpreted as pathogenic and called as mutation in the remaining manuscript, and 3) received at least one dose of pirfenidone or nintedanib.

Demographical data, clinical status, treatment continuation (as assessed by the physician in charge), adverse events (clinical and biological) and all lung function tests (LFT) results available were collected, at diagnosis, at antifibrotic treatment

initiation and during treatment follow up. Time zero was set to the date of first antifibrotic treatment.

In a first analysis, the impact of the first antifibrotic treatment on the evolution of FVC was analyzed by modeling the longitudinal FVC measurement with a mixed-effects model. The evolution after treatment was then compared to what a model fit with pre-treatment data only would predict. Confidence intervals were obtained by nonparametric bootstrap. In a second analysis, the post-treatment evolution was compared between groups of patients receiving pirfenidone or nintedanib using inverse probability of treatment weighting. The evolution of FVC was then compared between groups using a weighted linear mixed effects model, where the treatment effect on the slope of FVC was represented by the time by group interaction. All analyses were carried out using the R statistical software version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria), with the Hmisc, lme4, WeMix and boot packages.

We identified 89 patients with IPF, carrier of a TRG mutation: *TERT* (n=65), *TERC* (n=16), RTEL1 (n=5) or *PARN* (n=3). The mean age at diagnosis was 59.8 ± 9.4 years. At treatment initiation, median FVC was 84.3 % [70.0-94.2] of the predicted value and median DLCO was 45.8 [38.0-53.0]. At treatment initiation, patients treated with pirfenidone (n=55) or nintedanib (n=34) were similar in terms of age, smoking, mutation status, delay between diagnosis and treatment initiation or disease severity.

The median transplant-free survival was 64.9 months (3.0 months to 117.2 months) and the median duration of treatment was 22.0 months (13.5 months to 36.5 months) without significant difference between the 2 groups (figure 1A). No patient from this analysis received danazole. While being treated, 12 patients experienced an acute

exacerbation, 9 patients received a lung transplantation and 22 patients died. All deaths were caused by respiratory insufficiency due to progression of lung fibrosis.

During the follow up, 9 patients treated by pirfenidone (25.9%), 12 patients treated by nintedanib (32.4%) stopped the treatment due to gastro intestinal disorders. Three patients (3.7%) 2 with pirfenidone and 1 with nintedanib, showed an increase in liver enzymes , leading to treatment termination. Three patients treated with pirfénidone (5.6%) showed a skin related side effect leading to treatment cessation. Whilst 27 (30.3%) patients presented initially with blood abnormalities in the context of TRG mutation, none of the 89 patient experienced hematological adverse event.

To assess anti fibrotic efficacy, we collected 581 LFT results. The longitudinal change in FVC (liters), modeled in a linear mixed effects model, was significantly reduced compared to the predicted evolution of the FVC (Figure 1). The mean FVC decline was 39 mL / month (95% CI 23 to 55 mL/month) before treatment, and 22 mL / month (95% CI17 to 28 mL/month) in the next 30 months after treatment initiation ($p = 0.026$).

We compared the slope of FVC in patients receiving pirfenidone and nintedanib, with a weighting on the propensity score to correct any differences in baseline characteristics. After adjustment for confounders, the slope of FVC in the 30 months following treatment was 15 mL/month (5 to 24) with nintedanib and 25 mL/month (17 to 32) with pirfenidone ($p=0.12$).

In this multicenter European retrospective study, we observed that antifibrotic treatment was associated with a reduced decline of FVC in IPF patients with TRG mutations. We did not observe any unexpected adverse event, neither any difference between pirfenidone and nintedanib in terms of efficacy.

As previously reported in patients with TRG mutations [6],[7], patients in this cohort were younger and showed a more rapid decline of lung function as compared to previous cohorts of sporadic IPF patients [10]. Because of specific hematological and liver diseases associated with TRG mutation [11], we were concerned about an increased risk of liver toxicity and hematological adverse events [8, 9]. Fortunately, our data suggest that the side effect profile of pirfenidone or nintedanib in patients with TRG mutation is similar with those observed in the general IPF population [8, 9]. Twenty-seven patients (30.3%) had to stop the treatment due to a side effect, mostly gastro-intestinal. These results correspond to the usual proportion of patient stopping the antifibrotic drugs in reported retrospective and prospective cohorts of IPF patients [10], [12], [13], [14].

We observed that antifibrotic drugs were associated with a reduced decline of FVC in IPF patients carrying TRG mutations. Our results are in line with a post hoc analysis of INSPIRE, CAPACITY, and ASCEND trials in patients carrying a rare TRG variant. Patients with a rare TRG variant had a more rapid decline of FVC than IPF patients without a TRG variant [6], though pirfenidone was still associated with a reduced decline of FVC compared to placebo in this subpopulation [6].

Our study is the first to assess the benefit of nintedanib in this specific population, highlighted by the number of rare and unique mutation, and our results support the safety and efficacy of nintedanib in these patients.

This study has several limitations. Treatment continuation was self-reported by the patient, and we cannot ensure the presence of unreported adverse events due to the retrospective nature of the study. In addition, due to the limited number of patients included, we were neither able to evaluate the efficacy of antifibrotic according to the nature of the mutation status nor to compare the efficacy of pirfenidone and

nintedanib. With respect to the very limited number of patients who switched from pirfenidone to nintedanib, we didn't assess efficacy or safety of the second line of anti-fibrotic treatment. Finally and most importantly, we compared the observed decline of FVC to predicted decline, a comparison which can be suffer many biases, and which cannot replace a randomized control trial .

In conclusion, this study suggests that pirfenidone and nintedanib can be used safely in IPF patients with a TRG mutation and that both drugs reduce FVC decline. These results should be confirmed in a larger prospective study.

Figure legend

Figure 1:

a) Treatment continuation of pirfenidone and nintedanib

This figure shows the percentage of patients treated by pirfenidone (left panel) or nintedanib (right panel).

Blue column represents the percentage of patients treated by antifibrotic treatment at full dose (blue background) or reduced dose (hatched background). Red column represents the percentage of patients who stopped the treatment due to disease progression (including patients that died or received a lung transplantation) - (red background) or to a side effect (white background). At the bottom of the figures, the number of data available for each time.

b) Longitudinal FVC decline of IPF patients carrying TRG mutation treated by nintedanib or pirfenidone

The blue curves shows the FVC data collected for each patient, with a flexible model of the mean estimated by splines, in a mixed effects model. The curve represented by red dashes shows the predicted evolution of the FVC obtained with data before treatment only (until time zero)

Bibliography

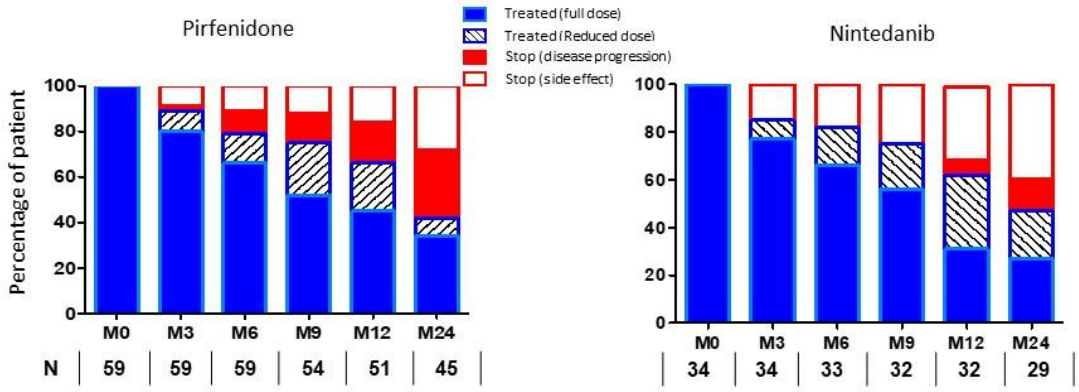
1. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, Swigris JJ, Taniguchi H, Wells AU. Idiopathic pulmonary fibrosis. *Nat. Rev. Dis. Primer* 2017; 3: 17074.
2. Borie R, Le Guen P, Ghanem M, Taillé C, Dupin C, Dieudé P, Kannengiesser C, Crestani B. The genetics of interstitial lung diseases. *Eur. Respir. Rev. Off. J. Eur. Respir. Soc.* 2019; 28.
3. Gable DL, Gaysinskaya V, Atik CC, Talbot CC, Kang B, Stanley SE, Pugh EW, Amat-Codina N, Schenk KM, Arcasoy MO, Brayton C, Florea L, Armanios M. ZCCHC8, the nuclear exosome targeting component, is mutated in familial pulmonary fibrosis and is required for telomerase RNA maturation. *Genes Dev.* 2019; 33: 1381–1396.
4. Kannengiesser C, Manali ED, Revy P, Callebaut I, Ba I, Borgel A, Oudin C, Haritou A, Kolilekas L, Malagari K, Borie R, Lainey E, Boileau C, Crestani B, Papiris SA. First heterozygous NOP10 mutation in Familial Pulmonary Fibrosis. *Eur. Respir. J.* 2020; .
5. Newton CA, Batra K, Torrealba J, Kozlitina J, Glazer CS, Aravena C, Meyer K, Raghu G, Collard HR, Garcia CK. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur. Respir. J.* 2016; 48: 1710–1720.
6. Dressen A, Abbas AR, Cabanski C, Reeder J, Ramalingam TR, Neighbors M, Bhangale TR, Brauer MJ, Hunkapiller J, Reeder J, Mukhyala K, Cuenco K, Tom J, Cowgill A, Vogel J, Forrest WF, Collard HR, Wolters PJ, Kropski JA, Lancaster LH, Blackwell TS, Arron JR, Yaspan BL. Analysis of protein-altering variants in telomerase genes and their association with MUC5B common variant status in patients with idiopathic pulmonary fibrosis: a candidate gene sequencing study. *Lancet Respir. Med.* 2018; 6: 603–614.
7. Justet A, Thabut G, Manali E, Molina Molina M, Kannengiesser C, Cadranel J, Cottin V, Gondouin A, Nunes H, Magois E, Tromeur C, Prevot G, Papiris S, Marchand-Adam S, Gamez AS, Reynaud-Gaubert M, Wemeau L, Crestani B, Borie R. Safety and efficacy of pirfenidone in patients carrying telomerase complex mutation. *Eur. Respir. J.* 2018; 51.
8. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, Gorina E, Hopkins PM, Kardatzke D, Lancaster L, Lederer DJ, Nathan SD, Pereira CA, Sahn SA, Sussman R, Swigris JJ, Noble PW, ASCEND Study Group. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 2014; 370: 2083–2092.
9. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, Cottin V, Flaherty KR, Hansell DM, Inoue Y, Kim DS, Kolb M, Nicholson AG, Noble PW, Selman M, Taniguchi H, Brun M, Le Maulf F, Girard M, Stowasser S, Schlenker-Herceg R, Disse B, Collard HR, INPULSIS Trial Investigators. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 2014; 370: 2071–2082.
10. Guenther A, Krauss E, Tello S, Wagner J, Paul B, Kuhn S, Maurer O, Heinemann S, Costabel U, Barbero MAN, Müller V, Bonniaud P, Vancheri C, Wells A, Vasakova M, Pesci A, Sofia M, Klepetko W, Seeger W, Drakopanagiotakis F, Crestani B. The European IPF registry (eurIPFreg): baseline characteristics and survival of patients with idiopathic pulmonary fibrosis. *Respir. Res.* 2018; 19: 141.
11. Armanios MY, Chen JJ-L, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA, Lansdorp PM, Greider CW, Loyd JE.

Telomerase mutations in families with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 2007; 356: 1317–1326.
12. Jo HE, Glaspole I, Grainge C, Goh N, Hopkins PMA, Moodley Y, Reynolds PN, Chapman S, Walters EH, Zappala C, Allan H, Keir GJ, Hayen A, Cooper WA, Mahar AM, Ellis S, Macansh S, Corte TJ. Baseline characteristics of idiopathic pulmonary fibrosis: analysis from the Australian Idiopathic Pulmonary Fibrosis Registry. *Eur. Respir. J.* 2017; 49: 1601592.
13. Cottin V, Koschel D, Günther A, Albera C, Azuma A, Sköld CM, Tomassetti S, Hormel P, Stauffer JL, Strombom I, Kirchgaessler K-U, Maher TM. Long-term safety of pirfenidone: results of the prospective, observational PASSPORT study. *ERJ Open Res.* 2018; 4: 00084–02018.
14. Crestani B, Huggins JT, Kaye M, Costabel U, Glaspole I, Ogura T, Song JW, Stansen W, Quaresma M, Stowasser S, Kreuter M. Long-term safety and tolerability of nintedanib in patients with idiopathic pulmonary fibrosis: results from the open-label extension study, INPULSIS-ON. *Lancet Respir. Med.* 2019; 7: 60–68.

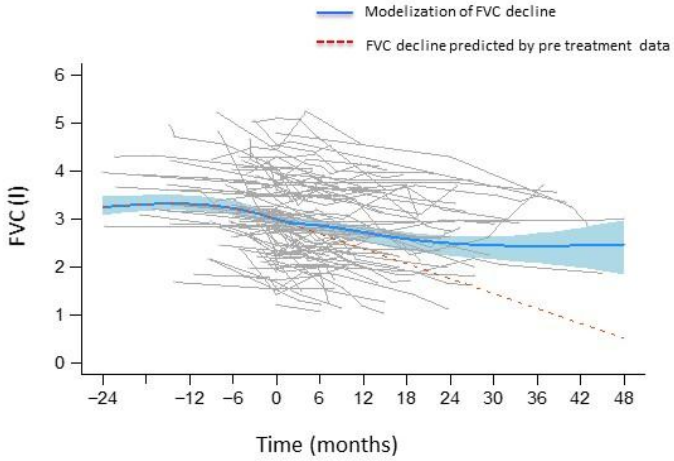
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A


Pirfenidone
Nintedanib
Treated (full dose)
Treated (Reduced dose)
Stop (disease progression)
Stop (side effect)
Percentage of patient
100
80
60
40
20
0
M0
M3
M6
M9
M12
M24
N
59
59
59
54
51
45
34
34
33
32
32
29

B


Modelization of FVC decline
FVC decline predicted by pre treatment data
FVC (l)
6
5
4
3
2
1
0
−24
−12
−6
0
6
12
18
24
30
36
42
48
Time (months)

Supplementary Table 1: Pathogenic telomerase related gene mutations

Patient	Gene	Mutation Type	cDNA	Amino acid	Segregation*	Frequency gnomAD$	Reference	Polyphen2&	CADD&	Splicing effect	Telomere length	Functional effect
1	*TERT*	Missense	c.2468+6T>G	NA	1/1	NR	Unpublished	NA	NA	Damaged donor Site (prediction of loss, weakening or activation of cryptic splice site)	NA	NA
2	*TERC*	Substitution	r.323C>G	NA	1/1	NR	Takeughi et al, 2007	NA	22,0	NA	NA	NA
3	*TERT*	Missense	c.2287-2A>G	NA	1/1	NR	Borie et al., 2016	NA	20,4	Loss of acceptor splice	7.56 kb 50th-90th	NA

										site		
4	*TERT*	Stop-gain	c.1215C>G	p.Tyr405*	2/2	NR	Unpublished	NA	35,0	No	NA	NA
5	*TERT*	Stop-gain	c.1215C>G	p.Tyr405*	2/2	NR	Unpublished	NA	35,0	No	NA	NA
6	*RTEL1*	Stop-gain	c.2956C>T	p.Arg986*	2/2	NR	Moriya et al, 2016	NA	NA	No		
7	*TERT*	Missense	c.3014T>C	p.Leu1005Pro	1/1	NR	Unpublished	0,60	25,8	No	<1st	NA
8	*TERT*	Missense	c.2011C>T	p.Arg671Trp	1/1	NR	Diaz de Leon et al., 2010, Snetselaar et al. 2017 plos one	0,79	21,6	No	Low	NA
9	*TERT*	Missense	c.3007A>G	p.Lys1003Glu	1/1	NR	Unpublished	0,72	18,2	No	NA	NA
10	*TERT*	Missense	c.1805C>T	p.Ser602Leu	1/1	0,000004	Unpublished	0,96	25,0	No	NA	No
11	*TERT*	Missense	c.2225G>A	p.Arg742His	1/1	NR	Petrovski, 2017	0,69	24,3	No	NA	NA
12	*TERC*	Substitution	r.23 G>C		1/1	NR	Unpublished	NA	8,29	No	NA	NA
13	*TERT*	Missense Frameshift	c.2224C>T c.2655-47_2659dup	p.Arg742Cys p.Leu887Argfs*16	3/3	NR	Unpublished	0,99	27,1	No	<1st	NA
14	*TERT*	Deletion	c.1646_1648del	p.Met549del	1/1	NR	Unpublished	NA	16,1	No	NA	NA

15	*TERT*	Missense	c.2321G>T	p.Arg774Leu	1/1	NR	Unpublished	0,55	23,8	No	NA	NA
16	*TERC*	Deletion	r.182delG	NA	2/2	NR	Thépault, rev Mal resp 2016	NA	23,0	NA	NA	NA
17	*TERT*	Missense	c.3286C>T	p.Leu1096Phe	1/1	NR	Unpublished	0,99	24,3	No	<1st	NA
18	*TERT*	Missense	c.1828C>T	p.Arg610Trp	1/1	0,000008	Nunes et al., 2017	0,72	24,0	No	NA	NA
19	*TERT*	Missense	c.2431C>T	p.Arg811Cys	1/1	0,000032	Marrone et al, 2007	0,91	25,4	No	NA	50%
20	*RTEL1*	Missense	c.2946C>G	p.His982Gln	1/1	NR	Unpublished	0,31	23,1	No	<10th	
21	*TERT*	Missense	c.2989G>A	p.Val997Met	1/1	NR	Unpublished	0,79	24,2	No	NA	NA
22	*TERT*	Missense	c.2542G>A	p.Asp848Asn	1/1	NR	Unpublished	0,99	23,7	No	NA	NA
23	*TERT*	Missense	c.2080G>A	p.Val694Met	1/1	NR	Yamaguchi et al., 2005	0,99	24,5	No	1^{st}-10th	NA
24	*TERT*	Missense	c.2516C>T	p.Thr839Met	1/1	0,000008	Unpublished	1,00	25,6	No	NA	NA
25	*TERT*	Missense	c.2516C>T	p.Thr839Met	1/1	0,000008	Unpublished	1,00	25,6	No	NA	NA
26	*TERT*	Missense			1/1	0,000	Unpublishe	1,00	25,6	No	NA	NA

			c.2516C>T	p.Thr839Met		008	d					
27	*TERT*	Missense	c.2287-2A>C	NA	1/1	NR	Borie et al., 2016	NA	20,4	Loss of acceptor splice site	7.56 kb 50th-90th	NA
28	*TERT*	Missense	c.1432T>C	p.Trp478Arg	1/1	NR	Nunes et al., 2017	1,00	24,9	No	NA	NA
29	*TERC*	Substitution	r.448A>U	NA	1/1	NR	Collopy et al, 2015	NA	16,0	NA	<1st	NA
30	*TERC*	Substitution	r.35C>U	NA	1/1	0,000023	Du et al, 2009	NA	9,0	NA	NA	NA
31	*RTEL1*	Missense	c.2903G>A	p.Cys968Tyr	2/2	NR	Unpublished	0,98	24,1	No	NA	NA
32	*TERC*	Substitution	r.235C>G	NA	1/2	NR	Unpublished	NA	13,0	NA	<25th	NA
33	*TERC*	Substitution	r.170C>A	NA	1/1	NR	Unpublished	NA	17,0	NA	NA	NA
34	*TERT*	Missense	c.2146G>A	p.Ala716Thr	1/1	NR	Parry et al., 2011, Borie et al., 2016, Snetselaar et al. 2017 Plos one	1,00	29,3	No	0.700, <1st[b]	4% WT
35	*TERT*	Missense	c.2005C>T	p.Arg669Trp	1/1	0,000032	Newton et al., 2015, van der Vis et al, 2020 chest,	0,36	24,9	No	0.717, 1-10th[b]	NA

							Snetselaar, et al. 2017 plos one					
36	*TERT*	Missense	c.1584T>G	p.Cys528Trp	1/1	NR	Snetselaar et al. 2017 plos one	0,86	10,0	No	0.829, >10th[b]	
37	*TERT*	Missense	c.2011C>T	p.Arg671Trp	2/2	NR	Diaz de Leon et al., 2010, Snetselaar et al. 2017 plos one	0,79	21,6	No	0.822, >10th[b]	NA
38	*TERT*	Deletion	c.1698_1700delCAC	p.Thr567del	4/4	NR	Snetselaar et al. 2017 plos one	NA	NA	No	0.577, <1st[b]	NA
39	*TERT*	Missense	c.299G>A	p.Gly100Asp	1/1	NR	Unpublished	0,99	26,3	No	0.821, >10th[b]	NA
40	*TERT*	Stop-gain	c.232A>T	p.Lys78*	2/2	NR	Unpublished	NA	35,0		0.813, >10th[b]	NA
41	*TERT*	Deletion	c.1698_1700delCAC	p.Thr567del	4/4	NR	Snetselaar et al. 2017 plos one	NA	NA	No	0.460 <1st[b]	NA
42	*TERT*	Missense	c.395G>A	p.Arg132Gln	2/2	NR	Borie et al., 2016	0,42	23,9	No	0.719, 1-10th[b]	NA
43	*TERT*	Missense	c.2456G>A	p.Arg819His	1/1	0,000008	unpublished	0,93	22,4		0.858, >10	NA

											th[b]	
44	*PARN*	Missense	c.98C>T	p.Pro33Leu	2/2	NR	Unpublished	1,00	31,0		0.666, 1-10 th[b]	NA
45	*TERT*	Missense	c.1729C>T	p.Arg577Trp	6/7✠	0,000007	unpublished	1,00	33,0	No	0.675, 1-10 th[b]	NA
46	*TERT*	Missense	c.2005C>T	p.Arg669Trp	2/2	0,000032	Newton et al., 2015, van der Vis et al. 2020, chest, Snetselaar, et al. 2017 plos one	0,36	24,9	No	0.722, 1-10 th[b]	NA
47	*TERC*	substitution	r.30G>A	NA	1/1	0,000004	Unpublished	NA	8,6	NA	0.788, >10 th[b]	NA
48	*TERT*	Missense	c.3199T>C	p.Ser1067Pro	1/1	NR	Unpublished	0,02	8,3		0.586, <1 st[b]	NA
49	*TERT*	Missense	c.299G>A	p.Gly100Asp	1/1		unpublished	0,99	26,3	No	0.567, <1 st[b]	NA
50	*TERT*	Missense	c.515G>A	p.Gly172Glu	1/1	NR	unpublished	0,99	24,7		0.693, 1-10 th[b]	NA
51	*RTEL1*	Deletion in-frame	c.2044_2046delCTC	p.Leu682del	2/2	NR	Unpublished	1 (Trp)	NA	No	0.728, <10 th[b]	NA
52	*PARN*	Missense	c.1214G>C	p.Ser405Thr	1/1	NR	Unpublished	0,18	22,0		0.734, 1-10	NA

											th[b]	
53	*TERT*	Missense	c.1882G>A	p.Asp628Asn	1/1	0,000004	unpublished	0,01	0,1		0.710, >10th[b]	NA
54	*TERT*	Missense	c.2005C>T	p.Arg669Trp	1/1	0,000032	Newton et al., 2015, van der Vis et al. 2020 Chest, Snetselaar, et al. 2017 plos one	0,36	24,9	No	0.868, >10th[b]	NA
55	*TERC*	Substitution	r.448A>G	NA	1/1	NR	Collopy et al, 2015	NA	16,0	NA	0.657, 1-10th[b]	NA
56	*TERC*	Substitution	r.91G>C	NA	1/1	NR	Unpublished	NA	22,1	NA	0.504, <1st[b]	NA
57	*PARN*	Missense	c.98C>T	p.Gly33Val	2/2	NR	Unpublished	1,00	31,0		0.755, >10th[b]	
58	*TERT*	Missense	c.3148A>G	p.Lys1050Glu	1/1	NR	Cronkhite et al., 2008 Diaz de Leon et al., 2010	0.640	24,2	No	0.741, >10th[b]	90-110% WT
59	*RTEL1*	Duplication	c.3493dupC	p.Gln1165Profs*22	2/2	NR	Kannengiesser et al, 2015	NA	NA	No		
60	*TERT*	Missense	c.446T>A	p.Leu149Gln	1/1	NR	Unpublished	0,81	23,7	No	NA	NA

61	*TERT*	Stop-gain	c.3216G>A	p.Trp1072*	3/3	NR	Borie et al., 2016	NA	41,0	No	10.62 kb >99th	NA
62	*TERT*	Missense	c.1864C>T	p.Arg622Cys	1/1	NR	Borie et al., 2016	1,00	28,9	No	7.54 kb 90th	NA
63	*TERT*	Missense	c.2935C>T	p.Arg979Trp	2/2	NR	Borie et al., 2016	0,98	32,0	No	4.73 kb 1st-10th	NA
64	*TERT*	Missense	c.2638G>A	p.Ala880Thr	1/1	NR	Borie et al., 2016	1,00	23,6	No		NA
65	*TERT*	Duplication	c.336dupC	p.Glu113Argfs*79	1/1	NR	unpublished	NA	22,7	No	NA	NA
66	*TERT*	Missense	c.1630T>C	p.Phe544Leu	2/2	NR	Borie et al., 2016	0,99	16,6	no	10th-50th	NA
67	*TERT* *TERC*	Digenism	TERC r.434G>U TERT c.2446C>G	NA, TERT p.His816Asp	7/7 TERT	NR	unpublished					NA
68	*TERT*	Stop-gain	c.2968C>T	p.Gln990*	2/2	NR	Borie et al., 2016	NA	39,0	Possible impact on splice site	7.33 kb 50th-90th	NA
69	*TERC*	Substitution	r.448A>G	NA	1/1	NR	Collopy et al, 2015	NA	16,0	NA	<1st	NA
70	*TERT*	Missense	c.2966T>G	p.Leu989Trp	2/2	NR	unpublished	1,00	25,3	No	NA	
71	*TERT*	Missense	c.2911C>T	p.Arg971Cys	1/1	NR	Borie et al., 2016	0,96	28,6	No	<1st	40-50%WT

72	*TERT*	Deletion	c.2851delC	p.Arg951Glyfs*30	2/2	NR	unpublished	NA	NA	NA	NA	NA
73	*TERT*	Missense	c.2267G>T	p.Arg756Leu	1/1	NR	Borie et al., 2016	0,13	23,4	No	3.79 kb <1st	75%WT
74	*TERT*	Missense	c.2225G>A	p.Arg742His	1/1	NR	Petrovski et al., 2017	0,69	24,3	No	NA	NA
75	*TERT*	Missense	c.2935C>T	p.Arg979Trp	1/1	NR	Vulliamy et al., 2005	0,98	32,0	No	Low	100%WT
76	*TERT*	Missense	c.2678A>T	p.Glu893Val	1/2	NR	Borie et al., 2016	1,00	25,9	No	50th-90th	No
77	*TERT*	Missense	c.2377G>A	p.Glu793Lys	2/2	NR	Unpublished	0,98	28,6	No	NA	No
78	*TERT*	Missense	c.2377G>A	p.Glu793Lys	2/2	NR	Unpublished	0,98	28,6	No	NA	No
79	*TERC*	Substitution	r.323C>G	NA	3/3	NR	Unpublished	NA	22,0	NA	NA	NA
80	*TERT*	Missense	c.1511C>T	p.Ser504Leu	1/1	NR	Borie et al., 2016	0,93	24,2	No	50th-90th	NA
81	*TERT*	Splicing	c.2843+1G>A		5/5			NA	NA	NA	NA	NA
82	*TERT*	Missense	c.2147C>T	p.Ala716Val	6/6	NR	Vulliamy 2011	1,00	27,7	NA	<1st	NA
83	*TERT*	Missense	c.1864C>T	p.Arg622Cys	1/1	NR	Borie et al., 2016	1,00	28,9	No	NA	NA
84	*TERC*	Substitution	r.164A>C	NA	1/1	0,000009	Unpublished	NA	13,0	NA	NA	NA

85	*TERT*	Missense	c.2594G>A	p.Arg865His	9/9	0,000011	Tsakiri et al., 2007, Diaz de Leon et al., 2010 Newton et al., 2016	1,00	29,1	No	<1st	30%WT
86	*TERT*	Duplication	c.1336dupC	p.Arg446Profs*93	1/1	NR	Collopy et al., 2015	NA	18,5	No	<1st	NA
87	*TERT*	Duplication	c.1336dupC	p.Arg446Profs*93	1/1	NR	Unpublished	NA	18,5	No	NA	NA
88	*TERC*	Delins	r.200_201delinsAG	NA	1/1	NR	Unpublished	NA	16,0	NA	NA	NA
89	*TERC*	Substitution	r.448A>U	NA	1/1	NR	Collopy et al, 2015	NA	16,0	NA	<1st	NA

✠ the patient not carrying the mutation was considered as a phenocopy