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Short title: Lung transplantation with telomere syndrome

Abstract

Background: Mutations in the telomerase complex (*TERT* and *TR*) are associated with pulmonary fibrosis and frequent hematological manifestations. The aim of this study was to characterize the prognosis of lung transplantation in patients with *TERT* or *TR* mutations.

Methods: Patients with documented *TERT* or *TR* mutations who received a lung transplantation in 2007-2013 in France were identified exhaustively through the lung transplantation network, one expert genetic laboratory, and the clinical research network on rare pulmonary diseases.

Results: Nine patients (7 men) with *TERT* (n=6) or *TR* (n=3) mutations received a single (n=8) or a double (n=1) lung transplantation for pulmonary fibrosis. Median age was 50 years [range, 35-61] at diagnosis and 52 years [37-62] at the time of lung transplantation. Seven patients had thrombocytopenia before lung transplantation. Six patients developed myelodysplasia and/or bone marrow failure after lung transplantation, directly contributing to death in 4 cases. Anemia and neutropenia were observed in 9 and 3 patients, respectively. The median survival after lung transplantation was 214 days [59-1709].

Conclusion: Patients with mutations of the telomerase complex are at high risk of severe hematological complications after lung transplantation namely bone marrow failure. Specific recommendations should be developed for appropriate guidance regarding the hematological risk assessment before transplantation and the management of post-transplantation immunosuppressive regimen.

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INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is associated with a median survival time of about 3 years [1]. Lung transplantation has been shown to improve survival in selected patients [2]. The prevalence of IPF is estimated at 1/2500 to 1/7000, and 2% to 20% of IPF patients have at least one first-degree family member with a fibrotic lung disease [3-4]. Genetic studies in familial forms of IPF led to the discovery of mutations within *TERT* (encoding the telomerase reverse transcriptase) and *TR* (encoding the telomerase RNA component), which are both required for normal telomerase activity [5]. Heterozygous mutations within *TERT* or *TR* are detected in 15% to 20% of the cases of familial forms of pulmonary fibrosis (PF), whereas they are very rarely detected in sporadic IPF (<3%) [5-7]. In addition to PF, mutations in the telomerase complex are associated with mucocutaneous abnormalities such as dyskeratosis congenita (abnormal skin pigmentation, nail dystrophy, oral leukoplakia), and possibly severe complications including liver cirrhosis and bone marrow failure. As the phenotype may be heterogeneous and the same *TERT* mutations can be associated with different phenotypes of lung disease even in the same family [8], individual risk prediction is challenging.

We recently evidenced a *TERT* mutation in a patient with PF, who developed bone marrow failure following lung transplantation, triggering the present study. The largest published experience only reports 8 patients with telomerase mutations and lung transplantation from 3 different countries [9]. Therefore we reviewed the results of lung transplantation in all the patients with PF associated with *TERT* or *TR* mutations in France, in order to determine whether these patients were at risk of hematological complications.

METHODS

PATIENTS

In this retrospective, observational, non-interventional study, all ten French lung transplant centers were contacted in order to identify patients with *TERT* or *TR* mutations referred for lung transplantation during the 2009-2013 period, as the laboratory of genetics began to perform these sequencing in 2009. In addition, cases were cross-identified through the only genetic laboratory in France that centrally assesses for telomerase mutations, and by the *Groupe d'Etudes et de Recherche sur les Maladies "Orphelines" Pulmonaires* (GERM"O"P), a collaborative group dedicated to research on rare (so-called "orphan") pulmonary diseases.

All the patients with PF with known mutations of *TERT* or *TR* who received a lung transplantation during the study period in France were included. The clinical charts of the patients were reviewed and data were collected on a standardized and anonymous collection form. Chest CT scans, biopsies and explants were systematically classified based on a multidisciplinary team discussion in each of the 4 expert centers involved in this study and classified according to the 2011 official ATS/ERS/JRS/ALAT statement for IPF [2, 10] and the revised classification of idiopathic interstitial pneumonia [11]. Haematogical manifestations including myelodysplastic syndromes were classified according to the 2008 WHO classification [12].

All patients or relatives gave signed consent for genetic analysis. The Institutional Review Board of the French learned society for respiratory medicine -*Société de Pneumologie de Langue Française*- approved this study (CEPRO 2012-016).

Survival and characteristics at the time of lung transplantation were compared to a control group of patients with PF, extracted from the database of A*gence de Biomedicine* that collects data from all organ transplants in France, for the same period (2009-2013) and same centers. During the 2009-2013 period, 9 patients with known *TERT* or *TR* mutations included in this series, and 196 patients with non-familial PF and unknown mutational status received lung transplantation. Physicians in charge of lung transplantation in corresponding centres were queried for occurrence of myelodysplastic syndrome following transplant.

TERT AND *TR* SEQUENCING

Exons, intron-exon junctions and promoters of *TERT* and *TR* were sequenced by bi-directional sequencing (primers sequences available on request) and compared to the reference sequences for *TERT* : NM_198253.2 and *TERC* : NR_001566.1.

TELOMERE LENGTH

The telomere length was evaluated by terminal restriction fragment assay. DNA from blood cells was extracted, digested by the Hinf1 and Rsa1 nucleases (which do not digest telomere DNA sequences), submitted to a Southern blot using a specific telomere probe, and compared to those found in healthy age-matched controls [13].

STATISTICAL ANALYSIS

Data for continuous variables are expressed as median (range) and were compared by the Mann-Whitney U test. Categorical variables were expressed as counts and proportions and compared by the Fisher's exact test. Survival was studied and compared with a Kaplan-Meier analysis. Categorical variables are expressed as number and percentage and were compared by

the Fisher's exact test. All tests were two-sided, with p < 0.05 indicating statistical significance. All analyses used the Graph-Pad Prism 5 software (La Jolla, Ca, USA).

RESULTS

Clinical characteristics at the diagnosis of pulmonary fibrosis

Nine patients with *TERT* or *TR* mutations received lung transplantation in France from January 2009 to October 2013. The main clinical characteristics of the patients at diagnosis are shown in **Table 1**. Interstitial lung disease was diagnosed as IPF in 7 patients, pneumoconiosis in 1 patient, and chronic hypersensitivity pneumonitis in 1 patient. Six patients had some degree of exposure to inhaled contaminants (**Table 1**). Six patients presented with extra-pulmonary manifestations suggestive of telomere syndrome: 5 with hematological abnormalities, 1 with isolated unexplained liver enzymes elevation, and 3 with mucocutaneous abnormalities: nail dystrophy (n=2), hypopigmented macules (n=1), premature hair graying (n=1), and mucosal leukoplakia (n=1).

Median age at PF diagnosis was 50 years [range, 35-61]. Patients had pulmonary symptoms for 6 months [1-59] prior to the diagnosis of lung fibrosis. Dyspnea was the most frequent symptom (n=8), although one patient was asymptomatic at the time of diagnosis. Pulmonary function tests (PFT) results showed a reduced vital capacity (VC) (63% [40-88] of the predicted value) with reduced diffusion capacity for carbon monoxide (DLCO) (38% [16-77] of predicted).

The CT scans were reviewed and classified as definite usual interstitial pneumonia (UIP) pattern in 5 cases, possible UIP pattern in 3 cases, and consistent with chronic hypersensitivity pneumonitis in 1 patient. A video-assisted surgical lung biopsy was performed before lung

transplantation in 5 patients; 3 samples were classified as a definite UIP pattern, 1 as a probable UIP pattern and 1 as unclassifiable fibrosis. Lung explants were classified pathologically as UIP pattern in 2 cases, probable UIP pattern in 5 cases (absence of fibroblast foci), not UIP pattern in 2 cases. The diagnosis was pneumoconiosis in one case; due to the presence of granuloma, the diagnosis of chronic hypersensitivity pneumonitis was suggested in one case.

The median follow-up between the PF diagnosis and lung transplantation was 24.6 [4.5-72.2] months. Before transplantation, all patients received corticosteroids, 5 patients had received immunosuppressants (azathioprine, n=3; cyclophosphamide, n=2), and 2 patients received pirfenidone (**Table 2**). None of the treatment was associated with any objective pulmonary improvement assessed either by CT scan or an increase in VC or DLCO by 10% of the predicted value or greater. At the last visit before transplantation, VC and DLCO were 42% [75-22] and 22% [15-33] of predicted, respectively.

Genetic analysis

Telomerase mutations were identified before (n=5), or after lung transplantation (n=4) (**Table 3**), including post-mortem in two cases. Indication of *TERT* and *TR* sequencing were a familial history of lung fibrosis (n=4), hematological abnormalities (n=2), or both (n=3). Two patients presented with both personal hematological abnormalities and a familial history of lung fibrosis and thrombocytopenia. Telomere lengths measurement was near or below 5kb, and considered as abnormally short compared to age-matched controls in all 5 tested cases [13].

Hematological abnormalities before transplantation

At the time of PF diagnosis and prior to any therapy, 4 patients had thrombocytopenia with a median platelet count of 59 G/L [50-66], and 1 patient had mild anemia (113 g/L) (**Figure 1**). During the pre-transplant period, 3 additional patients developed thrombocytopenia (53, 108 and 135 G/L, respectively), including 1 patient who received corticosteroids and pirfenidone alone, and 2 patients who were treated with azathioprine. Proband #5 received azathioprine before lung transplantation and did not develop cytopenia. Six patients had macrocytosis (mean corpuscular volume, 101 fL [101-103]) before lung transplantation, at the time of the lung fibrosis diagnosis in two patients, and during follow-up in four additional patients. Three of them had received azathioprine, but macrocytosis persisted after azathioprine withdrawal. During follow-up, only 1 patient developed macrocytic non-regenerative anemia (117 g/L). Neither neutropenia, nor monocytosis greater than 1 G/L developed in any patients. Two patients received cyclophosphamide before lung transplantation, and did not have hematological complications. Bone marrow cytology was available in 4 patients before lung transplantation (**Table 4**) considered as normal in 1 patient, and consistent with the diagnosis of refractory cytopenia with unilineage dysplasia in the 3 others. Karyotype was normal in all 4 tested cases.

General evolution in the post transplantation period

Eight patients received a single-lung transplantation and 1 patient received a double-lung transplantation. Five patients underwent high emergency lung transplantation. **Table 2** shows the post-transplantation therapies received. None of them received alemtuzumab or anti-thymocyte globulin. The median survival from the time of transplantation was 214 days (Figure

2). At the end of follow-up, 3/9 patients were alive, 310, 747 and 1709 days after transplantation. The causes of deaths are indicated in **Table 4**. Two patients with persistent pancytopenia received multiple platelets and blood transfusions, granulocyte-colony stimulating factor (G-CSF) and erythropoietin injections. Hematopoietic stem cell transplantation was not considered and palliative care was started. Finally, death occurred and was attributed to refractory pancytopenia. Two patients died from sepsis, related to *Staphylococcus haemolyticus* and *Enterobacter cloacae*, respectively; neutrophil counts were within the normal range, whereas platelet counts were reduced at 25 G/L and 9 G/L. In those patients, deaths likely occurred because of the lung transplantation complications and myelodysplastic syndromes. One patient died from pulmonary embolism unrelated to myelodysplastic syndrome. The cause of death remained unknown for one patient. Two of the 3 living patients have myelodysplastic syndromes.

Lung function tests were available after lung transplantation in 5 patients, all with single-lung transplantation. Forced expiratory volume in 1 second (FEV1) was 48% [38-64] and 40% [28-68] of the predicted values, 3 and 6 months after transplantation, respectively. The best FEV1 available was 50% [38-74] of the predicted values. In the 3 patients alive, the last follow-up visit showed a FEV1 of 44%, 53%, and 59% of the predicted values, respectively. During follow-up, three patients developed acute transplant rejection requiring increased doses of corticosteroids. The diagnosis of rejection was made after clinical suspicion and not on systematic biopsies. One patient developed a second episode of acute rejection. The severity of acute rejection was classified with transbronchial biopsies as A1B0 for 2 patients, and clinically

highly suspected for one patient but not confirmed by histology because of thrombocytopenia. Based on PFT, none of them developed bronchiolitis obliterans syndrome.

Comparison with a control group

Patients from the control group with pulmonary fibrosis and unknown mutational status (N=196) were more frequently women (25%, P=0.03). Their median age was 55.4+/-3.0 years (P=0.12 vs mutated patients). The diagnosis of myelodysplastic syndrome was given in 2 patients from the control group.

The median survival of the control group was 1301 days and was not statistically different from the mutated group (p=0.85). Lung transplantation was less frequently a high emergency procedure in controls than in mutated patients (25% vs 55%, P=0.04). In the control group, high emergency procedure was associated with poorer survival (167 days for high emergency procedure vs 1437 days without high emergency procedure, P=0.004). After excluding patients transplanted with high emergency procedures, survival was not different between the control and mutated groups.

Post-transplantation infections and hematological abnormalities

Due to the frequent use of extracorporeal circulation in the perioperative period, the nadir of thrombocytopenia was defined starting at day 7 post-transplantation. Thrombocytopenia worsened after lung transplantation in 5/7 patients who had thrombocytopenia prior to the transplantation, reaching a platelets count of 25 G/L [8-28]. The 4 other patients did not present with thrombocytopenia, including 1 patient previously treated with azathioprine (**Figure 1**). Two

patients who received azathioprine and one who received induction therapy with basiliximab, had worsening of thrombocytopenia (**Figure 1**). Two of the 3 patients treated with cyclosporine also had thrombocytopenia. Three patients received everolimus for a short duration without worsening of cytopenia. The thiopurine methyltransferase activity, which when low is associated with azathioprine toxicity [14], was available for 2 patients with severe pancytopenia and was normal.

All the patients developed anemia (**Figure 1**). Three patients had neutropenia with neutrophils counts of 0.1, 0.8 and 0.8 G/L, respectively. Two of them also had a thrombotic microangiopathy at that time. Bone marrow cytology was consistent with a myelodysplastic syndrome in 6 patients (**Table 4**): refractory anemia with excess blasts type 1 (n=1), refractory cytopenia with multilineage dysplasia (n=4), refractory cytopenia with unilineage dysplasia (n=1).

Severe infections occurred in 6 patients (**Table 4**). One patient had pulmonary infection with *Pneumocystis jiroveci* while co-trimoxazole was withdrawn due to cytopenia. Isolated CMV viremia developed in 3 patients, whose treatment by ganciclovir was limited by cytopenia.

Except thrombotic microangiopathy, which occurred in 2 cases, no patient presented acute renal failure. These patients died while they were still in need for dialysis. Only 1 patient had mildly elevated liver enzymes (below twice the upper limit of normal).

DISCUSSION

This is the largest report on outcomes of lung transplantation in a comprehensive series of patients with *TERT* or *TR* mutations. The main finding of the present study is that hematological

complications following lung transplantation were more frequent in this patient population than in a control group of lung transplant recepients for PF, with possible sepsis related to bone marrow failure, therefore warranting close attention of clinicians both for the pre-transplant evaluation, and in the post-transplant follow-up period.

Patients with familial PF are usually younger than 65 years, and may therefore be considered as adequate candidates for lung transplantation [8, 15]. Our series underscores that *TERT* and *TR* mutations may be revealed not only by familial history of PF but also by extra-pulmonary signs of telomere syndrome such as bone marrow failure or liver disease [16]. Moreover hematological complications, mainly thrombocytopenia, may be absent at the time of diagnosis and develop during follow-up particularly in subjects receiving cytotoxic drugs.

Our data indicate that cytopenia, and particularly thrombocytopenia, is a common complication in telomerase mutation carriers exposed to cytotoxic drugs. Compared to matched family members without mutation, patients with *TERT* mutations have lower platelets count although generally within normal limits [17]. The co-occurrence of bone marrow failure and PF in the same individual or family is highly predictive of telomerase mutation [18]. In a previous series [9], all patients with telomerase mutations and PF had thrombocytopenia after lung transplantation, with a majority of them requiring platelet transfusion. Nearly all our patients had anemia after lung transplantation, and neutropenia was frequent with cytotoxic drugs, however thrombocytopenia was the most prominent manifestation. Thrombocytopenia should be considered highly suggestive of telomerase mutations in this setting.

The median survival of 214 days following transplantation in this series was very low, compared to that reported by the International Society for Heart and Lung Transplantation (ISHLT) in

transplant recipients with PF, who have a median survival of 4.3 years [19]. However, mortality could be increased due to the high rate (55%) of high emergency lung transplantation and to the frequent use of extracorporeal circulation [20]. The survival time in our series also compares unfavourably with the study of Silhan et al., in which 7 of their 8 patients were still alive after a median follow-up of 1.9 years [9]. However, the small number of patients in the latter series (e.g. only 8 patients identified among transplant recipients from the USA, Australia, and Sweden), may suggest that results by Silhan et al., be biased by selection and recall bias, with possible underdiagnosis of PF related to telomerase mutations and/or underestimation of the severity of complications (early deaths are frequently underrepresented in retrospective series [21]). The present series may better and more realistically reflect the severity of complications in transplant recipients carrying telomerase mutations. In addition, differences in immunosuppressive regimen between the present series and that of Silhan et al., may have contributed to differences in outcome. Indeed, while most subjects in the Silhan study received a two-drug immunosuppressive regimen, all the patients from our cohort received a third additional immunosuppressive drug. Of note, data from the literature further suggest that azathioprine may be more toxic than mycophenolate mofetil in this setting [22], an hypothesis that needs to be evaluated. Azathioprine, mycophenolate mofetil and cyclophosphamide likely contributed to the haematological complications. In our study, all patients received drugs with established hematological toxicity co-trimoxazole (N=9), ganciclovir (N=4), or voriconazole (N=4) but none received linezolid.

In view of the poor results of transplantation, some patients from the current series might be considered contra-indicated for lung transplantation, especially those with myelodysplastic

syndrome. In the future, genetic testing might be considered in the pre lung transplantation work-up.

Two patients presented thrombotic microangiopathy, with low serum level of disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS13), and died. One patient from the current series presented low level of ADAMTS13 (<40% of the normal value) without thrombotic microangiopathy. Thrombotic microangiopathy has been previously reported after lung transplantation [23], and we speculate that ADAMTS13 synthesis might be reduced in patients with mutations even in the absence of cirrhosis.

Identifying telomerase mutation may be challenging in patients evaluated for lung transplantation since myelodysplastic syndromes may only appear after cytotoxic drugs exposure, and the clinical / biological presentation may vary. The high rate of cytopenia after exposure to cytotoxic drugs in mutation carriers illustrates the abnormal repair capacities after external injury in these patients, and the phenotype heterogeneity according to age and environmental exposure. This is consistent with the fact that mutation carriers are more prone to develop liver cirrhosis associated to hepatitis C virus infection or alcohol exposure [24]. Similarly, environmental risk factors including tobacco or inhaled contaminants are found with a high frequency (96%) in patients with PF and a *TERT* mutation [8]. Indeed some patients with *TERT* or *TERC* mutations, without specific trigger could remain asymptomatic [8]. However *TERT* or *TR* mutations in the general population are very rare (<1/10000).

All patients from this cohort fulfilled clinical telomere syndrome criteria. However some mutations have not been reported previously [9][25]. Three mutations were already published in the telomerase database and considered deleterious [26]. None of the novel mutations were

found in database of controls (Exome variant server; 1000 Genome project), and Polyphen (polymorphism phenotyping) predicted all of them to be probably damaging. Telomere lengths assessed in 5 patients were shorter than that of age-matched controls in every case [13].

Experience of organ transplantation in telomerase mutations carriers is very limited. In addition to series of lung transplantation (the series by Silhan et al. [9][25], and the present one), one report of liver transplantation has been published in a 63 year-old man with hepatocellular carcinoma, PF and *TERT* mutation, who experienced worsening of PF and died four months after the liver transplantation [27]. A patient with dyskeratosis congenita associated with a Telomeric Repeat-binding factor *TRF1-Interacting Nuclear Factor 2 (TINF2)* mutation, an additional mutation associated with the telomere syndrome [28], underwent lung transplantation for PF at the age of 13, ten years after receiving hematopoietic stem cell transplantation for severe aplastic anemia; he experienced neutropenia due to mycophenolate mofetil, which was withdrawn, and was reported to be doing well 21 months after lung transplantation with tacrolimus treatment.

From a hematological point of view, patients with telomerase dysfunction are at high risk of pulmonary complications after hematopoietic stem cell transplantation [29]. Therefore a procedure with reduced immunosuppression is currently proposed for patients with dyskeratosis congenita [29-31]. Some reports suggest that mycophenolate mofetil and azathioprine may be safely removed from the drug regimen following lung transplantation [28, 32]. Whether mycophenolate mofetil might be better tolerated than azathioprine in this setting warrants further study.

One limitation of the present study is its retrospective design. However, the use of different sources (the single genetic laboratory with expertise in telomerase genes in France; all transplantation centers in France; and over 200 physicians participating to a network of clinical research on rare pulmonary diseases) ensured exhaustive identification in France of all cases of PF diagnosed with a mutation within the telomerase complex. It is conceivable that some cases with telomerase mutations may have been missed in the control group, as it can be hypothesized in the 2 patients with a diagnosis of myelodysplastic syndrome. Indeed, the *TERT* and *TERC* mutational status could not be analysed retrospectively in the control group. However *TERT* mutations were previously found in no more than 1-3% of sporadic IPF cases [34-35]. The diagnosis of myelodysplastic syndrome in the control group was retrieved by querying physicians, possibly underestimating its frequency. A prospective study with mutational status known before lung transplantation would be the only way to obtain definitive conclusions. Exhaustive collection of hematological data and of infectious events was comprehensively and reliably obtained through electronic patients charts. We therefore consider that this series is representative of the reality of lung transplantation in this particular population of patients.

In conclusion, severe hematological complications are observed in patients with PF and mutations of the telomerase complex who receive a lung transplantation. We suggest thatlung transplantation should be discouraged in patients with myelodysplasia, and should be considered on a case-by-case basis in remaining cases. Specific recommendations should be developed for appropriate guidance regarding the hematological risk assessment before transplantation and the management of post-transplantation immunosuppressive regimen.

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Table 1 Characteristics at the time of pulmonary fibrosis diagnosis

Characteristics	Patients N=9
Male (%)	7 (77)
Age at diagnosis, years	50 [35-61]
Smoker ever/active	6 (66)/0(0)
Occupational or environmental exposure*	6 (66)
Extra-pulmonary symptoms of telomeropathy	6 (66)
• Hematological	5 (55)
- Platelets count <150G/L	4 (44)
- Anemia	1 (11)
- Macrocytosis	2 (22)
• Cutaneous	3 (33)
• Elevated liver enzymes	1 (11)

Data are presented as median [range] or n (%). Anemia was defined by WHO criteria. Macrocytosis was defined by a mean corpuscular volume (MCV) >100fL.

* Two patients worked as farmers, two worked as construction workers, one as carpenter and one as polisher

Table 2 Pre and post transplantation therapy and duration of treatment

Drugs	Pre-transplantation	Post-transplantation
Corticosteroids	9 (100)/ 625 days [128-2017]	9 (100)/ 214 days [59-1709]
Azathioprine	3 (33)/ 792 days [118-1219]	2 (22)/ 80-219 days
N-Acetylcysteine	2 (22)/ 594-625 days	0 (0)
Pirfenidone	2 (22)/ 59-790 days	0 (0)
Cyclophophamide	2 (22)/ 106-224 days	0 (0)
Proton Pump Inhibitor	4 (44)/ na	7 (77)/ na
Mycophenolate mofetil	0 (0)	9 (100)/ 79 days [13-905]
Tacrolimus	0 (0)	9 (100)/ 111 days [41-967]
Cyclosporin	0 (0)	3 (33)/ 111 days [13-112]
Everolimus	0 (0)	3 (33)/ 25 days [14-43]
Basiliximab (induction)	0 (0)	1 (11)/ 4 days

Data are presented as n (%).

Duration of treatment is expressed as median [range] or minimum-maximum for n<3. na, not available.

Table 3 Mutations in *hTERT, hTR* and telomere lengths in the 9 probands

Proband No.	Age at presentation (years)	Gender	Gene	Mutation	Previously described mutation	Telomere lengths (kb)
1	38	M	*hTERT*	c.2911C>T, p.Arg971Cys	No	ND
2	39	M	*hTERT*	c.2639C>T, p.Ala880Val	No	5.26
3	57	M	*hTERT*	c.1864C>T, p.Arg622Cys	No	ND
4	61	M	*hTERT*	c.1630T>C, p.Phe544Leu	No	4.32
5	35	F	*hTERT*	c.2935C>T, p.Arg971Cys	[1]	5.29
6	58	M	*hTERT*	c.1710G>C, p.Lys570Asn	[2]	ND
7	38	F	*hTR*	r.110_113delGACT	[3-4]	4.3
8	49	M	*hTR*	r.1-22C>T	No	3.3
9	55	M	*hTR*	n.135G>C	No	ND

Telomeres length was assessed by Southern blot and express in kb. Telomere lengths were always considered as telomere shortening

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2. Xin ZT, Beauchamp AD, Calado RT, et al. Functional characterization of natural telomerase mutations found in patients with hematologic disorders. *Blood* 2007; 109:524-532.
3. Marrone A, Sokhal P, Walne A, et al. Functional characterization of novel telomerase RNA (TERC) mutations in patients with diverse clinical and pathological presentations. *Haematologica* 2007; 92:1013-1020.
4. Vulliamy T, Marrone A, Dokal I, and Mason PJ. Association between aplastic anaemia and mutations in telomerase RNA. *Lancet* 2002; 359:2168-2170.

Table 4 Pre-transplantation bone marrow cytology and evolution after lung transplantation

Proband No.	Age at transplantation, years	Pulmonary diagnosis	ECMO/High emergency procedure	Pre-transplantation bone marrow cytology, karyotype	Post-transplantation bone marrow cytology, karyotype	Post-transplantation immunosuppression	Post-transplantation infections (no.)	Status	Follow-up (days)	Cause of Death
1	42	HP	Yes/no	Unilineage megakaryocytic dysplasia, normal	Multilineage dysplasia, 7% blasts, normal	Cs Aza MMF FK	Pneumocystosis	Alive	310	NA
2	40	IPF	Yes/yes	ND	Multilineage dysplasia, i(7q), i(3q)	Cs MMF Cyc FK	Bacterial Pneumonia (3) Candida sepsis (1) Bacterial sepsis (3)	Dead	197	Pancytopenia
3	59	IPF	Yes/yes	ND	Multilineage dysplasia, normal	Cs MMF Cyc FK	Bacterial Pneumonia (3) Bacterial sepsis (1)	Dead	59	Sepsis
4	62	Pneumoconiosis	Yes/yes	Unilineage megakaryocytic dysplasia, normal	ND	Cs MMF FK	Bacterial Pneumonia (2) Pyothorax (1)	Dead	79	Pulmonary embolism
5	39	IPF	No/no	ND	ND	Cs MMF FK	Tuberculosis	Alive	1709	NA
6	58	IPF	No/no	Normal, ND	Multilineage dysplasia, normal	Cs Aza MMF FK Eve	CMV reactivation Bacterial pneumonia (2) Bacterial sepsis (1)	Dead	214	Sepsis
7	45	IPF	No/no	Unilineage megakaryocytic dysplasia, normal	Multilineage dysplasia, ND	Cs MMF FK Eve Basi	CMV reactivation HHV6 encephalitis Bacterial Pneumonia (1) Bacterial sepsis (1)	Dead	84	Pancytopenia
8	52	IPF	Yes/yes	ND	Unilineage megakaryocytic dysplasia, ND	Cs MMF FK	Bacterial Pneumonia (3) Bacterial sepsis (1)	Alive	747	NA
9	58	IPF	No/yes	ND	ND	Cs MMF Cyc FK Eve	CMV reactivation	Dead	650	Unknown

NA, not applicable; ND, not done,; HP, chronic hypersensitivity pneumonitis; IPF, idiopathic pulmonary fibrosis; ECMO, use of extracorporeal circulation; Cs, corticosteroids; Aza, azathioprine; MMF, mycophenolate mofetil; FK tacrolimus; Cyc, cyclosporine; Eve, everolimus; Basi, basiliximab
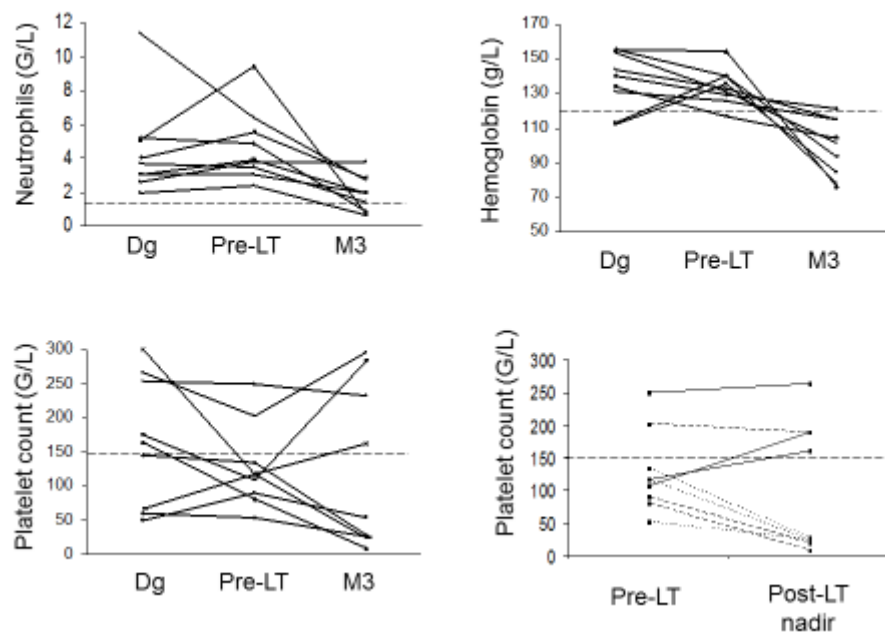
Figures

Figure 1

Figure 1 Evolution of platelet, neutrophil and hemoglobin counts at pulmonary fibrosis diagnosis, before lung transplantation (pre-LT) and 3 months after lung transplantation (M3) or last available data before death and post lung transplantation (post-LT) nadir by post-transplantation therapy. The horizontal dashed lines highlight the normal values. Because of the frequent use of extracorporeal circulation, nadir of thrombocytopenia was defined starting at day 7 post-transplantation. Dashed lines:

patients who received azathioprine or induction therapy (⋯⋯), patients who received cyclosporine (⋯——). Continuous

lines: patients who did not receive azathioprine, induction therapy nor cyclosporine (—■—).
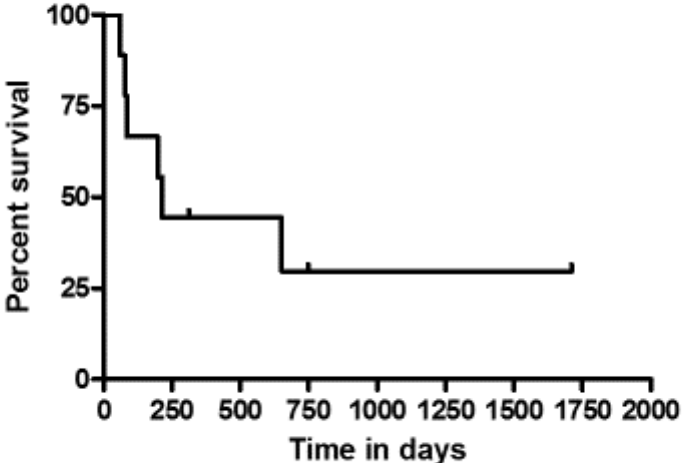
Figure 2

Figure 2 Kaplan-Meier estimation of overall survival after lung transplantation for 9 telomerase mutation carriers.