Telomere Shortening Is behind the Harm of Immunosuppressive Therapy in Idiopathic Pulmonary Fibrosis

Telomeres are crucial for cell function and tissue repair because these structures, capping the tip at the end of chromosomes, preserve genome integrity and cell life (1–3). Telomere length shortens during cell mitosis until it reaches a critical threshold that activates cell death or senescence (1). The telomerase complex catalyzes the addition of DNA sequences, thereby protecting telomeres (1). Several telomerase gene mutations that lead to telomerase dysfunction and telomere attrition have been described in different aging-related diseases, including pulmonary fibrosis (1–6). Reduced telomere length, the endotype that would lead to cell senescence and tissue degeneration, has been identified in a majority of patients with familial pulmonary fibrosis, in some sporadic cases of idiopathic pulmonary fibrosis (IPF), and, more recently, in other forms of pulmonary fibrosis (3, 4, 6–8). Telomere shortening is a strong predictive factor of poor prognosis (6–12): patients with IPF and short telomeres, especially middle-aged individuals, have more rapid disease progression and shorter lung transplant–free survival. Telomere dysfunction may affect other organs and systems; for instance, some abnormalities in bone marrow or the immune response have been found in some patients with telomeric IPF before and after lung transplantation, which increases the associated morbidity (10, 11, 13). Furthermore, short-telomere syndromes are multisystem disorders with widespread clinical manifestations that may be present in a minority of patients with familial pulmonary fibrosis (10). However, systemic dysfunction related to telomere shortening in patients with IPF may be overlooked and manifest clinically only after treatment with an immunosuppression medication required for lung transplantation (10, 13).

In this issue of the *Journal*, Newton and colleagues (pp. 336–347) describe a strong correlation between the presence of reduced telomere length below the 10th percentile and the harmful effect of immunosuppressive medication in patients with IPF from the PANTHER-IPF (Prednisone, Azathioprine, and *N*-Acetylcysteine: A Study That Evaluates Response in Idiopathic Pulmonary Fibrosis) clinical trial (14). They found that patients who were randomized to the triple therapy prednisone/azathioprine/*N*-acetylcysteine with telomere shortening (<10th percentile) had a significantly worse composite endpoint-free survival (death, transplant, hospitalization, and FVC decline) than those with telomere length >10th percentile. Findings of this *post hoc* analysis were replicated in patients with IPF who had been randomized to the placebo arm of the ACE (Anticoagulant Effectiveness) clinical trial and took prednisone or azathioprine. Similar results were also observed in patients with IPF from an independent longitudinal academic cohort that received at least one immunosuppressive medication (prednisone, azathioprine, or mycophenolate mofetil) for more than 6 months. Despite the limited number of subjects with available DNA from each cohort, after adjusting for other prognostic factors such as age and baseline FVC% predicted, the interaction between the harmful effect of prednisone and azathioprine and the presence of telomere shortening remained significant. Although prednisone is not a treatment for IPF, it is used in acute exacerbations and other pulmonary fibrotic diseases. The present study underlines the potential relevance of telomere length as a marker of danger regarding treatment with immunosuppressive drugs in pulmonary fibrosis. Currently, there is no specific treatment targeting telomere shortening that has demonstrated a benefit in patients with IPF (10). However, a recent *post hoc* analysis of the CAPACITY (Clinical Studies Assessing Pirfenidone in IPF: Research of Efficacy and Safety Outcomes) and ASCEND (A Study of Cardiovascular Events in Diabetes) trials showed the safety profile of pirfenidone, stratified by the presence of telomere shortening and in comparison with placebo, and a beneficial effect on FVC decline in patients with IPF and telomere gene mutations (15). Although scientific evidence regarding treatment in patients with the telomere shortening and IPF phenotype is limited, management should include initiation of antifibrotic agents, early referral to lung transplant evaluation, and efforts to balance the pros and cons when considering the use of corticosteroids and other immunosuppressive drugs in acute exacerbations or lung transplantation. Furthermore, prospective studies evaluating the effect of currently used immunosuppressive medications in patients with a non-IPF fibrotic disease and telomere shortening should be a priority.

In the present study, the authors used quantitative PCR of genomic DNA from blood leukocytes to measure telomere length. Although this methodology could cause overestimation of identified cases, the correlation using the standard method for analyzing telomere length (Southern blot) is very high (15). If only DNA is available and a laboratory uses well-defined standards and controls, this method of analysis is useful for detecting reduced telomere length (16). Flow cytometry–fluorescent *in situ* hybridization has been widely recommended for evaluating telomere length because it correlates better with Southern blot. However, this method requires live cells and more specialized technology, which makes it challenging to validate results and hypotheses in different cohorts and to translate the potential utility of telomere length screening to the clinic.

The high proportion (~50–60%) of subjects with telomere shortening from both included clinical trials could be attributed to the type of methodology used, but also to the possibility of a high

Originally Published in Press as DOI: 10.1164/rccm.201812-2330ED on January 9, 2019

rate of cases with family aggregation into the available DNA since they are more sensitive to consent for genetic studies. This clinical information was lacking in both of the clinical trials and the prospective cohort. Data about family history and other clinical signs of telomere abnormalities may be useful for interpreting the results of clinical trials. Evaluation of telomere length is not included in the diagnostic algorithm for IPF or other pulmonary fibrotic diseases because of the limited evidence regarding the utility of this test. Furthermore, telomere tests are only available in a minority of expert centers in interstitial lung diseases. However, the identification of clinical signs that increase the probability of telomere shortening is possible and could aid in diagnostic and treatment decisions (10).

Overall, this work provides insight into the biological bases of the detrimental effect of immunosuppressive drugs (prednisone and azathioprine) that was observed in IPF. This treatment could worsen disease behavior in subjects who have pulmonary fibrosis and telomere shortening, which is especially of concern considering the current treatments of many patients with a progressive non-IPF disease. Furthermore, this noteworthy study suggests a pharmacogenomics interaction related to an individual's endotype (telomere shortening) and his or her response to medications. However, telomere tests are being used only for research purposes. Therefore, prospective studies evaluating the clinical relevance of determining telomere length and telomerase gene mutations in subjects with IPF or progressive non-IPF diseases are required for translating into clinics. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Maria Molina-Molina, M.D., Ph.D.
Respiratory Department
Bellvitge University Hospital–Bellvitge Institute for Biomedical Research
Barcelona, Spain
and
CIBER of Respiratory Diseases
Madrid, Spain

ORCID ID: 0000-0002-1852-1723 (M.M.-M.).

References

1. Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett* 2005;579: 859–862.
2. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, *et al*. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356: 1317–1326.
3. Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, *et al*. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:729–737.
4. Armanios M, Alder JK, Parry EM, Karim B, Strong MA, Greider CW. Short telomeres are sufficient to cause the degenerative defects associated with aging. *Am J Hum Genet* 2009;85: 823–832.
5. Alder JK, Barkauskas CE, Limjunyawong N, Stanley SE, Kembou F, Tuder RM, *et al*. Telomere dysfunction causes alveolar stem cell failure. *Proc Natl Acad Sci USA* 2015;112:5099–5104.
6. Molina-Molina M, Borie R. Clinical implications of telomere dysfunction in lung fibrosis. *Curr Opin Pulm Med* 2018;24:440–444.
7. Newton CA, Batra K, Torrealba J, Kozlitina J, Glazer CS, Aravena C, *et al*. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J* 2016;48: 1710–1720.
8. Ley B, Newton CA, Arnould I, Elicker BM, Henry TS, Vittinghoff E, *et al*. The MUC5B promoter polymorphism and telomere length in patients with chronic hypersensitivity pneumonitis: an observational cohort-control study. *Lancet Respir Med* 2017;5: 639–647.
9. Stuart BD, Lee JS, Kozlitina J, Noth I, Devine MS, Glazer CS, *et al*. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *Lancet Respir Med* 2014;2:557–565.
10. Planas-Cerezales L, Arias-Salgado EG, Buendia-Roldán I, Montes-Worboys A, López CE, Vicens-Zygmunt V, *et al*. Predictive factors and prognostic effect of telomere shortening in pulmonary fibrosis. *Respirology* 2019;24: 146–153.
11. Newton CA, Kozlitina J, Lines JR, Kaza V, Torres F, Garcia CK. Telomere length in patients with pulmonary fibrosis associated with chronic lung allograft dysfunction and post-lung transplantation survival. *J Heart Lung Transplant* 2017;36: 845–853.
12. Kropski JA, Young LR, Cogan JD, Mitchell DB, Lancaster LH, Worrell JA, *et al*. Genetic evaluation and testing of patients and families with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2017;195: 1423–1428.
13. Popescu I, Mannem H, Winters SA, Hoji A, Silveira F, McNally E, *et al*. Impaired CMV immunity in idiopathic pulmonary fibrosis lung transplant recipients with short telomeres. *Am J Respir Crit Care Med* [online ahead of print] 8 Aug 2018; DOI: 10.1164/rccm.201805-0825OC.
14. Newton CA, Zhang D, Oldham JM, Kozlitina J, Ma SF, Martinez FJ, *et al*. Telomere length and use of immunosuppressive medications in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2019;200: 336–347.
15. Dressen A, Abbas AR, Cabanski C, Reeder J, Ramalingam TR, Neighbors M, *et al*. 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.
16. Aviv A, Hunt SC, Lin J, Cao X, Kimura M, Blackburn E. Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. *Nucleic Acids Res* 2011;39:e134.