

Precision Medicine in Idiopathic Pulmonary Fibrosis

The dawn of wishful thinking

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ABSTRACT

The conceptualization of precision medicine and the determinants of individuality in disease predisposition and treatment response represent a methodological revival of an emblematic figure of ancient Greece, called Hippocrates. The Human Genome Project truly revolutionized the quest for genomic anatomy and set the basis for molecular profiling of each individual in the context of the disease. Almost a year ago USA president Obama announced a research initiative that aims to revolutionize a new era in precision medicine mainly focusing in the area of oncology (www.whitehouse.gov/precisionmedicine). Despite all these cornerstone events precision medicine approaches in chronic lung diseases and particularly IPF have significantly lagged behind. For many chronic lung diseases we apply theoretically live-saving treatments without absolute-knowledge of their pathogenesis and without taking into consideration disease complexity and heterogeneity. As a consequence many of these therapeutic approaches lead to fatal side-effects. This short review article aims to summarize the current state of knowledge in the prognostic and therapeutic field of IPF, underline mistakes that have been applied in the field of clinical trials and have been carried out for many years and assess ways to optimize the use of "omics" in the everyday clinical practice in order to reformulate the Hippocrates commandment "to help, or do no harm". *Pneumon 2016, 29(3):217-223.*

INTRODUCTION

The conceptualization of precision medicine and the determinants of individuality in disease predisposition and treatment response represent a methodological revival of an emblematic figure of ancient Greece, called Hippocrates. Hippocrates, the father of western medicine, first coined out

the term “*idiosyncrasy*” to describe the uniqueness of the disease related to each patient. Thus he was mentoring his students that: “*we should treat the patient and not the disease and that diseases should be treated from their origin (Nature of Man)*”. For Hippocrates the comprehension of the individual’s biology was a major pillar of etiological treatment: “*...nobody can know medicine who is ignorant what a man is; he would treat patients properly must learn this.*” Another historical symbol of ancient Greece and father of Mathematics, Pythagoras introduced another paradigm of personalized medicine by forbidding the eating of “*fava beans*” because they contained the souls of the dead”. Therefore, accidentally or not, he was the first who described the hereditary abnormality of the red blood cell enzyme glucose-6-phosphate dehydrogenase (G6PD) deficiency, resulting in hemolytic anemia and jaundice. Following these examples, Karl Landsteiner, Nobel Prize awardee for the discovery of polio virus, reported, over a century ago, that “every blood transfusion guided by blood typing is a paradigm of genomic medicine”.

The Human Genome Project truly revolutionized the quest for genomic anatomy and set the basis for molecular profiling of each individual in the context of the disease. Almost a year ago USA president Obama announced a research initiative that aims to revolutionize a new era in precision medicine mainly focusing in the area of oncology (www.whitehouse.gov/precisionmedicine). Despite all these cornerstone events precision medicine approaches in chronic lung diseases and particularly IPF have significantly lagged behind. For many chronic lung diseases we apply theoretically live-saving treatments without absolute-knowledge of their pathogenesis and without taking into consideration disease complexity and heterogeneity. As a consequence many of these therapeutic approaches lead to fatal side-effects. This short review article aims to summarize the current state of knowledge in the prognostic and therapeutic field of IPF, underline mistakes that have been applied in the field of clinical trials and have been carried out for many years and assess ways to optimize the use of “omics” in the everyday clinical practice in order to reformulate the Hippocrates commandment “*to help, or do no harm. (Epidemics)*”.

CURRENT KNOWLEDGE

Functional and physiologic biomarkers

IPF has a median survival of 3-5 years after initial

diagnosis with a clinical course highly unpredictable and heterogeneous. Based on current functional and physiological indices three different IPF subpopulations can be described: slow progressors, rapid progressors and patients with relatively stable clinical course interposed by periods of rapid acceleration called acute exacerbations¹. So far, pulmonary functional tests (PFTs) and particularly forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLCO) represent the hallmark of disease prognostication. Studies have shown that an absolute decline $\geq 10\%$ in FVC and $\geq 15\%$ in DLCO constitute evidence of acute exacerbation² and therefore affect management decisions including prioritization for rapid enrolment into clinical trials and lung transplantation list. Moreover, they have been integrated into risk-stratification algorithms including GAP (Gender, Age and Physiology variables included) score³ and composite physiologic index (CPI)⁴ which have successfully stratified disease outcome in large cohorts of patients with IPF. Additionally, 6-minute walking test has been also tested as reliable prognosticator and as end-point in several clinical trials⁵⁻⁷. Nevertheless, they present with significant caveats that should be addressed cautiously. Both functional and physiological indices present with considerable technical variabilities including individual’s effort as well as inability of the majority of the patients with severe disease to perform single breath functional tests and 6 minutes of exercise. Furthermore results arising from these tests may be influenced by a variety of unrelated to cardiopulmonary status, including age, sex, height, and weight (i.e. there are no reliable predicted standard spirometry values for patients with short stature). 6-MWT can also be affected by musculoskeletal and nutritional status as well as cognitive function. More importantly, FVC is often overestimated by emphysema-associated lung hyperinflation which is present in almost a third of patients with IPF⁸. Finally, functional and physiological parameters provide no mechanistic insights and we have to wait months to obtain relative information. For all the reasons above, physiologic prognosticators are unlikely to identify distinct molecular endotypes of the disease⁹.

Lessons from clinical trials

The era of randomized, placebo-controlled clinical trials (RCTs) for IPF commenced 17 years ago, when Ziesche et al¹⁰ published in the New England Journal of Medicine that IFN- γ -1b may improve lung function in a small cohort comprised of 18 patients with IPF. Although this trial was performed before the 2000 ATS/ERS guidelines on IPF

diagnosis and treatment were published and therefore doubt has been cast on the validity of the IPF diagnosis; however, this study generated enormous but baseless enthusiasm and fueled two major RCTs investigating IFN- γ -1b efficacy in an overall of 1156 (330+826) patients with IPF involving more than 100 centers in 7 European countries, USA and Canada¹¹⁻¹³. Both trials failed to meet the primary end-point as they showed no impact on either progression-free-survival or overall survival. Despite the fact that little was known on IPF pathogenesis and IFN- γ mechanisms of action and based on statistical “*massaging*”, called intention-to-treat analysis, millions of dollars were spent and more importantly lives and precious time were lost on studying the effectiveness of a drug that has showed its inefficacy from preclinical models. Non randomized placebo controlled trials on the safety and efficacy of IFN- γ -1b have been also conducted; however rigid conclusions cannot be drawn¹³.

Same approach was followed on Bosentan, a dual endothelin-receptor antagonist. Again, there was no compelling evidence that endothelin was important in IPF pathogenesis; nevertheless an RCT (BUILD-1) involving 158 patients was conducted using as primary and secondary end-points of drug efficacy a physiological and a functional parameter, 6-MWT and FVC, respectively¹⁴. Moreover, although the BUILD-1 study showed no impact on both end-points; however, a subset statistical analysis revealed a beneficial effect in patients with a firm IPF diagnosis based on surgical lung biopsy. To this end, a second study (BUILD-3) was designed and as expected failed to meet the primary end-point¹⁵. Similarly, in another study (ACE) investigators demonstrated a detrimental effect of anti-coagulants in patients with IPF¹⁶. This trial exhibited major caveats in terms of design (high doses of warfarin were administered without any prophylactic coadministration of heparin) and patients' enrollment criteria (white Caucasian subjects with progressive disease). More importantly, while this study was designed based on data showing moderate efficacy of anticoagulants in a small cohort of Asian subjects with progressive IPF¹⁶; however, it did not take into consideration vital pharmacogenetic data suggesting that Asian and white subjects present with major differences regarding CYP2C9 and VKORC1 genes encoding the major enzymes responsible for metabolism of coumarin targets¹⁷. Even positive studies investigating the safety and efficacy of pirfenidone (CAPACITY, ASCEND)^{18,19} and nintedanib (INPULSIS)^{20,21} were designed and conducted based on limited in-vitro and in-vivo data regarding drug mechanisms. It is currently

unknown whether pirfenidone acts as an anti-fibrotic agent or it exerts immunomodulatory properties as well, while recently emerged data reveals that nintedanib inhibits more than 200 tyrosine kinases besides the three suggested ones (VEGF-R, FGF-R, PDGF-R)²²⁻²⁵. The list of negative clinical trials goes way further down. The scope of this review article is not to report each individual study of failure in IPF but to underline repetitive mistakes and lessons that need to be learned by using representative paradigms.

Another lesson that we should learn from all clinical trials, both positive and negative, is the fact that early phase trials have been large, long and most importantly dependent on clinical enrollment criteria and physiological efficacy end-points resulting into significantly high screening failure rates (64% for the pirfenidone trials^{18,19}, 29% for the nintedanib²¹ trials and 33% for the trials involving NAC administration²⁶). Furthermore, late phase trials have focused on subjects with mild to moderate disease leaving outside a significant proportion of patients with more severe disease that could potentially be in greater need for therapeutic interventions^{27,28}. These major issues should be addressed cautiously. Therefore: 1) End-points should focus on disease biology and mechanisms, 2) Studies may need biologic cohort enrichment, 3) Enrichment for patients at greater risk for the end-point or greater likelihood of response may also be necessary. Application of personalized medicine approaches by utilizing our “omics” tools seems to be the only way forward²⁹⁻³¹.

LESSONS FROM “FIBROMICS”

Genetics

Twenty years ago when the first RCT clinical trials in IPF were designed a dogma prevailed the research are of IPF: IPF is a chronic progressive fibrotic ILD of unknown etiopathogenesis with minimal genetic susceptibility. Three years ago genome-wide association studies (GWAS) in large cohort of patients with IPF revealed that almost 40% of IPF cases (sporadic and familial) can be explained by common or rare variants including MUC5B, TOLLIP, surfactant protein C and mutations in the telomere-telomerase complex (TERT, TERC, RTEL1 and shelterin)³²⁻³⁴. In particular, almost 35-60% of sporadic IPF cases carry the minor allele of a common variant located in the putative promoter region of the *MUC5B* gene (rs35705950)^{35,36}, that has been associated with the presence of subclinical interstitial lung abnormalities in the general population³⁷.

The same polymorphism is also relatively common in the general population since 20% of normal individuals carry this specific variant. In addition to increasing risk of developing IPF, the same "susceptibility allele" appears to confer a better prognosis, evidence that at present remains an open question³⁸. On the other hand mutations in the genes encoding telomerase, the multi-subunit enzyme that maintain telomere integrity, are rare in IPF^{39,40}, whereas shorter telomere length is a common finding in IPF patients compared to age-matched controls⁴¹. In addition, novel functional variants within *TOLLIP*, a toll-like receptor protein that regulates innate immunity, have been strongly correlated with increased disease susceptibility. Similarly, to what has been observed in *MUC5B* the same allele that appears to protect against the development of IPF, has been also associated with an increased risk of mortality⁴². More importantly, pharmacogenetic analysis identified *TOLLIP* genotypes that conferred different treatment response to N-acetylcysteine, evidence that highlights the amenable need for genotypic analysis before enrollment into clinical trials⁴³. In line with this concept, two multicenter lung transplantation clinical trials revealed that IPF carriers of telomerase mutations deserve further attention to specific post-transplantation hematologic complications potentially due to limited bone marrow reserves and the use of nephrotoxic immunosuppressants^{44,45}.

Epigenetics

Epigenetics study the gene-environment interactions and provide useful information that help investigators to fill the knowledge gap that lies between the genotype and the phenotype. Application of high-throughput screening tools identified that among differentially methylated and expressed genes were genes such as *TOLLIP* and *NOTCH1*, further confirming previous genomic data⁴⁶. An accumulating body of evidence has implicated microRNAs as major regulators of fibrogenesis through silencing of several pro-fibrotic or anti-fibrotic target genes. Among the most down-regulated and so called "anti-fibrotic" miRNAs, in patients with IPF compared to controls, were let-7d and miR-29, whereas miR-21 and miR-154 were found to be upregulated⁴⁷⁻⁵⁴. Among them, miR-29 has been used not only as a biomarker of disease progressiveness but also as a novel anti-fibrotic agent with therapeutic effects in experimental models of lung fibrosis^{50,55,56}.

Genomics

Application of high-throughput genomic platforms such as microarrays and nCounter (Nanostring) technology has accelerated progress towards better understanding of disease pathogenesis, identified novel disease prognosticators and therapeutic targets for pathway-specific treatment approaches. Kaminski and Selman were the first who managed to dissect disease activity IPF endotypes since they discriminated patients with IPF into rapid and slow progressors based on gene expression data¹. In a follow-up hallmark study, Herazo-Maya et al. identified 2 different endotypes of patients with IPF with distinct prognostic patterns based on a 52-gene signature including genes involved in the T-cell co-stimulatory pathway (such as *CD28*, *ICOS*, *LCK*, and *ITK*)⁵⁷. Authors suggested that patients with IPF that appear to be immunocompromised as assessed by down-regulation of genes related to adaptive-immunity responses present with unfavorable prognosis. Moreover, integration of the genomic data into prognostic algorithms encompassing functional and demographic data significantly improved the prediction of outcome. These data highlight the variability and complexity in IPF progression and may explain the difficulty in obtaining reproducible results in studies of therapeutic interventions by only using conventional demographic and physiologic criteria.

Proteomics

Proteomics represent the large-scale study of the structure and function of proteins in complex biological samples. This particular approach has the advantage of understanding the complex nature of the organism since it provides valuable information regarding the function of the genome, as well as dynamic processes including protein localization, trafficking, post-translational modifications and protein-protein interactions. More importantly, proteomics is the only technology that can reliably identify therapeutic targets for drug development. Several novel therapeutic compounds that are now in different stages of clinical trials have been identified from proteomics technology, including inhibitors of *LOXL2*, galectin, *CTGF*, *IL-13*, *NOX1/NOX4*, and *SHP1*. In addition, proteomics technology has advanced the prognostic field of IPF since several protein compounds, such as *MMP-7*, *CCL-18*, *CXCL13* and *MMP-degraded extracellular matrix proteins*, have been strongly associated with outcome prediction in several independent cohorts of patients with IPF^{31,58}.

FUTURE PERSPECTIVES AND LIMITATIONS

Despite the advent and approval of two novel drugs, pirfenidone and nintedanib, yet IPF represents a major bottleneck for physicians considering that both drugs only slow down disease progression, thus at the best case leaving patients with appreciable pulmonary disability. In addition, both of these drugs are associated with significant side-effects that although not fatal; however, hamper patients' quality of life. Furthermore, IPF complex biology reflects into a highly variable and unpredictable disease behavior that pose significant limitations to prioritize patients into lung transplantation lists, the only so far treatment that affects patients' survival. Current demographic and physiologic parameters are unable to phenotype disease patterns and identify relevant therapeutic targets. It's upon the hands of clinicians and researchers to convince the scientific, political and financial community that IPF is not an orphan disease and deserves equal attention as many types of cancer.

The President's Obama precision medicine initiative should not be only cancer-focused but should be enriched with diseases such as IPF which accounts for 40.000 deaths each year in the USA, the same as breast cancer where most recently a **70-gene signature assay kit** that predicts outcome and treatment response is now commercially available⁵⁹. This initiative will encourage and support the next generation of scientists to develop diagnostic tools to monitor biomarker levels that will help us identify distinct endotypes of IPF patients and thus apply targeted therapeutic interventions on a pathway-specific basis, i.e. treat with miR-29 only those patients with reduced miR-29 levels. Furthermore, implementation of pharmacogenetic-genomic approaches (MUC5B, TOLLIP) for enrolling patients into clinical trials is mandatory considering the robust information that we have generated the past 10 years supporting the presence of genotypes with distinct prognostic and treatment response profiles. Individually tailored treatments will not only enhance therapeutic benefits but most importantly eliminate the consequences of toxic or disfiguring therapies (i.e. corticosteroids or other immunosuppressants). Two ongoing studies, PROFILE (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) study from UK⁶⁰ and LGRC (Lung Genomics Research Consortium) study from USA, that aim to validate a panel of biomarkers to predict disease clinical course and treatment response represent the first efforts towards this direction. Ambitious projects like these will yield their greatest benefits years down the

road, but there should be some considerable short-term benefits. All we have to do is to be persistent and patient in order to launch IPF precision medicine into the same trajectory as breast and lung cancer.

REFERENCES

1. Selman M, Carrillo G, Estrada A, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS one* 2007;2:e482.
2. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *American Journal of Respiratory and Critical Care Medicine* 2011;183:788-824.
3. Ley B, Ryerson CJ, Vittinghoff E, et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. *Annals of Internal Medicine* 2012;156:684-91.
4. Wells AU, Desai SR, Rubens MB, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. *American Journal of Respiratory and Critical Care Medicine* 2003;167:962-9.
5. Swigris JJ, Wamboldt FS, Behr J, et al. The 6 minute walk in idiopathic pulmonary fibrosis: longitudinal changes and minimum important difference. *Thorax* 2010;65:173-7.
6. Han MK, Bach DS, Hagan PG, et al. Sildenafil preserves exercise capacity in patients with idiopathic pulmonary fibrosis and right-sided ventricular dysfunction. *Chest* 2013;143:1699-708.
7. Idiopathic Pulmonary Fibrosis Clinical Research N, Zisman DA, Schwarz M, et al. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. *N Engl J Med* 2010;363:620-8.
8. Wells AU. Forced vital capacity as a primary end point in idiopathic pulmonary fibrosis treatment trials: making a silk purse from a sow's ear. *Thorax* 2013;68:309-10.
9. Savale L, Jais X, Montani D, Sitbon O, Simonneau G, Humbert M. Treatment of pulmonary arterial hypertension in 2011: what's new since the 2009 ERS/ESC guidelines? *Presse Medicale* 2011;40(Suppl 1):1554-60.
10. Ziesche R, Hofbauer E, Wittmann K, Petkov V, Block LH. A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. *New Engl J Med* 1999;341:1264-9.
11. Raghu G, Brown KK, Bradford WZ, et al. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2004;350:125-33.
12. King TE, Jr., Albera C, Bradford WZ, et al. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. *Lancet* 2009;374:222-8.
13. Bouros D, Antoniou KM, Tzouveleki A, Siafakas NM. Interferon-gamma 1b for the treatment of idiopathic pulmonary fibrosis. *Expert Opinion on Biological Therapy* 2006;6:1051-60.
14. King TE, Jr., Behr J, Brown KK, et al. BUILD-1: a randomized placebo-controlled trial of bosentan in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2008;177:75-81.

15. King TE, Jr., Brown KK, Raghu G, et al. BUILD-3: a randomized, controlled trial of bosentan in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2011;184:92-9.
16. Noth I, Anstrom KJ, Calvert SB, et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2012;186:88-95.
17. Tzouveleki A, Margaritopoulos G, Loukides S, Bouros D. Warfarin in idiopathic pulmonary fibrosis: friend or foe, is it a matter of genes and heparin? *American Journal of Respiratory and Critical Care Medicine* 2013;187:213-4.
18. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet* 2011;377:1760-9.
19. King TE, Jr., Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2083-92.
20. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2071-82.
21. Richeldi L, Costabel U, Selman M, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. *N Engl J Med* 2011;365:1079-87.
22. Lehtonen ST, Veijola A, Karvonen H, et al. Pirfenidone and nintedanib modulate properties of fibroblasts and myofibroblasts in idiopathic pulmonary fibrosis. *Respiratory Research* 2016;17:14.
23. Inomata M, Nishioka Y, Azuma A. Nintedanib: evidence for its therapeutic potential in idiopathic pulmonary fibrosis. *Core Evid* 2015;10:89-98.
24. Iyer SN, Gurujeyalakshmi G, Giri SN. Effects of pirfenidone on transforming growth factor-beta gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. *The Journal of Pharmacology and Experimental Therapeutics* 1999;291:367-73.
25. Iyer SN, Gurujeyalakshmi G, Giri SN. Effects of pirfenidone on procollagen gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. *The Journal of Pharmacology and Experimental Therapeutics* 1999;289:211-8.
26. Idiopathic Pulmonary Fibrosis Clinical Research N, Raghu G, Anstrom KJ, King TE, Jr., Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. *N Engl J Med* 2012;366:1968-77.
27. Bouros D, Tzouveleki A. Idiopathic pulmonary fibrosis: on the move. *The Lancet Respiratory Medicine* 2014;2:17-9.
28. Bouros D. Pirfenidone for idiopathic pulmonary fibrosis. *Lancet* 2011;377:1727-9.
29. Spagnolo P, Sverzellati N, Rossi G, et al. Idiopathic pulmonary fibrosis: an update. *Annals of Medicine* 2015;47:15-27.
30. Tzouveleki A, Bonella F, Spagnolo P. Update on therapeutic management of idiopathic pulmonary fibrosis. *Therapeutics and Clinical Risk Management* 2015;11:359-70.
31. Spagnolo P, Tzouveleki A, Maher TM. Personalized medicine in idiopathic pulmonary fibrosis: facts and promises. *Current Opinion in Pulmonary Medicine* 2015;21:470-8.
32. Wei R, Li C, Zhang M, et al. Association between MUC5B and TERT polymorphisms and different interstitial lung disease phenotypes. *Translational research: the Journal of Laboratory and Clinical Medicine* 2014;163:494-502.
33. Blackwell TS, Tager AM, Borok Z, et al. Future directions in idiopathic pulmonary fibrosis research. An NHLBI workshop report. *American Journal of Respiratory and Critical Care Medicine* 2014;189:214-22.
34. Wolters PJ, Collard HR, Jones KD. Pathogenesis of idiopathic pulmonary fibrosis. *Annu Rev Pathol* 2014;9:157-79.
35. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med* 2011;364:1503-12.
36. Wang C, Zhuang Y, Guo W, et al. Mucin 5B promoter polymorphism is associated with susceptibility to interstitial lung diseases in Chinese males. *PloS one* 2014;9:e104919.
37. Hunninghake GM, Hatabu H, Okajima Y, et al. MUC5B promoter polymorphism and interstitial lung abnormalities. *N Engl J Med* 2013;368:2192-200.
38. Peljto AL, Zhang Y, Fingerlin TE, et al. Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. *JAMA* 2013;309:2232-9.
39. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007;356:1317-26.
40. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104:7552-7.
41. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2008;178:729-37.
42. Noth I, Zhang Y, Ma SF, et al. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *The Lancet Respiratory Medicine* 2013;1:309-17.
43. Oldham JM, Ma SF, Martinez FJ, et al. TOLLIP, MUC5B, and the Response to N-Acetylcysteine among Individuals with Idiopathic Pulmonary Fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2015;192:1475-82.
44. Silhan LL, Shah PD, Chambers DC, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *The European Respiratory Journal* 2014.
45. Borie R, Kannengiesser C, Hirschi S, et al. Severe hematologic complications after lung transplantation in patients with telomerase complex mutations. *The Journal of heart and lung transplantation: the official publication of the International Society for Heart Transplantation* 2015;34:538-46.
46. Yang IV, Pedersen BS, Rabinovich E, et al. Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2014;190:1263-72.
47. Milosevic J, Pandit K, Magister M, et al. Profibrotic role of miR-154 in pulmonary fibrosis. *American Journal of Respiratory*

- Cell and Molecular Biology 2012;47:879-87.
48. Huleihel L, Ben-Yehudah A, Milosevic J, et al. Let-7d microRNA affects mesenchymal phenotypic properties of lung fibroblasts. *American Journal of Physiology Lung Cellular and Molecular Physiology* 2014;306:L534-42.
 49. Pandit KV, Corcoran D, Yousef H, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* 2010;182:220-9.
 50. Montgomery RL, Yu G, Latimer PA, et al. MicroRNA mimicry blocks pulmonary fibrosis. *EMBO molecular medicine* 2014;6:1347-56.
 51. Parker MW, Rossi D, Peterson M, et al. Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *The Journal of Clinical Investigation* 2014;124:1622-35.
 52. Xiao J, Meng XM, Huang XR, et al. miR-29 inhibits bleomycin-induced pulmonary fibrosis in mice. *Molecular therapy: The Journal of the American Society of Gene Therapy* 2012;20:1251-60.
 53. Cushing L, Kuang PP, Qian J, et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. *American Journal of Respiratory Cell and Molecular Biology* 2011;45:287-94.
 54. Liu G, Friggeri A, Yang Y, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *The Journal of Experimental Medicine* 2010;207:1589-97.
 55. Tzouvelekis A, Kaminski N. Epigenetics in idiopathic pulmonary fibrosis. *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire* 2015;93:159-70.
 56. Pandit KV, Milosevic J, Kaminski N. MicroRNAs in idiopathic pulmonary fibrosis. *Translational research: The Journal of Laboratory and Clinical Medicine* 2011;157:191-9.
 57. Herazo-Maya JD, Noth I, Duncan SR, et al. Peripheral blood mononuclear cell gene expression profiles predict poor outcome in idiopathic pulmonary fibrosis. *Science Translational Medicine* 2013;5:205ra136.
 58. Tzouvelekis A, Herazo-Maya J, Sakamoto K, Bouros D. Biomarkers in the Evaluation and Management of Idiopathic Pulmonary Fibrosis. *Current Topics in Medicinal Chemistry* 2016;16:1587-98.
 59. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med* 2016;375:717-29.
 60. Maher TM. PROFILEing idiopathic pulmonary fibrosis: rethinking biomarker discovery. *European Respiratory Review: an official journal of the European Respiratory Society* 2013;22:148-52.