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 unicity 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 deads”. 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”.

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**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 exacerbations. 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 ≥10% in FVC and ≥15% in DLCO constitute evidence of acute exacerbation 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) score and composite physiologic index (CPI) which have successfully stratified disease outcome in large cohorts of patients with IPF. Additionally, 6-minute walking test has been also tested as reliable prognostic factor and as end-point in several clinical trials. 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 IPF. 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 disease.

**Lessons from clinical trials**

The era of randomized, placebo-controlled clinical trials (RCTs) for IPF commenced 17 years ago, when Ziesche et al 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 Canada. 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 drawn.

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, respectively. 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-point. Similarly, in another study (ACE) investigators demonstrated a detrimental effect of anti-coagulants in patients with IPF. 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 IPF; 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 targets. Even positive studies investigating the safety and efficacy of pirfenidone (CAPACITY, ASCEND) and nintedanib (INPULSIS) 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). 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 physiologically efficacy end-points resulting into significantly high screening failure rates (64% for the pirfenidone trials, 29% for the nintedanib trials and 33% for the trials involving NAC administration). 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. 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 forward.

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). 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), that has been associated with the presence of subclinical interstitial lung abnormalities in the general population.
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 question. On the other hand mutations in the genes encoding telomerase, the multi-subunit enzyme that maintain telomere integrity, are rare in IPF, whereas shorter telomere length is a common finding in IPF patients compared to age-matched controls. 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 mortality. 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 trials. 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.

**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 data. 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 upregulated. 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.

**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 data. 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). 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.
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 available. 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 with the robust information that we have generated 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.

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