Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-21-880

Reviewer A

The authors performed systematic review of the relationship between lung cancer and interstitial pneumonia, focusing on gene mutations, epigenetics, miRNA, and more. This study has clinical importance and are performed correctly. I have several comments. Sufficient improvements for those issues are needed for the acceptance to the Expert Review of Respiratory Medicine.

We really thank the Reviewer for the careful revision of our work, which is now improved in its scientific quality.

***Comment 1*:** In the section of 1.1 Background and Rationale and 3.1.2. 3.1.2 Epigenetics, please consider to add the following discussion. I do not consider that common factors of IPF and lung cancer associated with onset and DNA methylation is not only smoking but also environmental or occupational exposure, pathogen infection and persistent tissue damage.
Previous reports that SNPs in MUC5B promotor region (rs35705950) associated with prognosis of IPF may be related to the reduction of immunedefense mechanism of MUC5B are considered to be important (Molyneaux PL, et al. Respir Res. 2017; 18: 29. Roy MG, et al. Nature. 2014; 505: 412.).
I consider to be important that hypermethylation of the Thy-1 promoter region causes the loss of this molecule, which in more invasive behaviour of cancer and the transformation of fibroblasts into myofibroblasts within fibroblast foci in IPF.
Reply 1: *We agree with this issue and thank the Reviewer for his suggestions. We implemented the text coherently.*
Changes in the text: *"It should be remarked that not only smoke exposure can affect DNA methylation in both cancer and IPF. This issue is even more relevant for the cancer and the rare fibrotic cases that arise in non-smoker subjects. Indeed, environmental or occupational exposure, pathogen infection and persistent tissue damage. For instance, polymorphisms in CYP1A1 and GTSM1, xenobiotic metabolizing enzymes, have been reported to be associated to higher risk of lung cancer development whereas polymorphisms in MLH1, a mismatch pair enzyme should play a role in the onset of the disease in never smokers. Moreover, polymorphisms in genes involved in inflammatory cascade such as those encoding for interleukin (IL)-10, tumour necrosis factor (TNF), IL1-RN and IL-6 have been reported to be associated to lung cancer risk independently from cigarette smoke exposure. Within respect to IPF, previous reports suggested that SNPs in MUC5B promotor region (rs35705950) are associated with prognosis of IPF and this fact may be related to the reduction of immunedefense mechanism of MUC5B. Growing evidence suggests that lung microbiome plays a relevant role in maintaining lung immune homeostasis and that its alteration and disruption might be related to cancer onset by acting on epigenetic level such as by causing DNA damage, genomic instability, and inducing higher sensitivity to carcinogens. Environment factors that can alter*

lung microbiota might promote, mainly through production of bacterial toxins and other pro-inflammatory factors, cancer onset and progression. A number of recent observations suggests a role for lung bacteria in IPF onset as well]. First observation regarded the fact that bacteria (most often Streptococcus pneumoniae and Moraxella catarrhalis) are frequently isolated from broncholveolar fluid (BALf) from IPF patients and that patients enrolled in clinical trials have better outcomes in those arms encompassing treatment with antibiotics. Next generation analysis approaches more recently reported that changes in the lung…. Interestingly, hypermethylation of the Thy-1 gene promoter region causes the loss of this molecule, which in more invasive behaviour of cancer and the transformation of fibroblasts into myofibroblasts within fibroblast foci in IPF".

Reviewer B

Overall, it appears to be a well-written review article on the combined disease of IPF and lung cancer.
We thank the Reviewer for careful reading of the manuscript and the constructive remarks.

***Comment 1*:** In the introduction part, it is necessary to write in more detail the overall epidemiology (including prognosis) of patients with IPF and lung cancer to emphasize the importance of this article.
Reply 1: *We thank the Reviewer for this fruitful comment, and we have modified the manuscript accordingly*
Changes in the text*: "The concept that interstitial lung diseases represent a relevant risk factor for lung cancer development is well documented and known. Within respect to IPF, reports indicated a cumulative incidence of cancer in IPF patients varying from 3.3%, 15.4%, and 54.7% after 1, 5, and 10 years of follow-up for IPF to 41% and 82% at 1 and 3 years, respectively. Age and smoking habit act as known confounding variables since they impact on both lung cancer and IPF onset. Moreover, many occupational and environmental exposure toxics are common risks for the development of both the diseases. Notably, IPF patients are at higher risk of cancer development if compared to those affected by COPD (chronic obstructive pulmonary disease), another cancer predisposing pathologic entity. The Japanese Hokkaido registry data reports an unadjusted risk ratio of 7.8 for lung cancer in IPF patients vs COPD ones. Most often tumors in IPF context arise in peripheral lung, although these data need further confirmation. The mechanistic explanation and the association between IPF and cancer are discussed in detail in the next sections of the manuscript. However, several issues deserve to be here underline. It is conceivable that the pro-proliferative landscape that characterizes IPF, should promote the selection of those cells carrying oncogenic mutations.".*

Comment 2: An overall review of surgery, which is the main treatment other than chemotherapy and radiation therapy, is required.
Reply 2: *We thank the Reviewer for this suggestion; a dedicated section has been added.*

Changes in the text*: <u>Section 2.3 Surgery in lung cancer with IPF</u>*

Lung resection plays a role in the treatment of patients affected by IPF with resectable NSCLC. However, in this scenario two major issues influence significantly the surgical procedure and the survival outcomes: the high risk of postoperative acute exacerbations (AE) of IPF in the short-term, and the death due to cancer in the long-term. Surgery is a defined risk factor for AE in IPF patients and since its incidence in this group of patients is estimated to be approximately 9.3% and no preventive measure is known, it is crucial to carefully select the patients to properly refer treat the patients. In a study by T. Sato and colleagues, a simple scoring system to identify high risk patients for AE was derived in order to help in the decision-making process for surgery selection and predict the patients requiring intensive observation postoperatively. Among the surgical procedures of lung resection, wedge resection is associated to the lowest risks of postoperative AE compared to segmentectomy, lobectomy, bilobectomy and pneumonectomy, since AE risk increases according to the resected lung parenchyma volume. Death due to cancer is the major concern in the long-term: it represents the main cause of death in lung cancer patients affected by IPF, mostly attributable to cancer recurrence after surgery. Contrary to AE risk, lobectomy shows better results for death due to cancer in patients with stage IA, while wedge resection and segmetectomy were associated to poor outcomes. Lung resection in patients with IPF is challenging but required for several patients. The choice of surgical procedure must be tailored based on several criteria, such as pulmonary function, cancer stage and recurrence risk, postoperative AE risk, and the natural course of IPF.

Comment 3: In addition, a review of the relationship between pirfenidone and nintendanib, the main treatment for IPF, and lung cancer is also needed.

Reply 3: *We thank the Reviewer for raising this critical issue. The text has been implemented accordingly.*

Changes in the text*: Pirfenidone and nintedanib act as antifibrotic drugs through different mechanisms. The first essentially acts by deregulating a series of cytokines, including transforming growth factor (TGF)- β1, connective tissue growth factor (CTGF), platelet-derived growth factors (PDGF), and tumor necrosis factor (TNF)- α. Moreover, it behaves as scavenger of ROS and downregulate ACE expression. Nintedaninb is a multikynase inhibitor which also down-regulates protein and mRNA expression of extracellular matrix (ECM) proteins, fibronectin, and collagen 1a1 and inhibits (TGF)-β1–induced myofibroblast differentiation. Notably, both drugs inhibited collagen I fibril formation. It should be underlined that a relationship exists between theese main two treatments for IPF, namely pirfenidone and nintendanib, and lung as well. Several recent studies have shown a prophylactic effect of the use of pirfenidone perioperative setting against postoperative acute IPF exacerbations in patients with lung cancer. Notably therapy with pirfenidone seems to be associated to lower incidence of lung cancer in IPF patients if compared to non-pirfenidone treated cases, although this observation should be confirmed by more extensive analysis. Some recent observation also underlined a potential therapeutic role of pirfendone against lung cancer. In detail, it has been reported in vitro and in vivo that it could suppressed activation of NSCLC associated myofibroblasts, which are known to be involved in tumor progression and impairs EMT by acting on exogenous TGF-β1 and on paracrine TGF-β produced from NSCLC cells. Pirfenidone seems to play a synergic effect with conventional chemotherapy such as*

carboplatin, whereas studies evaluating effects of combination with immune checkpoint inhibitors are ongoing (the NCT04467723 trial evaluating the combination of pirfenidone with the PD-L1 and PD1 inhibitor atezolizumab in second-line and beyond NSCLC, website at www.clinicaltrials.gov). The antiproliferative effect of nintedanib derives to its ability to block the vascular endothelial growth factor (VEGF), the platelet-derived growth factor receptor (PDGF) and the fibroblast growth factor receptor (FGFR). Nintedanib in combination with docetaxel is approved as second-line therapy for advanced NSCLC. It also promotes antitumor immunity and antitumor activity in combination with PD□1 blockade in mice by targeting CAF thus attenuating the immunosuppressive tumor microenvironment on one hand and promoting intratumoural activation of antitumor CD8+ T cells. Although some reports suggesting a positive effect, it is still unclear if nintedanib could play an effective role against lung cancer aroused in IPF patients. When associated with corticosteroids, it seems to be able to attenuate targeted drug and immune checkpoint inhibitor-related pneumonitis in cancer patients.

Comment 4: line 56) Pulmonary idiopathic fibrosis -> Idiopathic pulmonary fibrosis (IPF)
Reply 4: *we thank the Reviewer for his careful reading. Text has been corrected*