Common pathways in idiopathic pulmonary fibrosis and cancer

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ABSTRACT Idiopathic pulmonary fibrosis (IPF) is marked by a very disappointing survival rate and still represents a clinical dilemma. According to the current pathogenic hypothesis, chronic damage of the alveolar epithelium is followed by abnormal tissue repair and impairment of the alveolar structure. This process is driven by pathogenic events very similar to cancer, including epigenetic and genetic changes, altered response to regulatory signals, abnormal expression of microRNAs and activation of specific signalling pathways. IPF also resembles cancer with regard to its poor response to medical treatment and prognosis, which is very often worse than many cancers. We have hypothesised that IPF might be assimilated to a neoproliferative disorder of the lung. Viewing IPF as a cancer-like disease may satisfy the need for a better understanding of the pathogenesis of IPF by exploiting the large amount of knowledge that cancer biology evokes. The recognition of common pathogenic pathways between the two diseases may stimulate new clinical trials with cancer drugs, different drug combinations and different lines of drugs, as already experimented in oncology. Moreover, the concept of IPF as a cancer-like disorder may improve the attention given to this dreadful disease on a public, political and healthcare level.

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Considering idiopathic pulmonary fibrosis as a cancer-like disorder may improve its treatment and investigation. http://ow.ly/mMHmH

Introduction

Despite the increasing number of studies investigating the pathogenesis of idiopathic pulmonary fibrosis (IPF) and the simultaneous increase in clinical trials dedicated to this disease, IPF is still marked by a very disappointing survival rate and remains, in many ways, a clinical and therapeutic dilemma. According to the commonly accepted pathogenic hypothesis, unknown environmental and/or occupational factors, cigarette smoking, viral infection or even tractional injury to the peripheral lung may cause, in susceptible individuals [1, 2], chronic damage of the alveolar epithelium. This triggers a series of events leading to abnormal tissue repair and, ultimately, to severe derangement of the alveolar structure. This altered "wound healing" process is driven by a variety of pathogenic events, which are for the most part described in other degenerative diseases and, interestingly, also in cancer. It is no coincidence that cancer is defined by some authors as a wound that does not heal [3–5], with an often unknown aetiology, risk factors similar to IPF and the presence of a specific genetic background considered important for the insurgence of the disease. IPF also resembles cancer with regard to its poor response to medical treatment and prognosis, which is very often worse than many cancers [6]. In addition to these obvious and circumstantial similarities, IPF and cancer also share a number of cellular and molecular aberrances, including epigenetic and genetic changes, delayed apoptosis, altered response to regulatory signals, abnormal expression of microRNAs (mRNAs), reduced cell-to-cell communication and activation of specific signalling pathways. Based on this evidence,

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we have hypothesised that IPF might be assimilated to a neo-proliferative disorder of the lung. Approaching IPF as a cancer-like disease may have some practical advantages, although it may be open to some criticism. The main purpose of this review is to analyse the main arguments either in favour of or against the intriguing hypothesis of the cancer-like nature of IPF.

Why we should not consider IPF a cancer-like disease

Over time, many IPF experts have generically compared IPF with cancer, although this concept has always remained rather vague and based mainly on the low-survival rate that characterises both diseases. There are three main arguments against the hypothesis of the cancer-like nature of IPF, as follows. 1) Monoclonality is a distinctive feature of cancer cells, and myofibroblasts within fibroblast foci are instead characterised by cytogenetic heterogeneity; 2) cancer is a disease that metastasises by invading other organs, whereas IPF is a disease limited to the lungs; and 3) cancer is always unilateral, whereas IPF is by definition bilateral. These arguments apparently exclude the cancer-like nature of IPF. However, these assertions about cancer are based largely on concurring opinions and often represent myths that need to be dispelled.

Monoclonality is a distinctive feature of cancer cells, and myofibroblasts within fibroblast foci are instead characterised by cytogenetic heterogeneity

A few years ago, COOL *et al.* [7] were the first to observe that ''fibroblast foci'' are not isolated areas of fibrosis, but are instead interconnected, infiltrating the tissue as a cancer. Based on this observation, they hypothesised that myofibroblasts within fibroblast foci could have a malignant nature. However, the absence of monoclonality of the fibroblasts infiltrating lung tissue led them to conclude that the origin of fibroblast foci is reactive and nonmalignant, abandoning the idea of the cancer-like nature of IPF [7]. Although it is common opinion that cancers are always of monoclonal origin, more recently, a number of studies have shown that only some cancers are monoclonal; instead, others are characterised by cytogenetic heterogeneity. Moreover, modifications of the clonal status of cancer during the course of the disease are anything but rare. Some cancers are initially monoclonal before acquiring clonal heterogeneity, others have a polyclonal origin becoming monoclonal over time, and some are first polyclonal, temporarily assuming a monoclonal pattern, that later reverts to polyclonality [8]. The existence of polyclonal cancers and occurrence of clonal convergence and clonal divergence brings into question the ''dogma'' of monoclonality as a distinctive feature of cancer, casting new light on the real nature of the fibroblast reticulum infiltrating the lung in IPF.

Cancer is a disease that metastasises by invading other organs, whereas IPF is a disease limited to the lungs

Despite what is generally thought, not all cancers metastasise; desmoid tumours, for instance, are fibroblastic/myofibroblastic cancers marked by aggressive local invasiveness and a lack of metastatic ability. This cancer, of unknown origin, is usually sporadic, sometimes familiar and, interestingly, few cases of bilateral involvement or recurrent multicentric, synchronous lesions of the same part of the body have been described. In desmoid tumours, similarly to other cancers, and in the same way as IPF, transforming growth factor (TGF)-β expression, fibroblast differentiation into myofibroblasts, collagen production, activation of the β-catenin/Wnt pathway and tyrosine kinase receptors deregulation are all events strictly related to the pathogenesis of this disease. Moreover, the diagnosis, based on clinical, radiological and histological criteria, may not be able to distinguish this disease from other fibrotic processes such as scars or other benign fibroblastic disorders [9]. The pathogenic and behavioural pattern of desmoid tumours, and the number of analogies between this fibroblastic cancer and IPF, lends further support to the possibility of considering IPF as a disease with cancer-like features.

Cancer is always unilateral, whereas IPF is by definition bilateral

Another firm belief about cancer is that this disease involves first one organ and, only at a later stage by metastasising, invades other tissues and organs. Several studies have instead demonstrated that the synchronous appearance of cancer in both lungs, breasts, kidneys, testicles, tonsils, ovary and parathyroids [10–13], although not frequent, is well described in 2–6% of cancers. This interesting observation confirms that bilateral and synchronous involvement of ''twin organs'', such as the lungs, may occur in both IPF and cancer.

Why we should consider IPF a cancer-like disease

The cytogenetic heterogeneity observed within myofibroblasts, the incapacity to metastasise and the contemporary involvement of both lungs are all arguments against the cancer-like nature of IPF, as these features are not believed to be exhibited in cancer. However, this is primarily based on the general belief that cancer is always monoclonal, involving only one of the ''twin organs'' before metastasising and invading other organs. Although this does not confirm in any way the cancer-like attitude of IPF, it does not preclude ''*per se*'' the concept that IPF may exhibit a cancer-like trait. In addition, a number of pathogenic features,

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such as epigenetic and genetic abnormalities, altered cell-to-cell communications, uncontrolled proliferation and abnormal activation of specific signal transduction pathways, are all biological hallmarks that characterise the pathogenesis of both IPF and cancer. These mechanisms have already been reported in detail in a previous review [6], and will be updated and summarised here (figs 1 and 2).

Epigenetic and genetic abnormalities in IPF and cancer

It is well known that methylation of tumour suppressor genes and/or hypomethylation of oncogenes has a fundamental role in carcinogenesis. These epigenetic alterations occurring in response to environmental exposures, tobacco smoke, diet or ageing have recently also been shown in IPF. In this disease, RABINOVICH *et al.* [14] have recently shown that the global methylation pattern is altered and in some way similarly to lung cancer. In IPF, hypermethylation of the Thy-1 promoter region causes a reduced expression of the glycoprotein Thy-1, which is normally expressed by fibroblasts [15, 16]. In IPF, the loss of this molecule, which in cancer is associated with a more invasive behaviour of the disease, is linked to the transformation of fibroblasts into myofibroblasts within fibroblast foci. Interestingly, the pharmacological inhibition of the methylation of the Thy-1 gene may restore the expression of Thy-1, suggesting a novel therapeutic approach for this disease. Specific mutation of genes considered to be a key factor in the origin and progression of cancer [12–17], such as p53, fragile histidine triad, microsatellite instability and loss of heterozygosity have been observed in ~50% of IPF patients and often described in the peripheral honeycomb areas characteristic of IPF [17–20]. Other mutations commonly associated with cancer initiation and development, such as telomere shortening and telomerase expression, have been described in familial and sporadic IPF [21–23]. More recently, the abnormal expression of mRNAs has also been associated with the pathogenesis of both cancer and IPF. mRNAs are short nonprotein-coding RNA strands that may regulate the expression of related target genes involved in the control of different processes linked to carcinogenesis, such as tumour growth, invasion and metastasis [24–26]. Recently, it has been shown that ~10% of mRNAs are abnormally expressed in IPF, some of them, such as let-7, miR-29, miR-30 and miR-200, are significantly downregulated, while miR-155 and miR-21 are instead upregulated. In both cases, their changes are linked to groups of genes related to fibrosis and capable of intervening in epithelial-mesenchymal transition (EMT) induction, regulation of apoptosis and extracellular matrix. Interestingly, some of the mRNAs that appear to be involved in IPF increase the expression of TGF-β, which in turn causes their altered expression, thus creating a ''vicious circle''. The involvement of mRNAs in the fibrotic process is supported by the observation that the miR-200 family members are downregulated in the lungs of mice with bleomycin-induced fibrosis and that restoration of miR-200 expression reverses lung fibrosis *via* the inhibition of the EMT induced by TGF-β [24]. The upregulation of miR-21 has also been related to bleomycin-induced fibrosis, whereas the administration of antisense nucleotides blocking miR-21 reduces

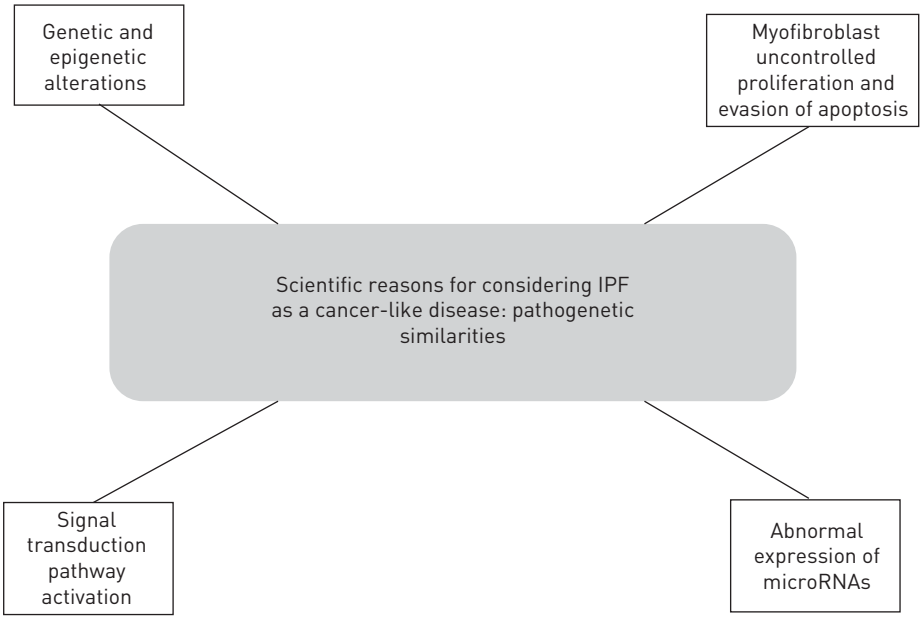

FIGURE 1 Scientific reasons for considering idiopathic pulmonary fibrosis (IPF) as a cancer-like disease.

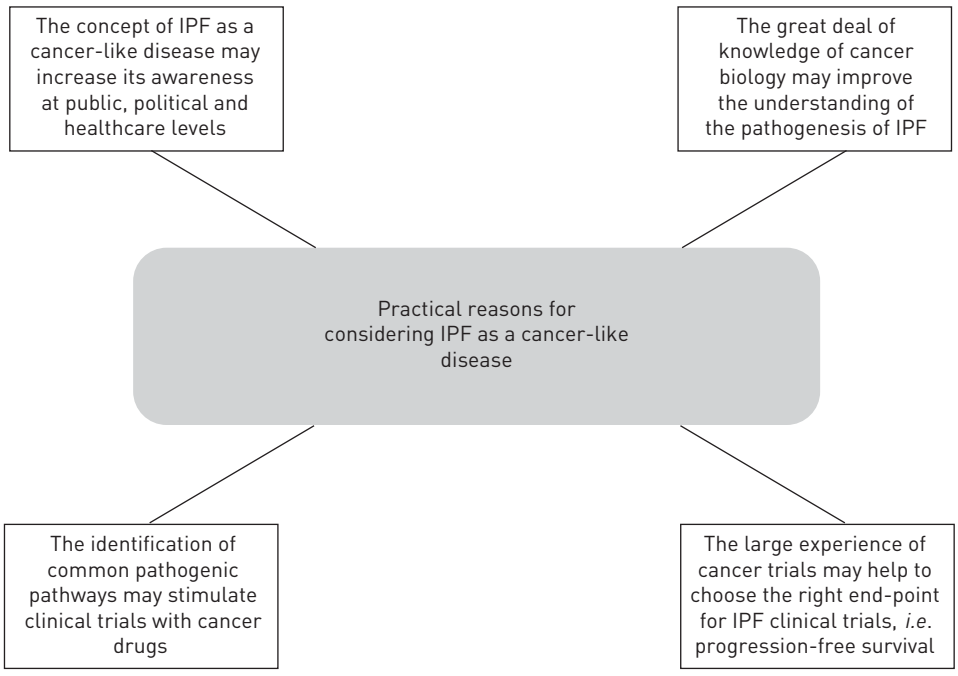

FIGURE 2 Practical reasons for considering idiopathic pulmonary fibrosis (IPF) as a cancer-like disease.

the fibrotic effect exerted by bleomycin, even when treatment started a week after bleomycin administration [27, 28]. In addition to mRNAs, cell-free DNA is also present in the blood of cancer patients and is considered by some authors to be a potential diagnostic and prognostic biomarker of cancer [29]. It is noteworthy that CASONI *et al.* [30] showed an increase of free circulating DNA in patients affected by IPF compared with healthy subjects or patients with other fibrotic diffuse lung diseases, such as nonspecific pulmonary fibrosis.

Altered cell-to-cell communication in IPF and cancer

Metabolic and electrical coupling of cells is normally provided by intercellular channels that connect the cytoplasm of adjacent cells, allowing the passage of ions and small molecules. These channels, formed by proteins called connexins (Cxs), are essential for the synchronisation of cell activities, such as proliferation and tissue repair [31]. It has been shown that Cx43, the most represented connexin on fibroblasts, is involved in the reparative process that takes place during wound healing. The downregulation, induced by antisense oligodeoxynucleotides, of the expression of this Cx accelerates wound repair by increasing cell proliferation and migration of keratinocytes and fibroblasts at skin wound sites. Moreover, an *in vitro* model of fibroblast wound healing has demonstrated that the reduction of Cx43 is linked to increased expression of TGF-β, collagen production, myofibroblast differentiation and, hence, to a faster healing process. In different contexts, the increased fibroblast proliferation and differentiation induced by Cx43 downregulation might be responsible for the loss of fibroblast proliferative control that characterises abnormal repair and/or fibrosis. Indeed, fibroblasts from keloids and hypertrophic scars compared with normal skin tissue express significantly lower levels of Cx43 [32]. It is interesting that cancer, a condition marked by the loss of cell proliferative control, is often associated with a diminished expression of Cxs and to the reduction of intercellular communication. Cancer cell lines from mouse and human lung carcinoma have low or absent levels of Cx43 expression [33]. Moreover, the deletion of the Cx43 gene results in higher susceptibility to the insurgence of cancer [34]. However, the transfection of human lung carcinoma cell lines with Cx43 cDNA reduces the proliferative ability of these cells [35]. In gastric cancer, the expression of Cx43 is directly related to the degree of differentiation of cancer cells; the less differentiated and aggressive the cancer is the lower the expression of Cx43 [36]. We have shown that in primary lung fibroblasts from IPF patients there is a reduced expression of Cx43 and, by means of a dye-loading technique, we assessed gap junctional activity and showed a reduced intercellular communication in fibrotic fibroblasts compared with normal cells. The reduced cell-to-cell communication described in IPF fibroblasts is very similar to what has been described in cancer cells and may explain both the release from the restraint of contact-inhibition and uncontrolled proliferation that is present in these diseases [37].

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The role of myofibroblasts in IPF and cancer

Abnormal wound healing and exaggerated myofibroblast activation are not specific features of IPF; other conditions including fibromatosis, inflammatory myofibroblastic tumours and myofibroblastic cancers, such as myofibromas and myofibroblastomas, are also characterised by uncontrolled proliferation of myofibroblasts [38]. In primary and metastatic cancers, TGF-β produced by cancer-derived epithelial cells is responsible for the emergence of myofibroblasts at the invasive front of the tumour and for protecting these cells from apoptosis. Myofibroblasts, encircling cancerous lesions, in turn produce additional TGF-β, inflammatory mediators and metalloproteinases that break apart the basement membrane of the surrounding tissues, thereby facilitating cancer invasiveness [39, 40]. In IPF, myofibroblasts, similarly to cancer cells, sustain their own growth through the autocrine production of the fibrogenic cytokines TGF-β and lose, at least in part, their ability to produce the anti-fibrotic prostaglandin E_2 (PGE_2) [41]. The controlling activity of PGE_2 is further diminished by the reduced expression in IPF tissues of the E prostanoid receptor 2 [42, 43]. The capacity of cancer cells to infiltrate the surrounding tissue is strictly related to the expression of a series of molecules that are able to facilitate cancer cell invasiveness, including laminin, heat shock protein (HSP)27 and fascin [44–46]. Interestingly, in IPF it has been shown that epithelial cells surrounding fibroblast foci express large amounts of laminin, fascin and HSP27. These molecules are exclusively expressed by bronchiolar basal cells layered between luminal epithelial cells on the one side and myofibroblasts on the other [47]. The expression of molecules so involved in both cell migration and invasion in bronchiolar basal cells adjacent to myofibroblasts and at the same time facing the luminal epithelium is very reminiscent of what has already been described in cancer, where these molecules are expressed at the invasive front of carcinomas.

Abnormal activation of specific signalling pathways in IPF and cancer

The Wnt/β-catenin signalling pathway regulates the expression of molecules involved in tissue invasion, such as matrilysin, laminin and cyclin-D1, and most importantly is involved in a biologically relevant cross-talk with TGF-β. It is well known that this pathway is abnormally activated in several human cancers, including lung cancer, mesothelioma and desmoid tumours [48]. More recently, an activation of the Wnt/β-catenin pathway has also been described in different fibroproliferative disorders of the liver and kidney [49]. With regard to this, CHILOSI *et al.* [50] have shown that the Wnt/β-catenin pathway is strongly activated in IPF lung tissues, as demonstrated by the presence of an intense immunoreactivity for β-catenin and a contemporary expression of high levels of two downstream genes of the Wnt/β-catenin pathway, cyclin-D1 and matrilysin. The Wnt pathway may also be activated by the fibrogenic cytokine TGF-β [51]. The transcription of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) target gene, induced by TGF-β, could also lead to a secondary activation of other signalling pathways, such as the phosphatidylinositol 3-kinase (PI3K)/Akt pathway that may regulate cell proliferation and apoptosis. We have demonstrated a role for the PI3K pathway in both proliferation and differentiation into myofibroblasts of normal human lung fibroblasts stimulated with TGF-β [52, 53]. More recently, we assessed the expression of class I PI3K p110 isoforms in IPF lung tissue, as well as in tissue-derived fibroblast cell lines also evaluating the effect of the selective inhibition of p110 isoforms on IPF fibroblast proliferation and fibrogenic activity. The expression of the p110γ isoform was increased in both IPF lung homogenates and *ex vivo* fibroblast cell lines. Myofibroblasts and bronchiolar basal cells in IPF lungs exhibited strong immunoreactivity for p110γ and a positive staining for the markers of proliferation, proliferating cell nuclear antigen and cyclin D1, within fibrolastic foci. Furthermore, both p110γ pharmacological inhibition and gene silencing were able to significantly inhibit proliferation, as well as α-smooth muscle actin expression in IPF fibroblasts. These data suggest that the PI3K p110γ isoform may have an important role in the pathogenesis of IPF and can be a specific pharmacological target [54]. In this regard, it has already been reported that oral administration of a p110γ inhibitor significantly prevents bleomycin-induced pulmonary fibrosis in rats [55]. These findings are also interesting, considering that in cancer the activation of the PI3K p110γ pathway is involved in lack of regulation of cell proliferation. Based on this knowledge, therapeutic inhibitors are being developed against the PI3K pathway, and their effect on tumour growth and survival in many cancers is being assessed [56].

Tyrosine kinases are key mediators of other signalling pathways involved in regulation of normal cellular processes, such as cell growth, differentiation, adhesion, motility and regulation of cell death. The activity of tyrosine kinase is mediated by specific transmembrane receptors, which mediate the activity of different ligands whose aberrant activity plays an important role in the development, progression and spread of several types of cancer [57]. More recently, the activity of these receptors has also been related to wound healing and fibrogenesis [58, 59]. Platelet-derived growth factor (PDGF), for instance, is a potent growth factor for fibroblasts *in vitro*, and there is enough evidence showing that fibrogenic mediators, such as TGF-β or basic fibroblast growth factor (FGF), have PDGF-dependent profibrotic activities. Indeed, PDGF protein and mRNA are increased in IPF and the inhibition of PDGF receptor attenuates the development of

pulmonary fibrosis in an animal model of fibrosis induced by radiation. Irradiated mice showed higher levels of PDGF (A–D) isoforms and a prolonged life span after treatment with a PDGF receptor inhibitor [60, 61]. Recent evidence has shown that vascular endothelial growth factor (VEGF) and FGF, two other ligands of the tyrosine kinase receptor, are largely involved not only in carcinogenesis, but are also responsible for fibrogenesis. FGF receptors, present on epithelial cell and fibroblasts, mediate EMT and fibroblast transition into myofibroblasts, whereas VEGF, whose role on vascular remodelling in IPF is still debated, may indirectly promote cell survival and proliferation through the activation of ERK1/2 and PI3K. Elevated levels of VEGF mRNA, expressed by endothelial progenitor cells, have been measured in IPF patients, although plasma levels were no different between IPF patients and control subjects. The antifibrotic profile of the multiple inhibitors of tyrosine kinase receptor has been evaluated on fibroblasts *in vitro* and, more interestingly, *in vivo* in a rat model of bleomycin-induced fibrosis. The contemporary inhibition of PDGF receptors, VEGF receptors and FGF receptors resulted in significant attenuation of fibrosis, even though the inhibitory drug was administered 10 days after intratracheal instillation of bleomycin, suggesting a novel therapeutic approach for the treatment of IPF [62–65]. During the past few years, tyrosine kinase receptor inhibitors have been proposed, and used as potential targets for the treatment of nonsmall cell lung carcinoma and other cancers. More recently, based on increasing *in vitro* and *in vivo* evidence, combined VEGF receptor, FGF receptor and PDGF receptor inhibitors have entered phase II and phase III clinical trials with promising results for the treatment of IPF [66].

Conclusions

Although the 5-year survival of IPF is worse than the majority of cancers, the severity of this disease is still underestimated and its diagnosis is often made when the disease is already in advanced stages [6]. The current pathogenic depiction of IPF, although satisfactory from a speculative point of view, in practical terms has still not been able to support the development of valid diagnostic and prognostic biomarkers, and we are still far from effective therapeutic approaches capable of stopping the disease, possibly making it chronic, or even reversing the disease. To further complicate the matter, the inadequate attention given to this dreadful disease prevents sufficient awareness of IPF at a public, political and healthcare level. Conversely, the awareness of cancer as a potentially fatal disease is widespread and the need for supporting cancer research is accepted at any level of public opinion. During the past three to four decades this has led to an incredible improvement in the diagnostic and therapeutic strategies against this disease. The concept of IPF as a neo-proliferative disorder of the lung may help in meeting the urgent need for a better understanding of the pathogenesis of IPF by taking advantage of the great deal of knowledge that cancer biology may suggest. More importantly, the identification of common pathogenic pathways between the two diseases may stimulate new clinical trials with cancer drugs and with different combinations or different lines of drugs, as has been intensively explored in cancer. Furthermore, clinical trials in IPF could take advantage of the large experience of oncologists, following the cancer model of trials of new treatments by using progression-free survival, if not ideal, as a reasonable, logical and clinically meaningful end-point.

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