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## **Clinical and Molecular Analysis of Lung cancers associated with fibrosing interstitial lung disease**

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### **Short title: Lung Cancer and Interstitial Lung Disease**

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**Key words:** lung cancer, interstitial lung disease, pulmonary fibrosis, next generation sequencing

**Abbreviations:** LC, Lung Cancer; ILD, Interstitial Lung Disease; AEILD, acute exacerbation of ILD; WHO, World Health Organization; CT, Computer Tomography; TNM, Tumors Nodes Metastases; PFS, Progression Free Survival; OS, Overall Survival; DLCO, diffusing capacity for carbon monoxide; IPF, Idiopathic Pulmonary Fibrosis; CTD-ILD, ILD associated with Connective Tissue Disease; SSC, Systemic Sclerosis; UIP, Usual Interstitial Pneumonia; NSIP, Non-Specific Interstitial Pneumonia; CI, Confidence Interval; NR, Not Reached; TMB, Tumour Mutational Burden; TGF- $\beta$ , Transforming Growth Factor beta; PDGFR, Platelet Derived Growth Factor Receptor; FGFR, Fibroblast Growth Factor Receptor; EGFR, Epidermal Growth Factor Receptor; MAPK, Mitogen Activated Protein Kinases; ERBB2, Erythroblastic Oncogene B2; PI3K/AKT, Phosphoinositide 3 Kinase/Protein Kinase B; NOTCH1, Notch Homologue 1

## **Abstract:**

**Background:** Interstitial lung disease is a heterogeneous group of diseases, some of which are known to present an independent risk factor for lung cancer. Its pathophysiological mechanism has not been fully elucidated and therapeutic management is also complex. We aim to both describe a cohort of patients with lung cancer associated with pre-existing fibrosing interstitial lung disease and to characterize their molecular profile.

**Methods:** We conducted a retrospective, single centre cohort study, at Toulouse University Hospital. Immunohistochemical (PD-L1, CD8) and molecular analysis was performed on archived tumour sample. Molecular signalling pathways involved were analysed with the Reactome Pathway Database.

**Results:** Forty-nine patients were analysed. Most common histology was adenocarcinoma (65,3%), followed by squamous cell carcinoma (30,6%). Idiopathic pulmonary fibrosis (30,6%) and interstitial lung disease associated with connective tissue disease (22,4%) were mostly diagnosed. Usual interstitial pneumonia dominated the scans patterns. A high proportion of early tumour stages was observed and overall survival was 34,5 months. In metastatic stages response rate to first line chemotherapy was 38% and overall survival was 11,2 months. Main cause of death was complex cancer progression. PD-L1 expression (n=23) was low (0%) to intermediate (1-49%). Tumour mutational burden was low in 69,2% of analysed cases (n=12) and microsatellite status was stable in all cases (n=13). Sample genotyping (n=14) showed frequent involvement of the TP53 gene and the implication of signalling pathways common to fibrotic processes such as TGFb and PI3K/AKT.

**Conclusion:** We suggest a particular phenotype of lung cancer associated with fibrosing interstitial lung disease that could provide the basis for specific therapeutic strategies.

## **Introduction:**

The link between LC and chronic ILD has been known for many years(1). It is now well established that the existence of ILD poses an independent risk factor for LC despite the frequency of confounding factors such as smoking and age(2). This relative risk has been assessed as ranging from 3.5 to 7.5(2) in various studies with an estimated incidence of 10% to 20% and a specific mortality of 15%(3,4). The issues involved in LC management in such situations are complex and marked by the specific risk of acute exacerbation of ILD (aeILD) and more common adverse effect to anti-cancer treatment(2). Current data suggest that the clinical link observed between fibrosing ILD and the onset of LC is supported by the presence of common changes in cell functions involving epithelial-mesenchymal transition and abnormal re-epithelialisation(5). The purpose of this work is to both describe a cohort of patients with lung cancer associated with fibrosing ILD and to characterize their molecular profile.

## **Patients and methods**

Our study is an observational, single centre, ambispective cohort study conducted at Toulouse University Hospital. Consecutive cases of LC associated with fibrosing ILD were identified between 2000 and 2018 from clinical databases in the Pulmonology Department, pathological databases in the Pathology Department and from the Regional Cancer Network. The patients enrolled in the study have been diagnosed with pulmonary fibrosis confirmed by thoracic imaging (honeycombing and/or reticulation with peripheral bronchiectasis or bronchiolectasis) regardless of the aetiology, and primary lung cancer diagnosed on the basis of a tumour biopsy sample. Diffuse interstitial pulmonary fibrosis must have been diagnosed before or concomitantly with LC. All primary lung histological subtypes according to the latest WHO Classification of Lung Tumours were accepted. The exclusion criteria were as follows: unavailable data (no CT imaging or tumour tissue); no evidence of fibrosis on imaging carried out at the time as cancer diagnosis or pulmonary fibrosis being developed after the introduction of first-line treatment; pulmonary metastases of extra-bronchial tumours. The main purpose of this study was the clinical, molecular (main oncogenes, microsatellite instability, tumour mutational burden) and immunohistochemical (expression of PD-L1 and CD8) characterisation of primary lung cancer being developed in the context of pulmonary fibrosis.

Aetiological diagnoses and images of interstitial lung diseases were confirmed following expert appraisals or multidisciplinary discussions in our Institution(6). Tumour stage was assessed based on the 8th edition of the TNM Classification of Lung Cancers. Formalin-fixed and paraffin-embedded tumour specimens were extracted from the archives of the Pathology Department of Toulouse University Hospital.

### *Molecular sequencing*

The genetic analysis of tumour tissues was carried out by the Foundation Medicine Inc. Laboratory using FoundationOne®CDx sequencing (synthesis sequencing, Illumina® HiSeq 4000).

### *Immunohistochemical analyses*

PD-L1 analysis was performed on an automated Bond Polymer Refine Detection System (Leica Biosystems®) after deparaffinisation (clone QR-1, 1-PR292-01, Diagnostics®). CD8 analysis was performed with the SP57 RabbitMono clone (Roche®) on the automated Ventana Benchmark Ultra System (Roche®). Results were expressed as a percentage of labelled cells and classified into three categories: 0%, 1-49% and ≥50% respectively.

### *Statistical analyses*

Clinical and biological characteristics were described using standard statistics. Quantitative variables were described by the median and range, and qualitative variables by frequency and percentage. Progression-free survival is defined as the period of time from the start date of the treatment until the disease progression date or date of death from any cause. Living and progression-free patients were censored on the date of the latest progress report or new line of treatment start date. Overall survival is defined as the period of time from the start date of the treatment to the date of death from any cause. Living patients were censored at the date of the latest progress report. Survival data were summarised using the Kaplan-Meier method. Statistical analyses were performed using STATA v16 software (StataCorp, Texas, USA). The genomic dataset obtained was analysed using the Reactome database (version 72), organised into signalling pathways.

## **Results**

A total of 49 patients diagnosed between 2000 and 2018, were retrospectively analysed between November 2017 and November 2019. Sufficient tumour material was archived to allow both immunohistochemical analysis of 23 tumours and molecular analysis in 14 patients. The patient selection process is summarised in **Figure 1**.

### *Clinical characteristics:*

Clinical characteristics are listed in **Table 1**. The population mainly comprised men (69.4%) with significant smoking exposure in 83.3% of patients. Impaired diffusing capacity for carbon monoxide (DLco) predominated (49%). We mainly found adenocarcinomas (65.3%) and mostly localised stages, I-II (51%).

Concerning ILD, 30.6% of the diagnoses performed were IPF and 22.4% were CTD-ILD, including SSc in 9/11 cases (81.8%). In 20.4% of cases, patients were not investigated for ILD (no pathological diagnosis). CT-Scan mainly revealed usual interstitial pneumonia (UIP) in 53.1% of cases (definite and probable UIP in 28.6% and 24.5% of cases, respectively). Non-specific interstitial pneumonia (NSIP) and indeterminate UIP accounted for 14.3% and 22.4% of cases, respectively.

The primary tumour lesion was located within fibrotic lesions in 77.6% of cases and emphysema lesions were present in 22.4% of cases. In 26% of cases, ILD was diagnosed concomitantly with LC on the basis of a CT-scan. In the remaining cases, a median 3-year follow-up period preceded LC onset.

In the sub-group of non-smoking patients (n=8) the most common diagnosis of ILD was connective tissue disease in 62.5% of cases [SSc, 50% (n=4); rheumatoid arthritis, 12.5% (n=1)], followed by IPF [25% (n=2)].

### *Therapeutic and progressive characteristics*

Forty-one patients (83.7%) received at least one line of treatment for LC. First-line treatment comprised chemotherapy in 58.5% of cases. Radiotherapy was administered to 17.1% of patients and in combination with chemotherapy for one patient (2.4%). Five patients (i.e., 71% of patients undergoing radiotherapy) received stereotactic radiotherapy exclusively. Finally, surgery was proposed in 41.5% of cases depending on individual cases. No patient was offered

immunotherapy or targeted therapy as first-line treatment. The response rate to first-line chemotherapy was 38.1% and the disease control rate was 81.0% respectively.

A total of five patients (10.2% of patients analysed) developed aeILD during follow-up with a specific mortality rate of 60%. Four occurred immediately after treatment: two cases after surgery, two were iatrogenic causes secondary to chemotherapy and one was idiopathic. Finally, two cases of grade > II radiation pneumonitis were documented in our cohort, one of which occurred after stereotactic irradiation, with a specific complication rate of 33%.

Median progression-free survival under first-line treatment was 12.7 months (95% CI [6.1; 29.7]) for localised stages (I-II), 5.1 months (95% CI [1.7; 9.7]) for locally advanced stages (III) and 4 months (95% CI [1.1; NR]) for metastatic stages (IV) (**Figure 2a**). Median overall survival was 16.7 months (95% CI [10.1; 34.5]) for all stages, 34.5 months (95% CI [14.1; 85.2]) for stages I-II, 6.1 months (95% CI [1.7; 17.2]) for stages III, and 11.2 months (95% CI [1.1; NR]) for stages IV (**Figure 2b**).

The cause of death was known in 50% of the patients who died at the time of the last progress report. 60% of deaths were related to a progressive complication of cancer. 15% were due to a complication of applied cancer treatments (including 2 acute exacerbations of ILD and 1 chronically progressive radiation pneumonitis). Finally, 20% of deaths were due to other causes (infection and cardiovascular problems in particular). Overall, 15% of these deaths were directly related to ILD.

#### *Immunohistochemical characteristics*

Twenty-three patients underwent immunohistochemical analysis. 65.2% of the specimens analysed showed no PD-L1 protein expression on tumour cells. Moderate expression (1 to 49%) was documented in 30.4% of patients. Tumour lymphocyte infiltration, as assessed by CD8 labelling, was deemed moderate (labelling between 1 and 49% of cancer cells) in all patients. The results are summarised in **Table 2**.

#### *Molecular characteristics*

Assessed in 14 patients, the tumour mutational burden (TMB) was considered low (0 - 5 Mut/Mb) or intermediate (6 - 19 Mut/Mb) in 69.2% and 30.8% of the analysed tumours, with a median of 3.78 Mut/Mb, respectively. All assessed patients showed a stable microsatellite status.

At least, one mutation was detected in 112 out of the 324 analysed genes. The most common mutations are shown in **Figure 3**, and are compared with the TCGA molecular database for lung cancer. TP53 was the most frequently altered gene (71.4%). These mutations were involved in 949 signalling pathways after analysis into the Reactome database. The 25 most significantly impacted signalling pathways are presented in **Table 3**. The signalling pathways shared by at least three patients are shown in **Figure 4**. Abnormalities in proliferation signal transduction by mutation of growth factor receptors or second messengers mainly concerned TGF- $\beta$  (transforming growth factor beta) pathway as well as PDGFR (platelet derived growth factor receptor) signalling pathways, FGFR (fibroblast growth factor receptor), EGFR (epidermal growth factor receptor, or ErbB1), Wnt, MAPK (mitogen-activated protein kinases), ErbB2 (erythroblastic oncogene B2, or HER2), PI3K/AKT (phosphoinositide 3-kinase/protein kinase B), and NOTCH1 (Notch homologue 1).

## **Discussion**

We analysed the pathological and clinical characteristics of LC associated with fibrosing ILD. Adenocarcinoma was the most frequent histological subtype in the assessed population, ahead of squamous cell carcinoma, with percentages comparable to the published data(7)(8). In the specific IPF subgroup, the squamous subtype was often described as predominant, especially in the basal and peripheral areas where fibrous mutilation is most significant(9–11). In our study, we found a higher proportion (40%) of squamous cell carcinomas in the IPF subgroup with 77% of primary lesions being located in the fibrotic zone.

As previously described(2), it seems that IPF and ILD associated with SSc are particularly related to LC onset. Only two patients included in our cohort were diagnosed with rheumatoid arthritis with fibrotic ILD and LC. In 2018, in a retrospective cohort of 24 patients presenting CTD-ILD and LC, Watanabe et al. found 54% cases of rheumatoid arthritis compared to only 29% cases of SSc. However, selection bias is extremely likely.

Notably, one in five patients (20.4%) presented typical ILD with fibrosis, which was confirmed by imagery. No aetiological diagnosis was given due to insufficient investigations. This may be attributed to the frequency of simultaneous ILD and LC diagnoses in this population (60%) and to the priority given to cancer treatment or a deterioration in the clinical condition requiring palliative management only (in 40% of those patients who underwent no further investigation).



UIP (definite or probable UIP) was mainly highlighted on CT-scans. These patients also had a particularly poor prognosis due to treatment-induced aeILD (2).

Overall survival was lower than the values generally reported in the overall population, regardless of the tumour stage (12). We nevertheless note major differences between tumour stages. Half of LC cases associated with ILD were diagnosed at a localised stage (stages I and II). This difference can be explained by the regular scan follow-up implemented in ILD management with fibrosis. In this population, the survival rate for surgically corrected stages 1 and 2 (34.5 months) is satisfactory and encourages early diagnosis and management for these patients. Regarding metastatic stages, the objective tumour response rate and the rate of disease control under chemotherapy were comparable to those observed in the general population based on previously published data(13,14). One notable feature is the overlap of the progression-free survival curves during first-line treatment in stage III and IV LC patients. This may be attributed to the under-representation of radiotherapy in the management of stage III LC associated with ILD. This is understandable given the much higher risk of aeILD following radiation pneumonitis in this population. In accordance with published data(2), tumour progression was the primary cause of death in our cohort, thus, it highlights the interest in specific treatments for LC associated with fibrosing ILD.

The data observed on PD-L1 expression on tumour cells, tumour lymphocyte infiltrate, tumour mutational burden or microsatellite instability in our cohort are consistent and indicative of anticipated low immunogenicity in these tumours. No prospective data are currently available to assess the efficacy and safety of immunotherapies in the context of fibrosing ILD. In a retrospective analysis of 16 patients with LC related to fibrosing ILD, Guyard et al. found that PD-L1 expression was absent (<1%) or moderate (between 1 and 49%) in 37.5% and 56% of patients respectively(8). These observations are consistent with a different distribution in terms of PD-L1 expression presenting a lower proportion of tumours strongly expressing PD-L1 (>50%) in this population(15). Contrastingly, squamous cell carcinomas associated with fibrosing ILD appeared to have significantly higher PD-L1 expression with a lower T-CD8 infiltrate (16). It should, however, be noted that there is currently no single biomarker predictive of immune checkpoint inhibitor activity, and these observations alone do not allow the efficacy of immunotherapy to be determined in this context. In the absence of a biomarker, their use is still not recommended, especially since recent observations show a high rate of severe pulmonary toxicity herein (52).

No molecular abnormality responsible for classic oncogene addiction (*EGFR*, *ALK*, *ROS1*, *BRAF* for example) was highlighted during the molecular analyses although some patients displayed gene mutation involving *KRAS* or *ERBB2*, for example - potential targets for therapies under clinical evaluation (17).

Gene *TP53* was the most commonly mutated one within our analysis (in 71.4% of patients). This is comparable to published LC data (18). *TP53* mutations also seem to be linked, in particular, to smoking-related LC (8). It is interesting to note that changes in *TP53* and *KRAS* characterise patients presenting IPF, in tissues devoid of tumour proliferation (5,19).

No mutations in *EGFR* gene were found, although it is frequently involved in about 10% of BPCs. This observation can probably be explained by the high proportion of male smokers in our cohort but has already been described in patients with IPF-related LC where the frequency of *EGFR* mutations is significantly lower (20,21). Similarly, no *ALK-EML4* translocation was detected. The frequency of this mutation does not appear to differ in patients presenting idiopathic ILD according to already published data (21), but no firm conclusion can be drawn given that it is relatively rare (approximately 4% of ADC in the general/overall population). Three isolated *ALK* mutations with unknown prognosis have nevertheless been identified.

Signalling pathway analysis provides quantitative results, highlights the level and extent of changes within the affected pathways. PI3K/AKT pathway was thus altered to considerable extent. It is known to be involved in fibroproliferative diseases, with a role in the proliferation of human pulmonary fibroblasts, and in cancer by ensuring cell survival(22). TGF $\beta$  pathway (Diseases of signal transduction by growth factor receptors and second messengers, **Table 3**), a major pro-fibrotic signalling pathway and promoter of epithelial-mesenchymal transition(5), was greatly impacted. This pathway also plays an important role in tumour proliferation (pro-mitotic effect) and progression (crossing the basal membrane and metastasis) and constitutes a research target for the development of NSCLC treatments (23). These observations, which are consistent with literature, suggest that the development of LC in a pulmonary fibrosis setting may be supported by the involvement of common biological mechanisms. An initial randomised trial to assess the merits of Nintedanib and Nab-paclitaxel combination therapy versus paclitaxel alone is on-going(19). This is the first randomised trial conducted specifically in this population. There may also be a prophylactic effect with a documented decrease in the incidence of LC in IPF treated with Pirfenidone(24). A pre-clinical study demonstrated that

blocking PI3K/AKT pathway prevented bleomycin-induced pulmonary fibrosis in rats(25). Omipalisib, a specific inhibitor of this pathway is currently being assessed in the treatment of IPF with a recently published phase 1 trial demonstrating acceptable drug tolerability in 17 patients and confirming pharmacodynamic activity on the basis of reduced tomographic fixation of pulmonary fibrosis zones (26). Finally, Wnt/ $\beta$ -catenin pathway (signal transduction, **Table 3**), featured in our analyses, is also known to be involved in the development of fibrosis in numerous organs. Specific blocking of Wnt/ $\beta$ -catenin pathway by a monoclonal antibody (OMP-131R10) is the subject of research with interesting pre-clinical results in pulmonary fibrosis models (27).

The main interest of this work is the clinical, immuno-histochemical and molecular description of LCs which was developed in the fibrotic ILD context on broad patient selection criteria facilitating analysis of real-life clinical practices in this area. To our knowledge, this is the first study combining immunohistochemical and molecular analyses with such a large cohort. However, several biases exist and unequivocal conclusions cannot, therefore, be drawn. There is a selection bias and, in particular, a "centre effect" one given that this is a single-centre study. There is also information bias in the data collection due to retrospective methodology, which is however more appropriate in this rare disease context. Finally, the absence of a control group, especially for molecular analyses does not allow any conclusion to be drawn with regard to the specific nature of the anomalies detected. Furthermore, the poor number of patients does lead to confusion bias.

## **Conclusion**

These observations suggest a specific phenotype of LC associated with fibrosing ILD. We found a clear majority of patients with idiopathic pulmonary fibrosis or interstitial lung disease associated with SSc, two conditions with a major risk of pulmonary neoplastic complications. These patients have a poor prognosis regardless of the tumour pathology stage. Nevertheless, our results demonstrate the merits of early detection and management of these neoplastic complications given the high, specific mortality rate associated with cancer. Overall, the predictive markers used to analyse the efficacy of immunotherapies do not suggest that such treatments are beneficial for the study population. The genomic study highlights an over-

representation in the tumour specimens of changes in signalling pathways known to be involved in pulmonary fibrosis. This observation suggests a continuum in the development of pulmonary fibrosis towards cancer, and may provide a basis for upcoming specific therapeutic strategies.

### **Declaration of Competing Interest**

All authors have completed the disclosure form. Julien Mazières benefits from funding Roche FMI tests. The other authors have no conflicts of interest to declare.

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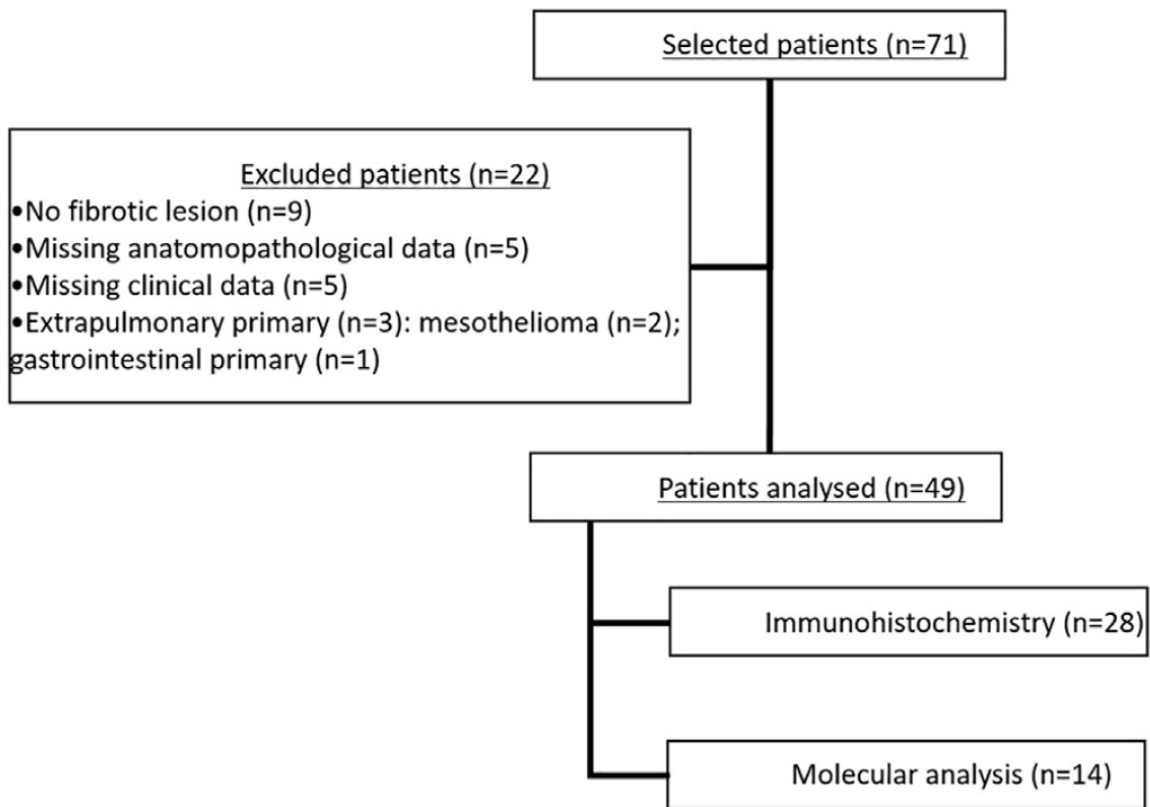


Fig. 1- Flow chart.

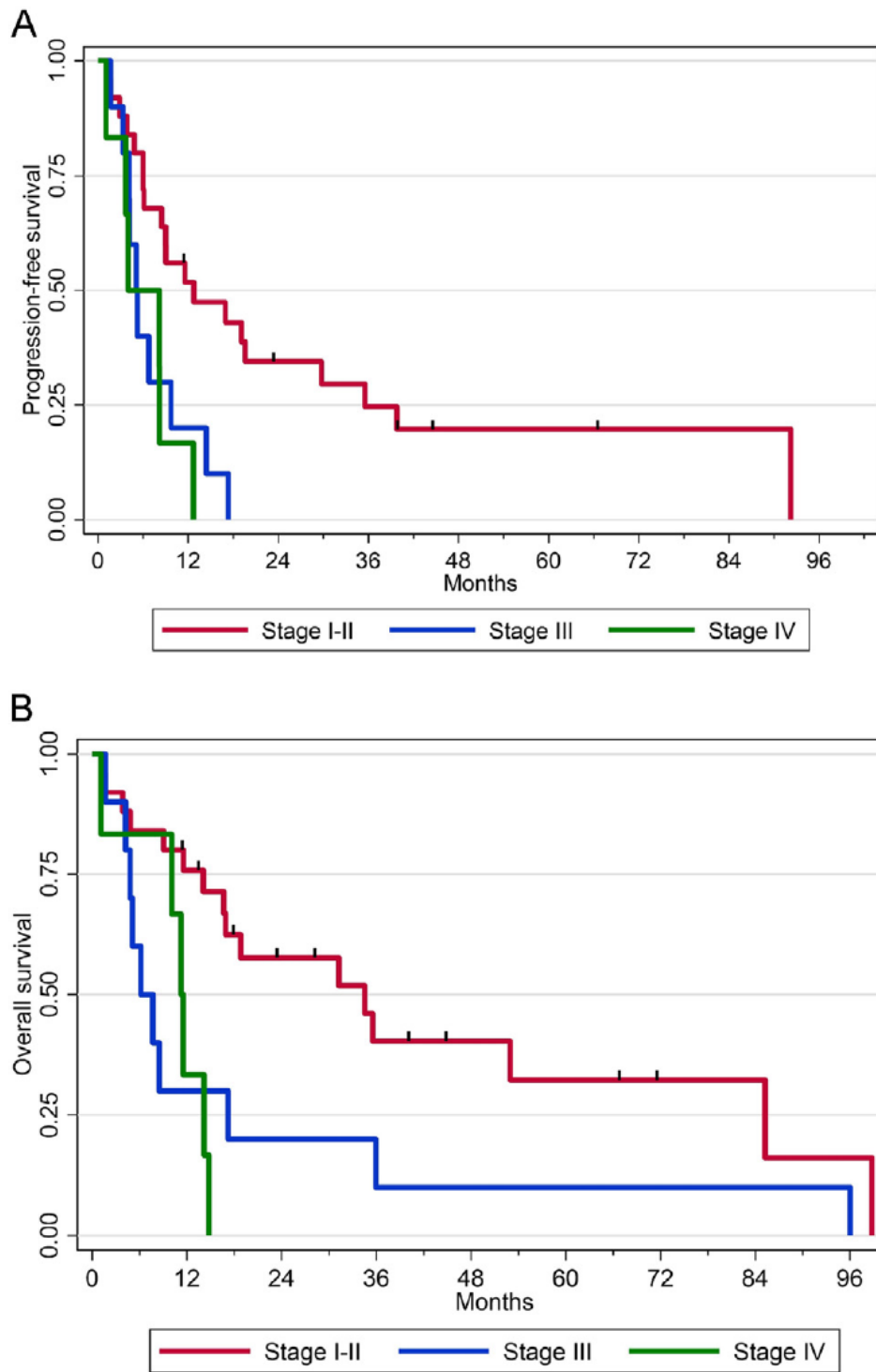
Variables	n=49	Missing - n
Gender, male – n (%)	34 (69.4)	-
Age - years (range)	68 (40 – 89)	-
Smoker		
Current or former – n (%)	40 (83.3)	1
Packs per year – n (range)	30 (1 -100)	7
Asbestos – n (%)	4 (8.7)	3
ECOG		5
0 – 1 – n (%)	31 (70.5)	-
≥2 – n (%)	13 (29.5)	-
FVC		-
Value – L	3.0 (1.3 – 4.5)	5
Predicted value percentage - %	89 (41 - 156)	2
FEV1		
Value – L	2.2 (1 – 4.2)	5
Predicted value percentage - %	94 (36 – 134)	3
DLco - predicted value percentage – (%)	49 (26 – 100)	5
Histology		
Adenocarcinoma – n (%)	32 (65.3)	-
Squamous cell carcinoma – n (%)	15 (30.6)	-
Small cell carcinoma – n (%)	2 (4.1)	-
Stage		
I, II – n (%)	25 (51)	-
III – n (%)	11 (22.4)	-
IV – n (%)	13 (26.5)	-
Median length of time to diagnosis – months (range)	2.5 (0 – 35.7)	7
Simultaneous diagnosis – n (%)	13 (26.5)	-
Length of time to onset of cancer – years (range)	3 (1-24)	-
LC diagnostic procedure		4
Bronchoscopy - n (%)	16 (32.6)	-
Transthoracic puncture – n (%)	15 (30.6)	-
Pleural puncture – n (%)	3 (6.1)	-
Mediastinoscopy – n (%)	2 (4.1)	-
Surgery – n (%)	5 (10.2)	-
Other – n (%)	4 (8.1)	-
Selected diagnosis for ILD		
IPF – n (%)	15 (30.6)	-
CTD-ILD <sup>†</sup> – n (%)	11 (22.4)	-
Other <sup>‡</sup> – n (%)	11 (22.4)	-
Non-classifiable ILD– n (%)	2 (4.1)	-
Without diagnosis – n (%)	10 (20.4)	-
CT scan pattern		
Definite UIP – n (%)	14 (28.6)	-
Probable UIP – n (%)	12 (24.5)	-
Undetermined for UIP – n (%)	11 (22.4)	-
NSIP – n (%)	7(14.3)	-
AEF – n (%)	3 (6.1)	-
Pneumoconiosis – n (%)	2 (4.1)	-
Anti-fibrotic treatment – n (%)	9 (18.4)	-
Immunosuppressant treatment – n (%)	9 (18.4)	-

**Table 1- Clinical Characteristics.**

<sup>†</sup> pneumoconioses and idiopathic ILD excluding IPF

<sup>‡</sup> ILD associated with connective tissue diseases

Abbreviations and symbols: ECOG: Eastern Cooperative Oncology Group, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, DLco: Diffusing capacity for carbon monoxide, ILD: Interstitial Lung Disease, IPF: Idiopathic Pulmonary Fibrosis, CTD-ILD: ILD associated with Connective Tissue Disease, CT: Computer Tomography, UIP: Usual Interstitial Pneumonia, NSIP: Non-Specific Interstitial Pneumonia, AEF: Airspace Enlargement with Fibrosis

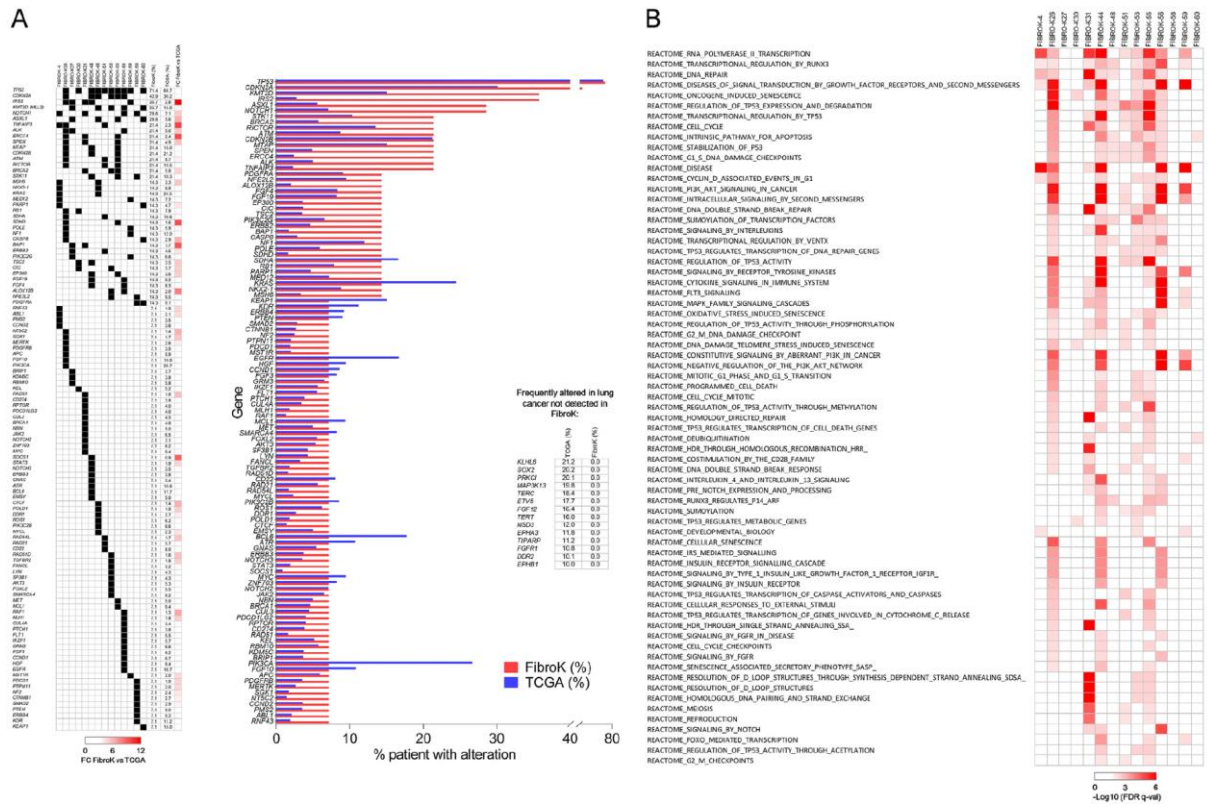


**Fig. 2-** Outcomes regarding tumour stage. (A) Progression free survival. (B) Overall survival.



Antigen	n = 23
PD-L1 (tumour cells)	
0% – n (%)	15 (65.2)
1 – 49% – n (%)	7 (30.4)
> 50% – n (%)	1 (4.3)
PD-L1 (Lymphocytes)	
0% – n (%)	15 (65.2)
1 – 49% – n (%)	8 (34.8)
> 50% – n (%)	0 (0)
CD8 (Lymphocytes)	
0% – n (%)	0 (0)
1 – 49% – n (%)	23 (100.0)
> 50% – n (%)	0 (0)
TMB	n = 14
High ( $\geq 20$ Mut/Mb) – n (%)	0 (0)
Intermediate (6-19 Mut-Mb) – n (%)	4 (30.8)
Low ( $\leq 5$ Mut-Mb)	9 (69.2)

**Table 2-** Immunohistochemical and molecular analysis.



**Fig. 3-** Molecular alteration and pathways involved. (A) Frequency of mutations detected. (B) Reactome pathways shared by  $\geq 3$  patients

Pathway Name	Entities Found	Entities Ratio	Entities p-value	Entities False Discovery Rate	Reactions Found	Reactions Ratio
Diseases of signal transduction by growth factor receptors and second messengers	43 / 484	0.033	1.11e-16	2.19e-14	273 / 358	0.028
Generic Transcription Pathway	77 / 1,553	0.107	1.11e-16	2.19e-14	341 / 824	0.065
RNA Polymerase II Transcription	78 / 1,692	0.117	1.11e-16	2.19e-14	346 / 885	0.07
Gene expression (Transcription)	79 / 1,850	0.127	1.11e-16	2.19e-14	352 / 996	0.078
PI3K/AKT Signalling in Cancer	22 / 134	0.009	1.11e-16	2.19e-14	7 / 21	0.002
PIP3 activates AKT signalling	29 / 316	0.022	6.44e-15	1.06e-12	68 / 86	0.007
Transcriptional Regulation by TP53	35 / 486	0.033	1.03e-14	1.45e-12	193 / 259	0.02
Intracellular signalling by second messengers	29 / 363	0.025	2.02e-13	2.48e-11	68 / 114	0.009
Negative regulation of the PI3K/AKT network	19 / 134	0.009	2.49e-13	2.71e-11	5 / 10	7.87e-04
Constitutive Signalling by Aberrant PI3K in Cancer	17 / 103	0.007	4.40e-13	4.31e-11	2 / 2	1.57e-04
FLT3 Signalling	26 / 311	0.021	1.44e-12	1.28e-10	32 / 61	0.005
MAPK family signalling cascades	27 / 348	0.024	2.84e-12	2.33e-10	31 / 86	0.007
PI5P, PP2A and IER3 Regulate PI3K/AKT Signalling	17 / 126	0.009	1.01e-11	7.56e-10	2 / 7	5.51e-04
Signalling by Receptor Tyrosine Kinases	35 / 622	0.043	1.09e-11	7.61e-10	440 / 698	0.055
Signal Transduction	94 / 3,364	0.232	1.30e-11	8.45e-10	961 / 2,347	0.185
MAPK1/MAPK3 signalling	24 / 297	0.02	2.21e-11	1.35e-09	27 / 46	0.004
RAF/MAP kinase cascade	23 / 290	0.02	8.50e-11	4.59e-09	25 / 39	0.003
TP53 Regulates Transcription of DNA Repair Genes	14 / 89	0.006	9.98e-11	5.09e-09	15 / 17	0.001
Cyclin D associated events in G1	11 / 48	0.003	2.21e-10	1.02e-08	12 / 14	0.001
G1 Phase	11 / 48	0.003	2.21e-10	1.02e-08	12 / 14	0.001
Signalling by Type 1 Insulin-like Growth Factor 1 Receptor (IGF1R)	12 / 73	0.005	1.37e-09	6.03e-08	8 / 19	0.001
Cytokine Signalling in Immune system	47 / 1,261	0.087	2.42e-09	1.02e-07	267 / 699	0.055
IRS-mediated signalling	11 / 65	0.004	5.01e-09	1.95e-07	4 / 9	7.09e-04
IRS-related events triggered by IGF1R	11 / 69	0.005	9.18e-09	3.40e-07	6 / 12	9.45e-04

**Table 3-** Molecular characteristics.