# Biomarkers in ILDs
# Are they useful ?

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# Conflicts of interest

| Research | Consultancy |
| --- | --- |
| **Boehringer Ingelheim** | **Astra Zeneca** |
| **CARDIF** | **Boehringer Ingelheim** |
| **Intermune, Inc** | **InterMune, Inc** |
| **LVL** | **Sanofi** |
| **MedImmune** | **Stallergenes** |

# Definition of a biomarker

- “A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”

Atkinson AJ, Clin Pharmacol Therap 2001; 69:89-95

# Are Biomarkers of interest in ILDs ?

1. Diagnosis
2. Prognosis : predict disease progression
3. Treatment tailoring
4. A better understanding of pathophysiology

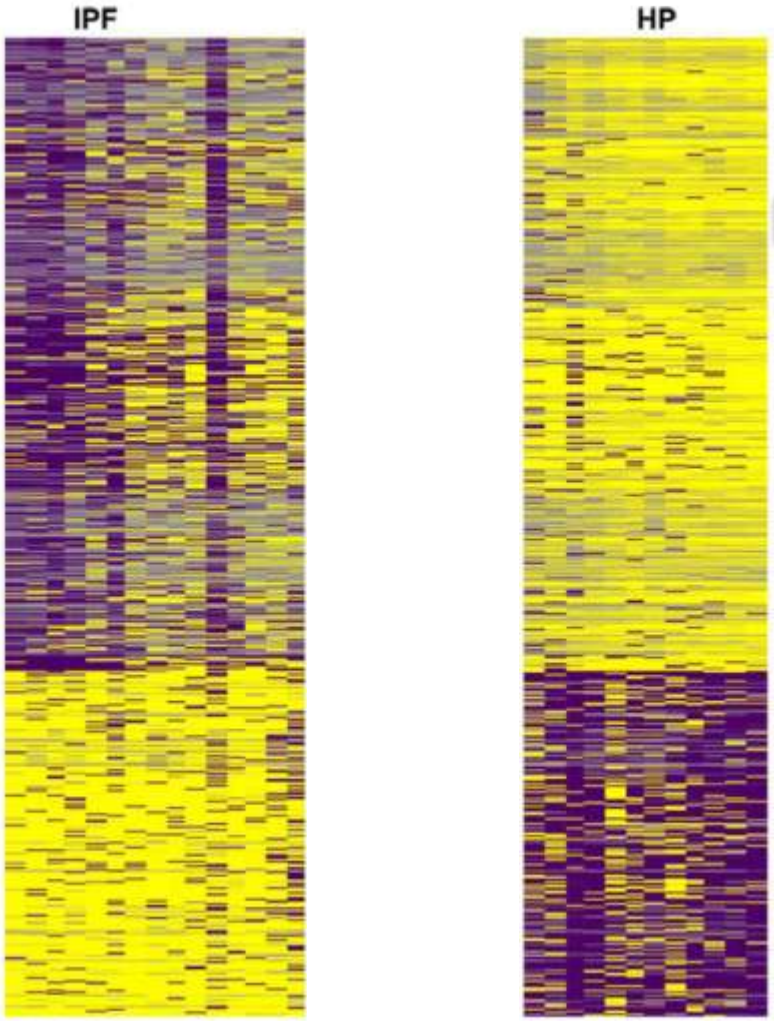
# Are Biomarkers of interest in ILDs ?

- **Diagnosis**
  - Idiopathic/non idiopathic
    - e.g. IPF vs Chronic HP

    *(≠t precipitins : marker of exposure)*

  - Identify pathological patterns : e.g. NSIP/UIP
    - Avoid surgical lung biopsy in Possible UIP/Probable NSIP

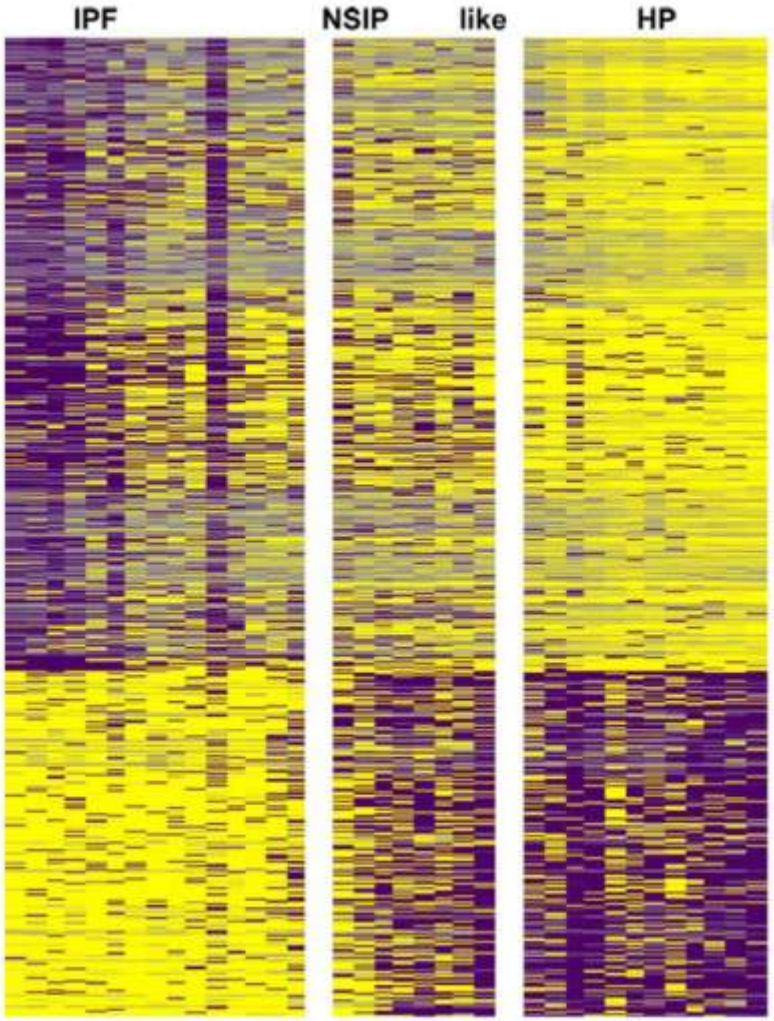
# Lung transcriptome- to differentiate IIPs/nonIIPs




**IPF**
Tissue remodeling, epithelial, and myofibroblast genes.
**HP gene expression signature**
inflammation, T-cell activation, and immune responses.

Selman, AJRCCM 2006

# Lung transcriptome-
# to differentiate IIPs/nonIIPs
# UIP/NSIP




Selman, AJRCCM 2006

**IPF**
Tissue remodeling, epithelial, and myofibroblast genes.
**HP gene expression signature**
inflammation, T-cell activation, and immune responses,
**NSIP**
Two cases : IPF-like gene expression,
One : HP like
Others : specific pattern

**Unresolved controversy :**

- Yang et al. found slight minimal differences between UIP/NSIP pattern

Yang, AJRCCM 2007

**Lung transcriptome, miRNome, proteome**

**=**

**Proof of concept**

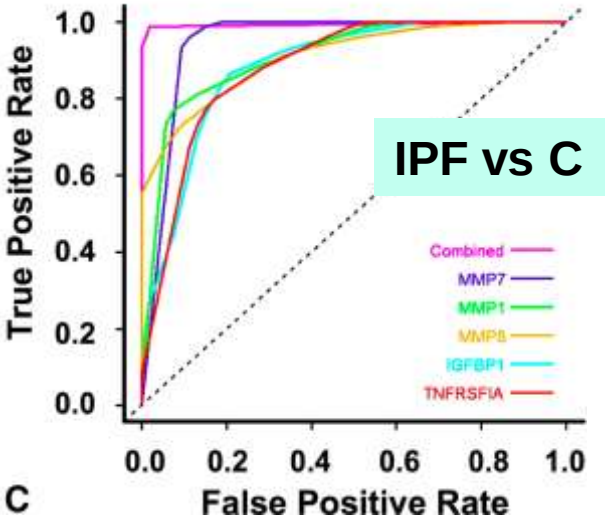
**More readily available material**

**Blood, Exhaled Air, Urine…**

**Plasma**

## Multiplex analysis

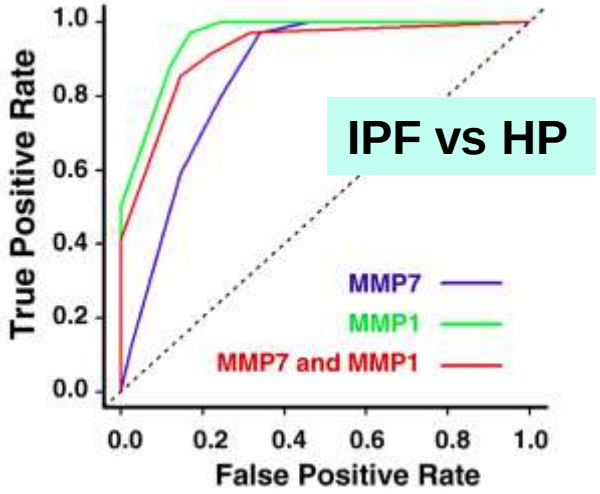
*48 proteins :*
*cytokines, chemokines, MMPs, GFs*




**MMP1 (>2.15 ng/mL) +MMP7 (>1.99 ng/mL)** :

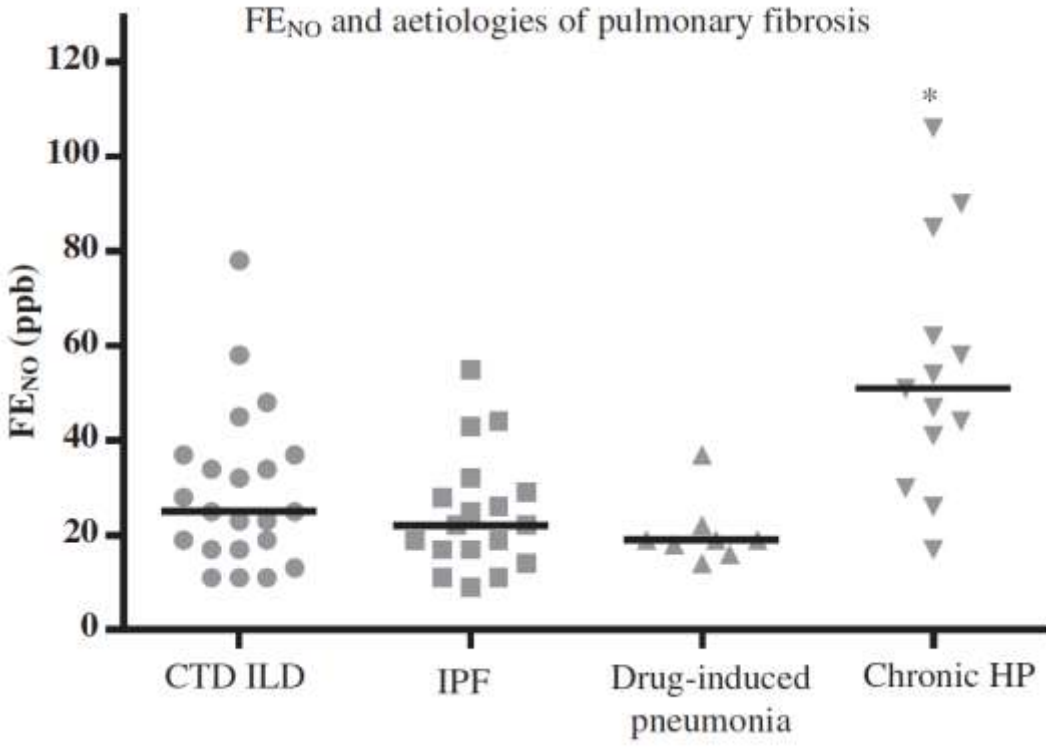
- Excludes all controls (Sp 100%)
- Not increased in COPD or sarcoidosis
- Differentiate IPF/HP (s/a, chr)
  (Se 96.3%, Sp 87.2%)

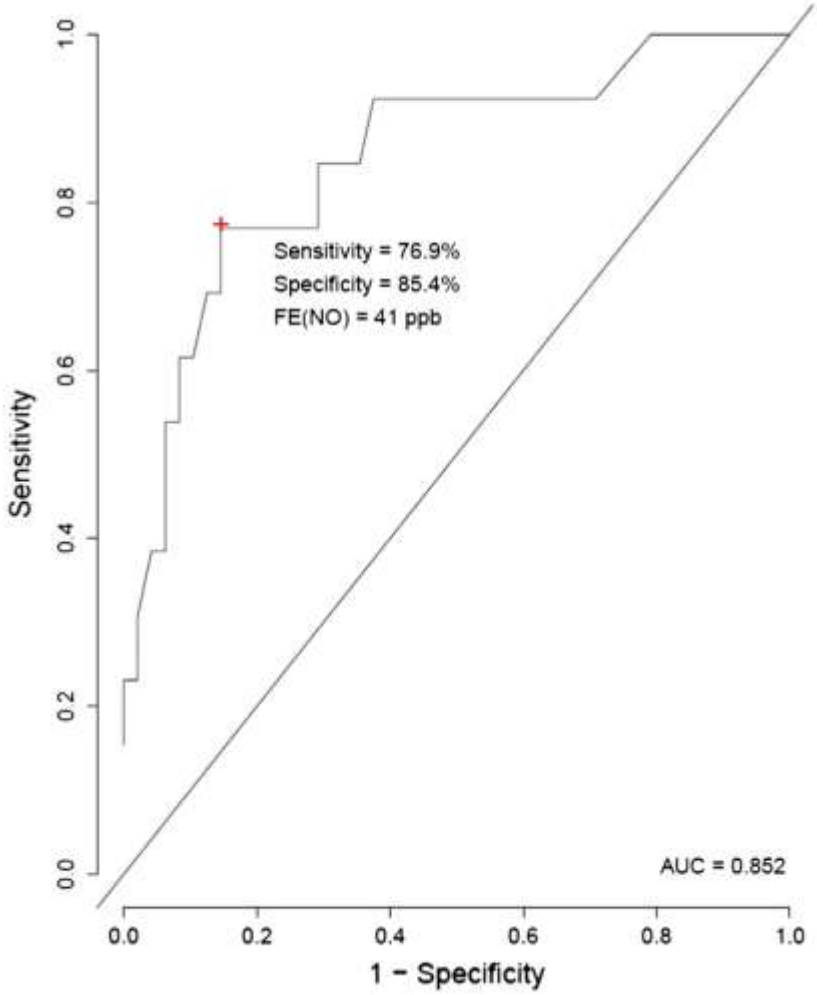
**Confirmed in a validation cohort**




Rosas, PLoS Med 2008

# FENO for the diagnosis of chronic HP

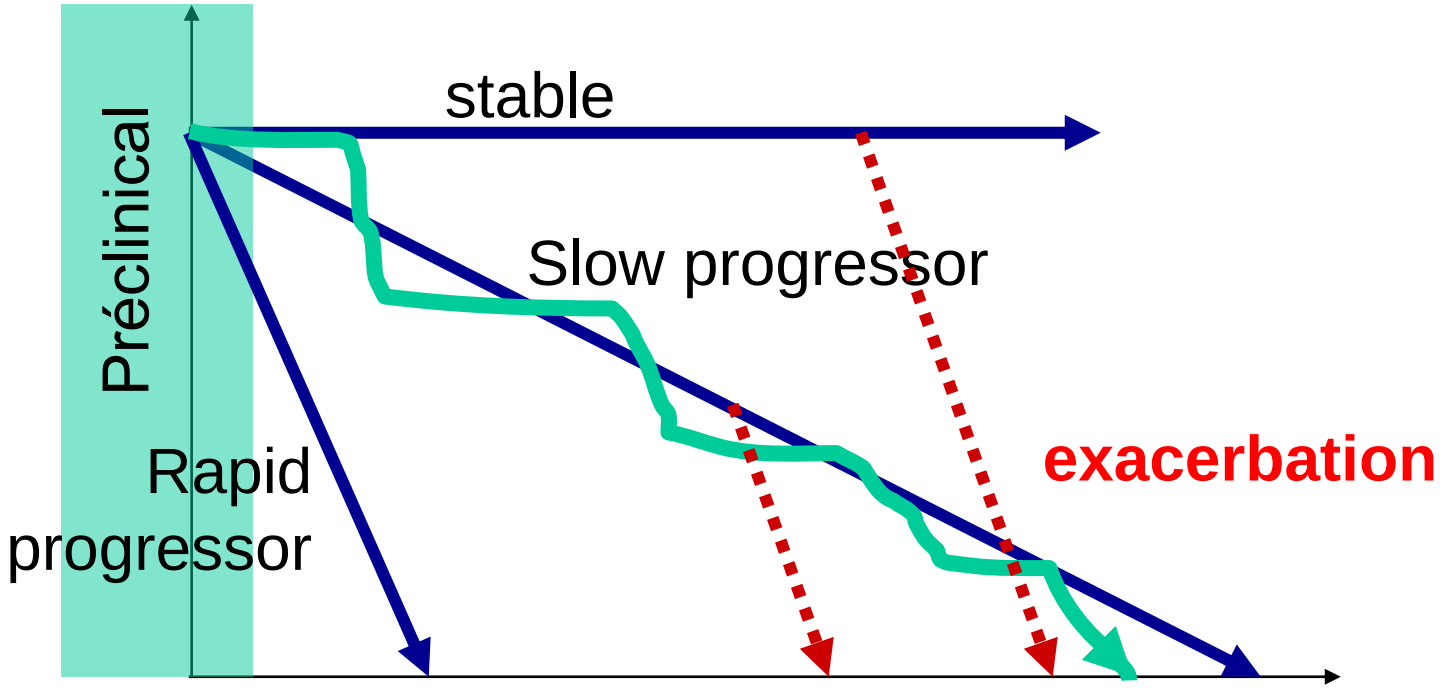



*(Guilleminault…Marchand-Adam, Respir Med 2013)*

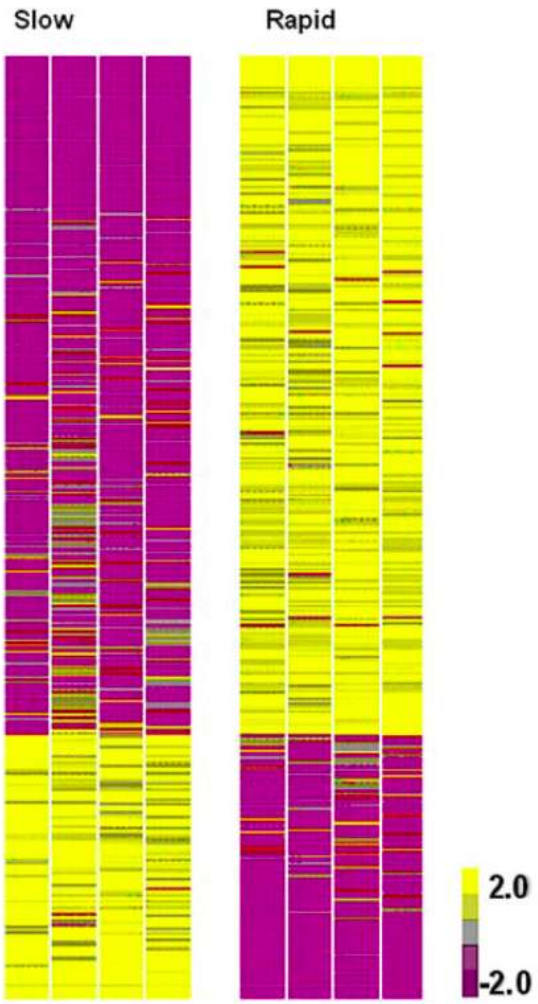
# Are Biomarkers of interest in ILDs ?

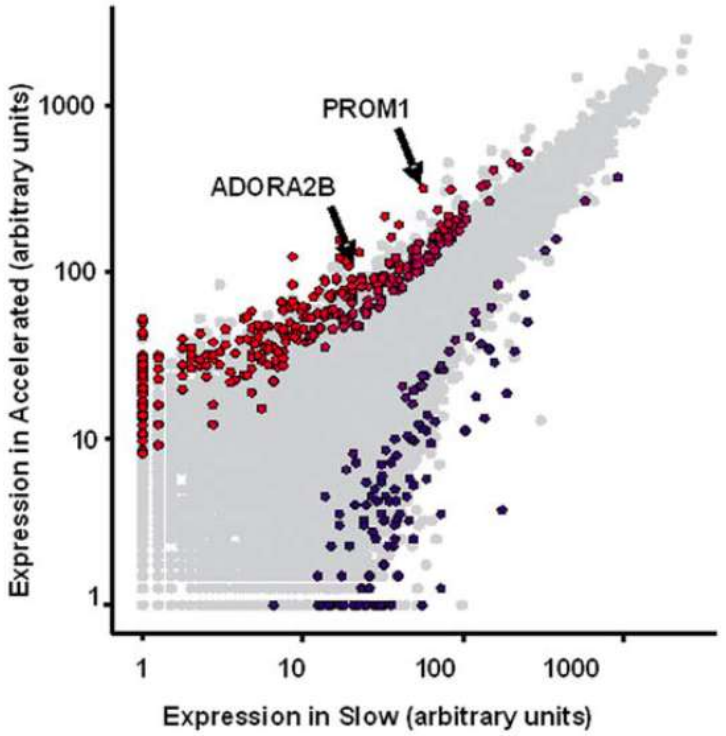
- **Prognosis : predict disease progression**




Adapted from Ley, Collard and King, AJRCCM 2011

# Lung transcriptome-
# Slow vs rapid progressors




(Selman, PLoS ONE, 2007; Boon, PLoS ONE 2009)

# Lung miRNA-
# Slow vs rapid progressors

## miRNA

- Small non coding RNAs (21-25 nts) N≅1500
  - bind to the 3'-untranslated region of target mRNAs
- 10% are differentially expressed in IPF/Control
- A defect in lung miRNA processing in rapidly vs slowly progressing IPF
- Some miRNA control fibrogenesis
  - ↓ let-7d, miR-200, miR-31
  - ↑ miR-21

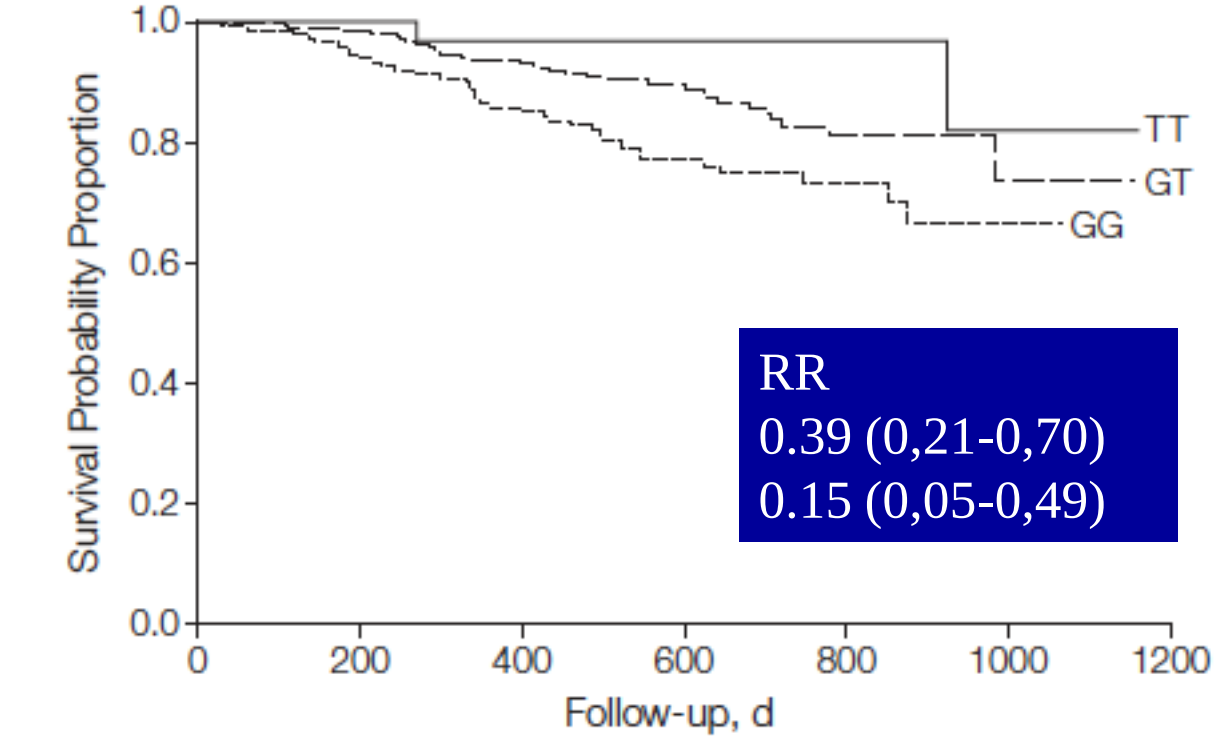
**Increased (n=5) and decreased (n=1) miRNA in rapid/slow progressors**

| miRNA | Fold increase/decrease |
|---|---|
| miR-302c | 10.56 |
| miR-423-5p | 7.97 |
| miR-210 | 4.50 |
| miR-376c | 3.84 |
| miR-185 | 2.94 |
| miR-423-3p | −97.68 |

(Oak, PLoS ONE 2011

(Pandit, AJRCCM 2010; Xu, J Exp Med 2010; Oak, PLoS ONE 2011)

# Survival according to MUC5B genotype




| No. at risk | | | | | | |
|---|---|---|---|---|---|---|
| TT | 31 | 26 | 21 | 11 | 3 | 0 |
| GT | 255 | 224 | 122 | 55 | 8 | 0 |
| GG | 140 | 115 | 67 | 36 | 5 | 0 |

Peljto, JAMA 2013

# Biomarkers for outcome in blood

| | | |
|---|---|---|
| **KL-6 (MUC1)** | **IPF, Survival, Progression, Disease severity** | Ohnishi, AJRCCM 2002; Ishii, Thorax 2003; Satoh, J Intern Med 2006; |
| **SP-A, SP-D,** | **IPF, Survival, Progression, Disease severity** | Takahashi, AJRCCM 2000; Ohnishi, AJRCCM 2002; Greene, Eur Respir J 2002; Ishii, Thorax 2003; Kinder, Chest 2009 |
| **CCL18** | **Survival, Progression, Disease severity** | Prasse, AJRCCM 2009 |
| **YKL-40** | **Survival** | Furuhashi, Respir Med 2010; Korhagen, Respir Med 2011 |
| **Periostin** | **Progression** | Okamoto, Eur Respir J 2011 |
| **VEGF** | **Progression, Disease severity** | Ando, Lung 2010 |
| **Free DNA** | **IPF** | Casoni, Int J Biol Markers 2011 |
| **% fibrocytes** | **Survival** | Moeller, AJRCCM 2009 |
| **%CD4(+)CD28null** | **Survival** | Gilani, PLoS ONE 2010 |

**Plasma**

## Multiplex analysis

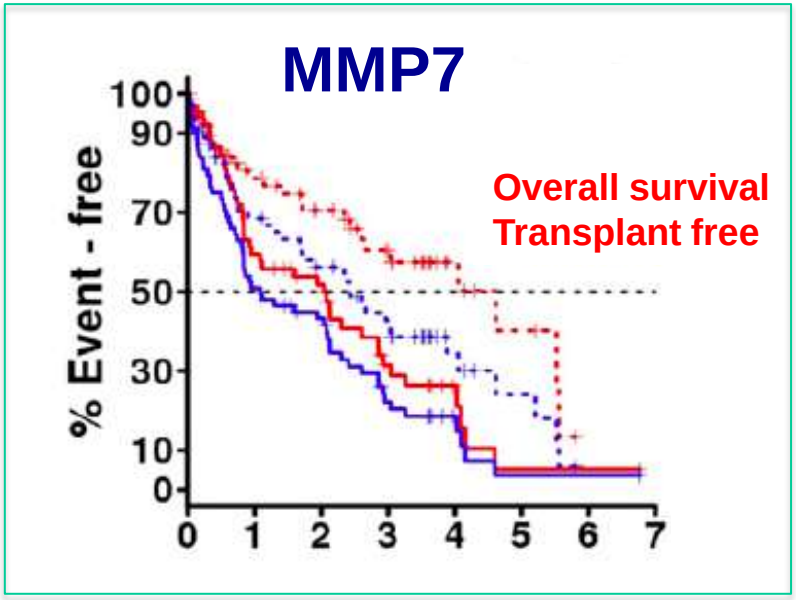
**95 proteins**

*Cytokines, chemokines, MMPs, markers of apoptosis and epithelial injury*

**Derivation cohort : N=140 IPF**

↑ MMP7, ICAM-1, VCAM-1, IL-8, S100A12
→↓ survival, transplant free survival, progression free survival

*(Independent of age, gender, LFTs)*




**Validation cohort : N=101 IPF**

- All 5 five proteins : transplant free survival
- MMP7, ICAM-1, IL-8 : overall survival
- ICAM-1 : progression free survival

Richards, AJRCCM 2012

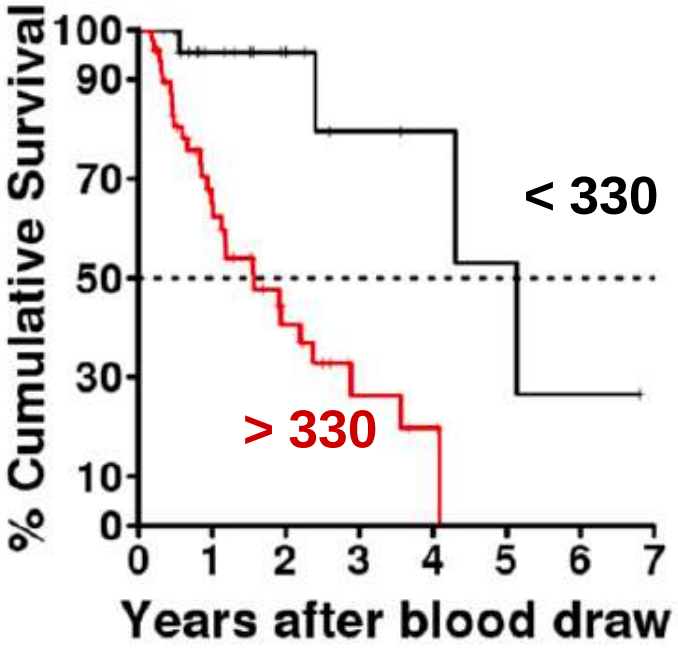
**Plasma**

**Personal Clinical and mortality Index (PCMI):**

- Gender
- FVC
- DLCO
- MMP7

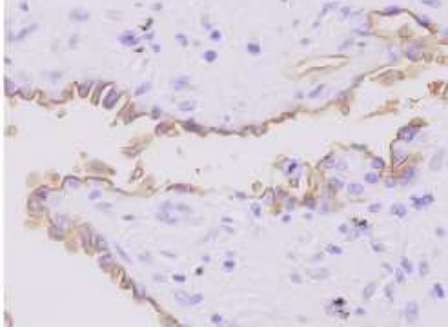
→predicts survival in the derivation and validation cohorts

**Survival according to index < or > 330**




Richards, AJRCCM 2012

# Krebs von den lungen-6 (KL-6)-MUC1

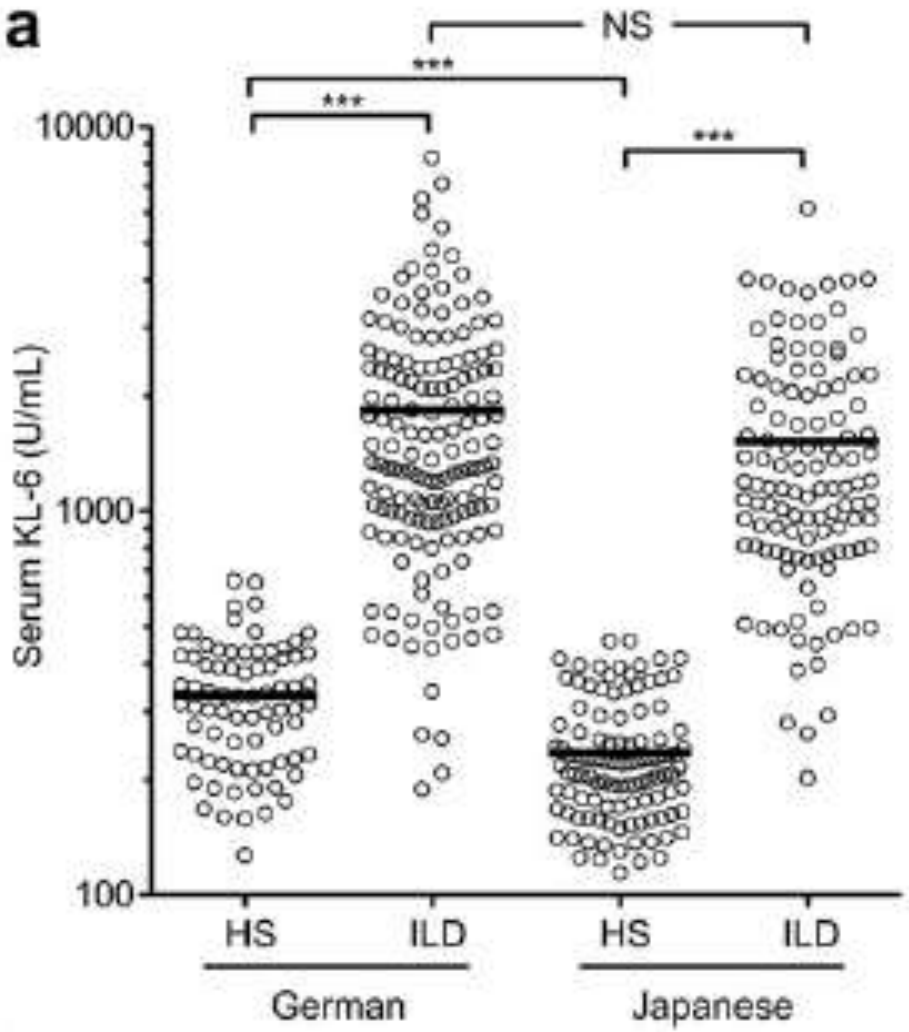


- Primary source : Regenerating type 2 pneumocytes
- Serum levels are genetically determined (common polymorphism)
- Increased in most patients with most ILDs
  - including IIPs, CVD-ILDs, HP, radiation pneumonitis, drug-induced ILDs, pulmonary sarcoidosis, and pulmonary alveolar proteinosis
- And many other diseases
  - ARDS, tuberculosis, metastatic adenocarcinomas…
- Used routinely in Japan for disease monitoring
  - > 2.000.000 assays/year in Japan

*(Ishikawa, Respir Invest 2012; Horimasu… Costabel, Respir Med 2012)*

# Krebs von den lungen-6 (KL-6)-MUC1

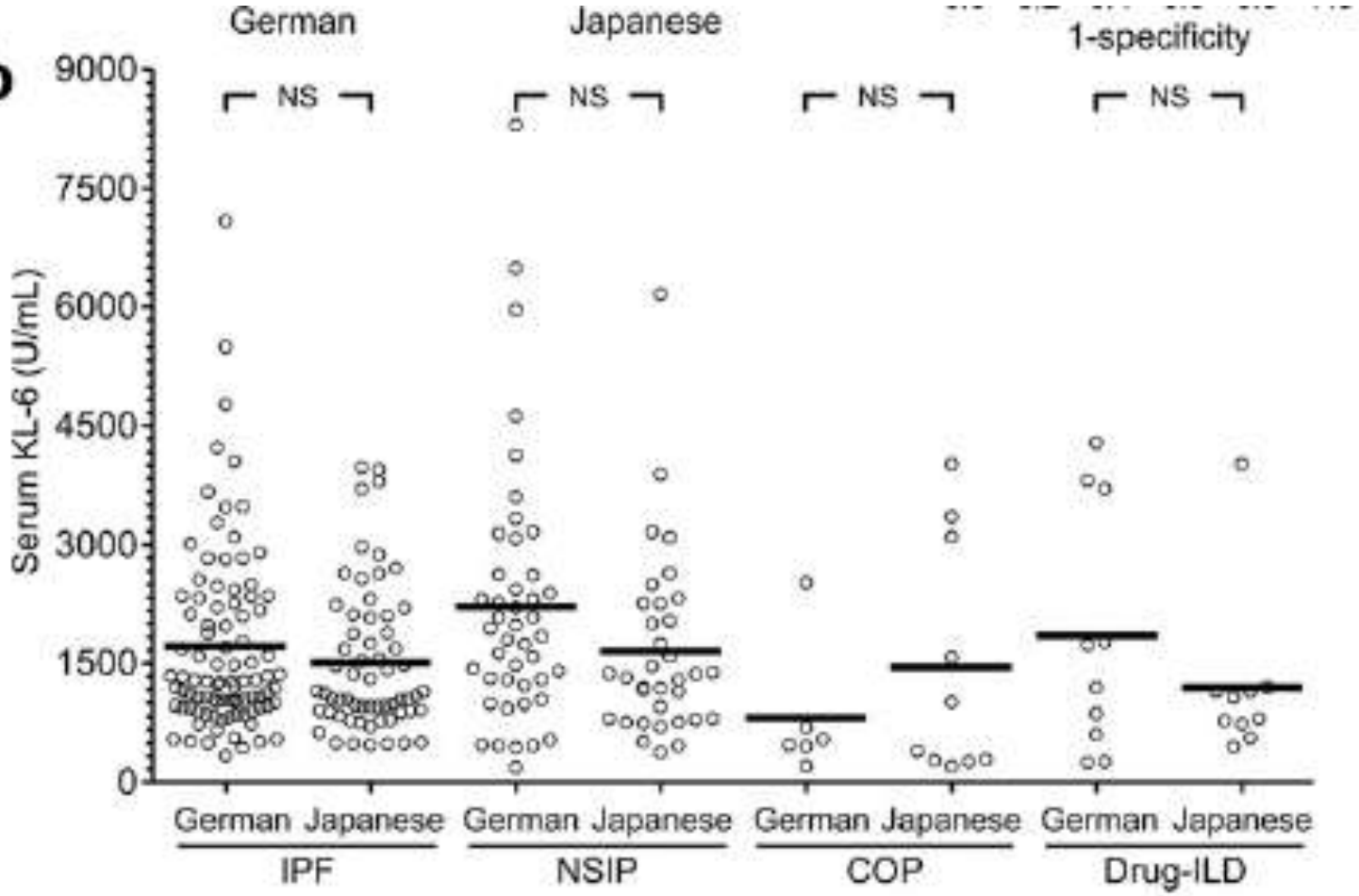
**Differentiates healthy/diseased**




*(Horimasu… Costabel, Respir Med 2012)*

# Krebs von den lungen-6 (KL-6)-MUC1

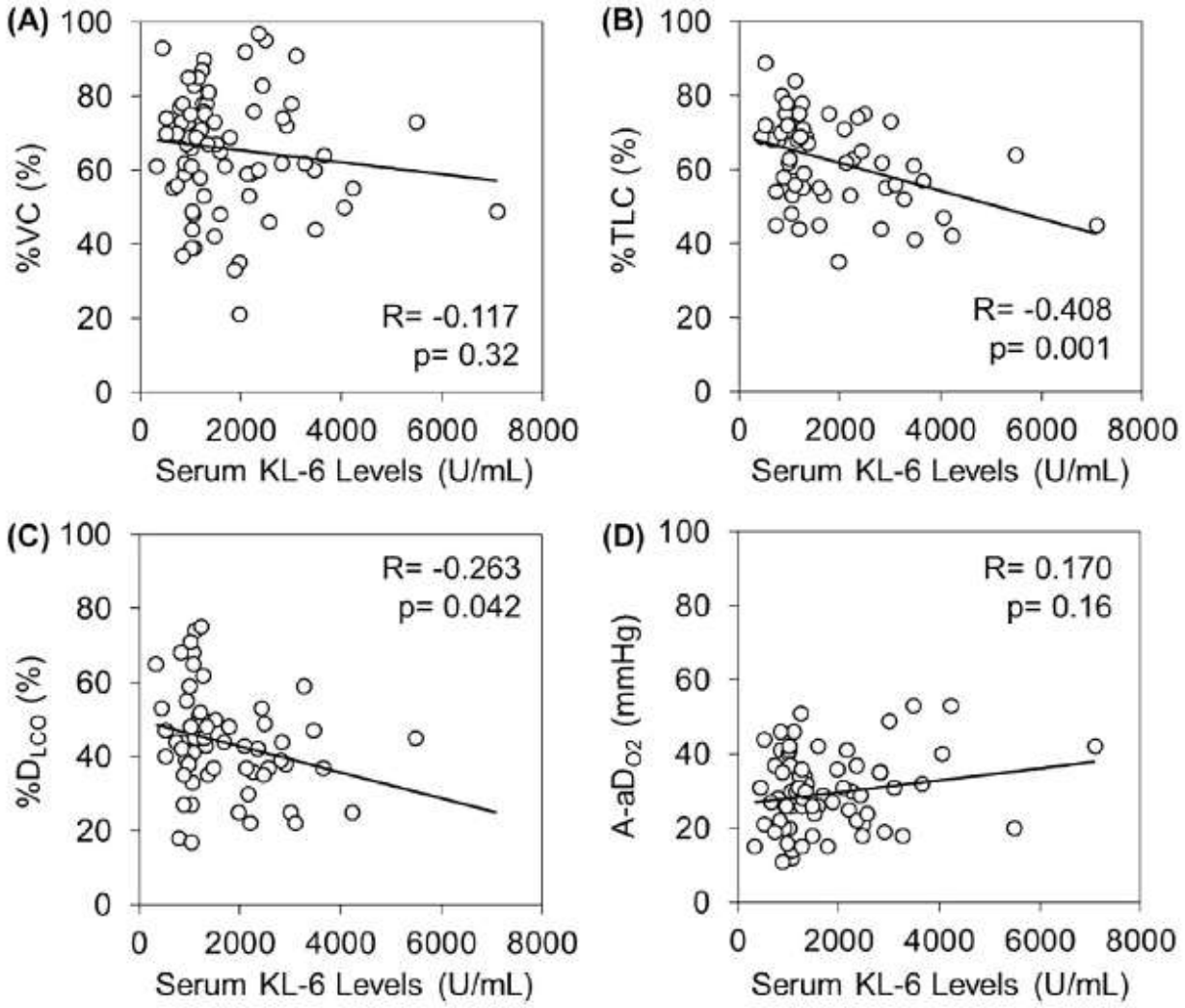
**BUT**
**Not useful for ≠tial diagnosis**




*(Horimasu… Costabel, Respir Med 2012)*

# Krebs von den lungen-6 (KL-6)-MUC1

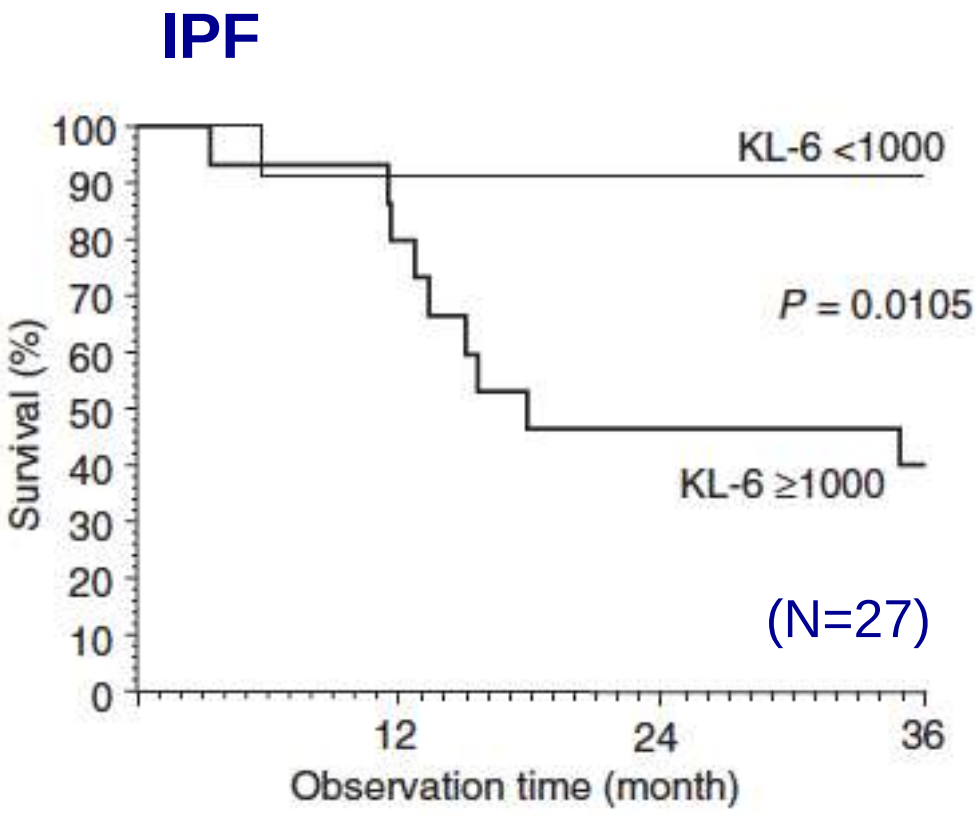
**Limited correlation with lung function**




*(Ohshimo… Costabel, Respir Med 2014)*

# Krebs von den lungen-6 (KL-6)-MUC1

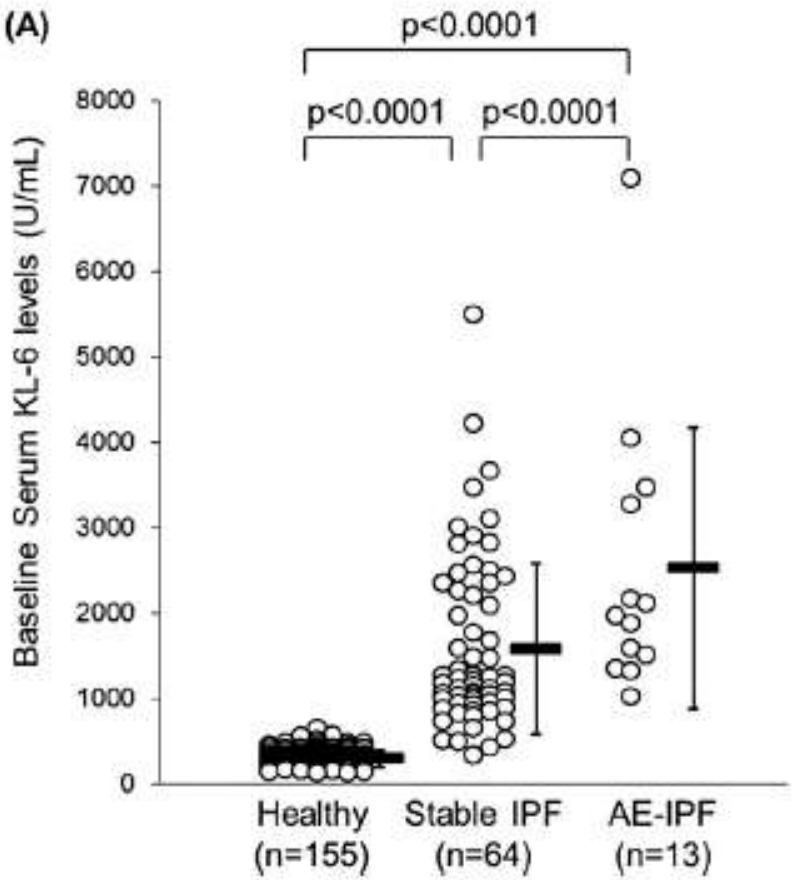
- Serum levels vary with disease changes
- The highest KL-6, the lowest the survival
- Predicts survival independently of usual variables for severity in small series (VC, DLCO)




*(Yokoyama, Respirology 2006)*

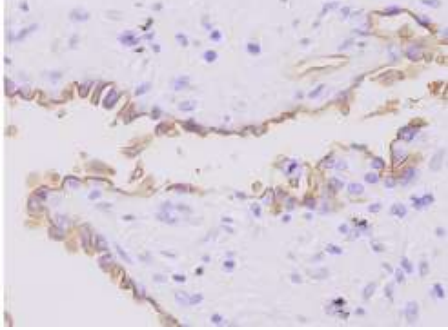
# Krebs von den lungen-6 (KL-6)-MUC1

- **Higher levels in patients with exacerbations**




*(Ohshimo… Costabel, Respir Med 2014)*

# Krebs von den lungen-6 (KL-6)-MUC1

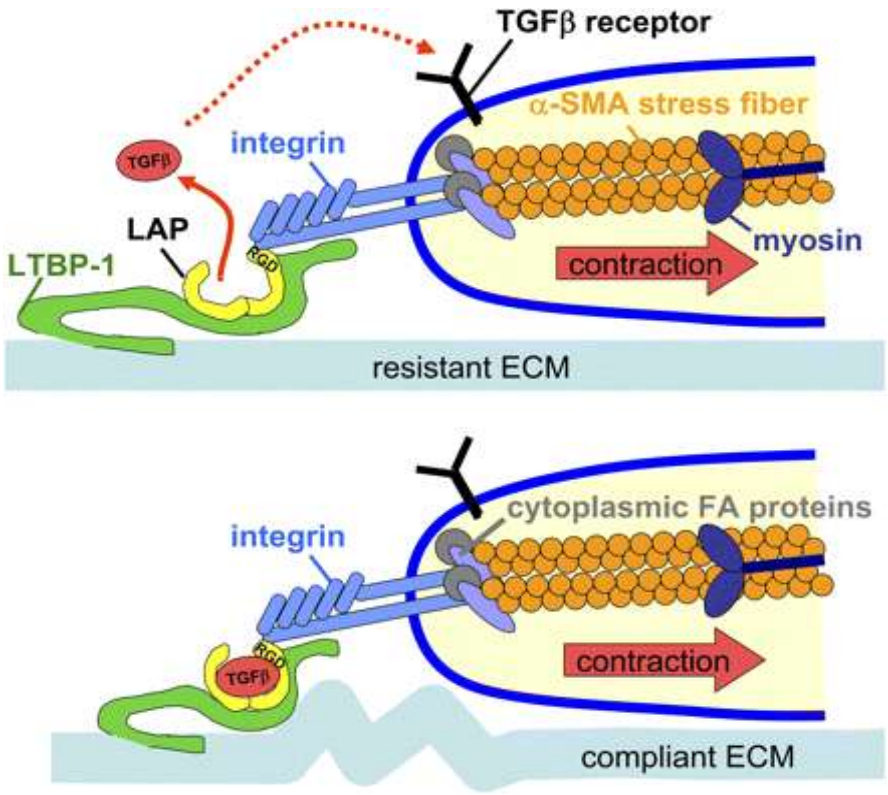


- **Yet to demonstrate that it is useful for management of patients with ILDs, including IPF**
- **Not accepted as a clinical test in my institution**

# Are Biomarkers of interest in ILDs ?

- **Treatment tailoring**
  - Identify treatment responders
    - pirfenidone, nintedanib
  - Short term read-outs for proof of concept studies
  - Disease stratification for tailored targeted therapies

# Matrix stiffness modulates the phenotype of fibroblasts



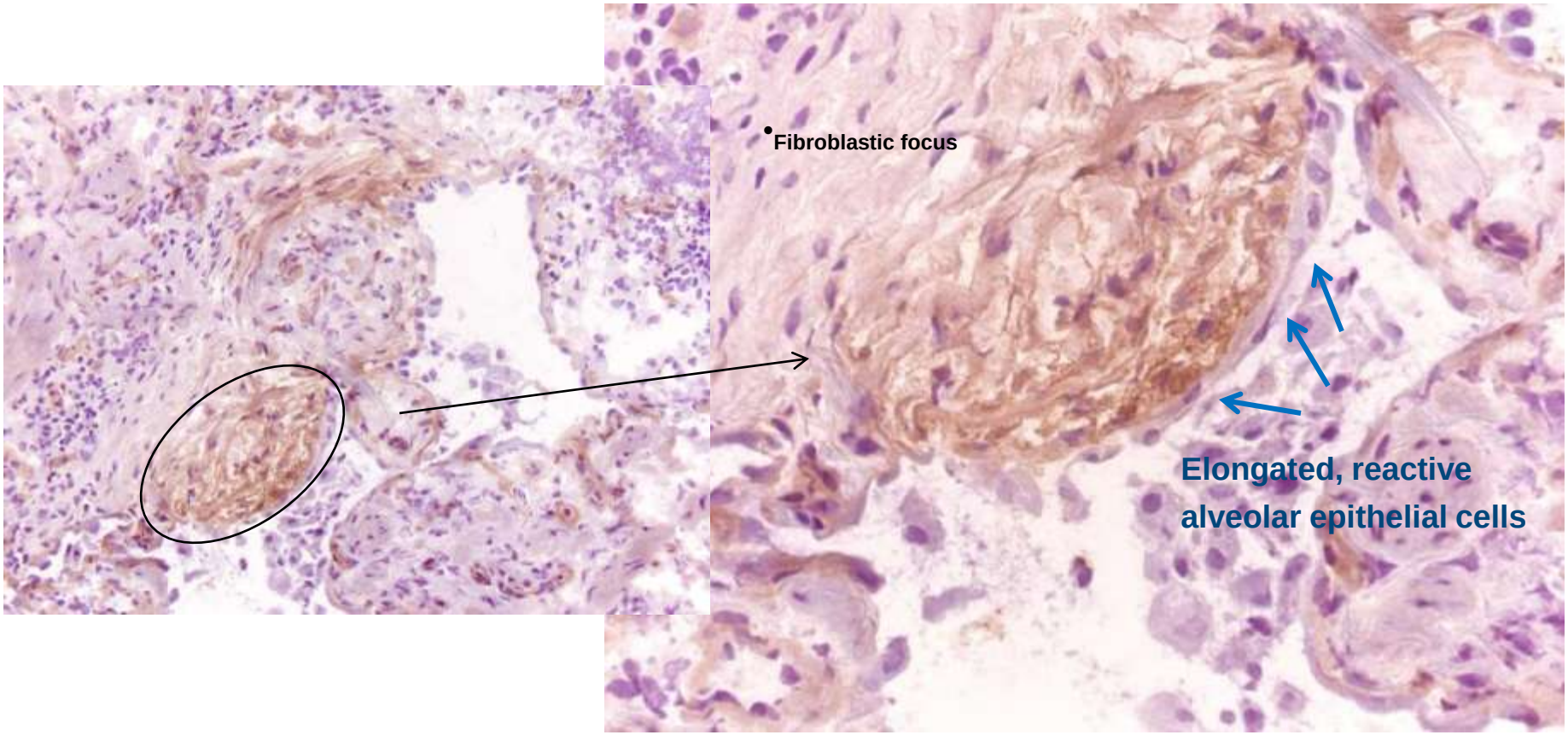

- **Cross-linking enzymes modulate ECM stiffness**
- **Cross-linking enzymes inhibition reduces fibrosis *in vivo :***

➢ Lysyl oxidase-like 2 (LOXL2)
  - Barry-Hamilton, Nat Med 2010

➢ Transglutaminase 2
  - Olsen, AJRCCM 2011;
  - Oh J Exp Med 2011

*Wipff, J Cell Biol 2007;79:1311-1323*

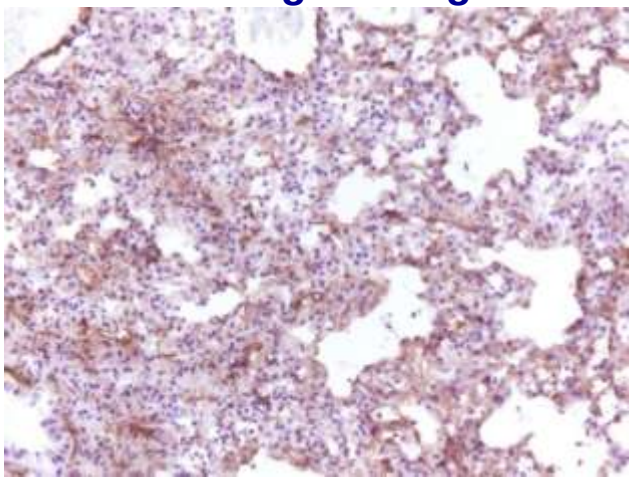
# LOXL2 in Lung Biopsies from IPF Patients

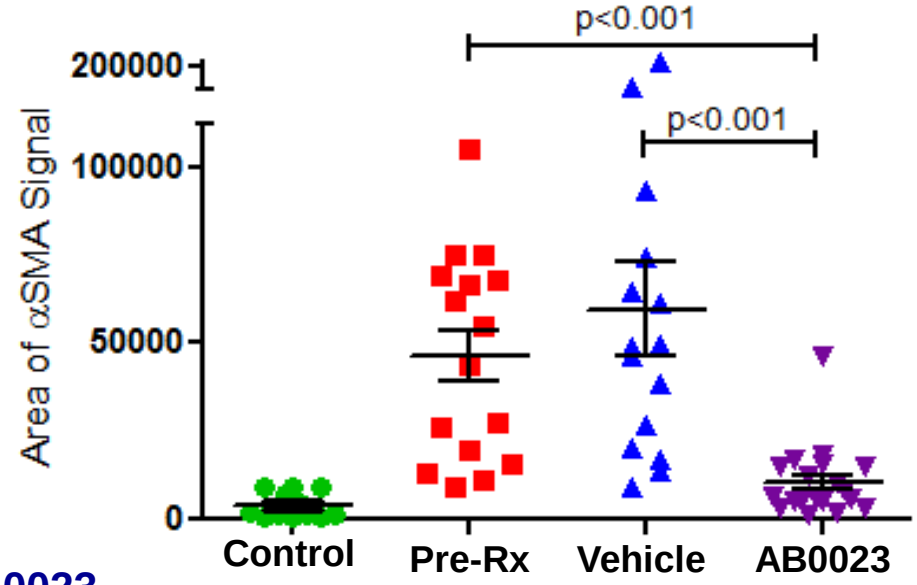



*Slide courtesy of Dr Jason Chien, Gilead*

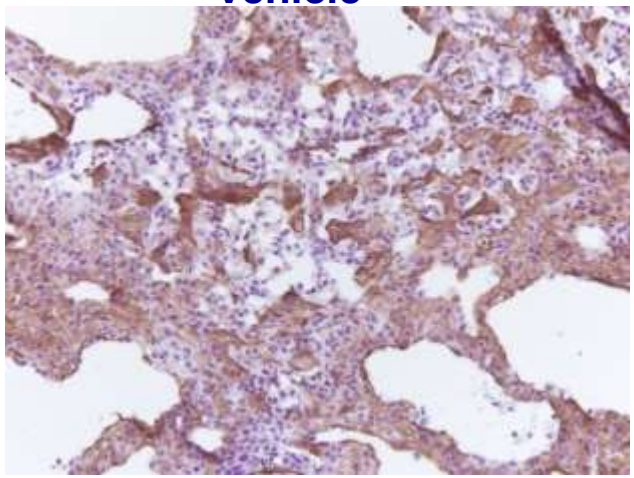
# LOXL2 antibody (AB0023) inhibits bleomycin-induced Pulmonary Fibrosis

**Damaged Lung**

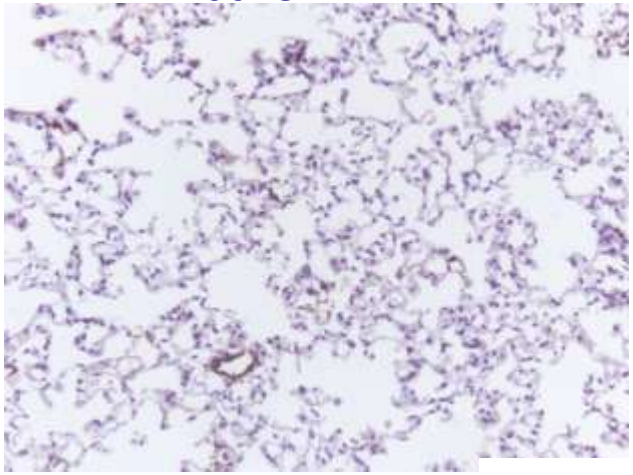





**Vehicle**

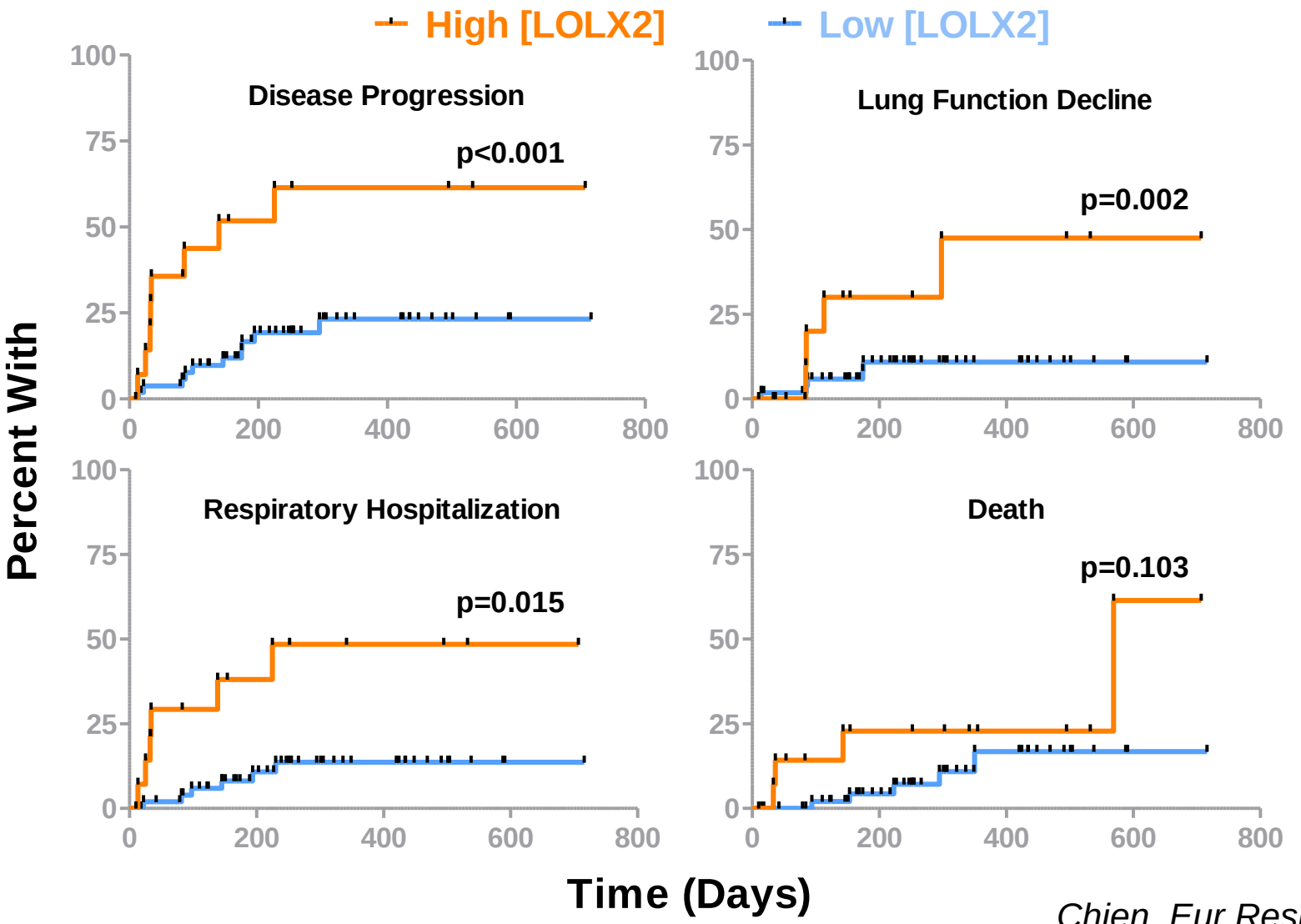


**AB0023**



Barry-Hamilton et al, Nature Medicine, 2010

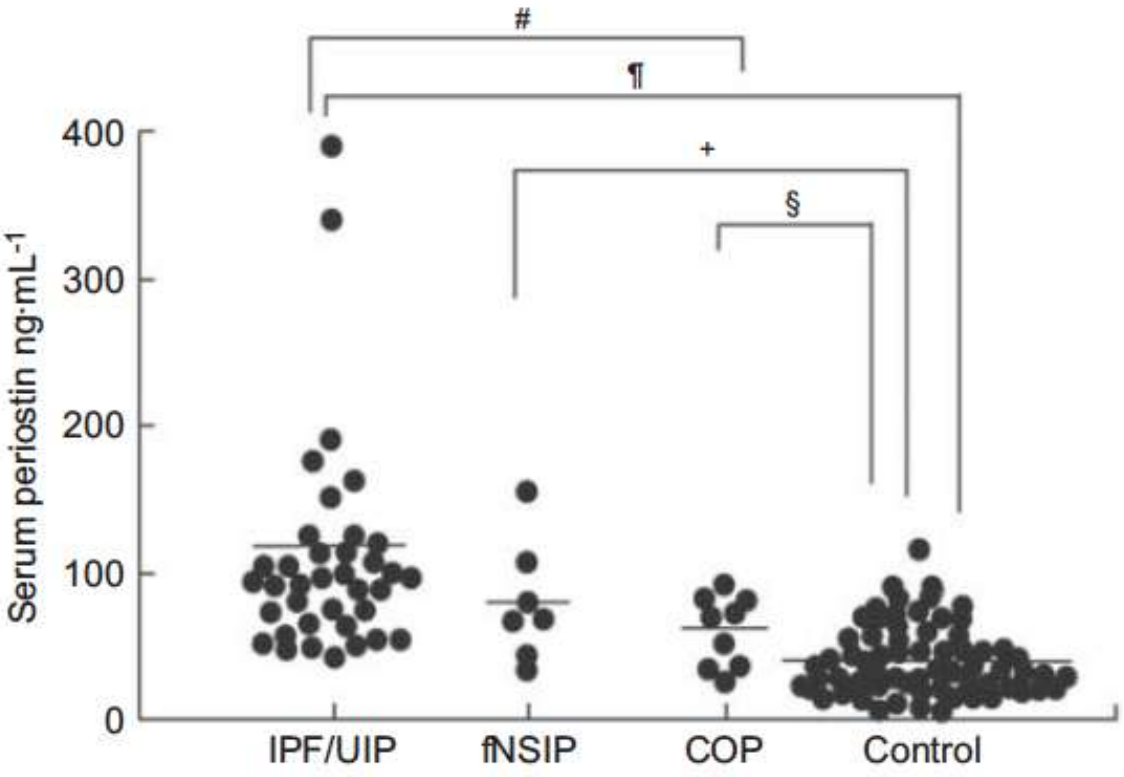
# High LOXL2 (>800 pg/ml) is Associated with Significantly More Disease Progression Events




*Chien, Eur Respir J 2013*

- **Anti-LOXL2 antibody currently evaluated in a phase 2/3 trial (RAINIER)**

# Periostin : a marker of IL-13 activation




*Okamoto, Eur Respir J 2011*

**Periostin**

-Increased in IPF patients

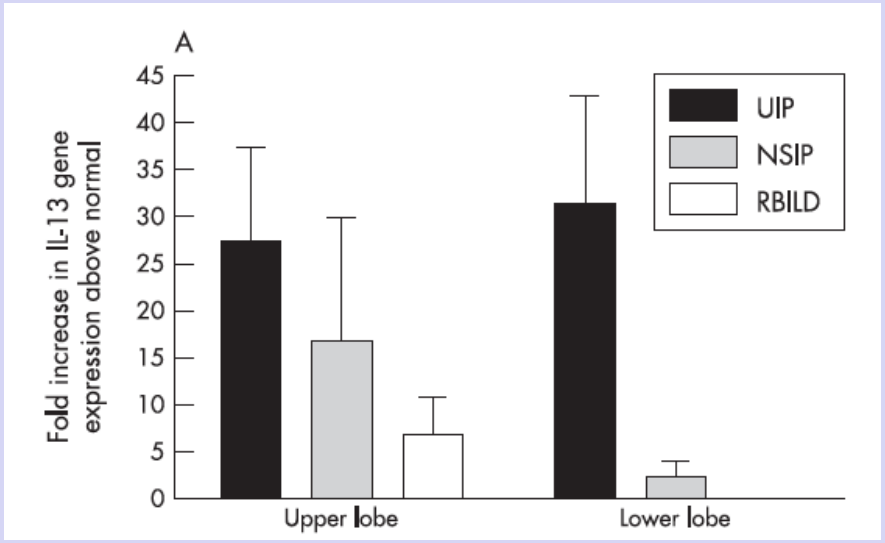
-Higher levels associate with lung function decline

*Okamoto, Eur Respir J 2011*

Naik, Am J Physiol Lung 2012

# An IL-4/IL-13 profibrotic pathway in IPF

- Th2 immune response in the fibrotic lung
- Increased IL-13 production by AMs in IPF
- Increased expression of IL-4R and IL-13R in IIPs
- IL-13 overexpression in mice induces fibrosis
- Il-13 inhibition reduces Bleo-induced lung fibrosis in mice
- IL-4 and IL-13 target lung fibroblasts




*Jakubzick, J Pathol 2004*

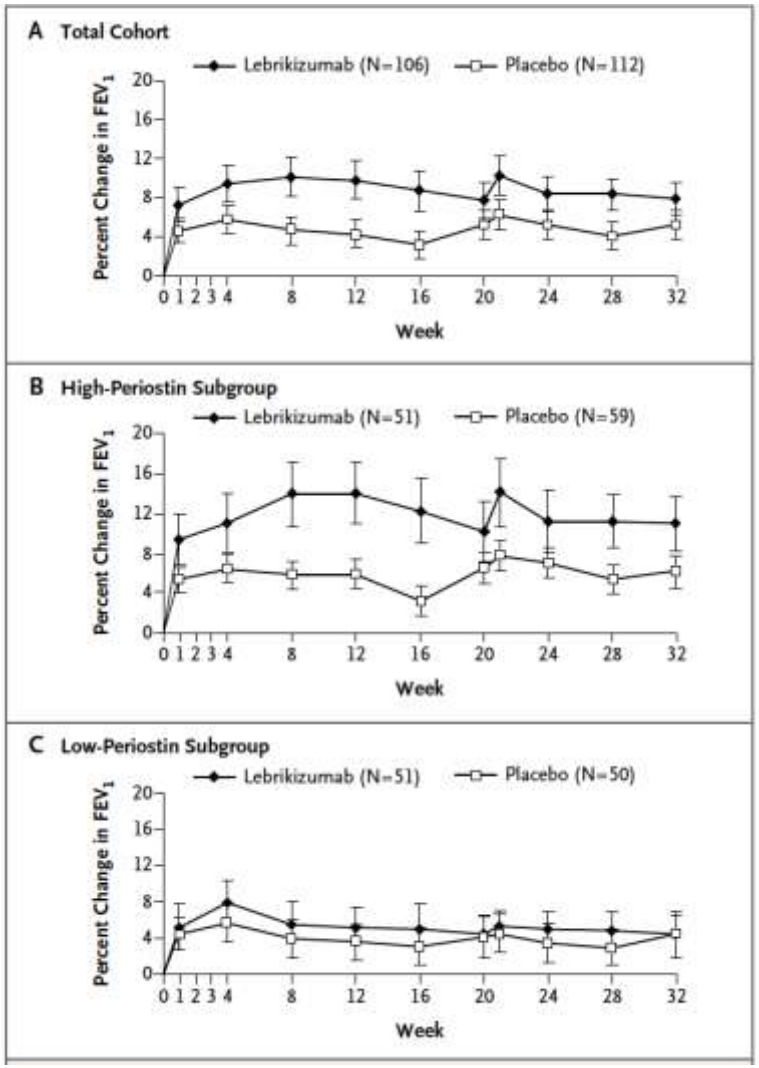
*Wallace, Clin Exp Immunol 1995; Hancock, AJRCMB 1998; Zhu, JCI 1999; Lee, J Exp Med 2001; Belperio, AJRCMB 2002 ; Jakubzick, J Pathol 2004*

# An IL-4/IL-13 profibrotic pathway in IPF

**Targeting the IL-4/IL-13 pathway improves lung function in asthma in "high periostin subgroup"**

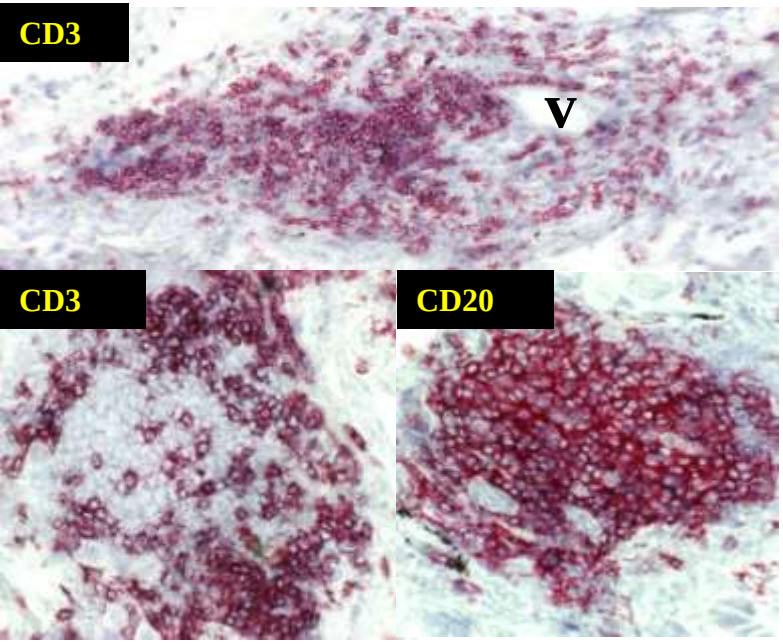
**? In IPF ?**

**Ongoing clinical trials**




*Corren, NEJM 2011*

# Evidence for B cell activation in IPF




Marchal-Somme J, *J Immunol*, 2006

**Blood**

- Anti-periplakin [1]
- Anti-HSP70 [2]
- CXCL13 [3]

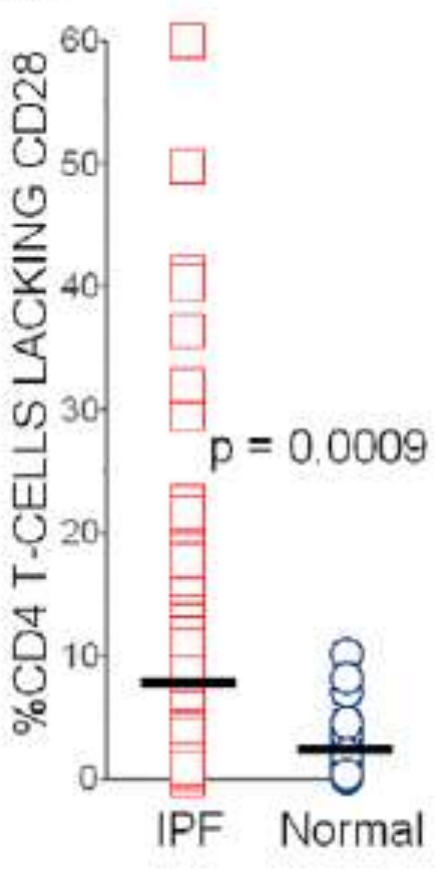
[1]Taillé, AJRCCM 2011; [2]Kahloon, AJRCCM 2013; [3]Vuga, AJRCCM 2014

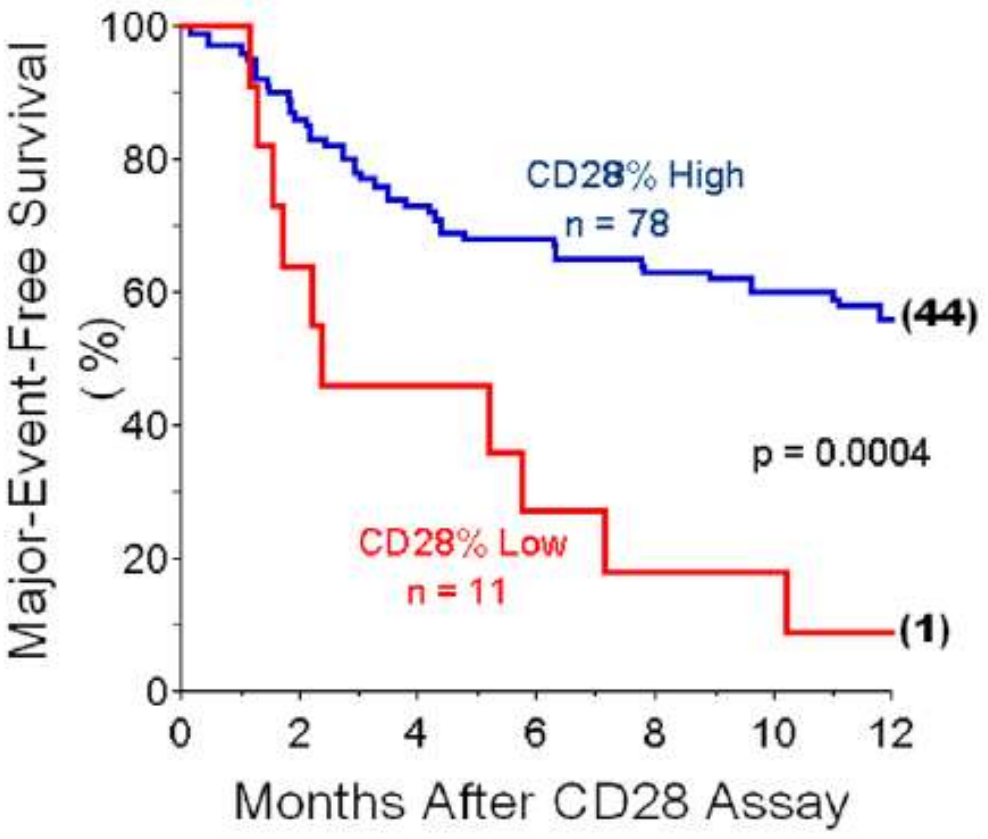
**Targeting B cells in ILDs :**
e.g. AE-IPF, HP, CTD-ILD

Keir, Eur Respir J 2012; Keir Respirology 2014; NCT01524068 (Univ. Pittsburgh)

# Down regulation of CD28 on circulating CD4 cells

***a result of repeated antigen-driven proliferation***



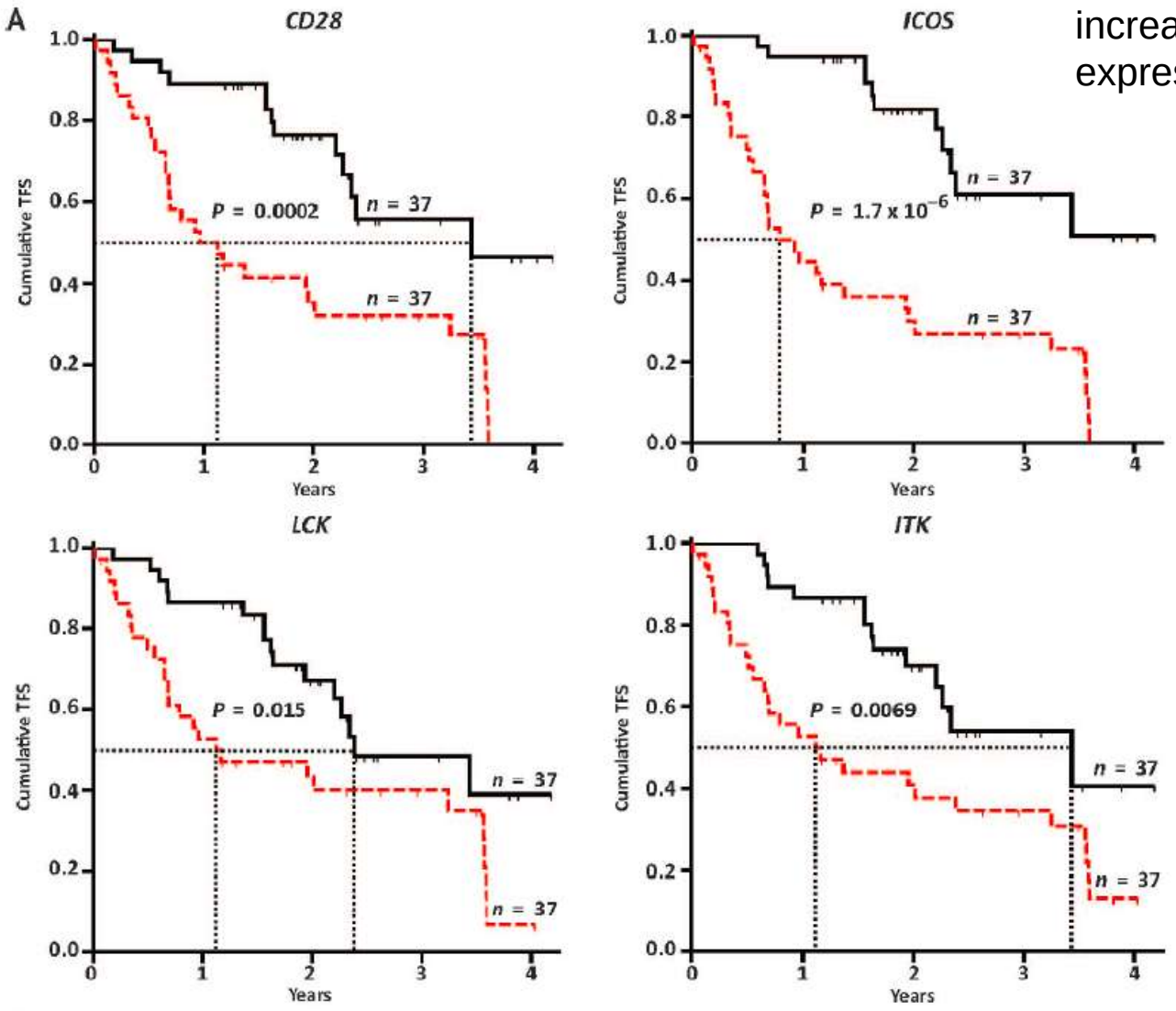




Gilani, Plos One 2010;e8959

# Blood mononuclear cells predict prognosis

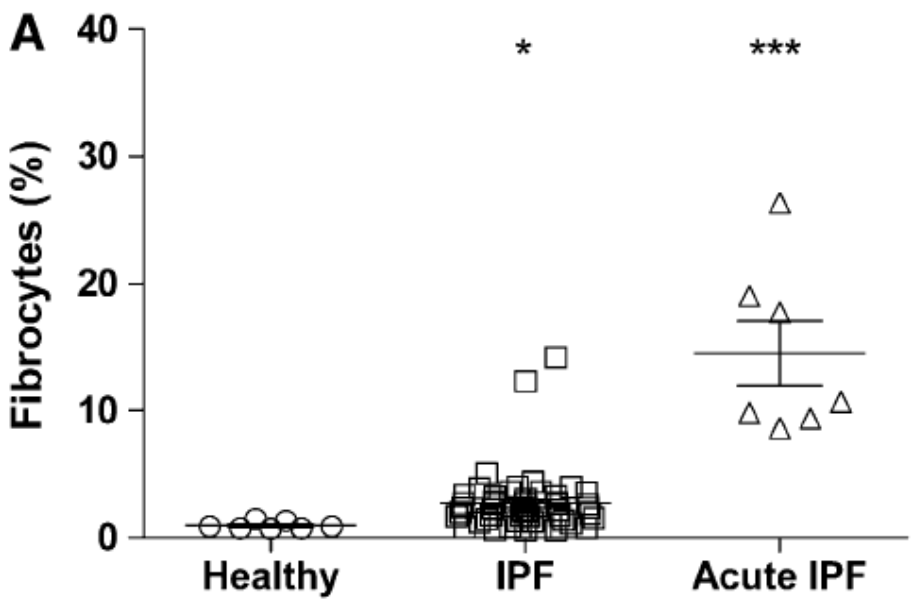
Black line : increased expression

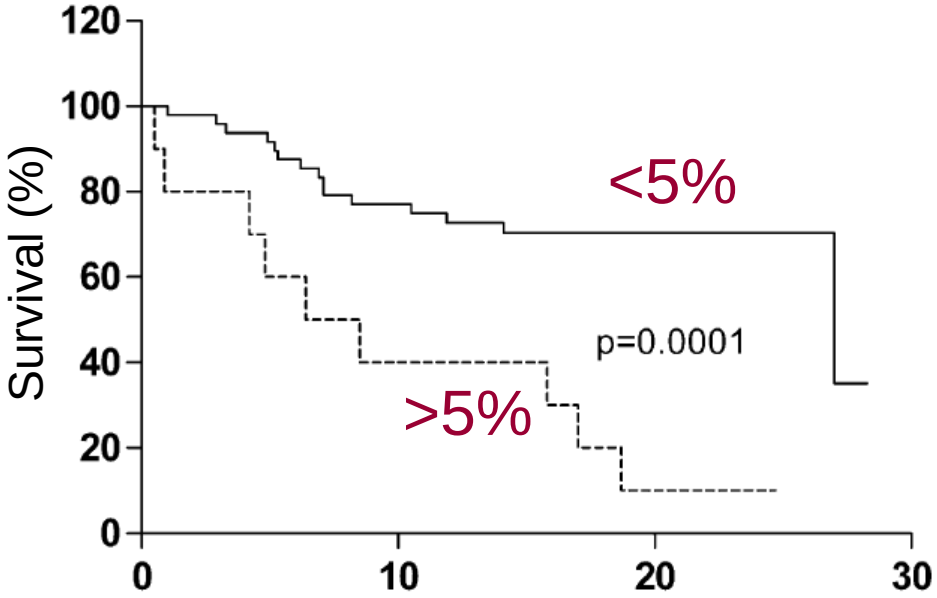
Underexpression of CD28, ICOS, LCK and ITK, associates with decreased transplant free survival




*(Herazo-Maya, Sci Translat Med 2013)*

# Blood Fibrocytes in IPF



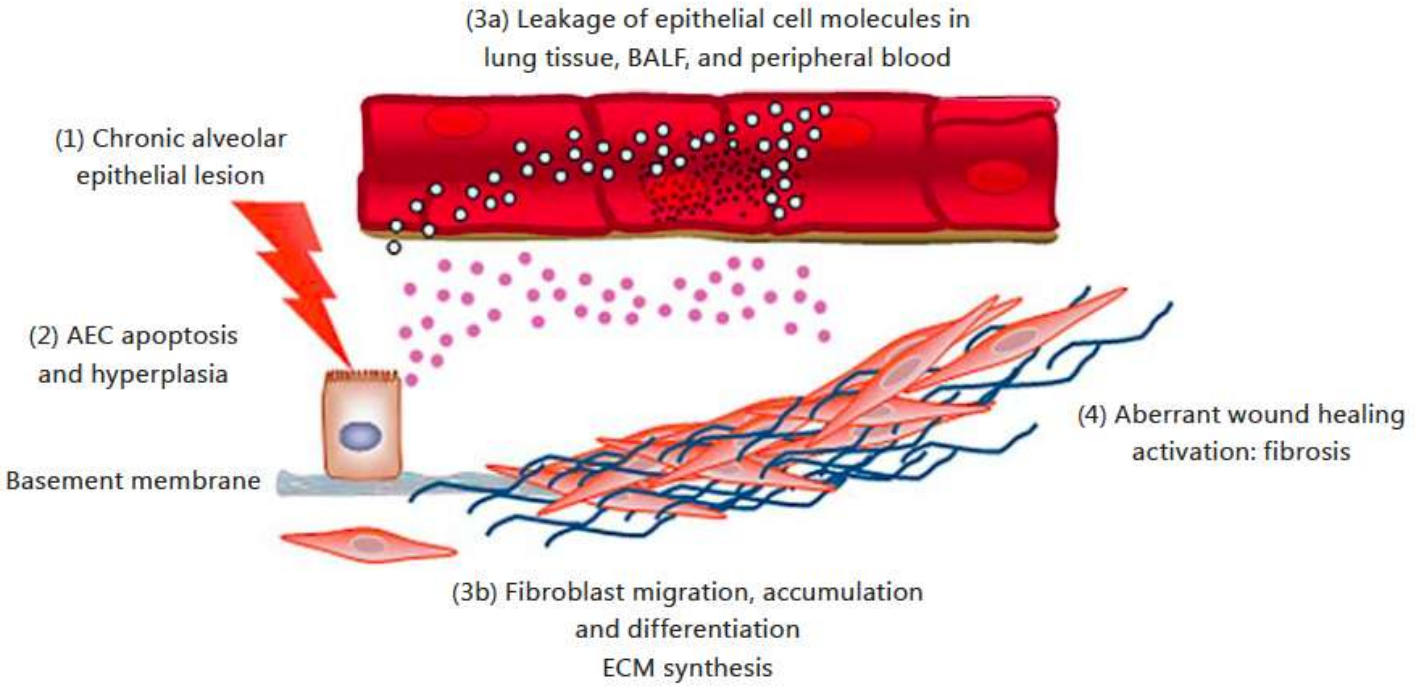

- Fibrocyte % did not correlate with LFTs or HRCT fibrosis score
- Predictive value to be determined in larger prospective studies

*(Moeller, AJRCCM 2009)*

# Are Biomarkers of interest in ILDs ?

- **A better understanding of pathophysiology**

KL-6 (MUC1)
SP-A
SP-D
CA15-3
CEA




Reviewed in Borensztajn, Crestani and Kolb, Respiration 2013

# Conclusion

**The search for the Holy Graal still running**

- Even more needed than before
(Responders to effective antifibrotic drugs)

- Failed because of methodological reasons
  - Small retrospective cohorts
  - Mixed populations
  - Without independent validation
  - Inadequate statistics

- Take advantage of “Big cohorts” / modern validation methods/multicentric studies