Mechanisms of pulmonary fibrosis
Need for an integrated approach

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A complex situation

- Genetic susceptibility
  - Environmental factors
- Patient w/o symptoms
- Genomics/Genetics/Ethics
  - Risk prediction/Lifestyle
- Clinical and molecular phenotype,
  - Animal models of disease
- Patient with symptoms
- Clinical and translational medicine, Systems biology
- Phenotyping assays,
  - Individual responses
- Stratification
  - Individualized therapy
    - (defined by disease stage)
- In vitro studies
  - Systems biology
Expression profiles from tissue, BAL, serum, cells

Expression profiles, in vitro response rates

Systems biology, surrogate outcomes

Parallel universes

Mouse model

Patient with symptoms

Experimental intervention

Stratification

Assessment of individualized therapeutic efficacy

Individualized therapy (defined by disease stage)
Idiopathic Pulmonary Fibrosis (IPF)

ATS/ERS definition 2011: “IPF is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause limited to the lungs, occurring primarily in older adults, and associated with a histopathologic and/or radiologic pattern of UIP. IPF diagnosis requires exclusion of other causes of interstitial pneumonias.”
The challenge:
IPF – an elusive disease

- IPF is a progressive and fatal interstitial lung disease (ILD)
- The natural history of IPF has literally been the same for the last 20 years
- Improving disease understanding has not yet led to translation into clinical practice
- Clinical trials have been unsuccessful (but 3)
- Despite impact of guidelines and consensus recommendations (ATS/ERS 2002), IPF remains a challenge in terms of heterogeneity
Lung function decline in IPF

4-year-survival of 84 SLB proven IPF patients
- stable disease at 6 months (n=38)
- marginal decline of FVC (5-10\% ) at 6 months (n=23)
- significant decline of FVC (>10\% ) at 6 months (n=23)

p<0.005

Zappala et al., Eur Respir J, 2010; 35:830-835
Idiopathic Pulmonary Fibrosis (IPF)

- small alveolar septae, little ECM deposition
- smooth muscle actin expression restricted to the circumference of airways and vessels

- *inhomogeneous* picture
- small alveolar septae next to massively thickened septae
- interstitial ECM deposition
- non-restricted smooth muscle actin expression
Idiopathic Pulmonary Fibrosis (IPF)
Peripheral blood proteins are predictive of outcome

140 patients, 92 markers
(A) MMP7
(B) ICAM1
(C) IL8
(D) VCAM1
(E) S100A12

Richards et al., AJRCCM 2011
microRNAs are differentially expressed in IPF

Platform: Agilent
10 IPF and 10 control tissues

Pandit et al., AJRCCM 2010
let-7d inhibition causes alveolar septal thickening and collagen deposition in the lung

Pandit et al., AJRCCM 2010
Bleomycin-induced Pulmonary Fibrosis
Bleomycin-induced Pulmonary Fibrosis

DAPI, αSMA, Aq5

small airway

alveoli
Fibrosis quantification by software analysis
Quantification method

- Each image is analyzed separately upon conversion to 8-bit
- Whole lung is selected for area measurement
- Percentage of tissue density is obtained
Unbiased software based fibrosis quantification

Day 14

![Graph showing % of Air space and % of Tissue density for PBS and Bleo groups.](image)
Air space percentage quantification and airway resistance correlation

![Graph showing the correlation between % of Air space and resistance (cmH2O/ml). The graph indicates a significant negative correlation with a Pearson's r² of 0.6249 and p-value < 0.0001.]

**Note:**
- PBS: Black circles
- Bleo: Red squares

**Legend:**
- r² = 0.6249
- p < 0.0001
What about fibroblast phenotypes?
Idiopathic Pulmonary Fibrosis (IPF)

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1. TGFβ1 and EGF enhance the invasion capacity of CCL206 fibroblasts

- Prepare collagen matrices (3.2mg/ml)
- Seed cells (1% FCS)

1h

- Treatment (TGFβ1, EGF) (1% FCS)

24h

72h invasion

96h

- Fix cells + DAPI staining
- LSM confocal microscopy, analysis IMARIS

![Graph showing the fraction of cells in gel (%) for A549 [untreated], untreated, EGF [50ng/ml], and TGFβ1 [5ng/ml].]
Parallel universes

- Mouse model
- Experimental intervention
- Assessment of individualized therapeutic efficacy

- Patient with symptoms
- Stratification
- Individualized therapy (defined by disease stage)

- Expression profiles from tissue, BAL, serum, cells
- Expression profiles, in vitro response rates
- Systems biology
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With this,
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