I BIOMARCATORI

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«UOS dedicata allo studio e cura della fibrosi polmonare idiopatica e delle altre interstiziopatie polmonari»

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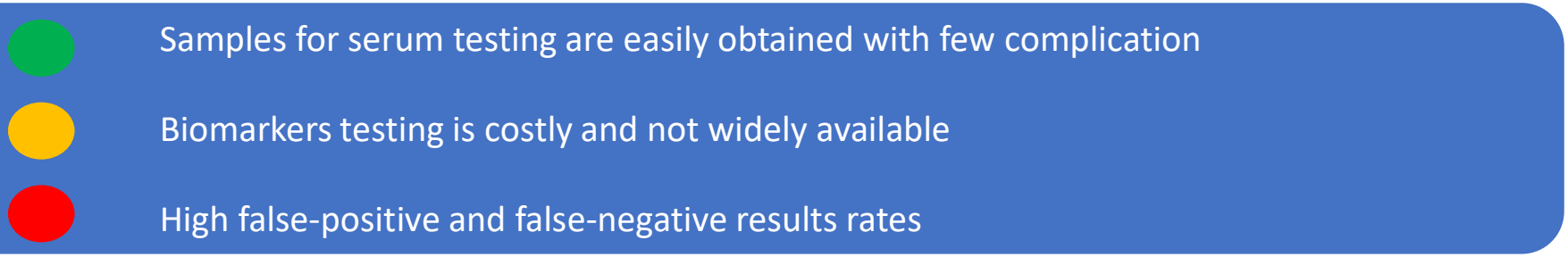
Diagnosis of Idiopathic Pulmonary Fibrosis

An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline

Question 8: Should Patients with Newly Detected ILD of Unknown Cause Who Are Clinically Suspected of Having IPF Undergo Serum Biomarker (MMP-7, SPD, CCL-18, KL-6) Measurement for the Purpose of Diagnosis?

- We recommend NOT measuring serum MMP (matrix metalloproteinase)-7, SPD (surfactant protein D), CCL (chemokine ligand)-18, or KL (Krebs von den Lungen)-6 for the purpose of distinguishing IPF from other ILDs (*strong recommendation, very low quality of evidence*).



Am J Respir Crit Care Med Vol 198, Iss 5, pp e44–e68, Sep 1, 2018

Definition of biomarker

A characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic process, or of pharmacological responses to therapeutic interventation

Ideally, a biomarker is normal in the absence of disease, dysregulated in disease and normalized with effective treatment

Also, a biomarker should be easy and widespread measurable in non invasively collected body samples

Searching for the optimal biomarker in IPF: critical issues

Low incidence disease (<10 cases/100.000/yr)

High morbidity and mortality

Lack of a diagnostic gold standard (*working diagnosis*)

Clinical heterogeneity

Comorbidities

Unmet therapy needs

Why we need a biomarker?

Ameliorate the diagnostic process (early case identification, no/less need of invasive procedures)

Facilitate clinical phenotyping

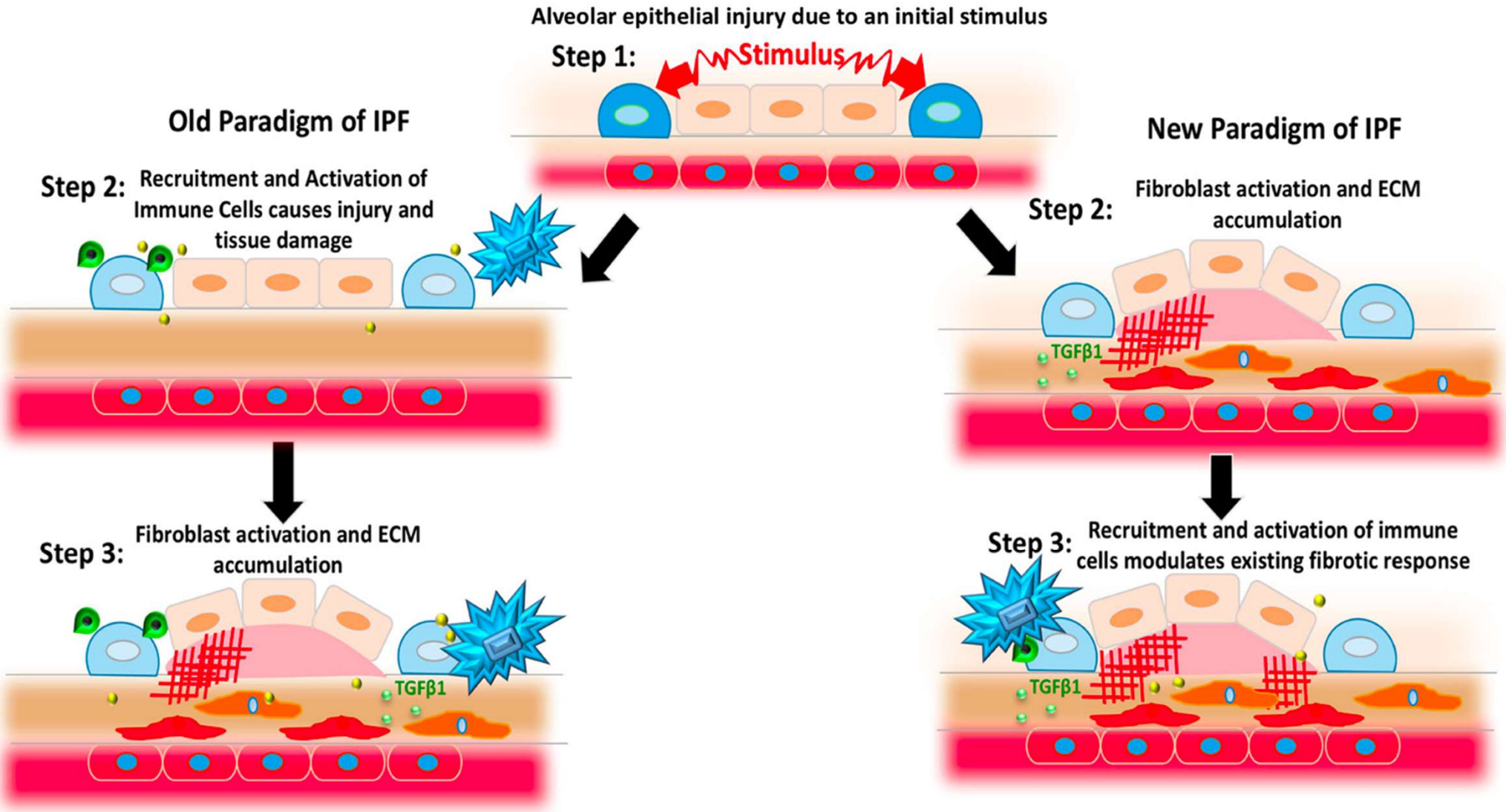
Discriminate patients according to disease severity and behaviour

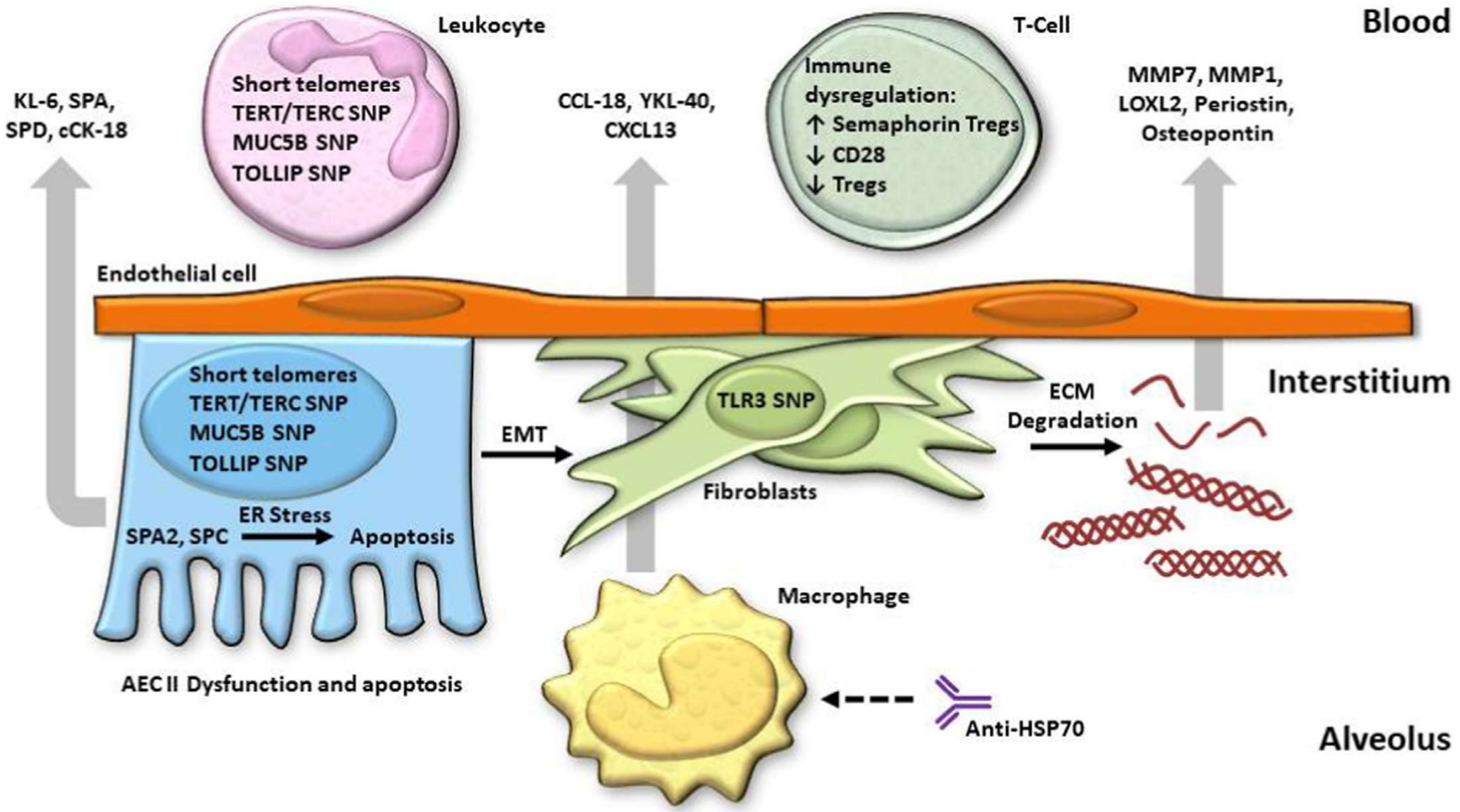
Improve accurate disease monitoring

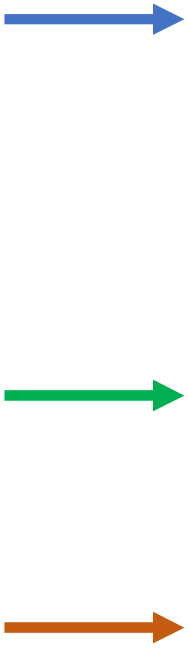
Predict responsiveness to pharmacological therapies

Identify potential therapeutic targets

Stratify patients for clinical trails


Alveolar epithelial injury due to an initial stimulus
Step 1:
Stimulus
Old Paradigm of IPF
Step 2:
Recruitment and Activation of Immune Cells causes injury and tissue damage
Step 3:
Fibroblast activation and ECM accumulation
TGFβ1
New Paradigm of IPF
Step 2:
Fibroblast activation and ECM accumulation
TGFβ1
Step 3:
Recruitment and activation of immune cells modulates existing fibrotic response
TGFβ1


Blood
Leukocyte
Short telomeres
TERT/TERC SNP
MUC5B SNP
TOLLIP SNP
T-Cell
Immune dysregulation:
↑ Semaphorin Tregs
↓ CD28
↓ Tregs
KL-6, SPA, SPD, cCK-18
CCL-18, YKL-40, CXCL13
MMP7, MMP1, LOXL2, Periostin, Osteopontin
Endothelial cell
Interstitium
Short telomeres
TERT/TERC SNP
MUC5B SNP
TOLLIP SNP
EMT
TLR3 SNP
Fibroblasts
ECM Degradation
ER Stress
SPA2, SPC
Apoptosis
AEC II Dysfunction and apoptosis
Macrophage
Anti-HSP70
Alveolus



Biomarker	Diagnosis of IPF/disease susceptibility	Differential diagnosis from other ILDs	Disease prognosis (progression/ mortality)	Treatment response
Associated with alveolar epithelial cell dysfunction				
KL-6	+	–	+	–
SP-A	+	+	+	–
SP-A genetic variants (*SFTPA2*)	+	–	–	–
SP-C genetic variants (*SFTPC*)	+	–	–	–
SP-D	+	+ as part of a biomarker index	+	–
CA 19-9	+	–	+	–
CA-125	+	–	+	–
Mucin5B genetic variants (*MUC5B*)	+	–	+	–
cCK-18	+	+	–	–
Telomere length and telomerase mutations (*TERT*, *TERC*)	+	–	+	–
Associated with ECM remodeling and fibroproliferation				
MMP-7	+	+	+	–
MMP-1	+	+	–	–
LOXL2	+	–	+	–
Fibrocytes	+	–	+	–
Periostin	+	+	+	–
Osteopontin	+	+	–	–
Associated with immune dysfunction				
CCL-18	+	–	+	–
YKL-40	+	–	+	–
TLR3 genetic variants	–	–	+	–
Toll interacting protein genetic variants (*TOLLIP*)	+	–	+	+
S100A12	–	–	+	–
Anti-HSP70	+	–	+	–
a-Defensins	+	–	+	–
CXCL13	+	–	+	–
Anti-vimentin Abs	+	–	+	–
CD4 + CD28+	–	–	+	–
Tregs	+	–	+	–
Microbiome	+	–	+	–
mtDNA	+	–	+	+
52-gene signature	+	+	+	+

Multiple biomarker signature

SP-D, MMP-7, Osteopontin	diagnostic accuracy
MMP-7, MMP-1, MMP-8, IGFBP-1, TNFRSF1A	(IFS vs ILDs other than IPF)
KL-6, CCL18, ICAM1, SP-D, SP-A, MMP-7, HE-4, prostatin	
Degradation products of ECM	
miRNA-302, miRNA-423, miRNA-210, miRNA-376C, miRNA-185	disease phenotyping and
SP-D, CA19.9, CA125	behaviour
Degradation products of ECM	(slow *vs* fast)
KL-6, SP-D	prognostic accuracy
SP-A, SP-D	(progression and mortality)
Gender, FVC, DLCO, MMP-7	
MMP-7, SP-A, KL-6, FVC, DLCO, age, ΔFVC_{6m}	
Degradation products of ECM	
52-gene signature (Scoring Algorithm for Molecular Subphenotypes)	

Biomarkers in Medicine

Oxidative stress-linked biomarkers in idiopathic pulmonary fibrosis: a systematic review and meta-analysis

Executive summary

Idiopathic pulmonary fibrosis & oxidative stress

- The idiopathic pulmonary fibrosis (IPF) is characterized by increased systemic oxidative stress (OS).
- We conducted, for the first time, a systematic review and meta-analysis of studies investigating the relationship between the OS biomarkers and presence of IPF.

Studies selected

- Fifteen studies were included in the meta-analysis, involving 293 IPF patients (191 males and 102 females, mean age 48.1 $\pm$ 24.3 years) and 234 healthy controls (149 males and 85 females, mean age 62.9 $\pm$ 8.7 years).
- Two studies evaluated thiobarbituric acid reactive substances, hydroperoxides and isoprostanes in blood, two isoprostanes in expired breath condensate, three glutathione in epithelial lining fluid and four protein carbonyls in bronchoalveolar lavage fluid.

Results

- Pooled systemic hydroperoxides and thiobarbituric acid reactive substances concentrations were significantly higher in IPF patients when compared with controls.
- A significant decrease in epithelial lining fluid-glutathione concentrations was observed in IPF patients compared with controls.
- Bronchoalveolar lavage fluid carbonyl proteins concentrations were significantly higher in IPF than in controls.
- Isoprostane expired breath condensate levels were significantly higher in IPF than in controls.

Conclusion

- This meta-analysis demonstrated a significant reduction in antioxidant markers and a consistent increase in the concentrations of OS markers in IPF, independent of the biological sample examined.

Biomark. Med. (Epub ahead of print)

Breath biomarkers in idiopathic pulmonary fibrosis: a systematic review

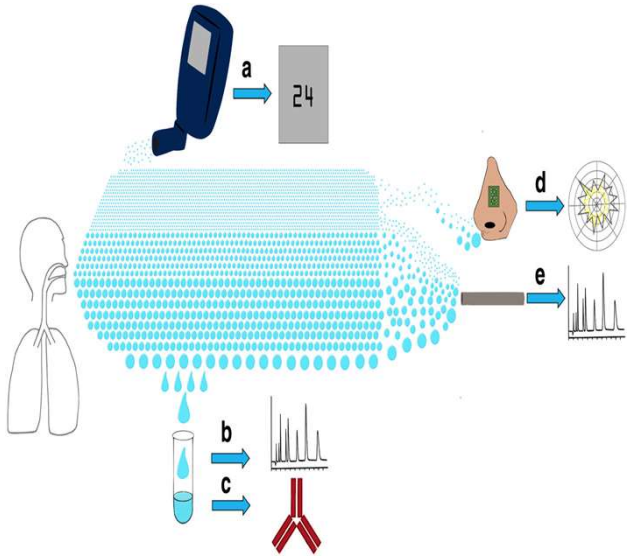
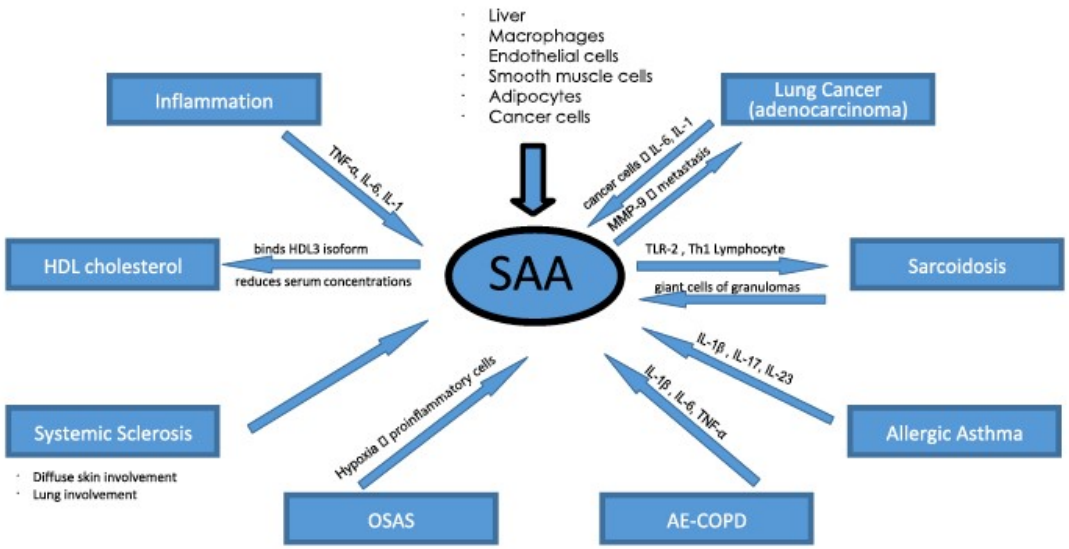


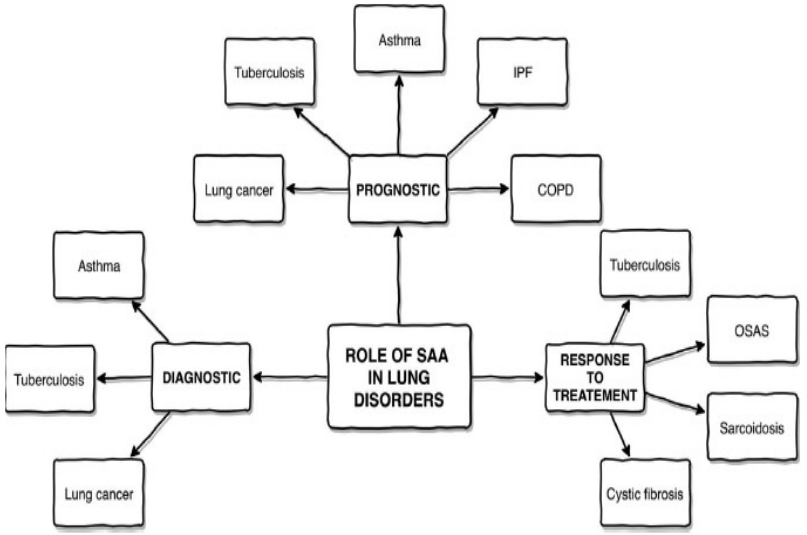
Table 2 Biomarkers reported to discriminate between IPF patients and healthy controls. Direction of discrimination and reported *p*-value. [a]$C_{alv}NO$. [b]$FeNO_{50}/FeNO_{100}/FeNO_{150}/C_{alv}NO$

Biomarker	Sample Medium	Discrimination	*p*-value	References
Nitric Oxide	Exhaled breath	Higher in IPF	0.0001, < 0.0001	[62][a], [65][b]
8-isoprostane	EBC	Higher in IPF	0.02, < 0.05	[58], [62]
Hydrogen Peroxide	EBC	Higher in IPF	0.003	[58]
Nickel	EBC	Higher in IPF	< 0.05	[59]
Chromium	EBC	Higher in IPF	< 0.05	
Silicon	EBC	Higher in IPF	< 0.05	
Cobalt	EBC	Lower in IPF	< 0.05	
Iron	EBC	Lower in IPF	< 0.05	
Copper	EBC	Lower in IPF	< 0.05	
Selenium	EBC	Lower in IPF	< 0.05	
Molybdenum	EBC	Lower in IPF	< 0.05	
Nitrite	EBC	Higher in IPF	< 0.01	[60]
Nitrate	EBC	Lower in IPF	< 0.01	
22:4 LPA	EBC	Higher in IPF	0.001	[63]
Unidentifiable metabolite	EBC	Higher in IPF	≤0.01	[64]
p-cymene	Exhaled breath	Lower in IPF	< 0.001	[66]
Acetoin	Exhaled breath	Higher in IPF	< 0.001	
Isoprene	Exhaled breath	Higher in IPF	< 0.001	
Ethylbenzene	Exhaled breath	Higher in IPF	< 0.001	
Unidentified VOC	Exhaled breath	Higher in IPF	< 0.001	

IPF idiopathic pulmonary fibrosis, *EBC* exhaled breath condensate, *22:4 LPA* Docosatetraenoyl lypophosphatidic acid, *VOC* volatile organic compound, $C_{alv}NO$ alveolar nitric oxide concentration, $FeNO_{50/100/150}$ fractionated exhaled nitric oxide at 50 ml/100 ml/150 ml per second

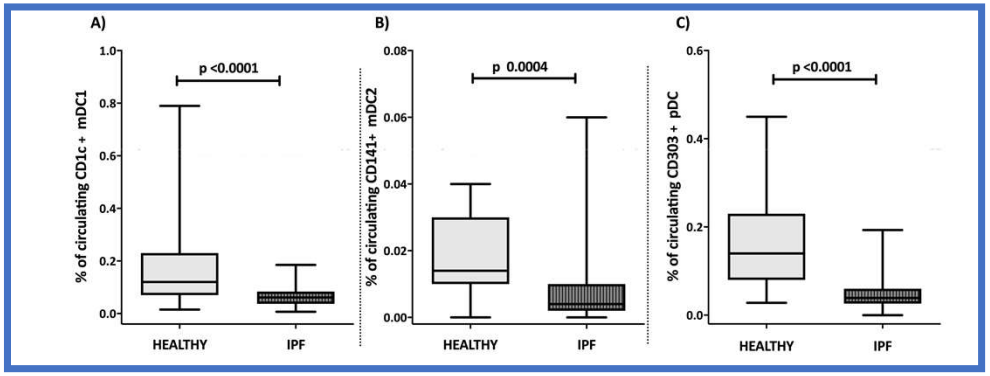
Serum amyloid A: A potential biomarker of lung disorders

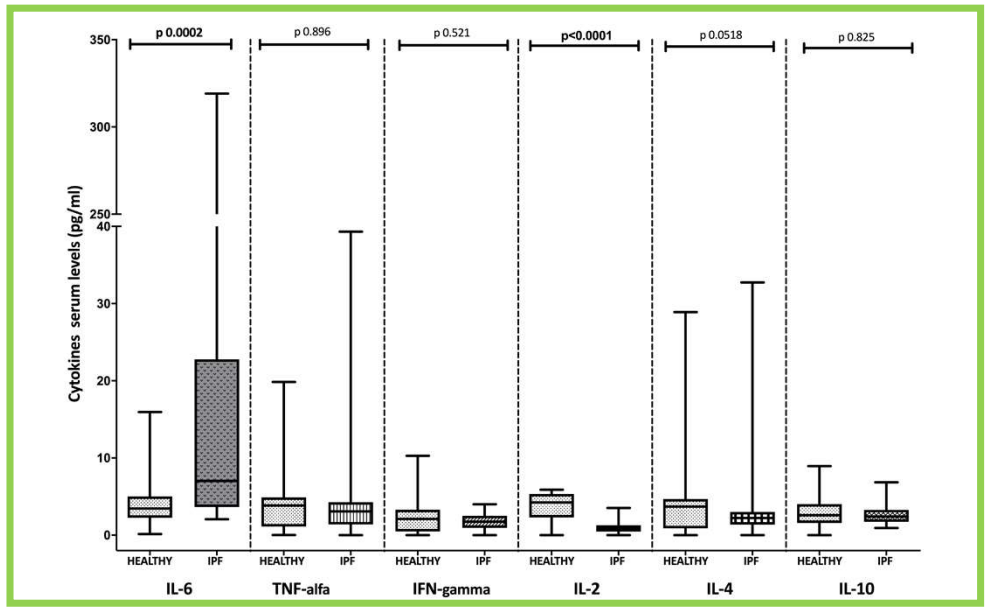


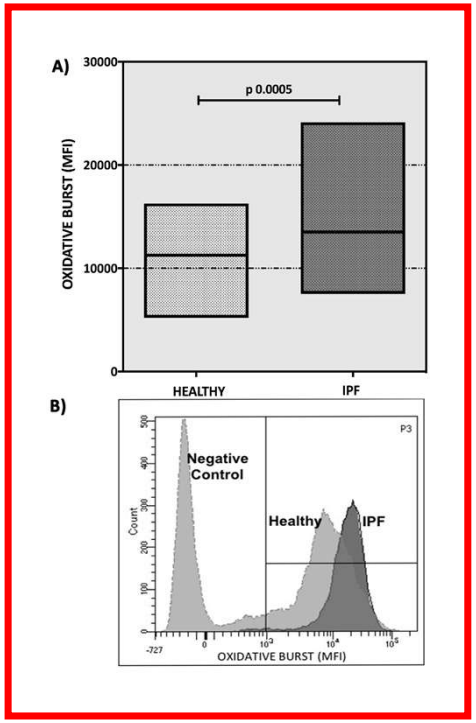


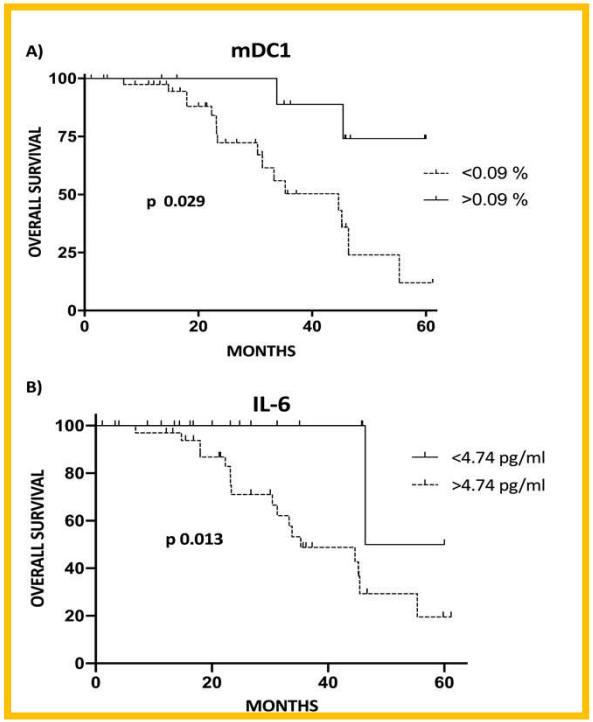
Respiratory Investigation

Peripheral frequencies of CD1c⁺ dendritic cells and serum levels of interleukin-6 are prognostic biomarkers in idiopathic pulmonary fibrosis patients



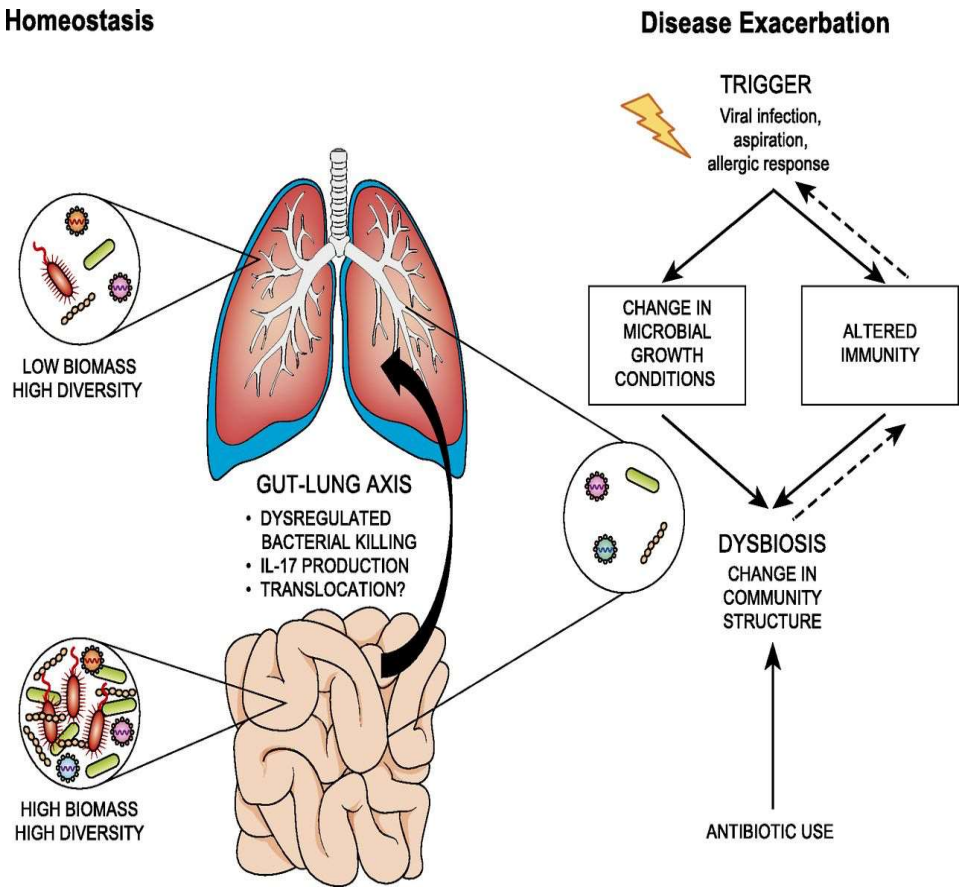


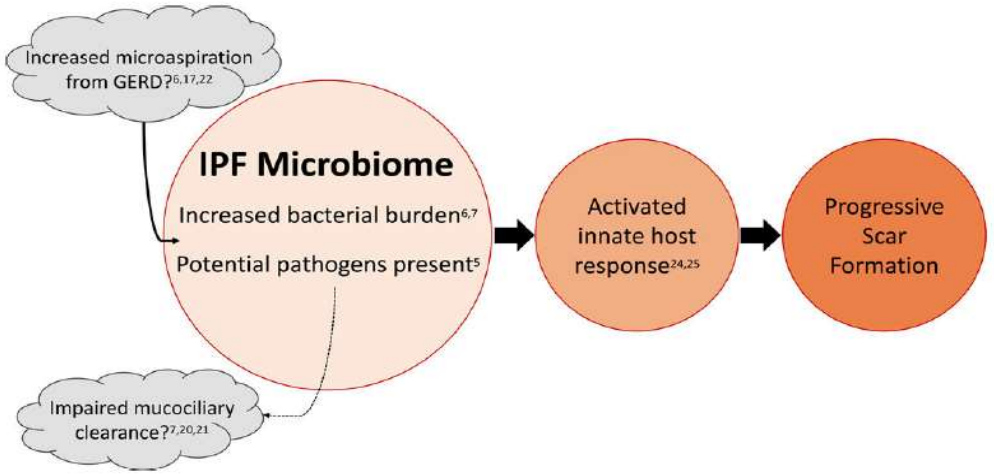




Circulating DC subsets, peripheral levels of oxidative stress and serum concentrations of IL-6 are not modulated by currently used anti-fibrotic drugs

M Bocchino, et al., *submitted manuscript*





KEY POINTS

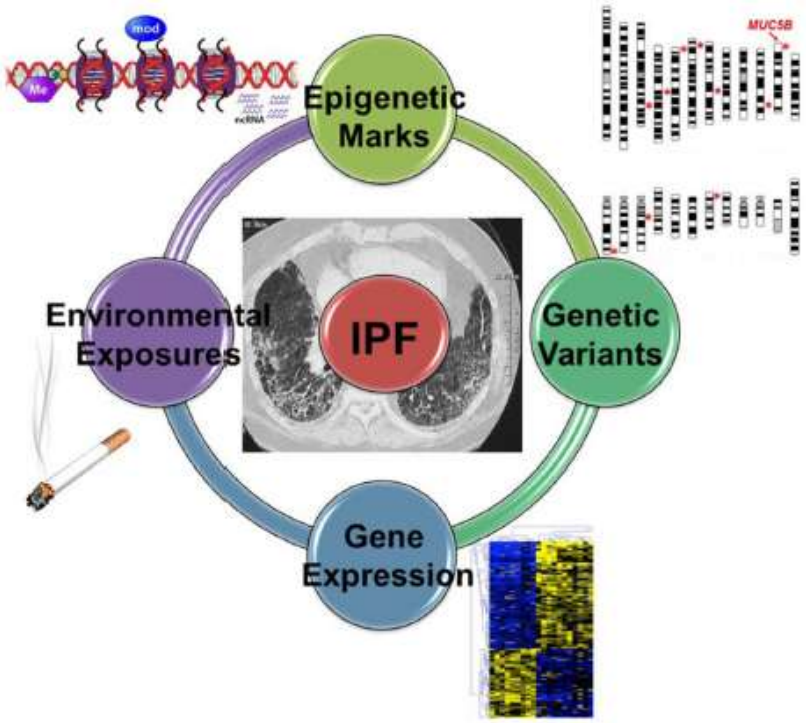
- Advances in molecular sequencing technology in the last decade have allowed study of the role of the microbiome in health and disease.
- The lung contains a dynamic community of microbes in health, and patients with interstitial lung disease may have systematic derangements in bacterial community composition.
- Existing evidence suggests that knowledge of lung microbiome composition in IPF may serve as a prognostic biomarker, a therapeutic target, or provide an explanation for disease pathogenesis.

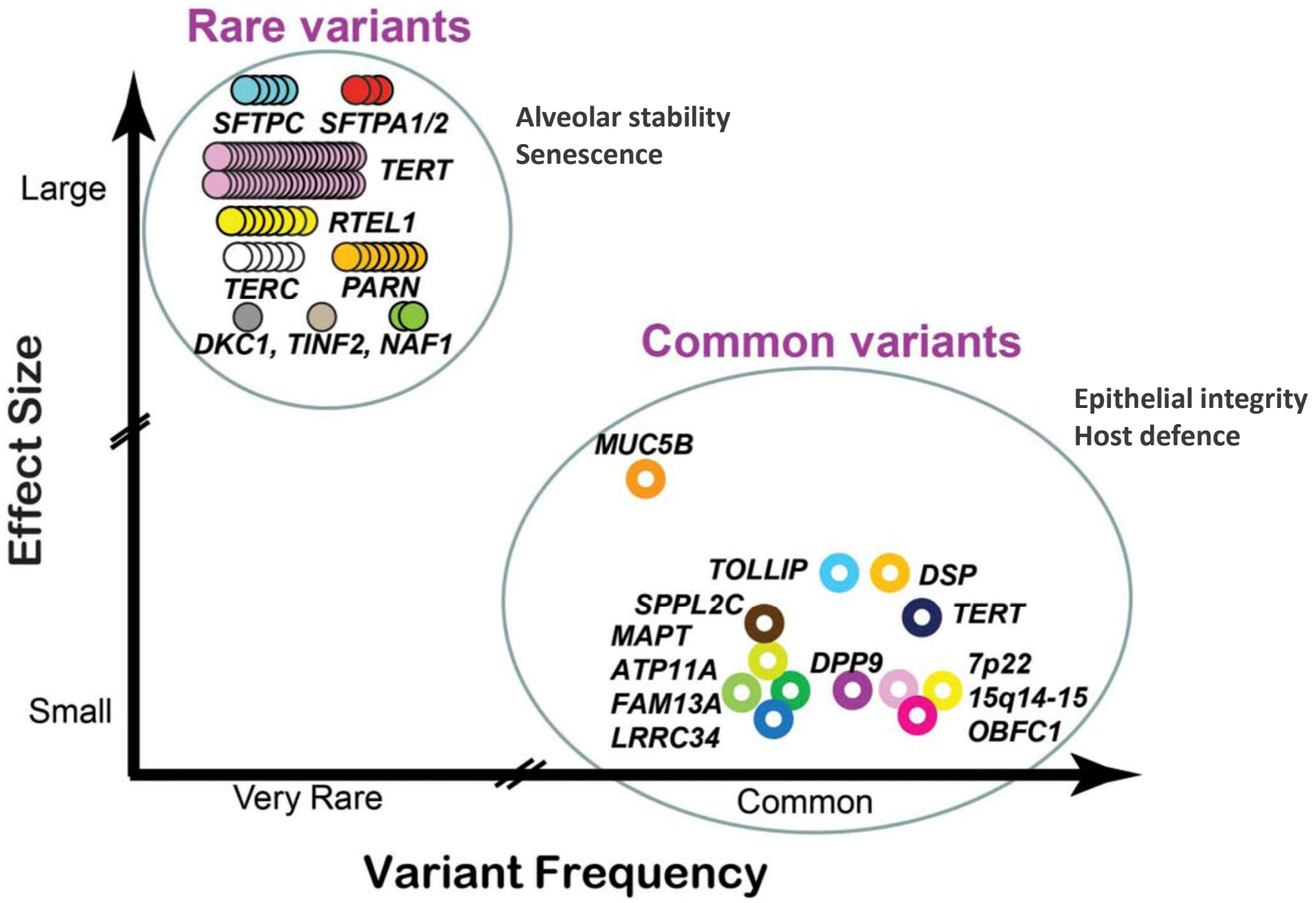
The Role of Immune and Inflammatory Cells in Idiopathic Pulmonary Fibrosis

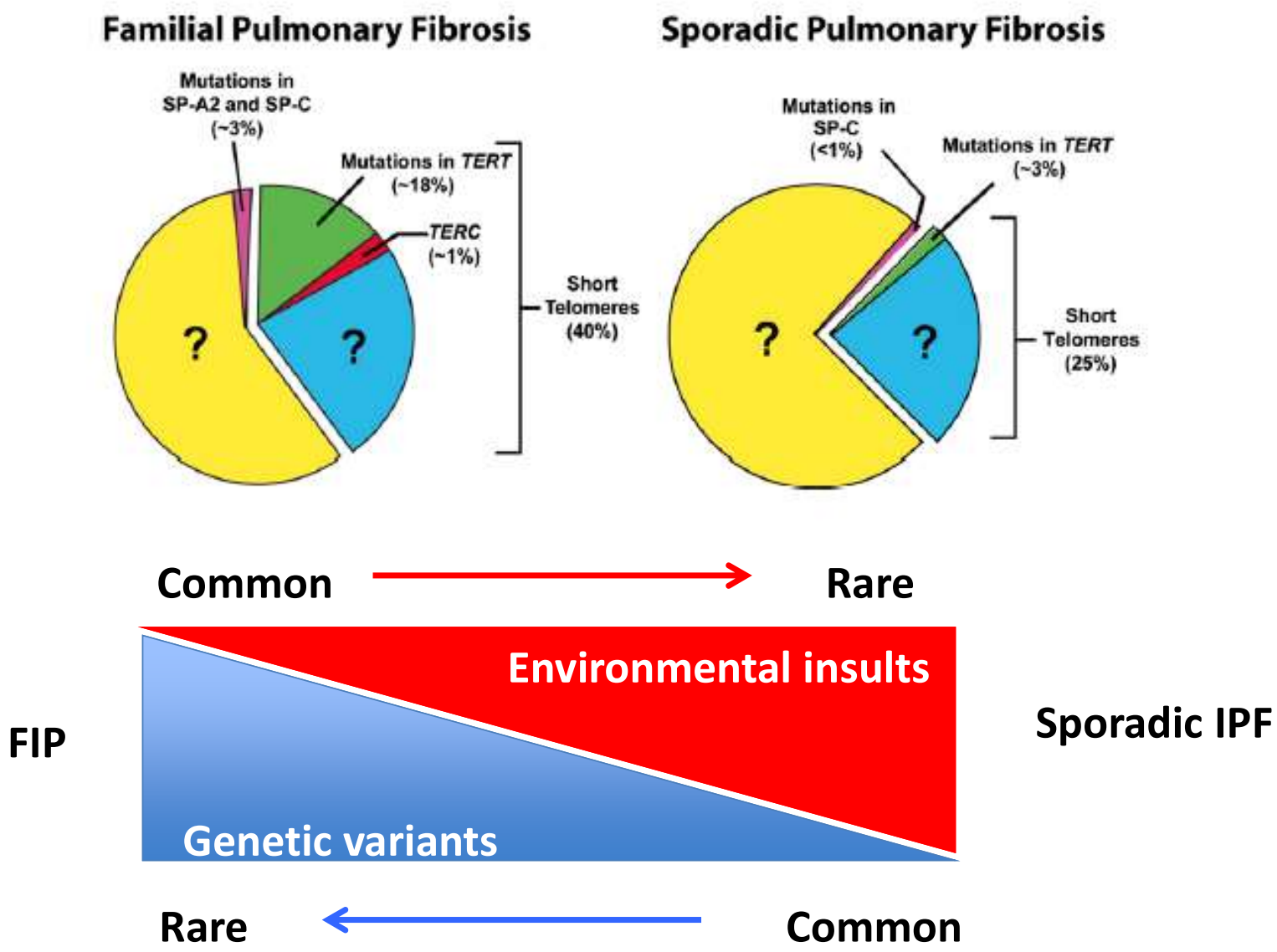
Unanswered questions regarding the immune and inflammatory cells in idiopathic pulmonary fibrosis (IPF).

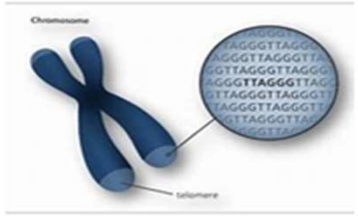
- To what extent do data obtained from mouse models reflect the situation in the fibrotic human lung? Can mimetics be developed that more accurately simulate the IPF disease state?
- Do events in the peripheral blood truly reflect events occurring in the diseased lung?
- Do the innate immune abnormalities seen in IPF represent a unique form of immunosenescence?
- Can therapies targeting macrophage activation stabilize or restore lung function in patients with IPF?
- Does the altered microbiome cause pathogen-associated molecular pattern-driven innate immune activation in IPF and are antimicrobial therapies efficacious in IPF?
- Does perpetuated microinjury cause danger-associated molecular pattern (DAMP)-driven innate immune activation in IPF and are therapies targeting DAMPs and their receptors efficacious in IPF?
- Are neutrophil extracellular traps an important part of IPF pathogenesis?
- What is the role of fibrocytes and myeloid-derived suppressor cells in IPF?
- Do innate lymphoid cells participate in IPF?
- How does the relative balance of T-helper cells participate in IPF and can this contribution be targeted in a safe and efficacious manner?
- Are B cells involved in the development of IPF?
- Can immune events detected in the circulation be used to guide personalized therapies in IPF?

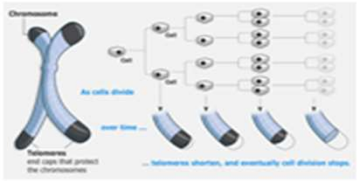
Is IPF a genetic disease?

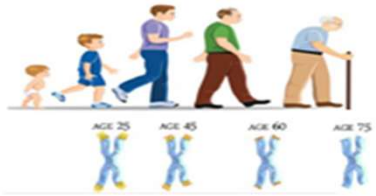


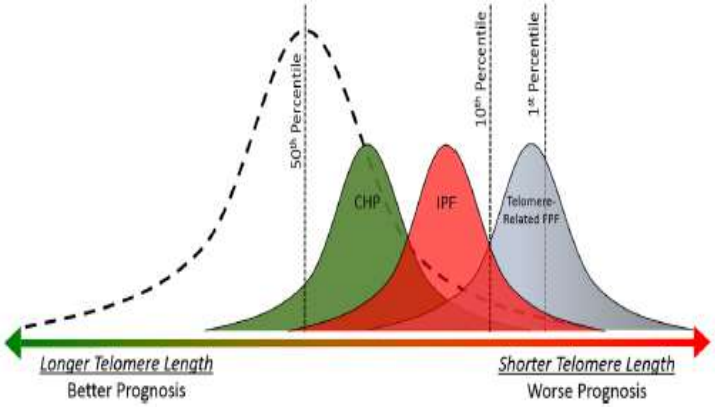

Rare variants
SFTPC
SFTPA1/2
TERT
RTEL1
TERC
PARN
DKC1, TINF2, NAF1
Alveolar stability
Senescence
Common variants
MUC5B
TOLLIP
DSP
SPPL2C
TERT
MAPT
ATP11A
FAM13A
LRRC34
DPP9
7p22
15q14-15
OBFC1
Epithelial integrity
Host defence
Large
Small
Effect Size
Very Rare
Common
Variant Frequency


Familial Pulmonary Fibrosis
Mutations in SP-A2 and SP-C (~3%)
Mutations in TERT (~18%)
TERC (~1%)
Short Telomeres (40%)
?
?
Sporadic Pulmonary Fibrosis
Mutations in SP-C (<1%)
Mutations in TERT (~3%)
Short Telomeres (25%)
?
?
Common
Rare
Environmental insults
Genetic variants
FIP
Sporadic IPF
Rare
Common



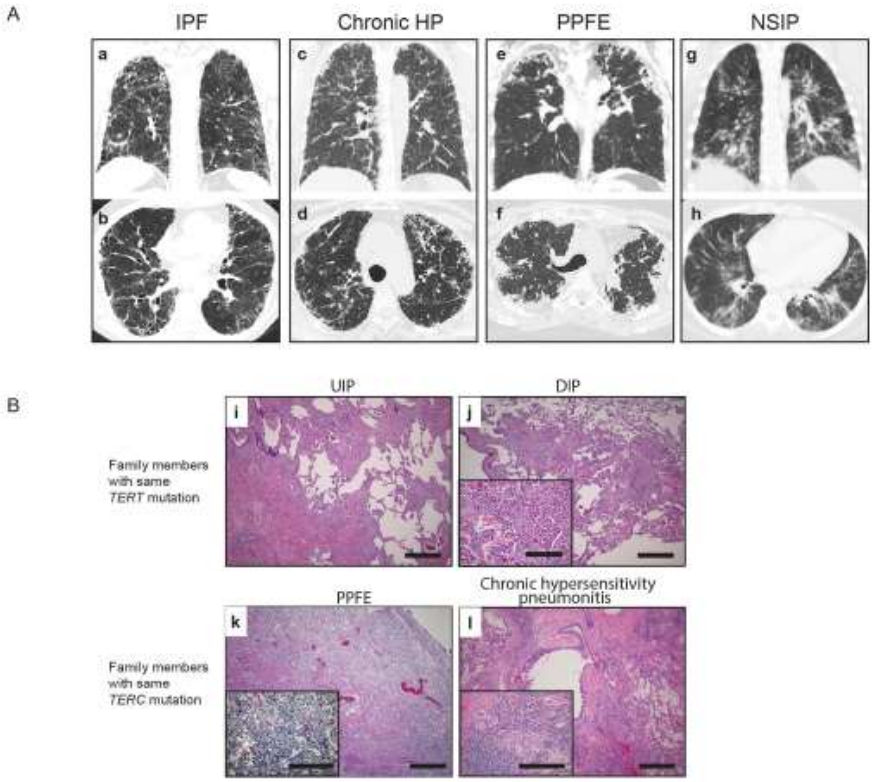






Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive

Chad A. Newton[1,2], Kiran Batra[3], Jose Torrealba[4], Julia Kozlitina[1], Craig S. Glazer[2], Carlos Aravena[5], Keith Meyer[6], Ganesh Raghu[7], Harold R. Collard[5], and Christine Kim Garcia[1,2]



Eur Respir J, 2016

A Common *MUC5B* Promoter Polymorphism and Pulmonary Fibrosis

Max A. Seibold, Ph.D.,

N Engl J Med 2011 April 21; 364(16): 1503–1512.

The ORs for heterozygous and homozygous individuals are 6.8 and 20.8 for FIP and 9.0 and 21.8 for sporadic IPF, respectively.

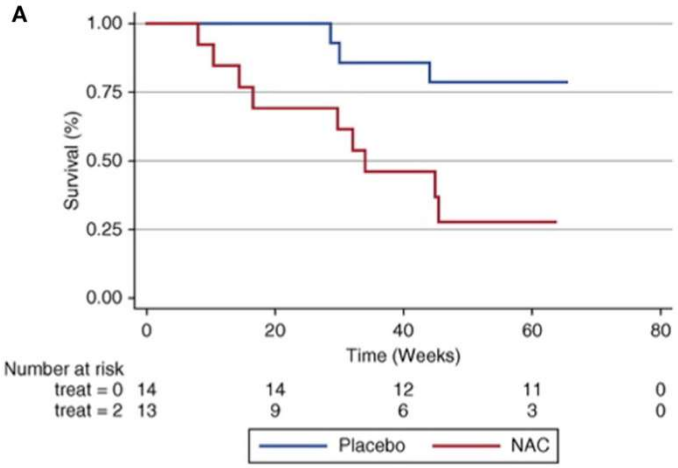
MUC5B promoter polymorphism (rs 35705950):

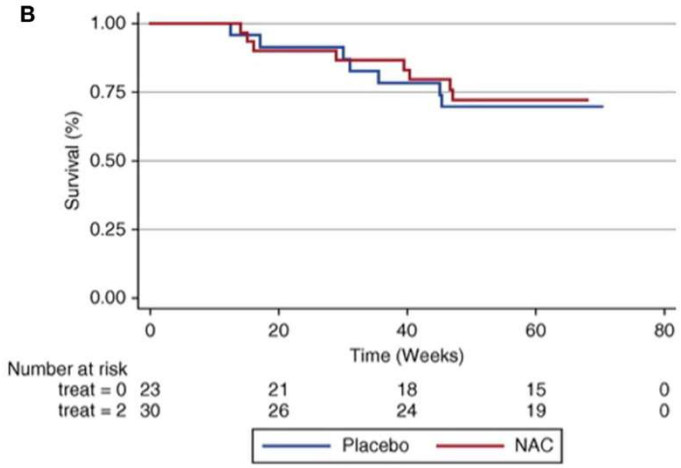
- seems to be specific to IIPs
- is associated with radiographic evidence of ILA (Framingham cohort) and their progression (age and copy number)
- is associated with the CT *UIP pattern* in the setting of fibrotic IIPs

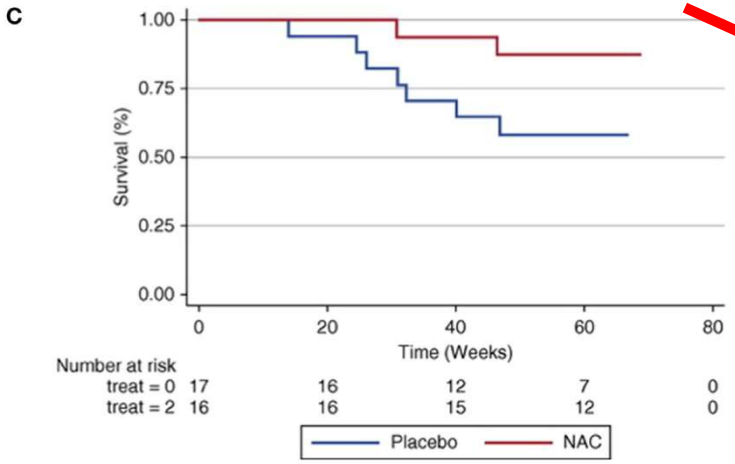
ORIGINAL ARTICLE

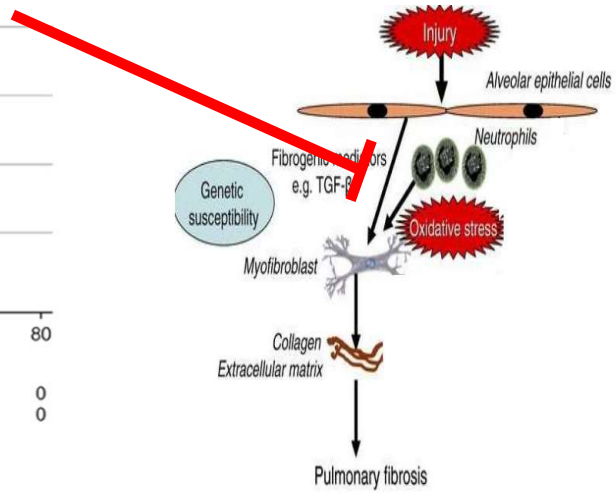
TOLLIP, *MUC5B*, and the Response to *N*-Acetylcysteine among Individuals with Idiopathic Pulmonary Fibrosis

Justin M. Oldham[1*], Shwu-Fan Ma[1*], Fernando J. Martinez[2], Kevin J. Anstrom[3], Ganesh Raghu[4], David A. Schwartz[5], Eleanor Valenzi[1], Leah Witt[1], Cathryn Lee[1], Rekha Vij[1], Yong Huang[1], Mary E. Strek[1], and Imre Noth[1]; for the IPFnet Investigators







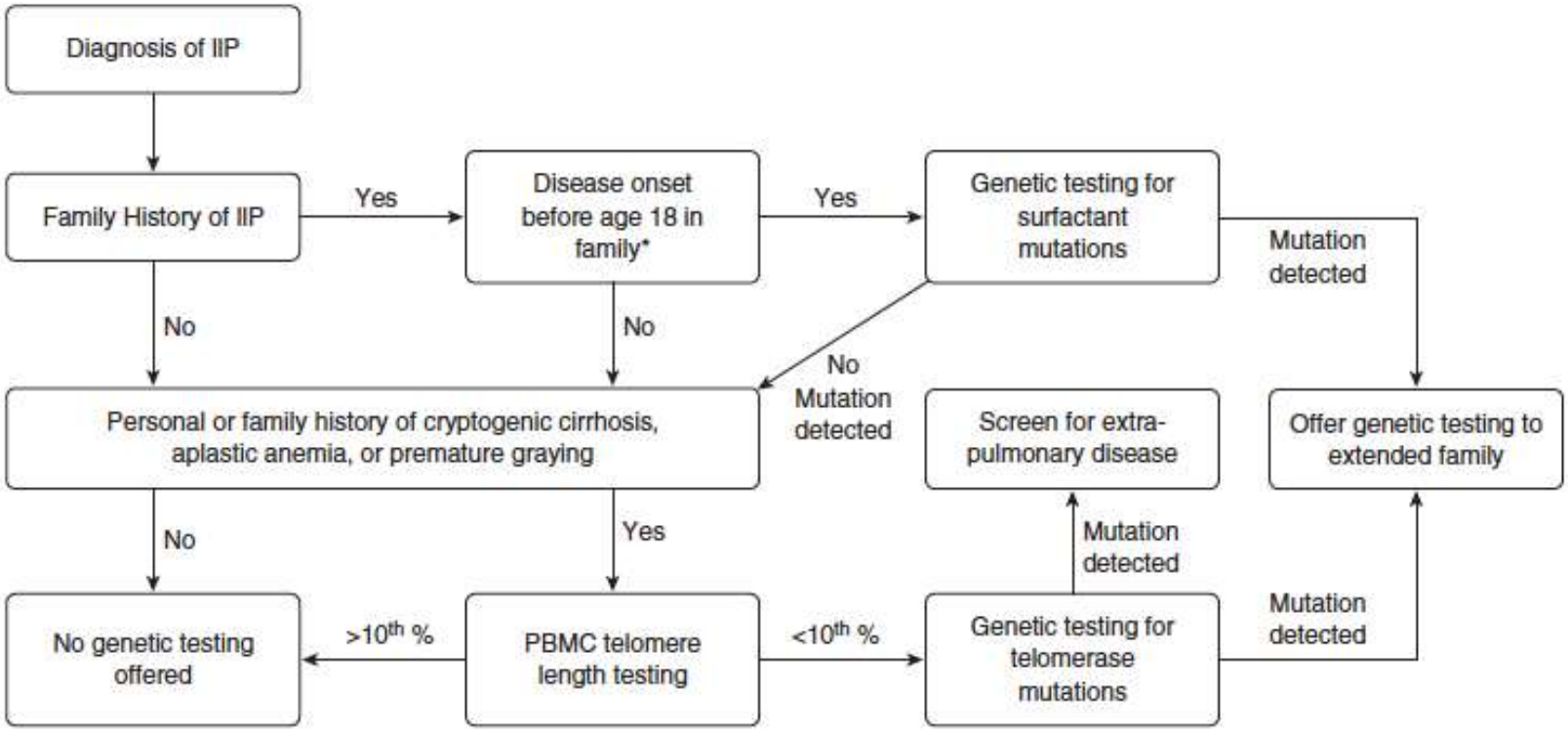


rs3750920 (TT and CC genotype)

WARNING!

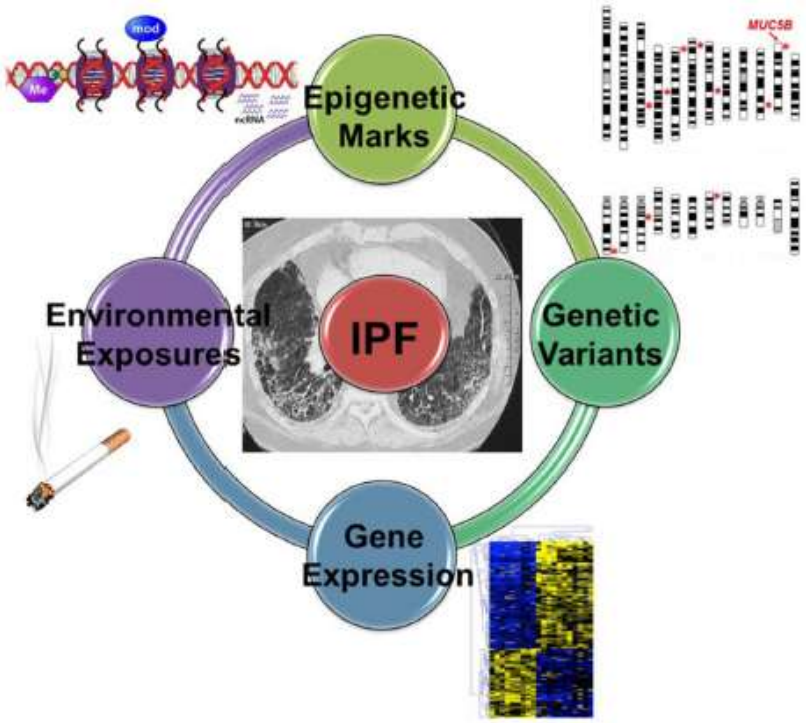
At this time there are no clinical guidelines suggesting genetic testing in the routine care and counseling of IPF patients

Genetic testing in FIP/IPF: a proposed flowchart



Kropski JA et a., *Am J Respir Ctit Care Med*, 2017

Epigenetics: a new frontier in IPF



Exosomal miRNAs in Lung Diseases: From Biologic Function to Therapeutic Targets

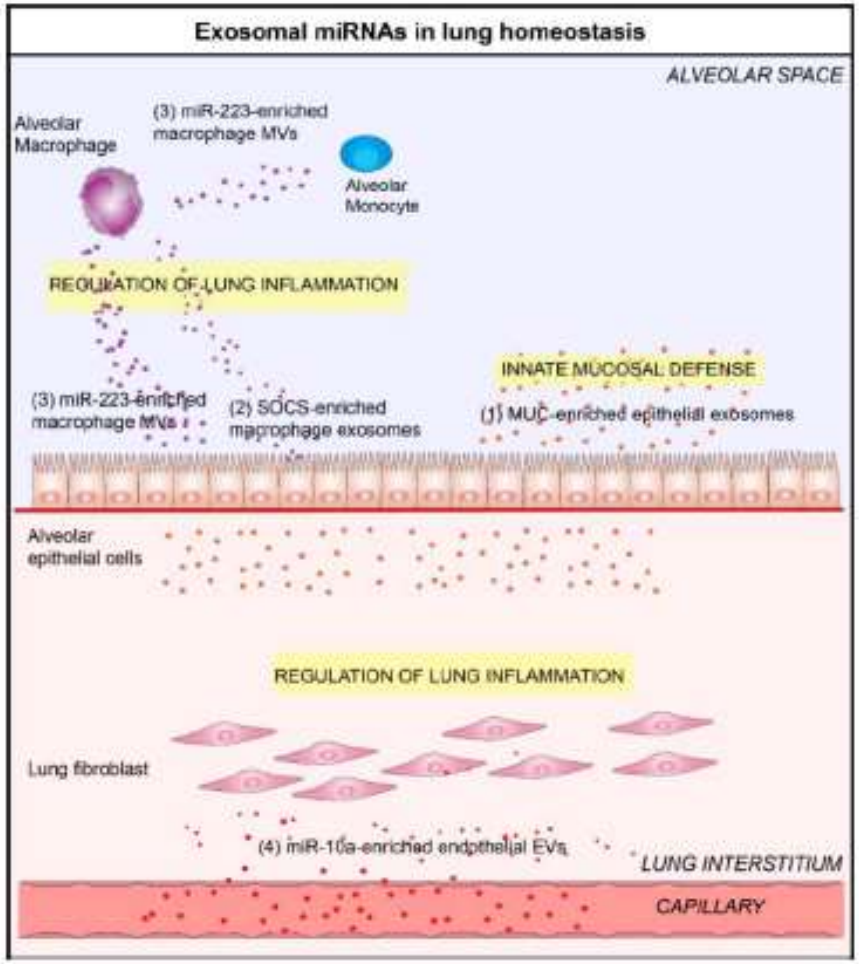
Julien Guiot [1,2,*], Ingrid Struman [2,3], Edouard Louis [2,4], Renaud Louis [1,2], Michel Malaise [2,5] and Makon-Sébastien Njock [1,2,4,5,*]

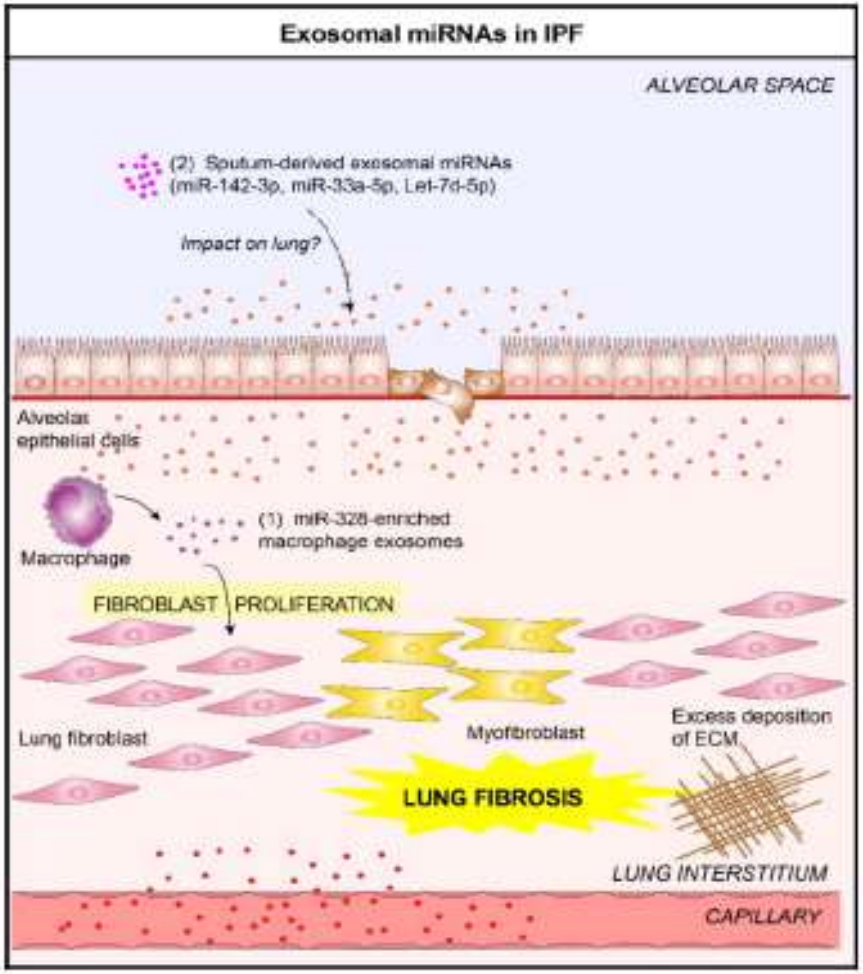
Characteristics	Exosomes	MVs	ABs
Size (nm)	30–150	100–1000	1000–5000
Morphology	Cup–shaped	Heterogeneous	Heterogeneous
Density (g/mL)	1.13–1.19	Undetermined	1.16–1.28
Origin	MVBs	Plasma membrane	Plasma membrane
Biogenesis	Fusion of MVBs with plasma membrane	Budding and scission of plasma membrane	Cell fragmentation /blebbing
References	[9–12]	[9,13,16,17]	[9,14,15]

Abbreviations: ABs, apoptotic bodies; MVs, microvesicles; MVBs, multivesicular bodies.

Journal of *Clinical Medicine*

2019, *8*, 1345; doi:10.3390/jcm8091345


Exosomal miRNAs in lung homeostasis
ALVEOLAR SPACE
Alveolar Macrophage
(3) miR-223-enriched macrophage MVs
Alveolar Monocyte
REGULATION OF LUNG INFLAMMATION
INNATE MUCOSAL DEFENSE
(3) miR-223-enriched macrophage MVs
(2) SOCS-enriched macrophage exosomes
(1) MUC-enriched epithelial exosomes
Alveolar epithelial cells
REGULATION OF LUNG INFLAMMATION
Lung fibroblast
(4) miR-10a-enriched endothelial EVs
LUNG INTERSTITIUM
CAPILLARY


Exosomal miRNAs in IPF
ALVEOLAR SPACE
(2) Sputum-derived exosomal miRNAs (miR-142-3p, miR-33a-5p, Let-7d-5p)
Impact on lung?
Alveolar epithelial cells
Macrophage
(1) miR-328-enriched macrophage exosomes
FIBROBLAST PROLIFERATION
Lung fibroblast
Myofibroblast
Excess deposition of ECM
LUNG FIBROSIS
LUNG INTERSTITIUM
CAPILLARY

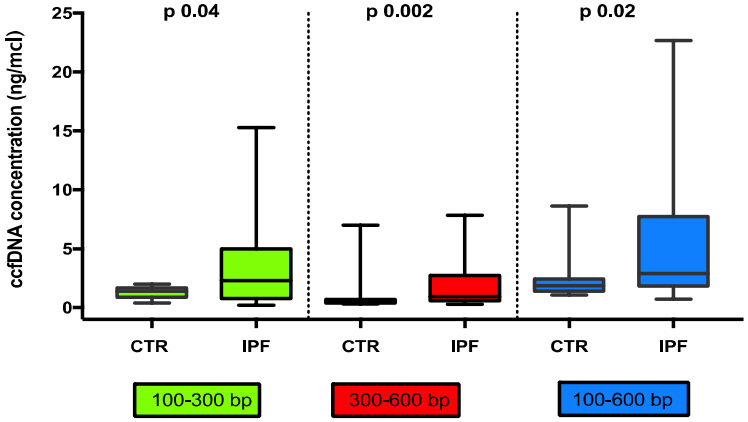
Lung Diseases	Biofluids	EVs	miRNAs	Expression in Lung Disease (vs. Controls)	References
COPD	Plasma	Circulating miRNAs	miR-1 miR-499 miR-133 miR-206	Upregulated Upregulated Upregulated Upregulated	[67]
	BALF	Exosomes	miR-223-3p miR-223-5p miR-338-3p miR-1469 miR-204-5p miR-618	Upregulated Upregulated Upregulated Upregulated Upregulated Upregulated	[66]
	Serum	Exosomes	miR-21	Upregulated	[30]
	Plasma	MVs	let-7d miR-191 miR-126 miR-125a	Upregulated Upregulated Upregulated Upregulated	[17]
ASTHMA	Sputum	SputummiRNAs	miR-142-3p miR-629-3p miR-223-3p	Upregulated Upregulated Upregulated	[47]
	BALF	Exosomes	miR-21 miR-1268 miR-658 Let-7a miR-24 miR-26a miR-99a miR-200c	Upregulated Upregulated Downregulated Downregulated Downregulated Downregulated Downregulated Downregulated	[24]
	Serum	Exosomes	miR-128 miR-140-3p miR-196-5p miR-468-5p	Upregulated Upregulated Upregulated Upregulated	[48]
IPF	Sputum	Exosomes	miR-142-3p miR-33a-5p Let-7d-5p	Upregulated Upregulated Downregulated	[25]

Abbreviations: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; EVs, extracellular vesicles; IPF, idiopathic pulmonary fibrosis; miRNAs, microRNAs; MVs, microvesicles.

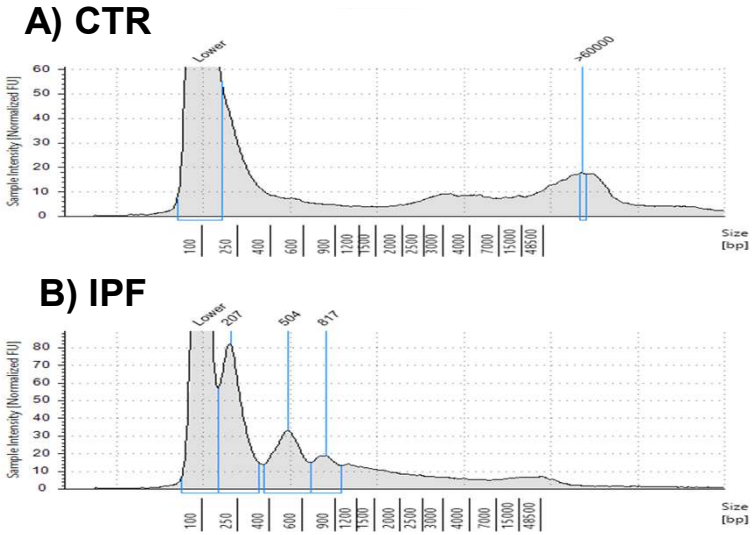
Liquid biopsy, that allows the isolation of circulating cell-free (ccf) DNA from blood, is an emerging non-invasive and convenient tool for cancer biomarker discovery. Specifically, ccfDNA in patient-derived plasma/serum samples contains DNA fragments released from the tumor cells that carry both genetic and epigenetic information.

Recent ccfDNA-based studies have provided promising results with potential clinical application in early cancer detection, diagnosis and prognosis.

In 2010, Casoni *et al.* first suggested that serum ccfDNA may help discriminate patients affected by idiopathic pulmonary fibrosis (IPF) from those with other interstitial lung diseases.



Comparisons between study groups were performed with the two-tailed Mann Whitney test for unpaired data.
A p value <0.05 was considere significant.



ccfDNA (100-300 bp) concentrations were negatively correlated with $DLCO_{sb}$

p=0.017; Spearman's rho= -0.35
(95% CI= -0.50 to 0.05)

M. Bocchino, et al., *ERS International Congress* 2019, Madrid
manuscript in preparation

The «biomarker pipeline» in IPF: conclusions and perspectives

Evidence for the definite use of a specific biomarker in real life clinical practice is inconclusive

Critical issues include single center studies, retrospective design, small number of patients, heterogeneity of sample collection and analytic procedures

Better understanding of disease pathogenesis and disease behaviour

Identification of new players and drug targets

Standardization of sample collection and analytical procedures

Need of prospective longitudinal studies (combination with clinical parameters and genotyping)

Best assessment of timing and benefit of therapies

Personalized medicine in IPF