Molecular mechanism and non Steroidal treatment of Lung Fibrosis

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DM Seminar

Pathology of Pulmonary Fibrosis

- Histopathological lesion is UIP
- Normal lung alternates with fibrosed portion
- Fibrosis: alveolar septal thickening with sub-pelural distribution

Observations

- Whorls of fibroblasts in ECM on the edge of dense scars
- Minimal interstitial inflammation
- Hyperplasia of type II pneumocytes is found in areas of fibrosis
- No alveolar exudates and hyaline membranes

Pathological categories of UIP

1. UIP pattern
2. Probable UIP
3. Possible UIP
4. Not UIP

UIP pattern

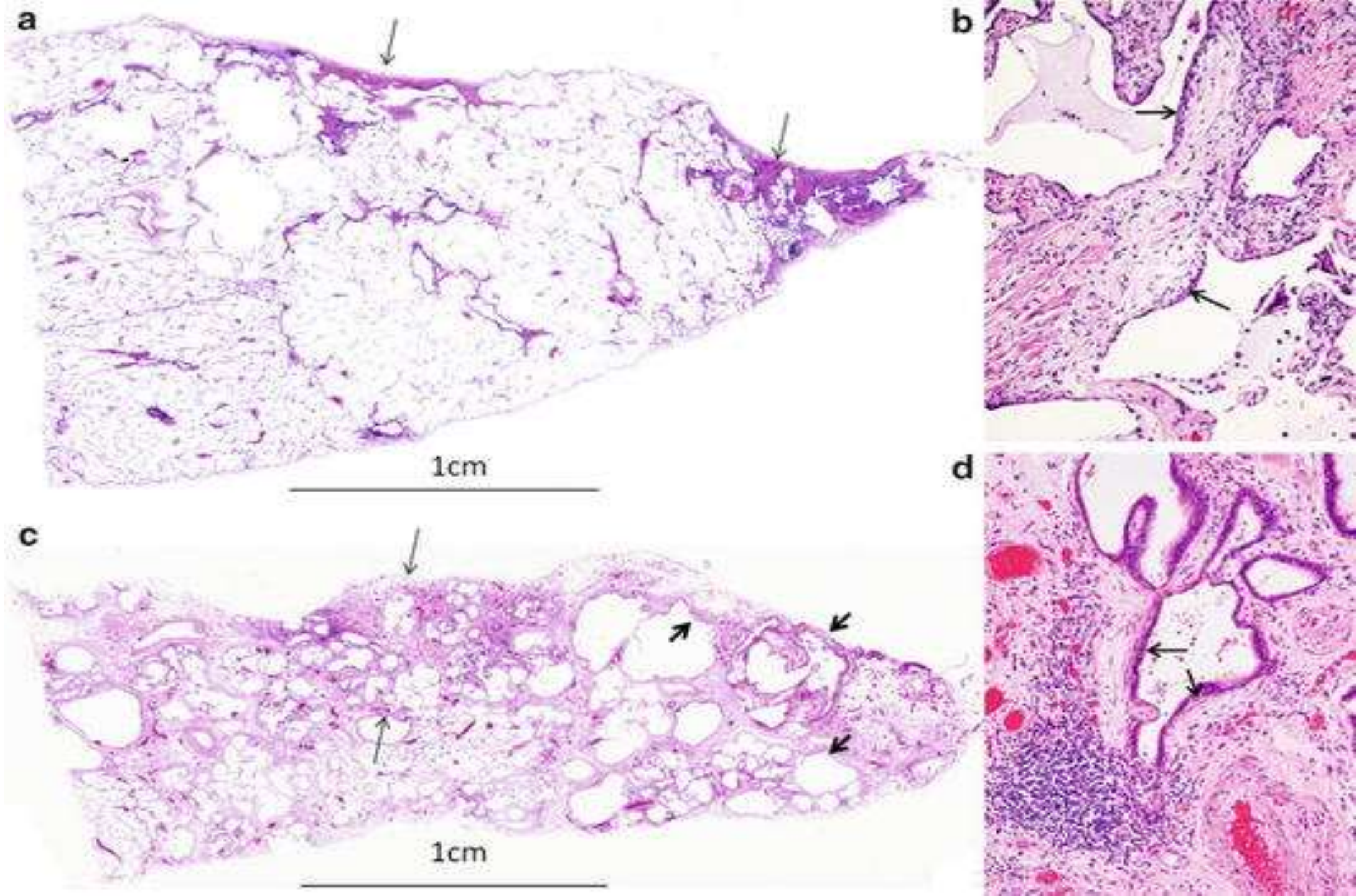
1. Fibrosis with architectural distortion
2. Patchy involvement of fibrosis
3. Fibroblastic foci
4. Absence of OP, Granuloma, Airway centered pathology, hyaline membranes

Probable UIP

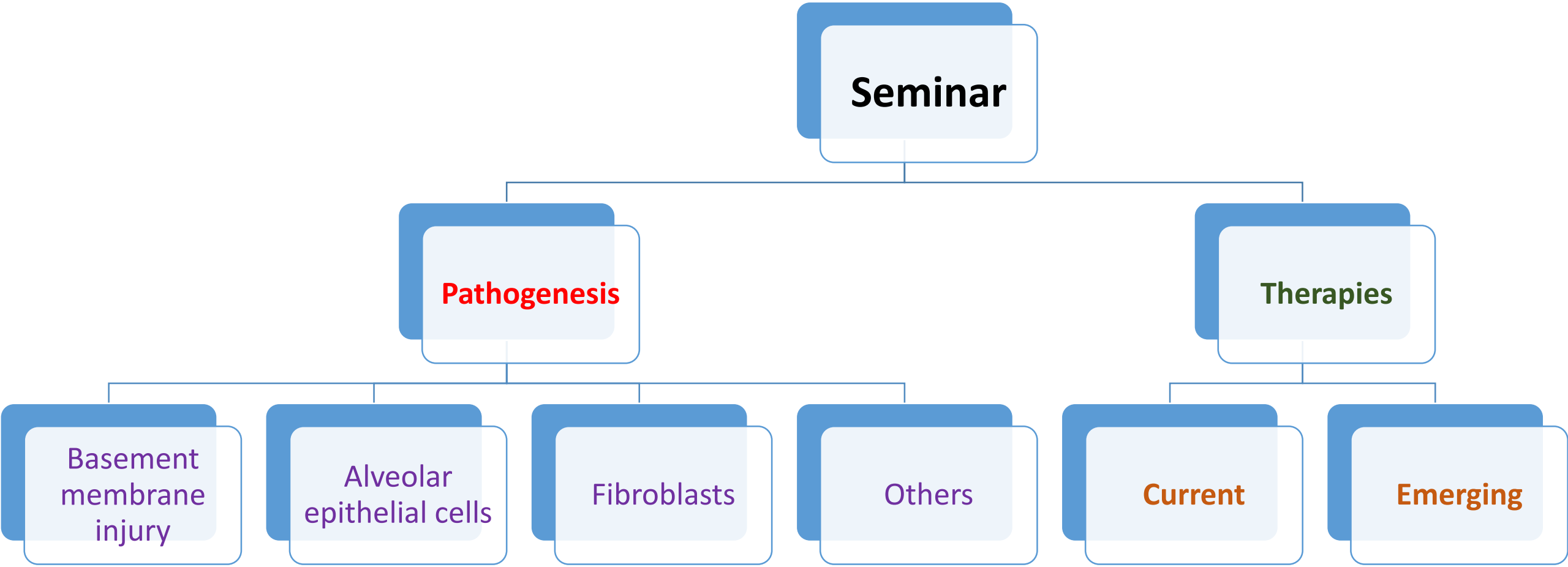
1. Marked fibrosis with architectural distortion
2. Presence of patchy involvement or fibroblastic foci (one of these only)
3. Absence of other features

Possible UIP

1. Patchy or diffuse fibrosis within pulmonary parenchyma
2. In the absence of other UIP criteria
3. Absence of other patterns (4)

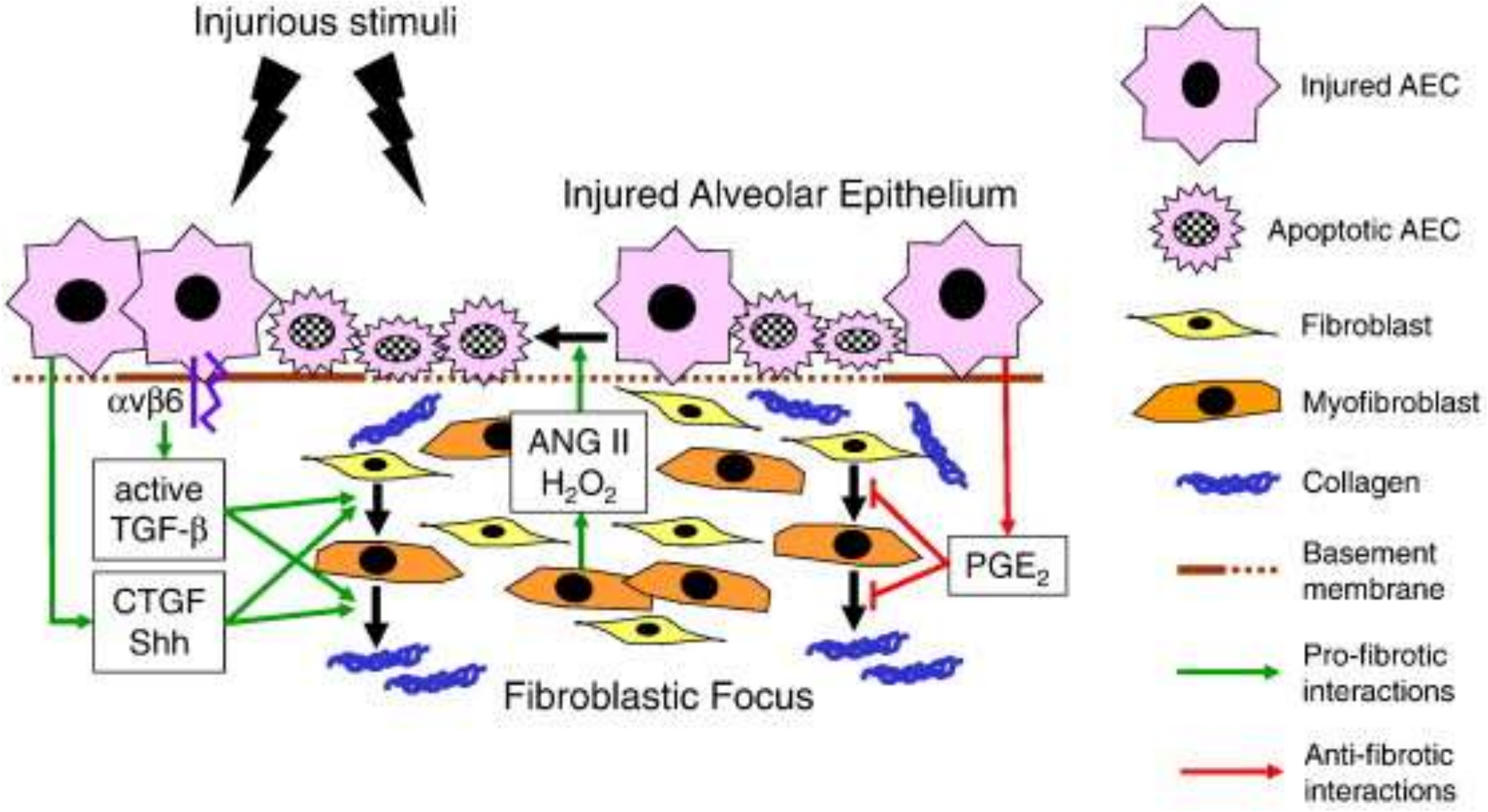

a
b
1cm
c
d
1cm

Content


Seminar
Pathogenesis
Therapies
Basement membrane injury
Alveolar epithelial cells
Fibroblasts
Others
Current
Emerging

1. Basement membrane injury

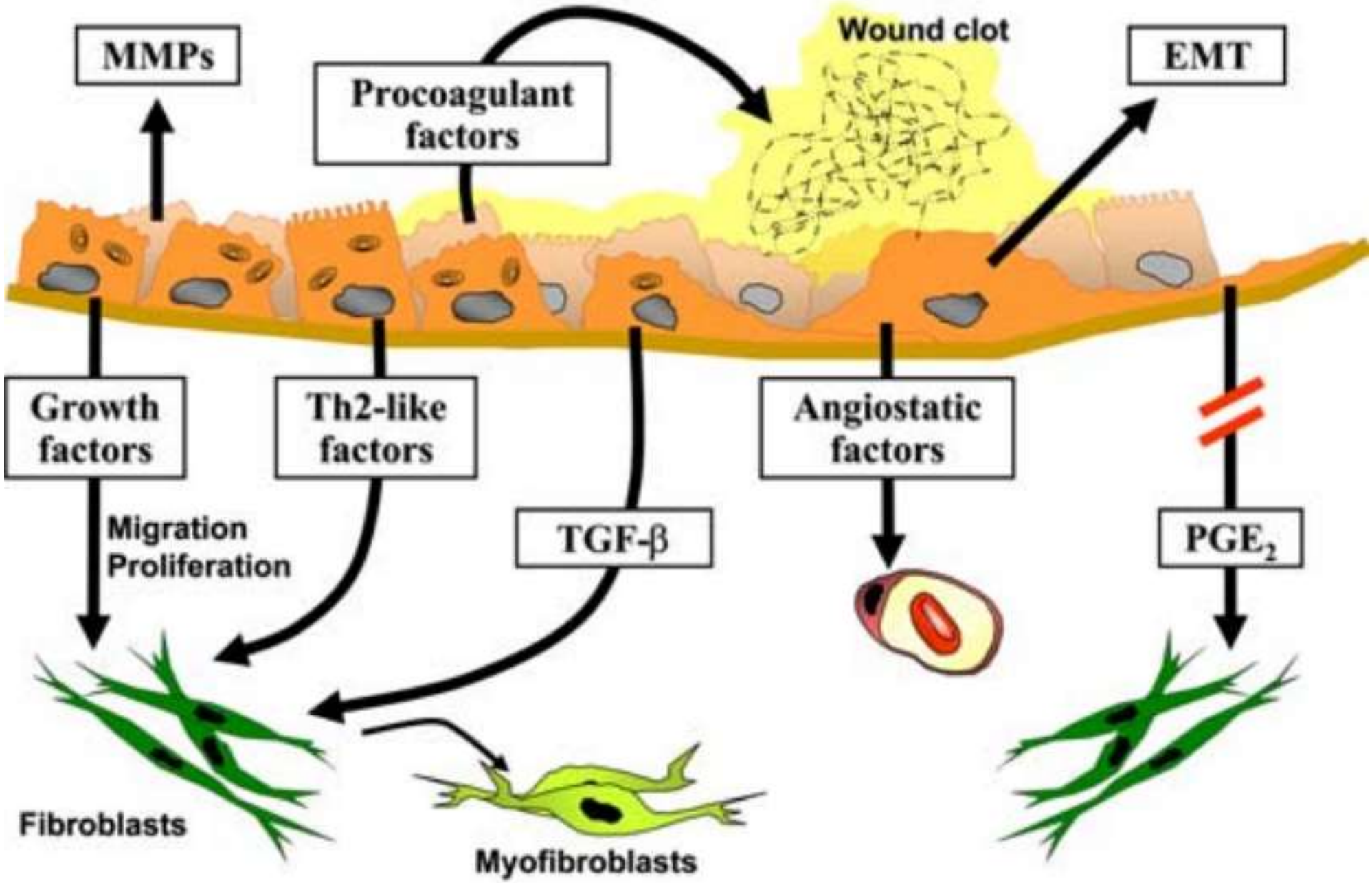
- BM loss of integrity has been demonstrated
- Usual sites of breakdown are beneath type I pneumocytes
- Exposed BM provides a continuous signal for epithelial cell proliferation and fibroblast infiltration
- *Newer* type II cells are unable to send feedback sample to termination of epithelial cell proliferation

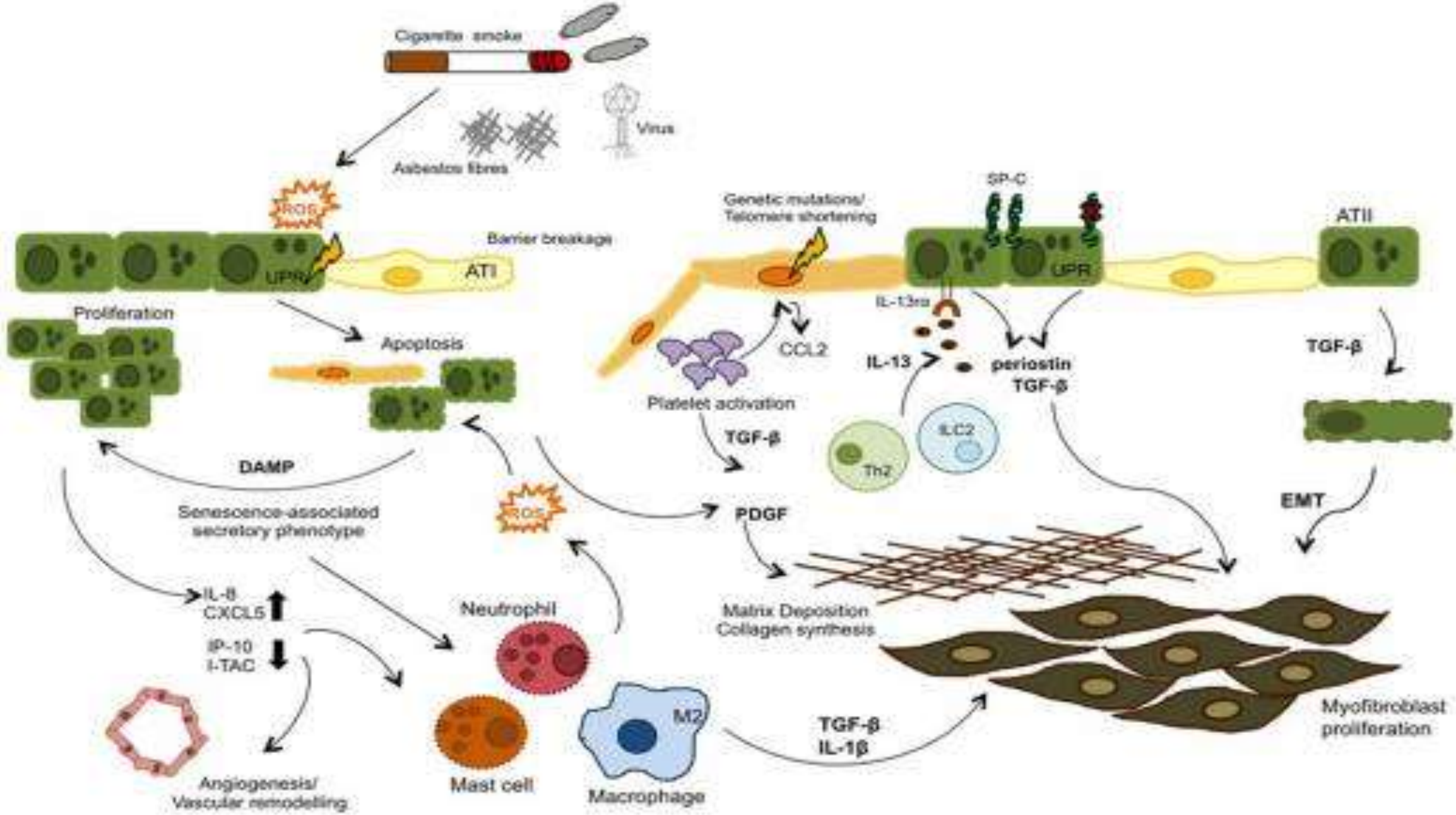

Injurious stimuli
Injured Alveolar Epithelium
αvβ6
active TGF-β
CTGF Shh
ANG II
H_2O_2
PGE_2
Fibroblastic Focus
Injured AEC
Apoptotic AEC
Fibroblast
Myofibroblast
Collagen
Basement membrane
Pro-fibrotic interactions
Anti-fibrotic interactions

2. Alveolar Epithelium

- Currently the most accepted hypothesis: Low level *repeated* injury to a *vulnerable* epithelium
- Epithelial cell apoptosis: multiple agents have been implicated to cause increased loss of type I pneumocytes like TGF-β, Angiotensin, ROS, TNF-α
- Knockout of Individual genes for these agents have been shown to be protective against BILI Mice model.

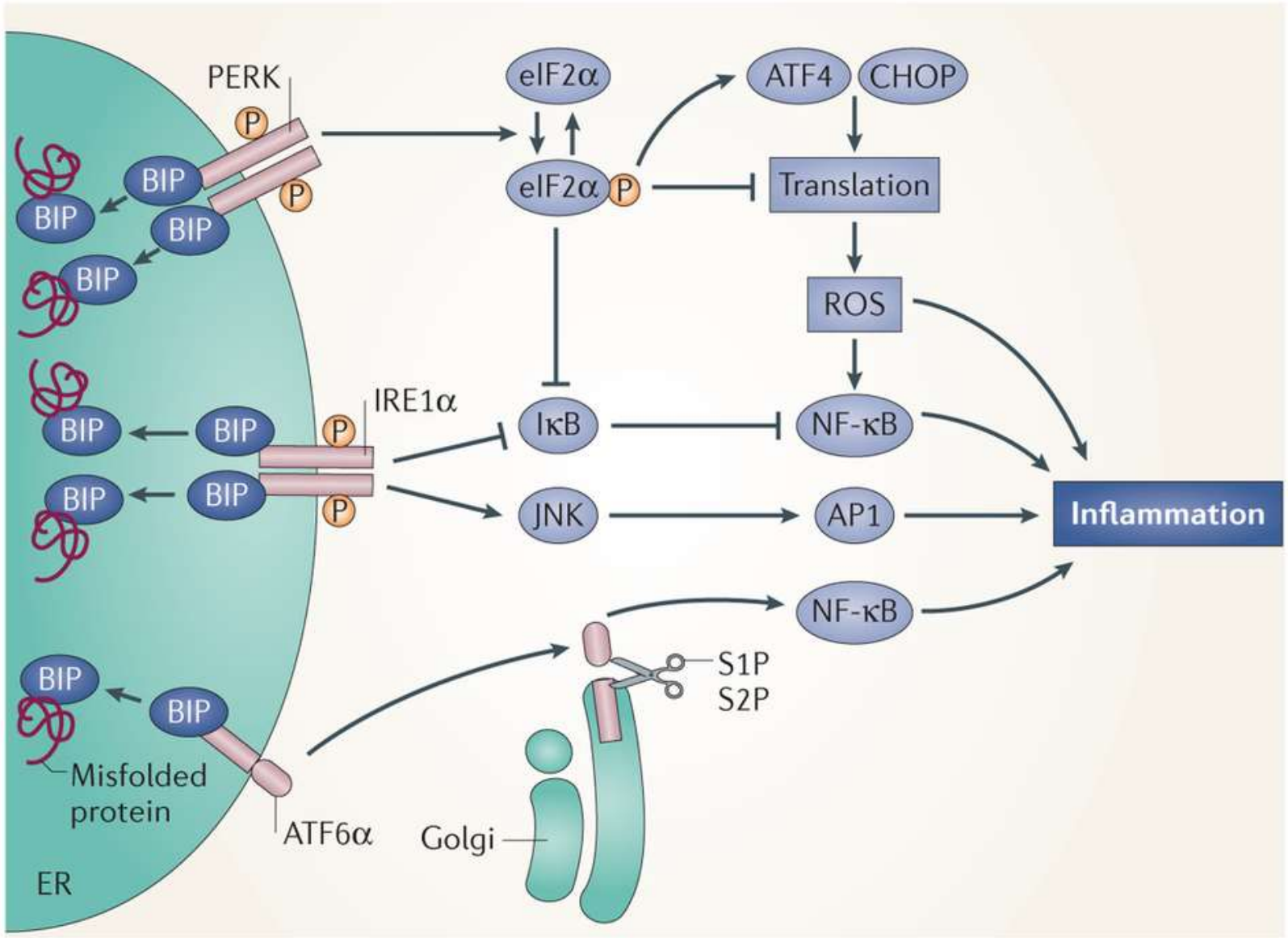
- After loss of Type I pneumocytes there is chaotic hyperplasia of type II pneumocytes
- Once Injured there is downhill cascade: epithelial cells secrete cytokines and molecules to recruit fibrocytes and increase ECM


MMPs
Procoagulant factors
Wound clot
EMT
Growth factors
Th2-like factors
TGF-β
Angiostatic factors
PGE2
Migration
Proliferation
Fibroblasts
Myofibroblasts


Cigarette smoke
Virus
Asbestos fibres
ROS
UPR
Barrier breakage
ATI
Proliferation
Apoptosis
DAMP
Senescence-associated secretory phenotype
ROS
IL-8
CXCL5
IP-10
I-TAC
Neutrophil
Mast cell
M2
Macrophage
Angiogenesis/
Vascular remodelling
Genetic mutations/
Telomere shortening
SP-C
ATII
UPR
IL-13rα
CCL2
Platelet activation
TGF-β
IL-13
periostin
TGF-β
Th2
ILC2
TGF-β
EMT
PDGF
Matrix Deposition
Collagen synthesis
TGF-β
IL-1β
Myofibroblast
proliferation

Role of ER stress in epithelial Cells

- Mutations of surfactant proteins A2 and C have been associated with IPF
- Protein misfolding in post translational process has been associated with increased ER stress causing Unfolded Protein Response
- UPR predisposes towards second hit like viruses, smoking or acid reflux to incite inflammations, apoptosis and fibrosis


PERK
P
P
BIP
BIP
BIP
BIP
BIP
BIP
BIP
BIP
IRE1α
P
P
BIP
BIP
Misfolded protein
ATF6α
ER
eIF2α
eIF2α
P
ATF4
CHOP
Translation
ROS
IκB
NF-κB
JNK
AP1
Inflammation
NF-κB
S1P
S2P
Golgi

3. Fibroblasts

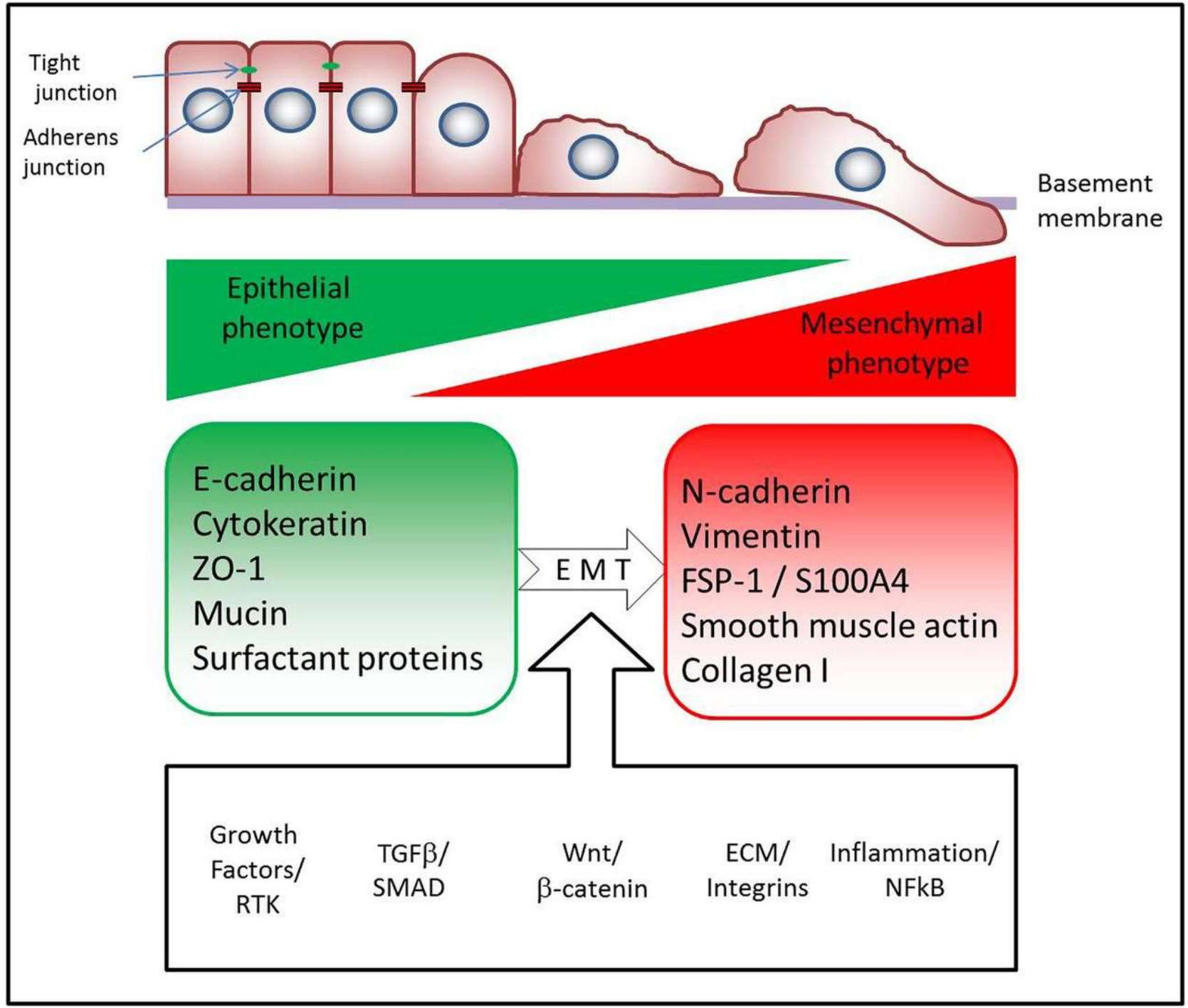
- Main culprits of pulmonary fibrosis
- Source: *Fibrocytes* (released from bone marrow and recruited into lungs), *Epithelial cells* (via EMT), *Indigenous fibroblasts*
- Fibroblastic foci: areas intense fibrosis seen on histopathological examination

Fibrocytes

- CD45 and CD 34 positive
- Bone marrow derived cells which produce collages at injured sites after recruitment
- Culprit cytokines: CXCL12, CCL2, CCL3, IL-10, TGFβ

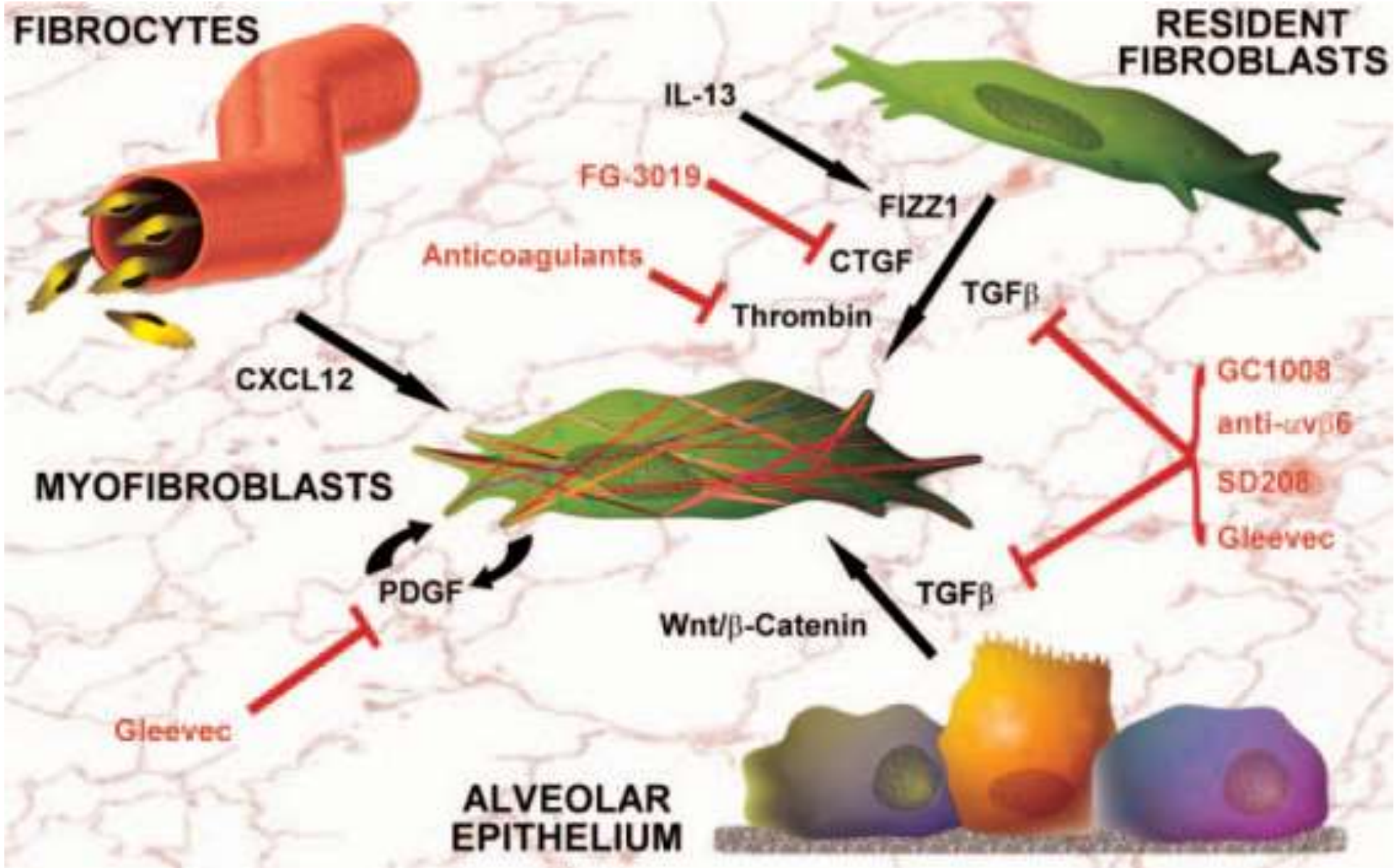
EMT: epithelial to mesenchymal transition

- Transformation of epithelial cells into parenchymal cells is known and important step in wound healing process
- Three types
  - Type 1: Embryonic
  - Type 2: Fibrotic
  - Type 3: Malignant


Tight junction
Adherens junction
Basement membrane
Epithelial phenotype
Mesenchymal phenotype
E-cadherin
Cytokeratin
ZO-1
Mucin
Surfactant proteins
E M T
N-cadherin
Vimentin
FSP-1 / S100A4
Smooth muscle actin
Collagen I
Growth Factors/ RTK
TGFβ/ SMAD
Wnt/ β-catenin
ECM/ Integrins
Inflammation/ NFkB

Myofibroblast

- αSMA positive cells
- Produces new collages
- Abundantly seen in fibroblastic foci


FIBROCYTES
RESIDENT FIBROBLASTS
IL-13
FG-3019
FIZZ1
CTGF
Anticoagulants
Thrombin
TGFβ
CXCL12
GC1008
anti-αvβ6
SD208
Gleevec
MYOFIBROBLASTS
PDGF
TGFβ
Wnt/β-Catenin
Gleevec
ALVEOLAR EPITHELIUM

Fibroblast functions in pulmonary fibrosis

- TGF-β is the most profibrotic agent in Lung fibrosis
- Increases fibrocytes by recruitment, EMT and proliferation of resident fibroblasts
- Increases expression of myofibroblast via NOX4
- Integrins are critical to the activation of latent fibroblasts
- Through various inflammatory and non inflammatory cytokines procollagen and excessive ECM is secreted

Other mechanisms

1. Genes related to pulmonary fibrosis
2. Coagulation pathway
3. Reactive oxygen species
4. Role of Integrins
5. Fas-FasL
6. Inflammation

1. Genetic susceptibility for IPF

- FIP: most common genes involved are surfactant associate protein C (SFTPC) and less frequently SFTPA2
- These are the genes whose mutations are needed in EMT, apoptosis and protein misfolding
- Telomerase regulator genes mutations have also been implicated in development of FIP

Rops et al Eur Respir J. 2015 Jun;45(6):1717-27

Genes in Sporadic IPF

- Highest genetic risk factor is variant in promotor region of MUC5B
- MUC5B encodes mucin and is found in approximately one third patients in IPF
- Other genes which are commonly mutated in IPF are
  - DSP (Desmoplakin)
  - DPP6 (dipeptidyl peptidase 9),
  - Telomere specific genes like TERT, TERC, OBFC1, RTRL1, PARN
  - SNPs mutates like TOLLIP (Toll interacting protein)

Rops et al Eur Respir J. 2015 Jun;45(6):1717-27

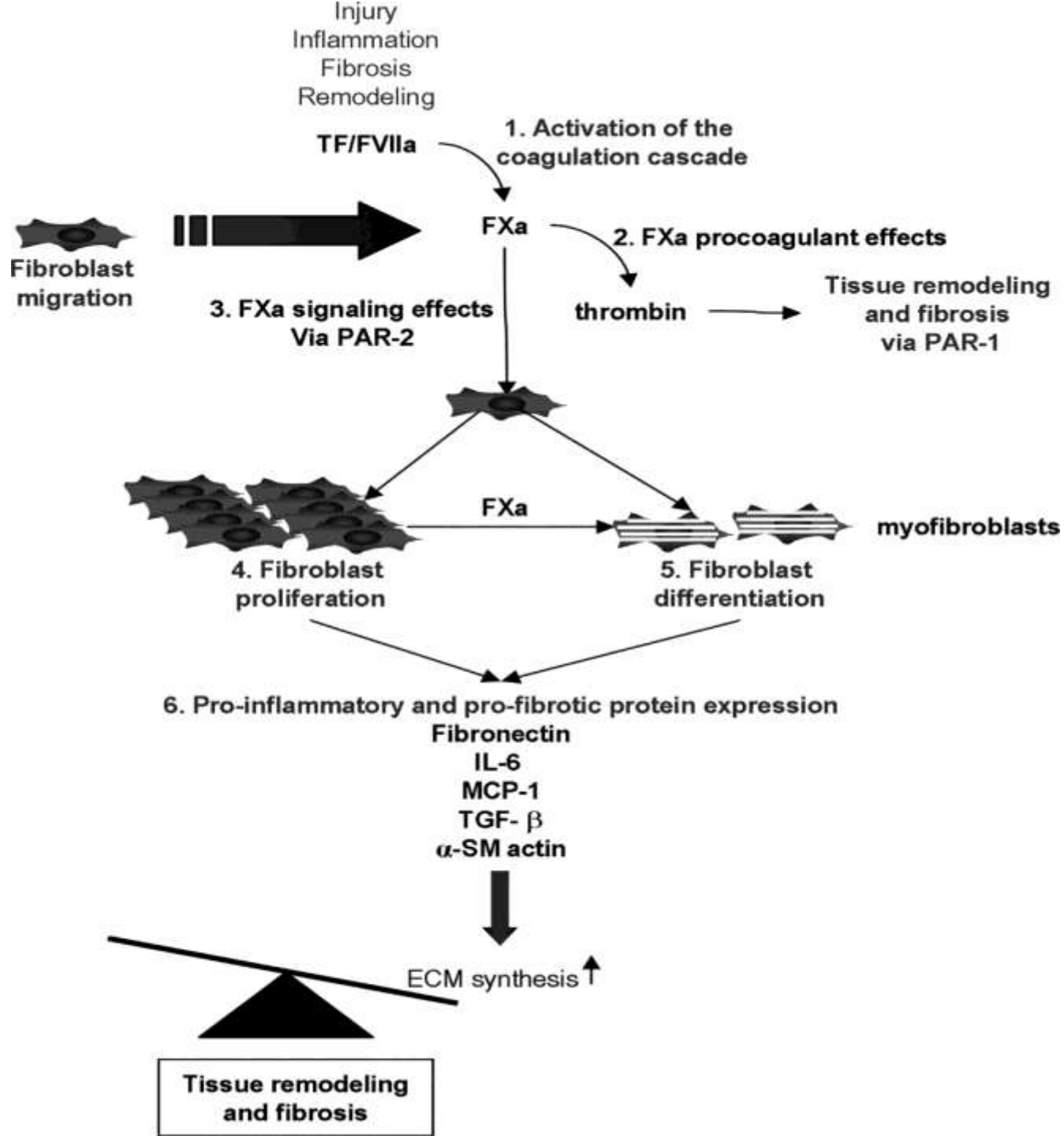
2. Coagulation pathway

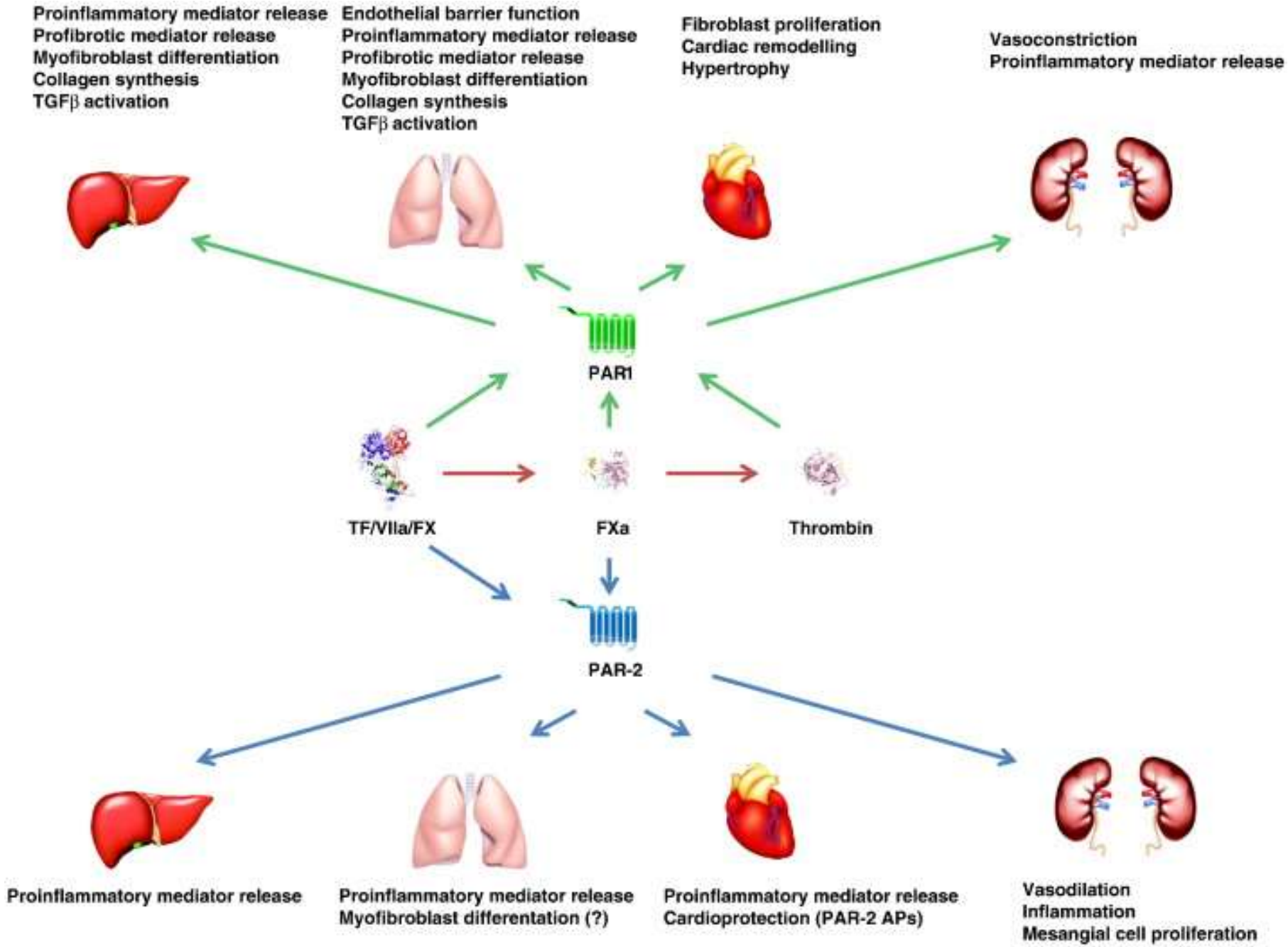
- Thrombin is a known mitogen for fibroblasts
- It also increases the production of procollagen
- Also binds with PAR (proteinase activated receptor)
- PAR are transmembrane protein which have a unique mechanism of activation when one of the part of the PAR protein gets cleaved off and acts as a Ligand for PAR
- Activation of PAR causes multiple downstream effects like inflammation, epithelial and mesenchymal cell function and TGF-β

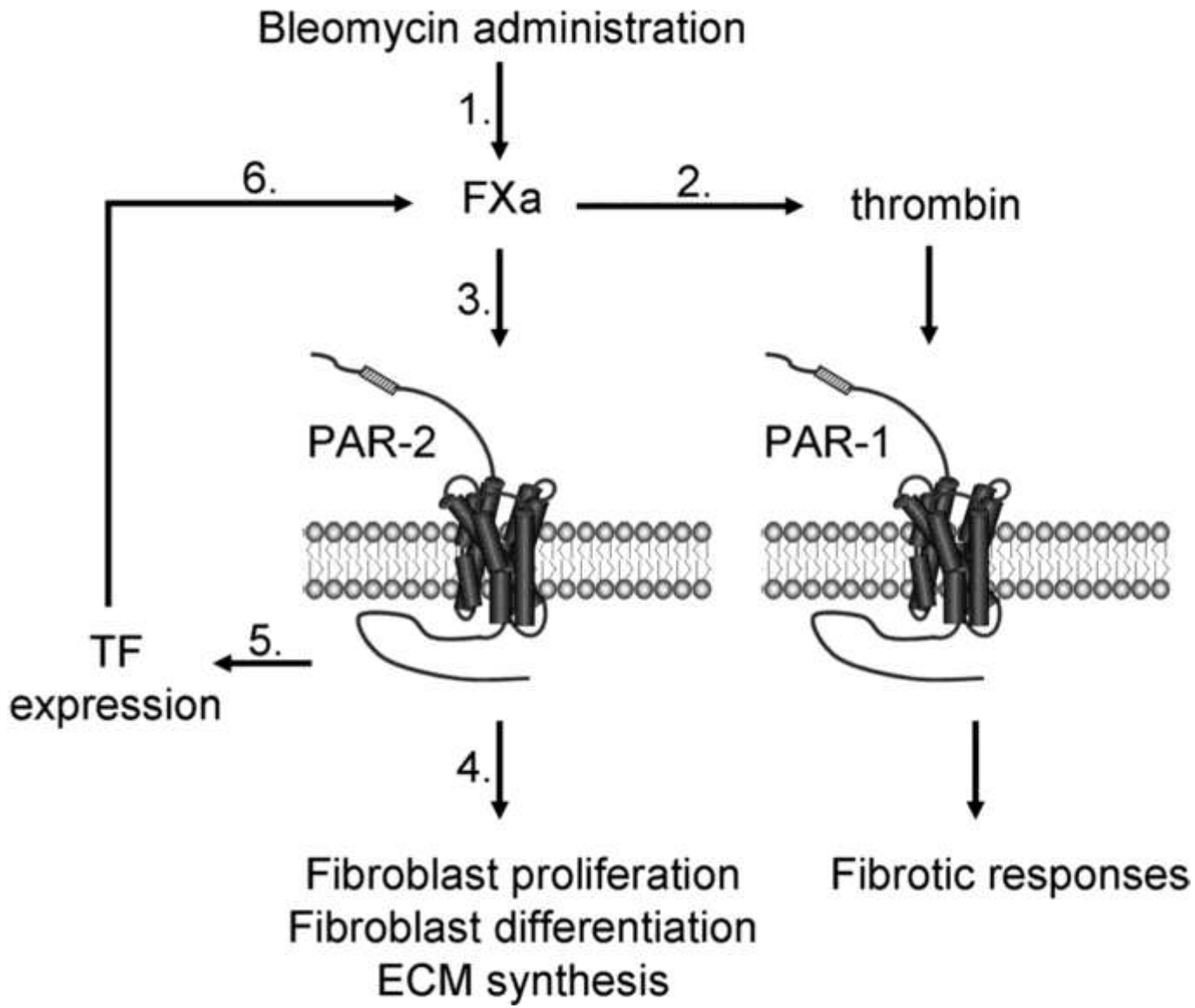
Lon et al J Thromb Haemost. 2017 Apr;15(4):597-607

- Thrombin also mediates differentiation into Myofibroblast phenotype from fibroblast
- Thrombin and PAR 1 also increased expression of PDGFβ1
- PAR2 is increased in expression by TGFβ1 and is activated by its ligand VIIa
- PAR2 has mitogenic effects on human fibroblasts
- Other coagulation factors also participate in profibrotic activities like factor Xa, Va, fibrin & VIIa

Lon et al J Thromb Haemost. 2017 Apr;15(4):597-607


Injury
Inflammation
Fibrosis
Remodeling
TF/FVIIa
1. Activation of the coagulation cascade
FXa
2. FXa procoagulant effects
Fibroblast migration
3. FXa signaling effects Via PAR-2
thrombin
Tissue remodeling and fibrosis via PAR-1
FXa
myofibroblasts
4. Fibroblast proliferation
5. Fibroblast differentiation
6. Pro-inflammatory and pro-fibrotic protein expression
Fibronectin
IL-6
MCP-1
TGF- β
α-SM actin
ECM synthesis
Tissue remodeling and fibrosis


Proinflammatory mediator release
Profibrotic mediator release
Myofibroblast differentiation
Collagen synthesis
TGFβ activation
Endothelial barrier function
Proinflammatory mediator release
Profibrotic mediator release
Myofibroblast differentiation
Collagen synthesis
TGFβ activation
Fibroblast proliferation
Cardiac remodelling
Hypertrophy
Vasoconstriction
Proinflammatory mediator release
PAR1
TF/VIIa/FX
FXa
Thrombin
PAR-2
Proinflammatory mediator release
Proinflammatory mediator release
Myofibroblast differentation (?)
Proinflammatory mediator release
Cardioprotection (PAR-2 APs)
Vasodilation
Inflammation
Mesangial cell proliferation


Bleomycin administration
1.
6.
FXa
2.
thrombin
3.
PAR-2
PAR-1
TF
expression
5.
4.
Fibroblast proliferation
Fibroblast differentiation
ECM synthesis
Fibrotic responses

3. Reactive Oxygen Species

- Superoxide Anions, Hydrogen Peroxide and Hydroxyl Radical
- NOX family of enzymes (NADPH oxidase)
- Protective entities: Superoxide dismutase, Glutathione, Catalase

Rochter et al Cell Tissue Res. 2016; 365(3): 591–605.

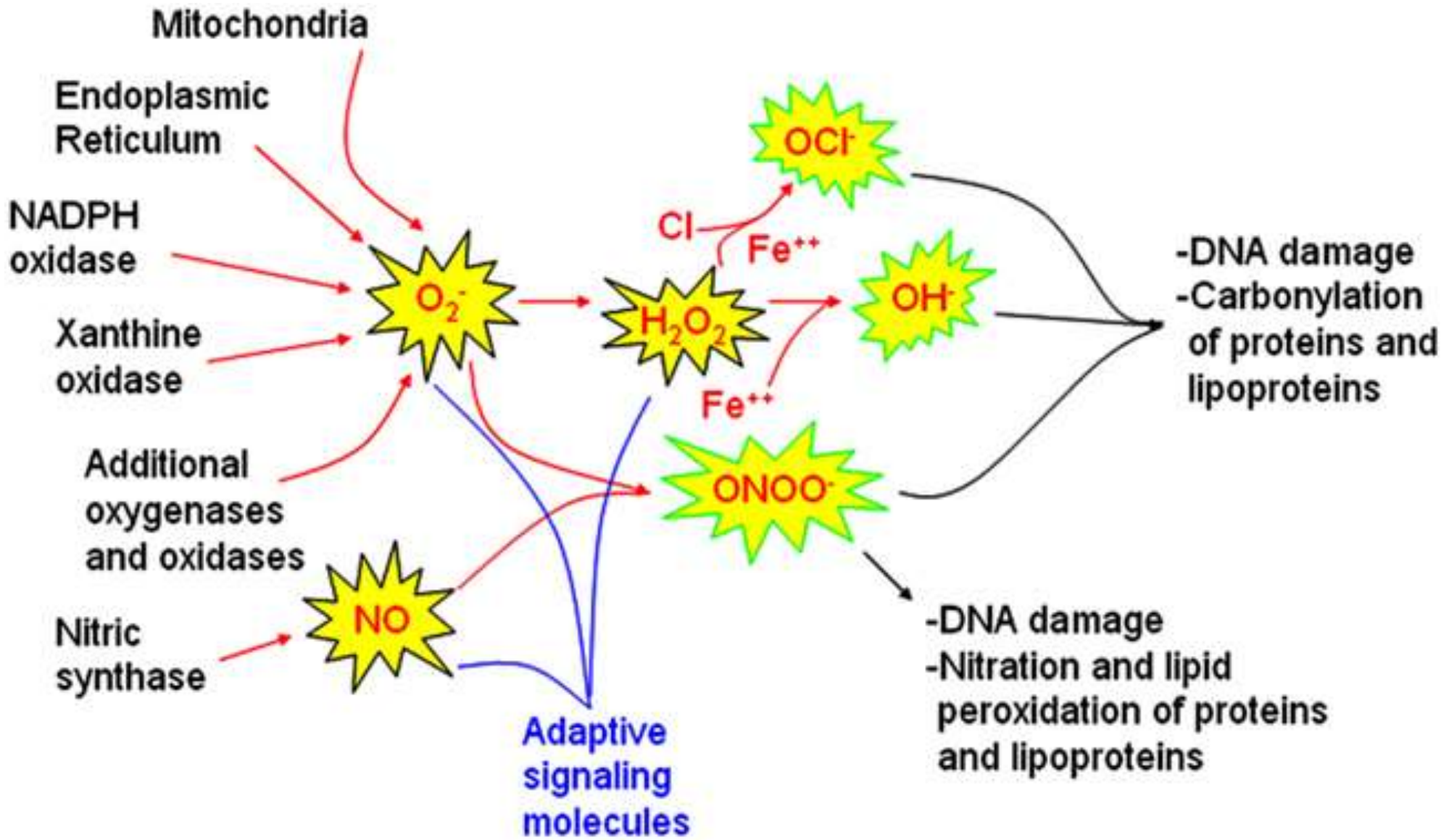
IPF observations: ROS

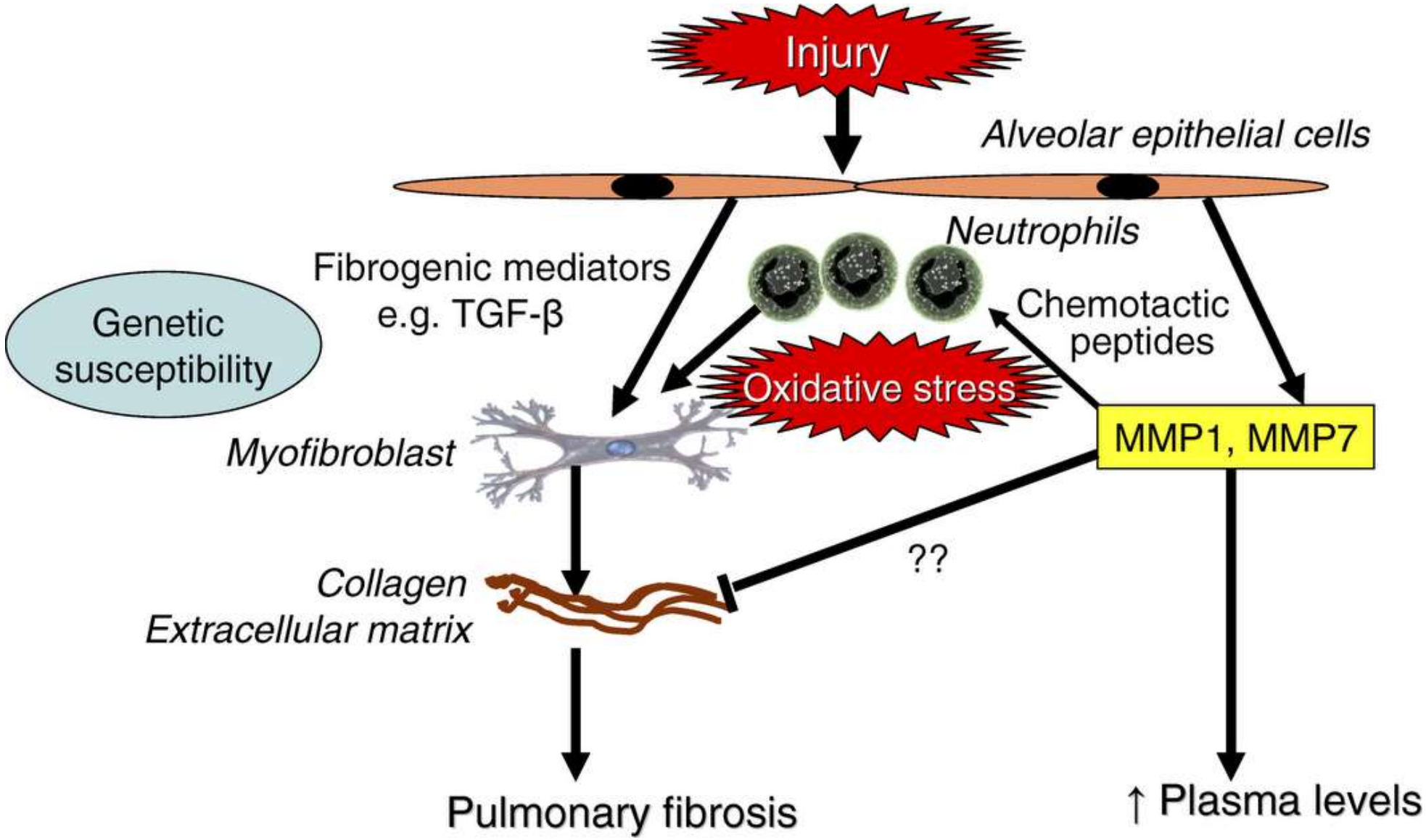
- Fibroblasts from IPF patients have been found to generate high amount of ROS
- IPF lungs contain significantly lower level of GSH in biopsy specimen, BAL fluid and epithelial lining fluid
- Extracellular SOD was absent in fibroblastic foci of IPF patients
- NOX 4 expression is increased in fibroblastic foci
- Intraperitoneal instillation of MnTBP (metalloporpohyrin) decreased Bleomycin induced lung fibrosis in Mice models along with decrease in ROS

Rochter et al Cell Tissue Res. 2016; 365(3): 591–605.

- ROS has been shown to activate latent TGF-β and TGF-β has been shown to increase NOX4 and ROS
- Similarly inhibiting the synthesis of GSH has been implicated in increased in TGF-β levels
- TGF-β and ROS have been implicated in the EMT
- TGF-β increased expression of NOX4, superoxide anion, αSMA, CTGF and fibronectin

Rochter et al Cell Tissue Res. 2016; 365(3): 591–605.


Mitochondria
Endoplasmic Reticulum
NADPH oxidase
Xanthine oxidase
Additional oxygenases and oxidases
Nitric synthase
O_2^-
NO
H_2O_2
OCl^-
Cl
Fe^{++}
OH^-
$ONOO^-$
Fe^{++}
-DNA damage
-Carbonylation of proteins and lipoproteins
-DNA damage
-Nitration and lipid peroxidation of proteins and lipoproteins
Adaptive signaling molecules


Injury
Alveolar epithelial cells
Neutrophils
Fibrogenic mediators
e.g. TGF-β
Genetic
susceptibility
Chemotactic
peptides
Oxidative stress
MMP1, MMP7
Myofibroblast
??
Collagen
Extracellular matrix
Pulmonary fibrosis
↑ Plasma levels

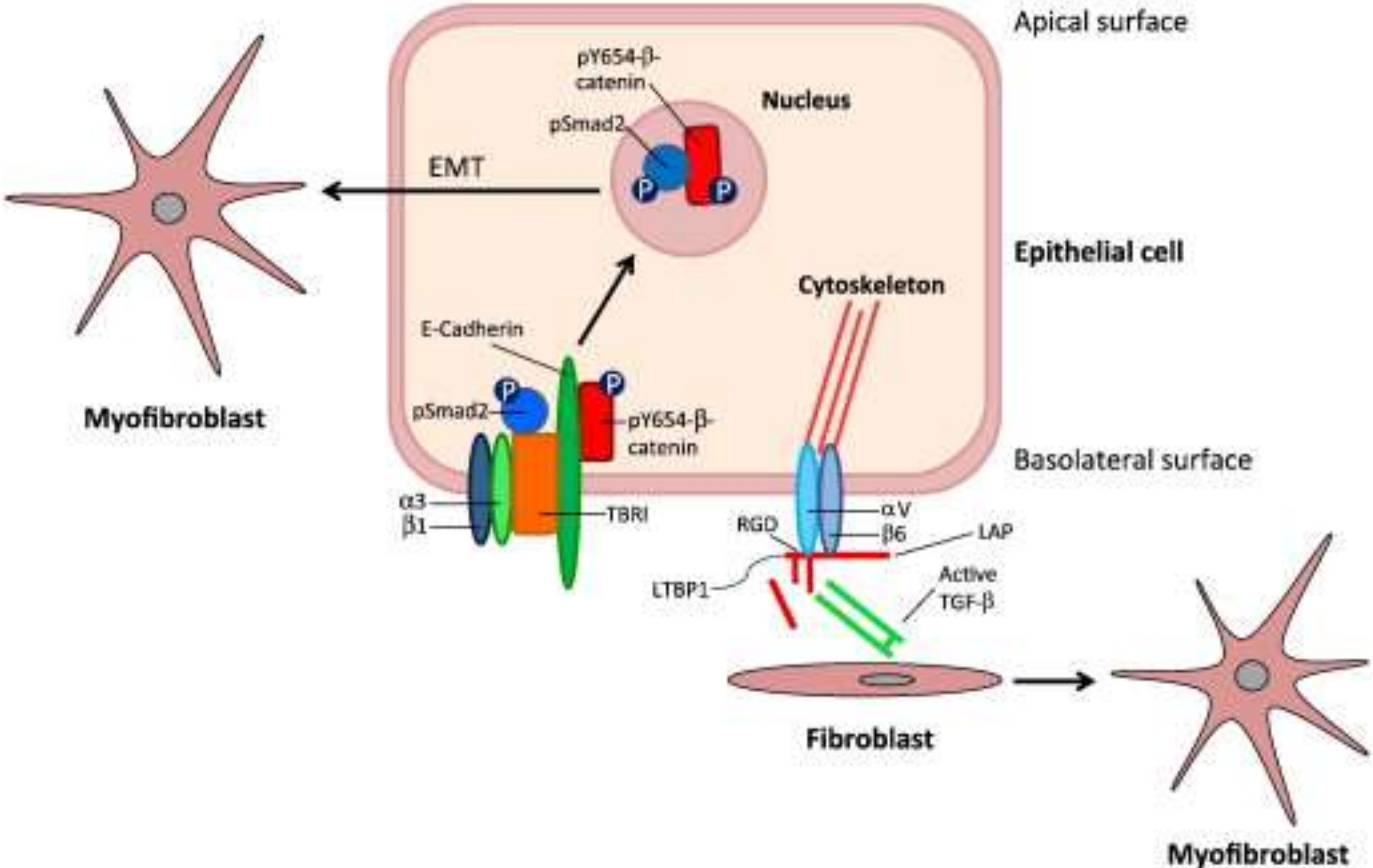
4. Role of Integrins

- Integrins are transmembrane molecules that primarily mediate cell-cell and cell- ECM adhesion
- 18 α subunits and 8 β subunits to form 24 integrins
- Integrin α*v*β6 has the ability to activate TGFβ
- Lack of the β6 subunit significantly reduces pulmonary fibrosis and β6 is upregulated in mice models of BILI
- α*v*β6 is systemic sclerosis has been associated with UIP pattern as compared to NSIP pattern

Sheppard et al European Respiratory Review 2008 17: 157-162

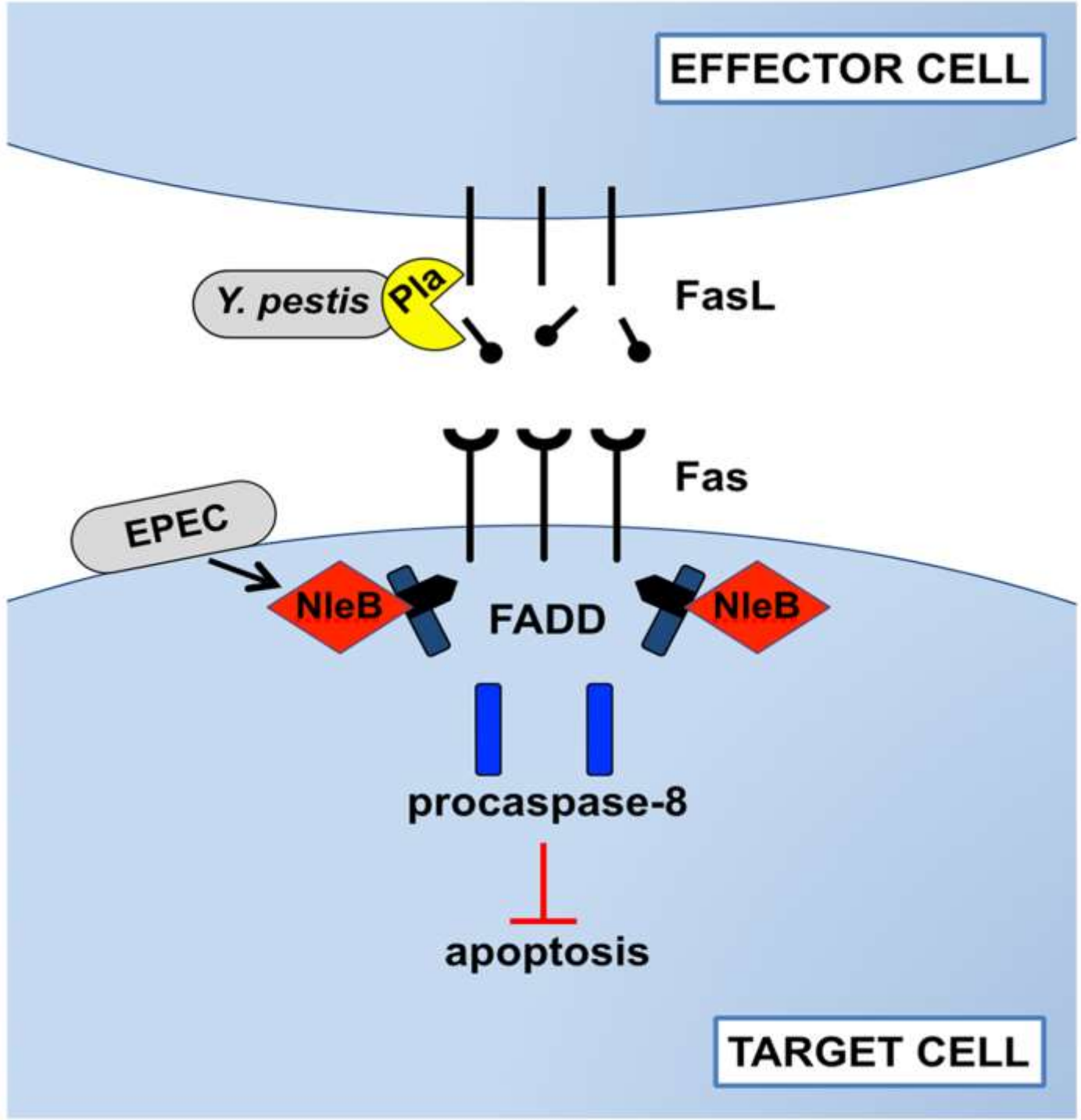
- α*3*β1 integrin acts as a laminin receptor and is protective against fibrosis
- Loss of α3 subunit expression is associated with inability to form βcatenin/Smad 2 complexes which have been implicated in EMT

Sheppard et al European Respiratory Review 2008 17: 157-162


Apical surface
pY654-β-catenin
Nucleus
pSmad2
EMT
Epithelial cell
Cytoskeleton
E-Cadherin
pSmad2
pY654-β-catenin
Basolateral surface
α3
β1
TBRI
RGD
αV
β6
LAP
LTBP1
Active TGF-β
Myofibroblast
Fibroblast
Myofibroblast

5. *Fas-FasL*

- Bronchiolar and alveolar epithelial cell apoptosis is consistent finding in BILI model
- Genes related to this pathway are upregulated
- Agonistic antibody use had been shown to increase apoptosis, inflammation, collagen deposition and upregulation of TGFβ
- Mice deficient in *Fas* or *FasL* have substantially reduced tissue inflammatory cells, apoptosis and fibrosis


EFFECTOR CELL
Y. pestis
Pla
FasL
Fas
EPEC
NleB
FADD
NleB
procaspase-8
apoptosis
TARGET CELL

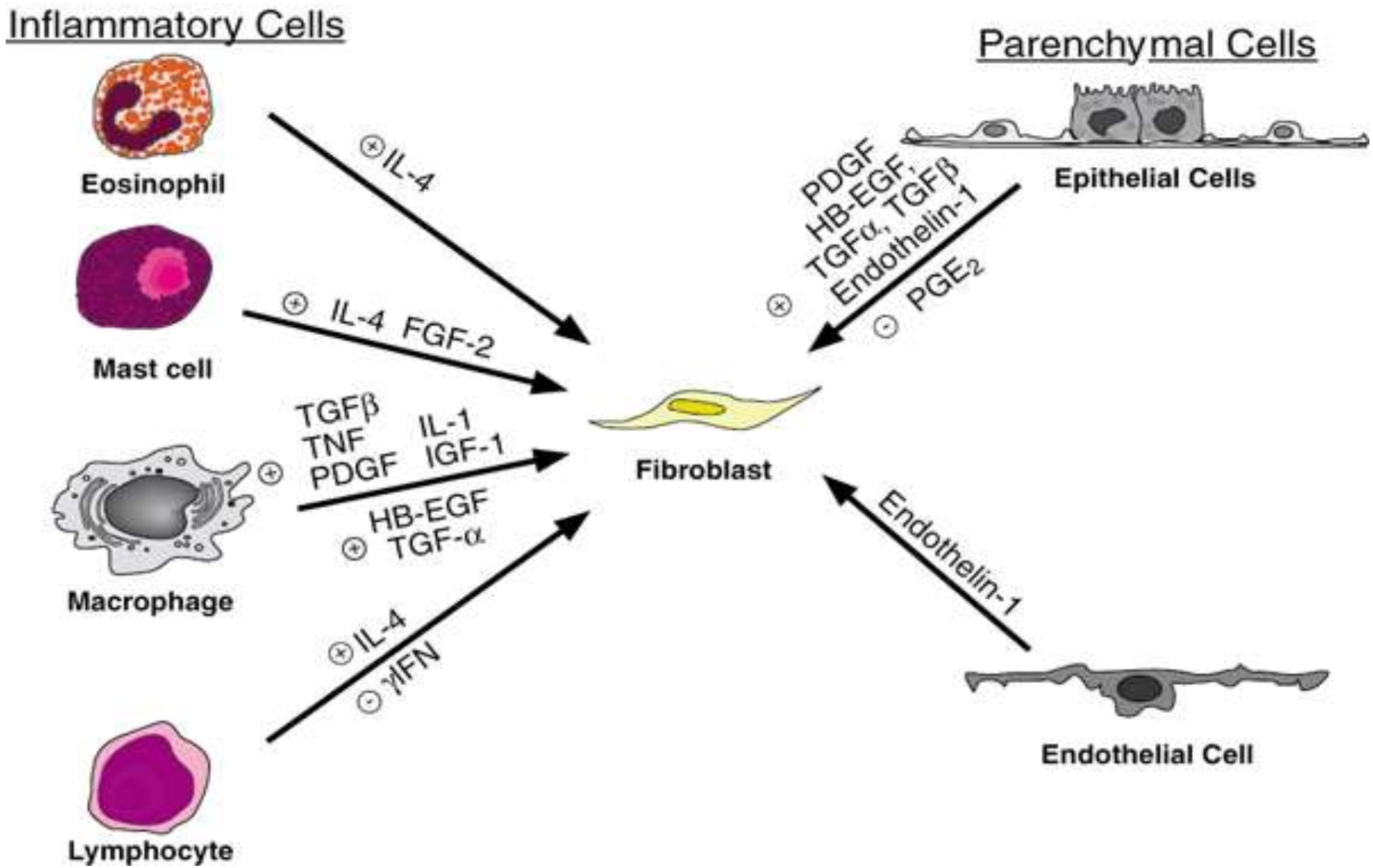
6. Inflammation & immune mechanism

- Histologic analysis showed varied amount of lymphocytes, macrophages and neutrophils in SLB specimens
- Inflammation related cytokines are also increased

Against

- Anti-inflammatory therapies have been proved to be uniformly ineffective
- Upcoming hypothesis or injury and repair of epithelial cells

Pulmonary Fibrosis



Role of cytokines

- TGF-β: major prothrombotic growth factor
  - Stimulates fibroblast ECM production
  - EMT differentiation
  - Production of reactive oxygen species
  - Myofibroblast differentiation

- PDGF: fibroblast proliferation and chemotaxis
  - Overexpression in vivo
  - Inhibition reduces fibrosis
  - Upregulated in human fibrotic diseases

- IFN-γ: proinflammatory cytokine
  - Enhances fibroblast apoptosis
  - Inhibits ECM production in vivo
  - In vivo models of Bleomycin induced lung fibrosis have shown to decrease fibrosis

- IL-1β and TNF- α: stimulate fibroblast proliferation and chemotaxis
  - Upregulated in Bleomycin model
  - Overexpression induces inflammation and subsequent fibrosis

- IL-17: proinflammatory cytokine
  - Upregulated in bleomycin model
  - Exogenous administration induces fibrosis
  - Fibrosis likely mediated through TGF-β

- Oncostatin M:
  - In fibroblasts stimulates proliferation and ECM production
  - Inhibits apoptosis
  - Overexpression and administration induces fibrosis
  - Works independent of TGF-β

- IL 10: anti inflammatory cytokine
  - Inhibits fibroblast proliferation
  - Upregulation is protective in bleomycin model of fibrosis

Chemokines

- CCL 2: pro-inflammatory
  - Stimulates fibroblasts for ECM production
  - Inhibits apoptosis
  - Inhibition in animal models reduces fibrosis
  - Recruits BM derived Fibrocytes in lung

- CXCL 12:
  - Upregulated in bleomycin model
  - Recruits fibrocytes into the lung
  - Increased in BAL fluid of IPF patients
  - Inversely correlates with physiological parameters

Conclusion

- Multiple pathways
- Inter-correlation of cytokines
- Inhibition of single cytokines has not been proved to reduce fibrosis
- Small molecules like serotonin, endothelin, leptin and angiotensin have also been studied but their roles are still exploratory

Current therapies

Pirfenidone

- Pirfenidone is an antifibrotic agent that inhibits TGF-β stimulated collagen synthesis, decreases the extracellular matrix, and blocks fibroblast proliferation in vitro.
- Anti-inflammatory
- Decreases level of TGF- β by 33%
- Suppresses over expression of pro-collagen I and III genes
- Pirfenidone inhibits pulmonary fibroblast expression of heat shock protein 47, a collagen-specific molecular chaperone

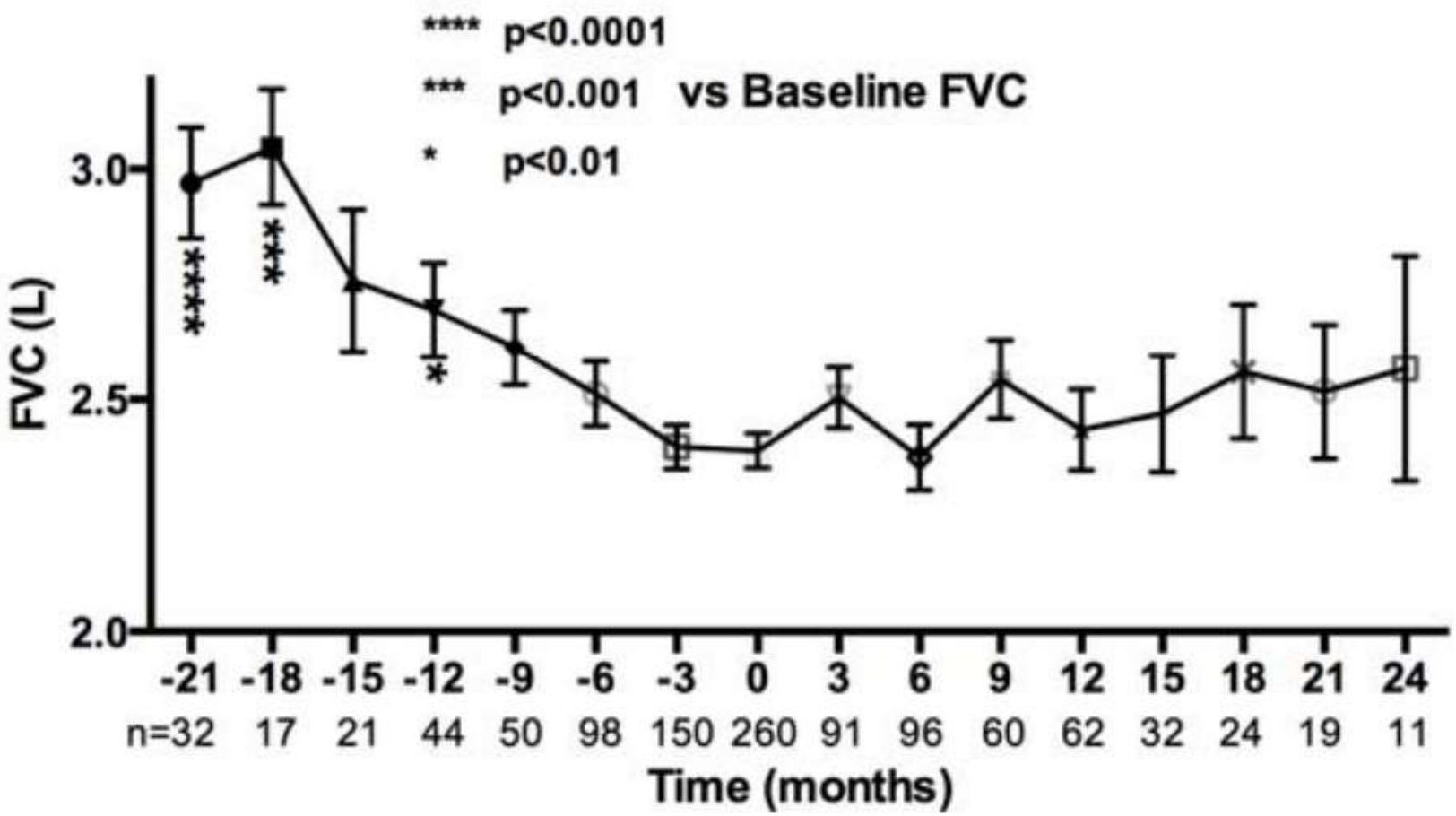
Cho et al Expert Opin Investig Drugs. 2010 Feb; 19(2): 275–283

Name	N	Follow up	Primary end point	Result
CAPACITY 004 (2:1:2)	435	52w	Change in FVC	Pirfenidone reduced decline in FVC (p=0·001)
CAPACITY 006 (1:1)	344	72w	Change in FVC	Difference between groups in FVC change at week 72 was not significant (p=0·501)
ASCEND	555	52w	Change in FVC	P<0.001 for FVC change at week 52 P<0.001 for progression-free survival P=0.04 for 6-MWT distance change at week 52
Eur Respir J. 2010;35:821–829	275	52w	Change in FVC	Significant improvement in PFS and rate of decline of VC (in High dose group)

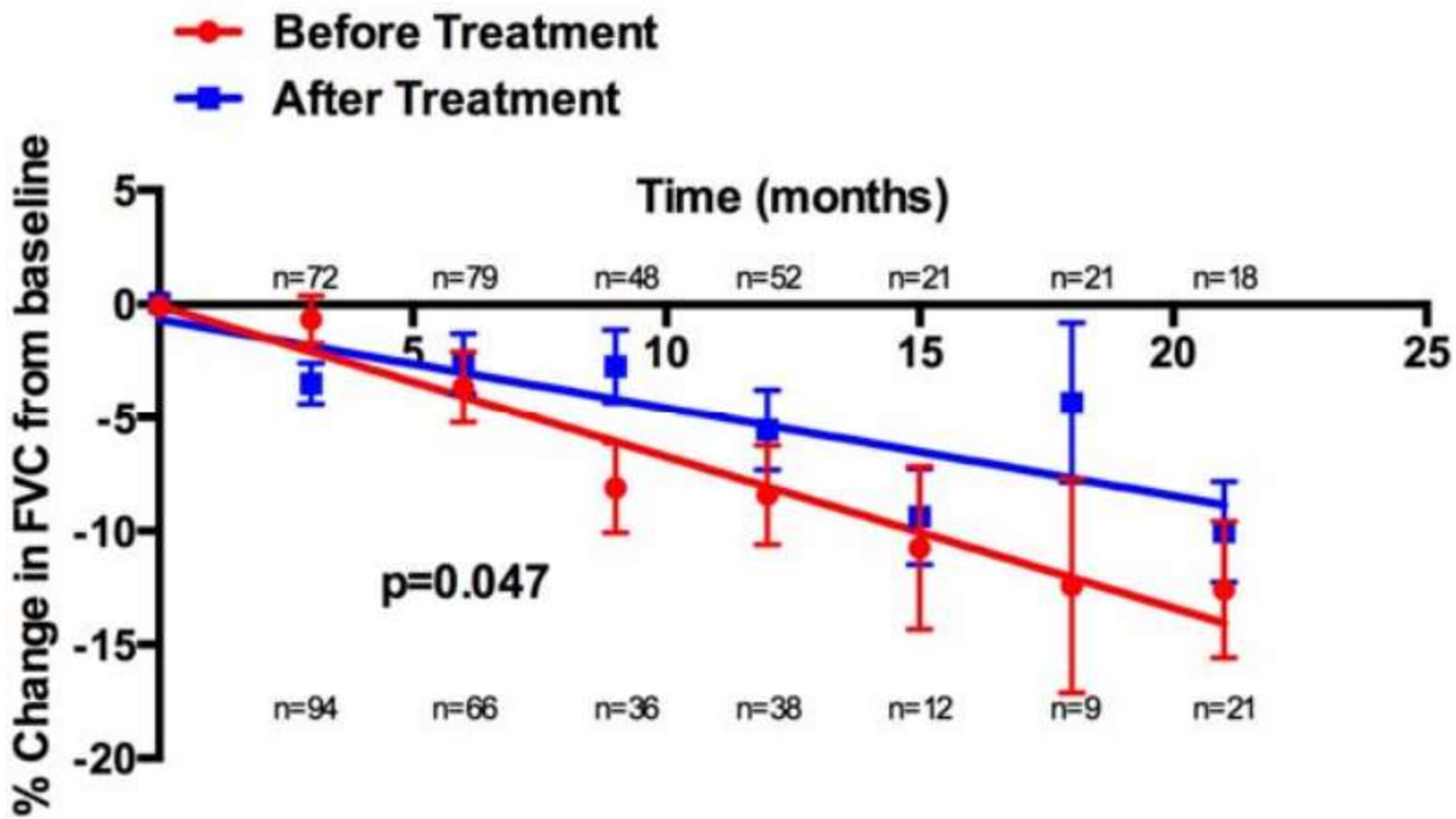
Inclusion criteria

- Diagnosis via HRCT or SLB
- Age 40–80 years
- FVC >50% but <90% pred
- DLCO >35% but <90% pred
- 6-MWT distance >150 m

Real world scenario (n=514)

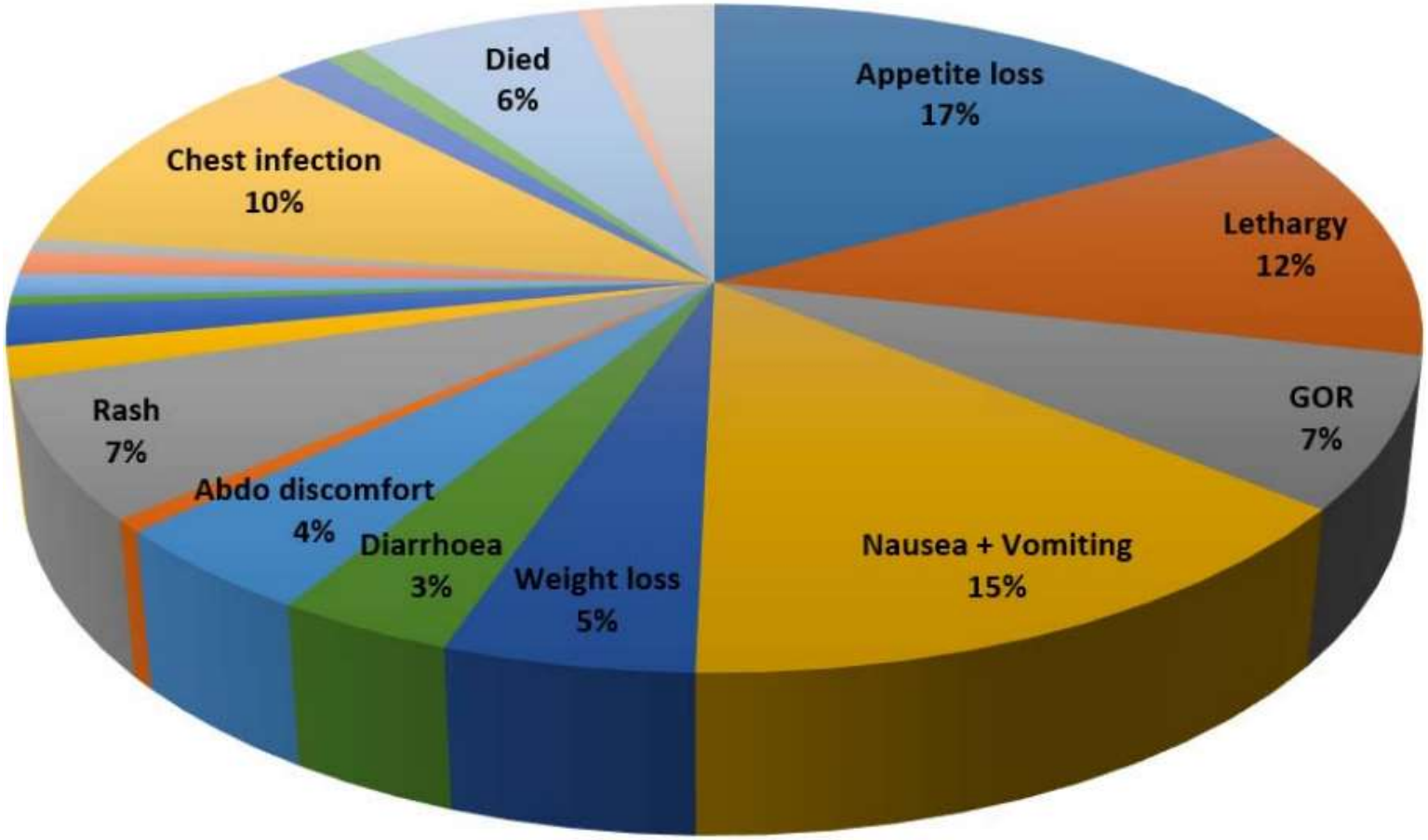


Hughes et al *J. Clin. Med.* 2016, *5*(9), 78;



Hughes et al *J. Clin. Med.* 2016, *5*(9), 78;

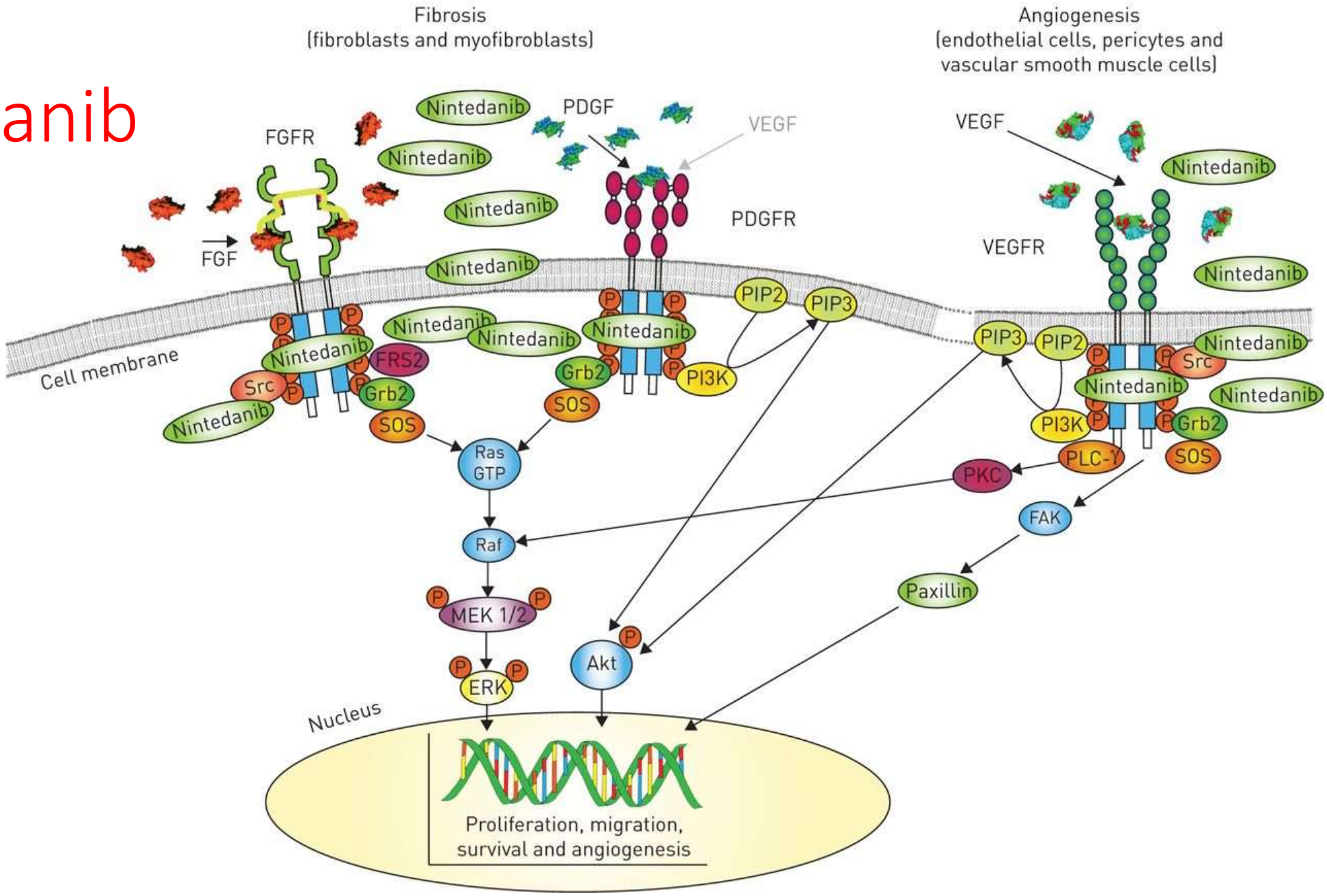
Adverse effects



Caution

- Obtain liver function testing prior to initiation, monitor regularly
- Advise minimizing or avoiding sun exposure
- Kidney function impairment: use with caution; avoid with ESRD

Nintedanib

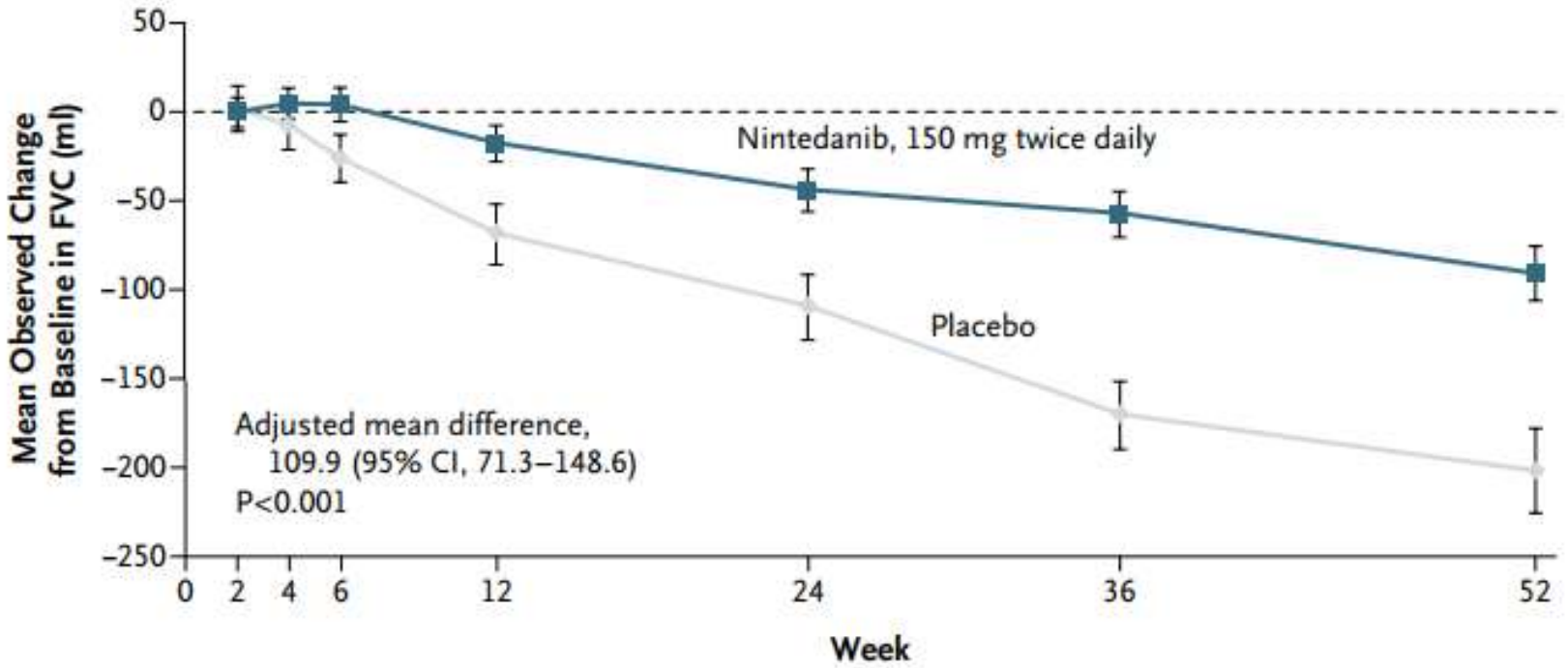

Fibrosis
(fibroblasts and myofibroblasts)
Angiogenesis
(endothelial cells, pericytes and
vascular smooth muscle cells)
Nintedanib
PDGF
VEGF
FGFR
PDGFR
VEGFR
FGF
Cell membrane
PIP2
PIP3
FRS2
Src
Grb2
SOS
PI3K
Ras
GTP
Raf
MEK 1/2
ERK
Akt
PKC
PLC-γ
FAK
Paxillin
Nucleus
Proliferation, migration,
survival and angiogenesis

Evidence

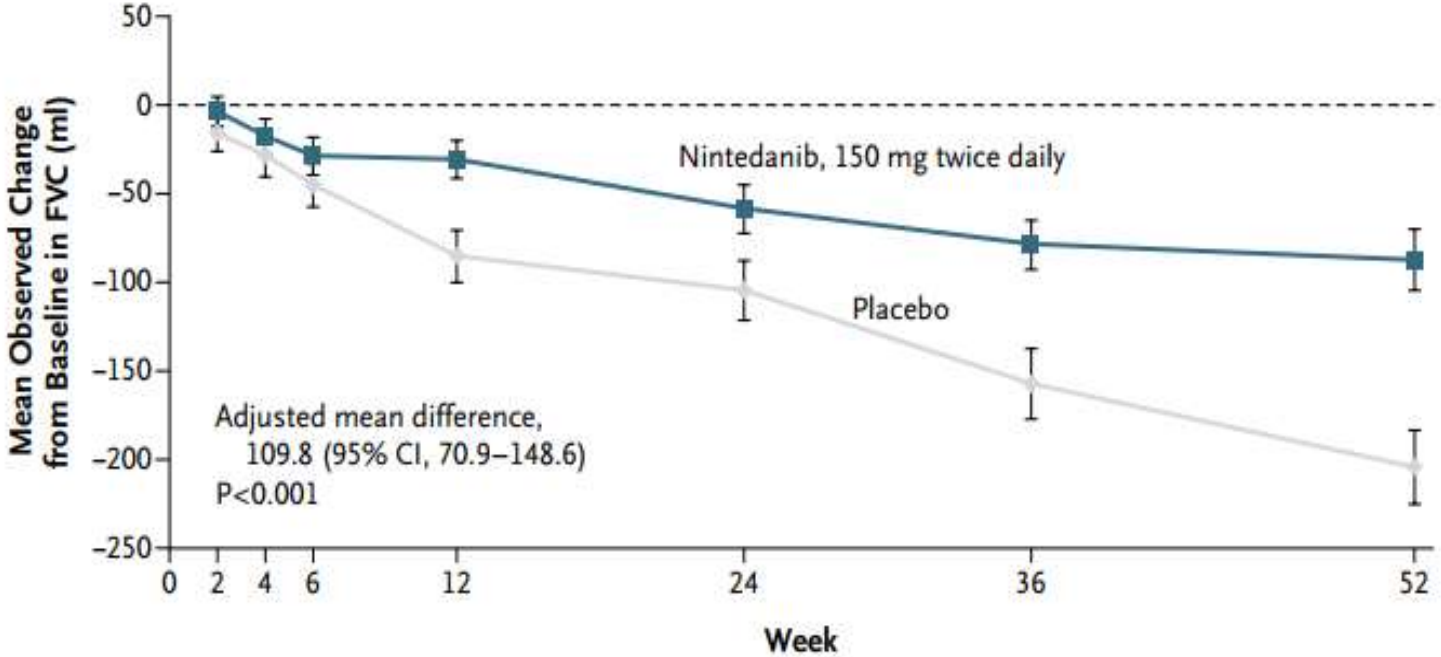
	N	Follow up	Primary end point	Comments
INPULSIS 1	515	52w	annual rate of decline in FVC	Significant improvement in the rate of decline of FVC
INPULSIS 2	551	52w	annual rate of decline in FVC	Significant improvement in the rate of decline of FVC

Racheld N Engl J Med. 2014 May 29;370(22):2071-82.

INPULSIS-1

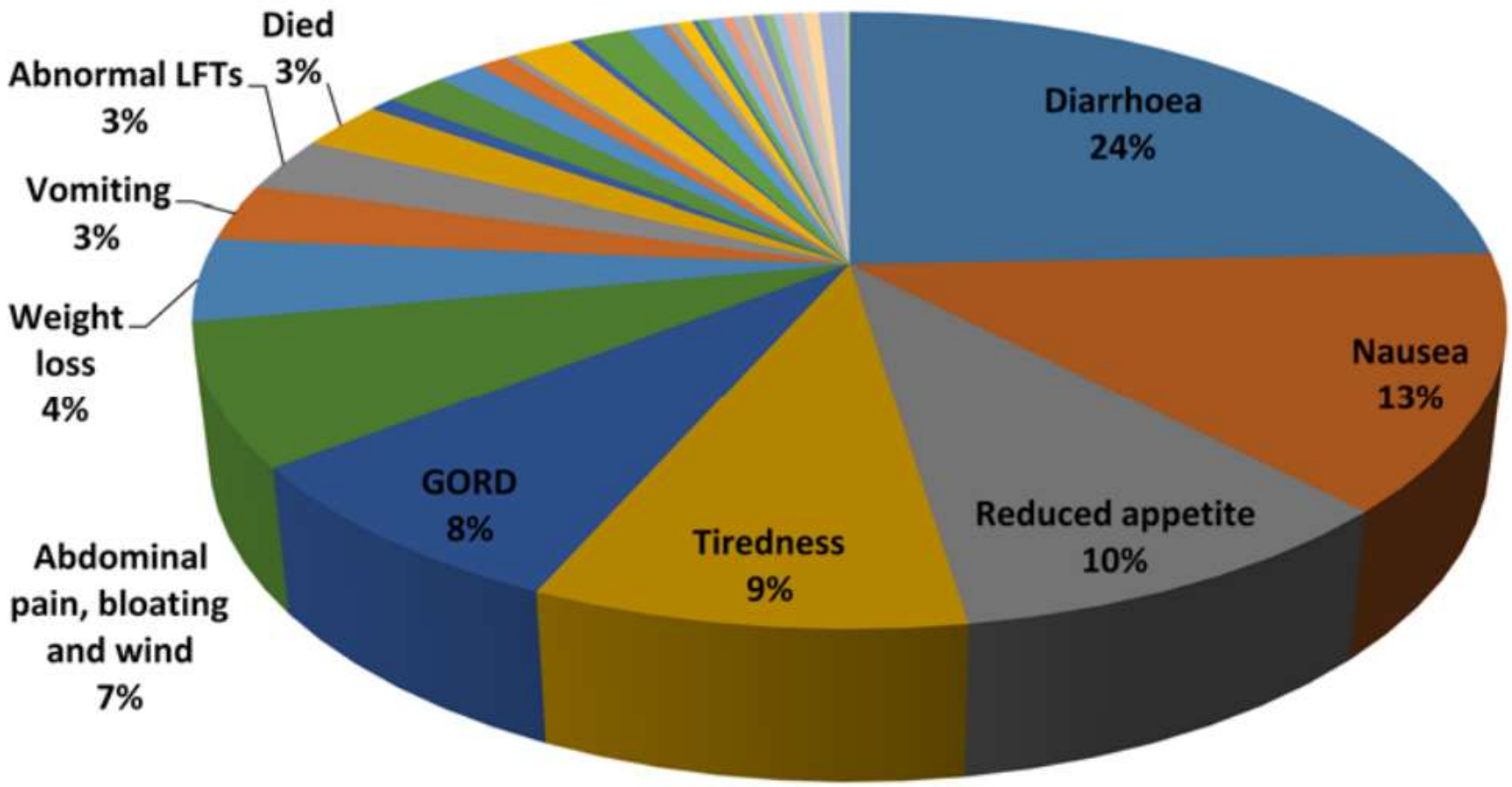


INPULSIS-2



Richeldi et al N Engl J Med. 2014 May 29;370(22):2071-82

Adverse effects Nintedanib



N-acetylcysteine

- PANTHER IPF: Harmful effect of triple therapy were highlighted (Prednisolone, azathiprine and NAC)
- Increased rate of death (8 vs. 1, P=0.01) and hospitalization (23 vs. 7, P<0.001)
- Theoretically *N*-acetylcysteine increases the synthesis of glutathione, a potent antioxidant, and decreases the fibrotic response.
- A second hypothesis is that *N*-acetylcysteine-mediated downregulation of lysyl oxidase activity alleviates bleomycin-induced pulmonary fibrosis in rats

Martnez et al N Engl J Med. 2014 May 29; 370(22): 2093–2101.

Evidence

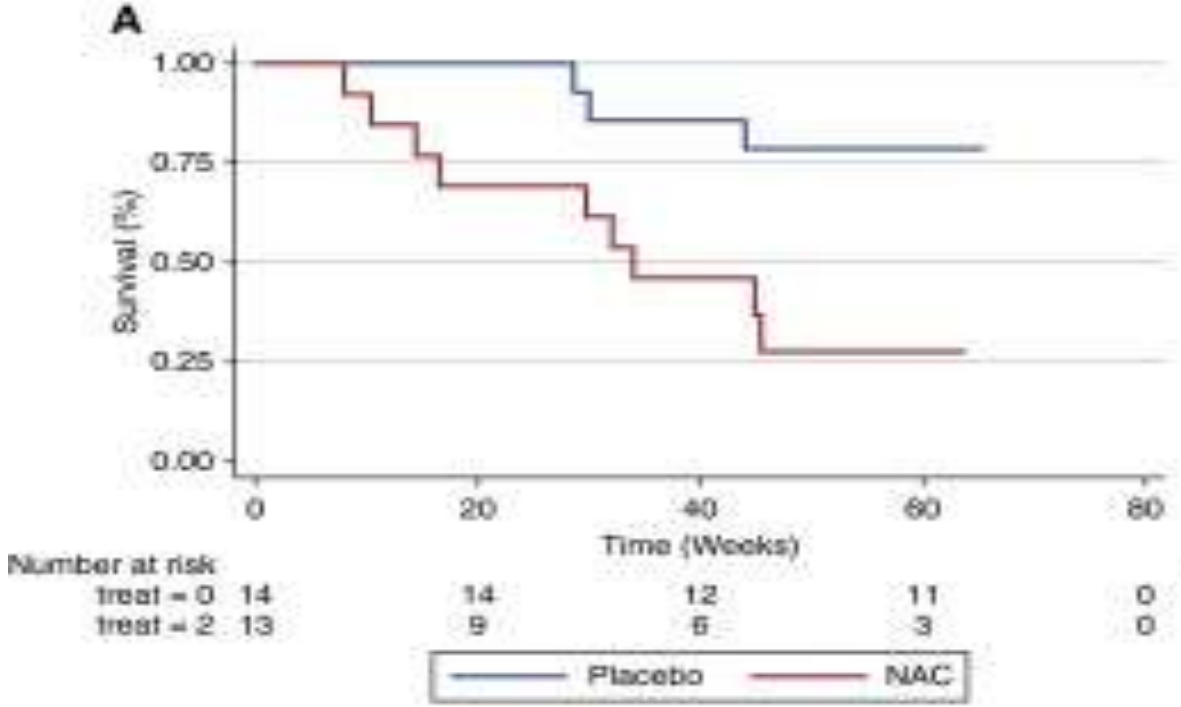
- Double blind, placebo controlled, multicenter trial
- 3 groups, triple therapy vs NAC alone Vs Placebo
- First group removed in middle of trial due to safety concerns of triple regimen
- Results: No difference in FVC decline in two groups, death or AE
- Increased Walking distance, D_{LCO} measurements and mental well-being associated with the use of NAC
- Favorable but insignificant benefits
- Similar results of PANORMA trial (Pirfenidone plus NAC)

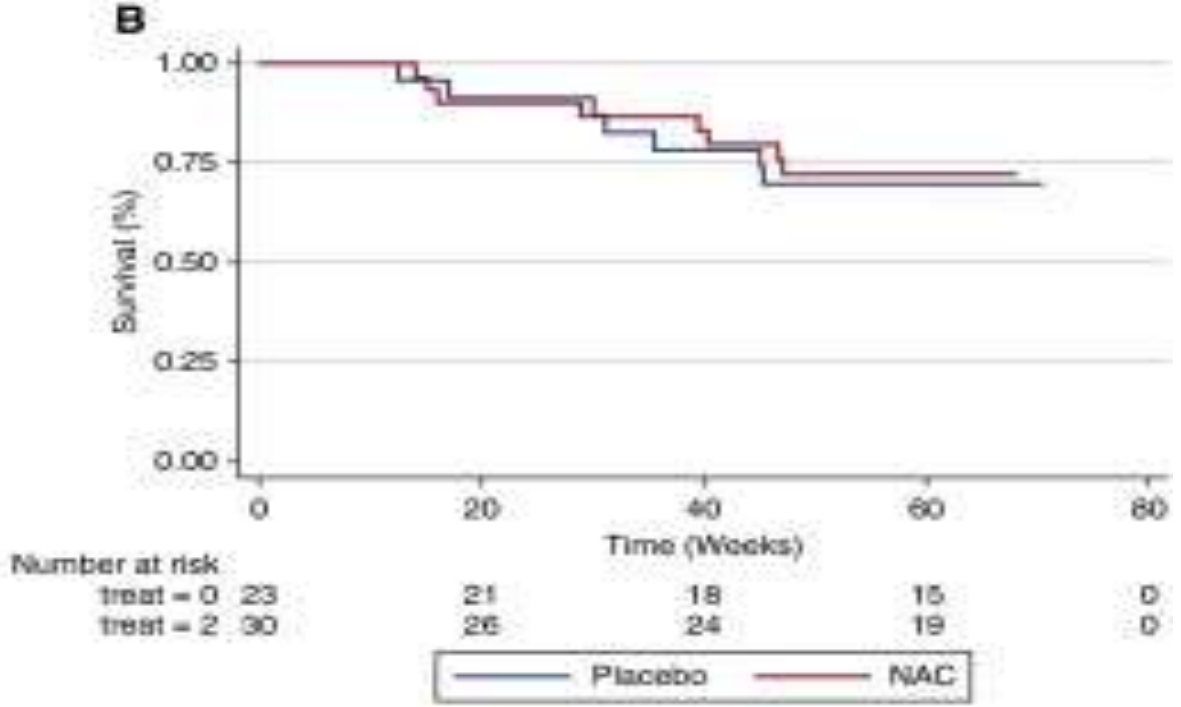
Martine et al N Engl J Med 2014; 370: 2093–2101.
Behr et al Lancet Respir Med 2016; 4: 445–453.

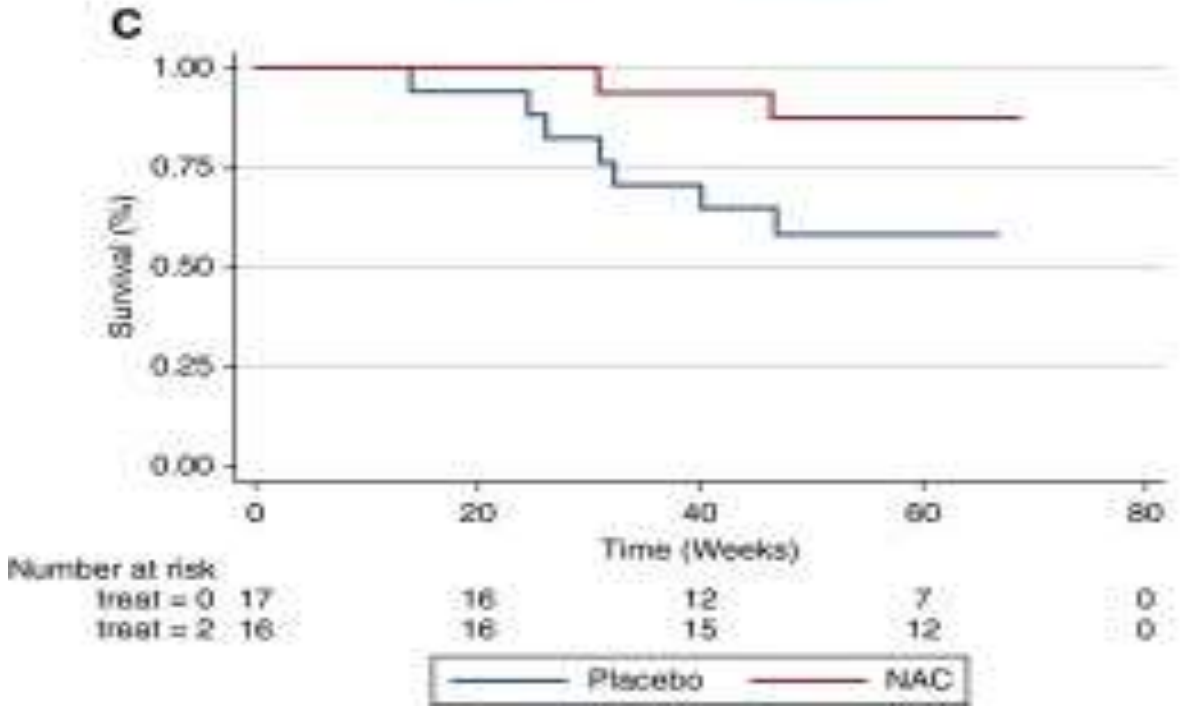
But:

- *post hoc* studies suggested potential therapeutic effects with NAC monotherapy in a subgroup of patients with genotypes for TOLLIP,
- The potential benefit of NAC monotherapy was observed in IPF patients carrying a particular TOLLIP and not a MUC5B genotype

Oldham et al Am J Respir Crit Care Med 2015 Dec 15; 192(12): 1475–1482.







Oldham et al Am J Respir Crit Care Med 2015 Dec 15; 192(12): 1475–1482.

- In pt with a CC genotype, NAC is associated with worse survival than placebo (*P* = 0.01; [HR], 3.23; 95% [CI], 0.79–13.16; *P* = 0.10).
- In those with a CT genotype (*B*), survival is similar between groups (*P*= 0.82; HR 0.76; 95% CI 0.27–2.19; *P* = 0.62).
- In those with a TT genotype (*C*), NAC therapy is associated with improved survival compared with placebo (*P*= 0.06; HR 0.14 ; 95% CI 0.02–0.83; *P* = 0.03).

Oldham et al Am J Respir Crit Care Med 2015 Dec 15; 192(12): 1475–1482.

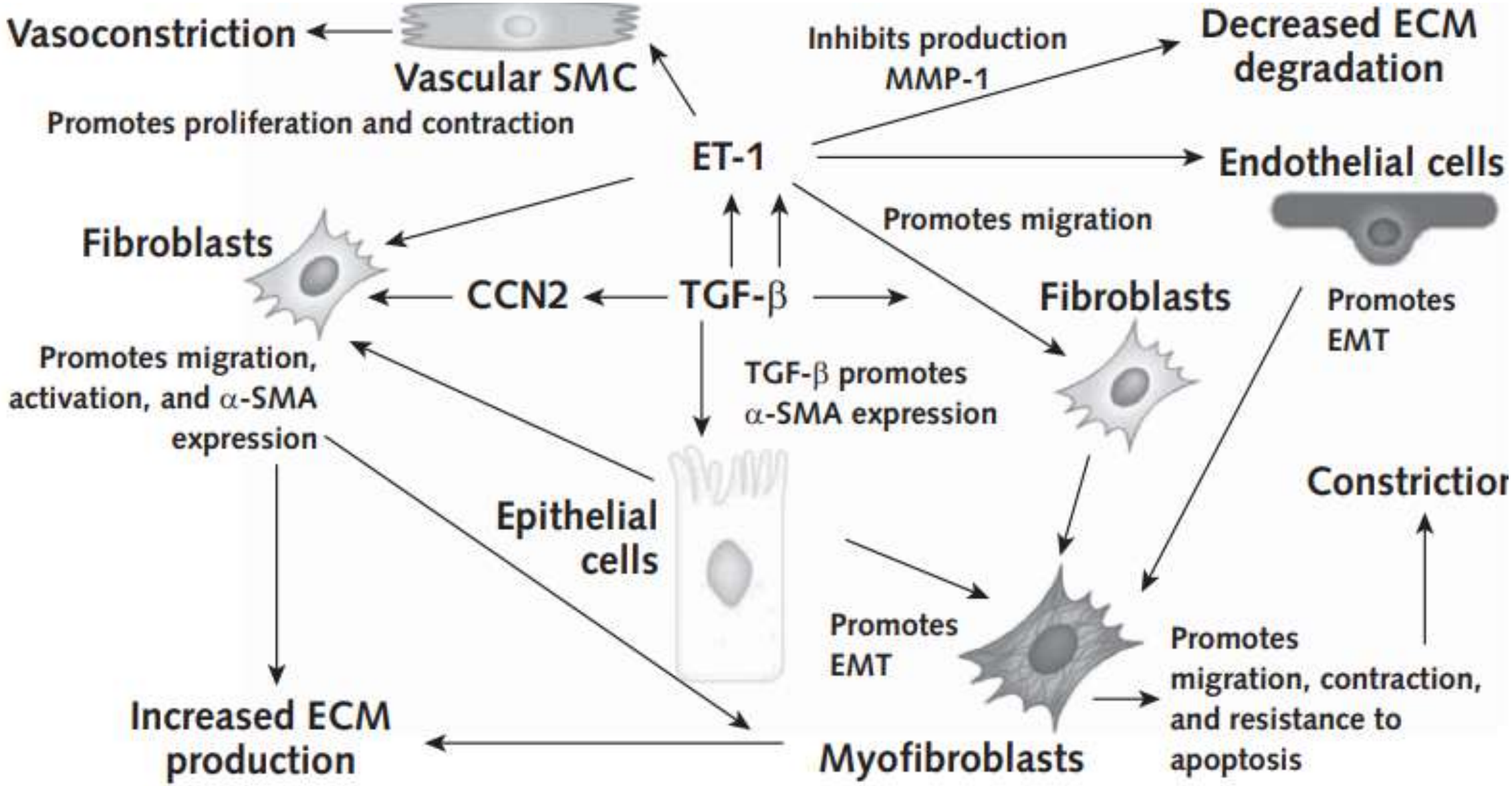
Warfarin in IPF: ACE-IPF trial

- Double-blind, Randomized, placebo-controlled trial
- Due to a low probability of benefit and an increase in mortality observed in the subjects randomized to warfarin (14 warfarin versus 3 placebo deaths; P = 0.005) an independent Board recommended stopping the study
- The mean follow-up was 28 weeks.

Noth et al Am J Respir Crit Care Med. 2012 Jul 1;186(1):88-95.

Ambrisentan in IPF

- Endothelin-1 is one of many profibrotic cytokines
- It acts in an autocrine and paracrine manner through endothelin A (ETA) and endothelin B (ETB) receptor subtypes.
- Contractile activity of activated lung fibroblasts and endothelin-1–induced lung fibroblast proliferation are blocked by ETA antagonism
- ETA receptor can also promote epithelial–mesenchymal transition via TGFβ
- Data from preclinical models suggest that antagonism of endothelin receptors may decrease the severity of pulmonary fibrosis


Vasoconstriction
Vascular SMC
Promotes proliferation and contraction
Inhibits production MMP-1
Decreased ECM degradation
ET-1
Endothelial cells
Promotes migration
Fibroblasts
CCN2
TGF-β
Fibroblasts
Promotes EMT
Promotes migration, activation, and α-SMA expression
TGF-β promotes α-SMA expression
Epithelial cells
Constriction
Promotes EMT
Promotes migration, contraction, and resistance to apoptosis
Increased ECM production
Myofibroblasts

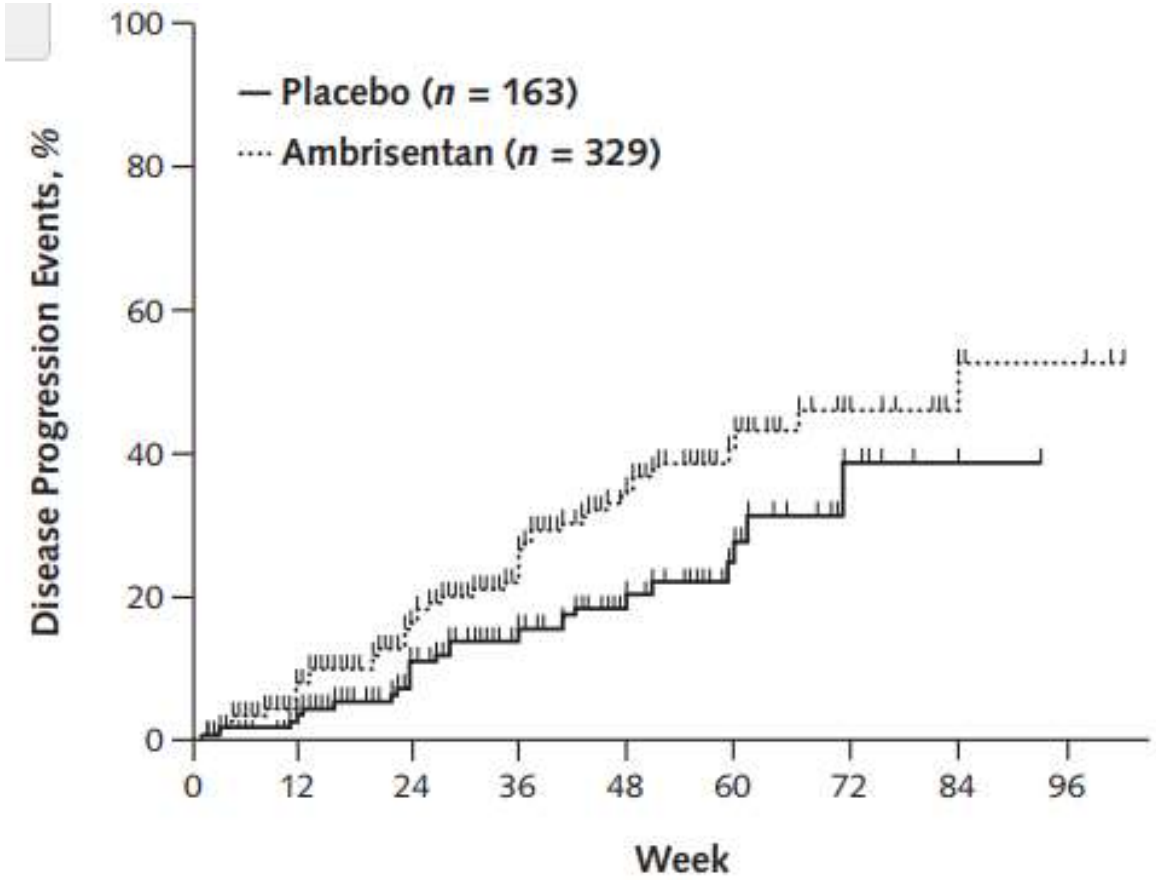
Evidence- ARTEMIS-IPF

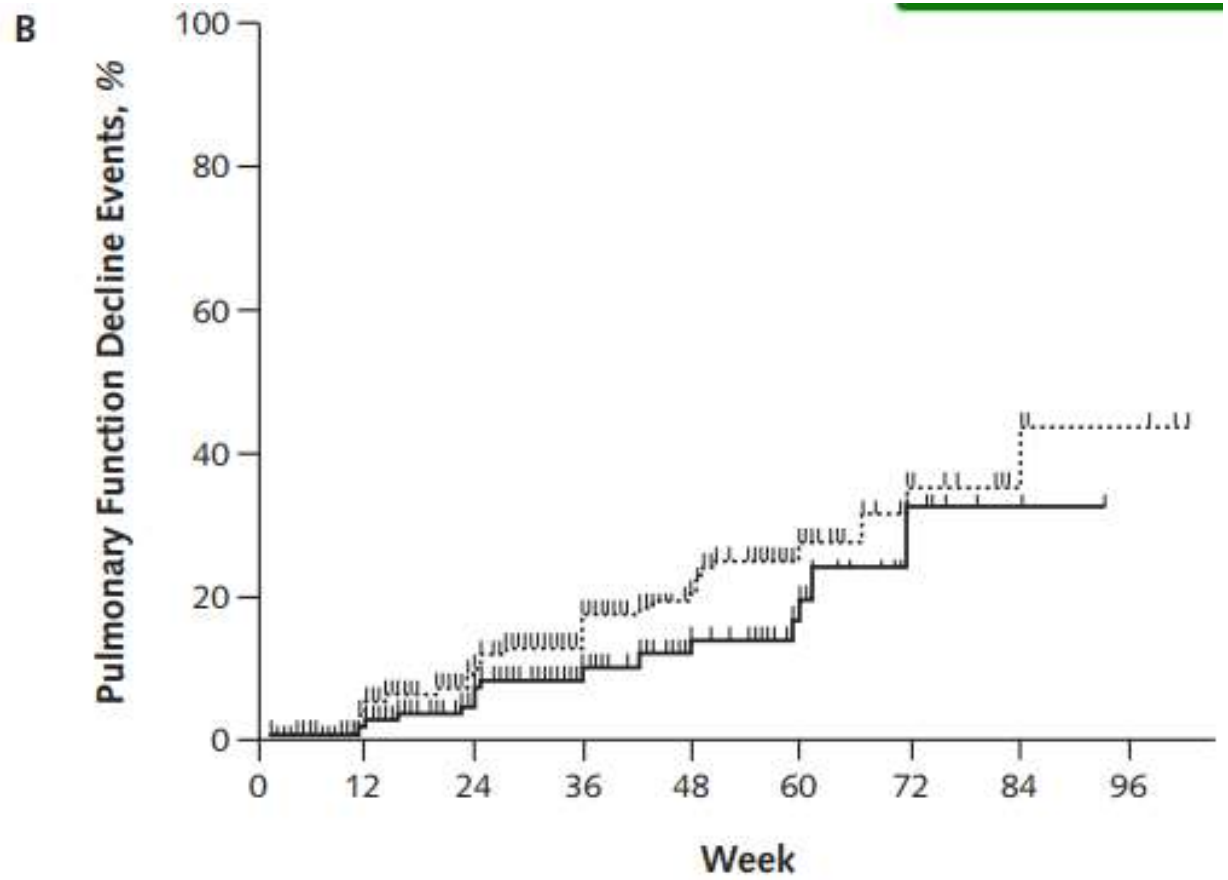
- Eligible patients
  - Aged 40 to 80 years
  - Diagnosis of IPF for at least 3 months.
  - Patients with greater than 5% honeycombing on HRCT scans were excluded

Raghu et al Ann Intern Med. 2013 May 7;158(9):641-9

Intervention

- Randomly assigned in a 2:1 ratio to receive Ambrisentan or placebo.
- Ambrisentan at 5 mg/d and titrated to 10 mg/d after 2 weeks





Raghu et al Ann Intern Med. 2013 May 7;158(9):641-9

Antacids in IPF

- The incidence of GERD is higher in patients with IPF (8–87%) compared with the general population
- This may be due to shared risk factors, including age and smoking
- Current treatment guidelines give a conditional recommendation for AAT in patients with IPF, albeit with very low confidence in estimates of effect

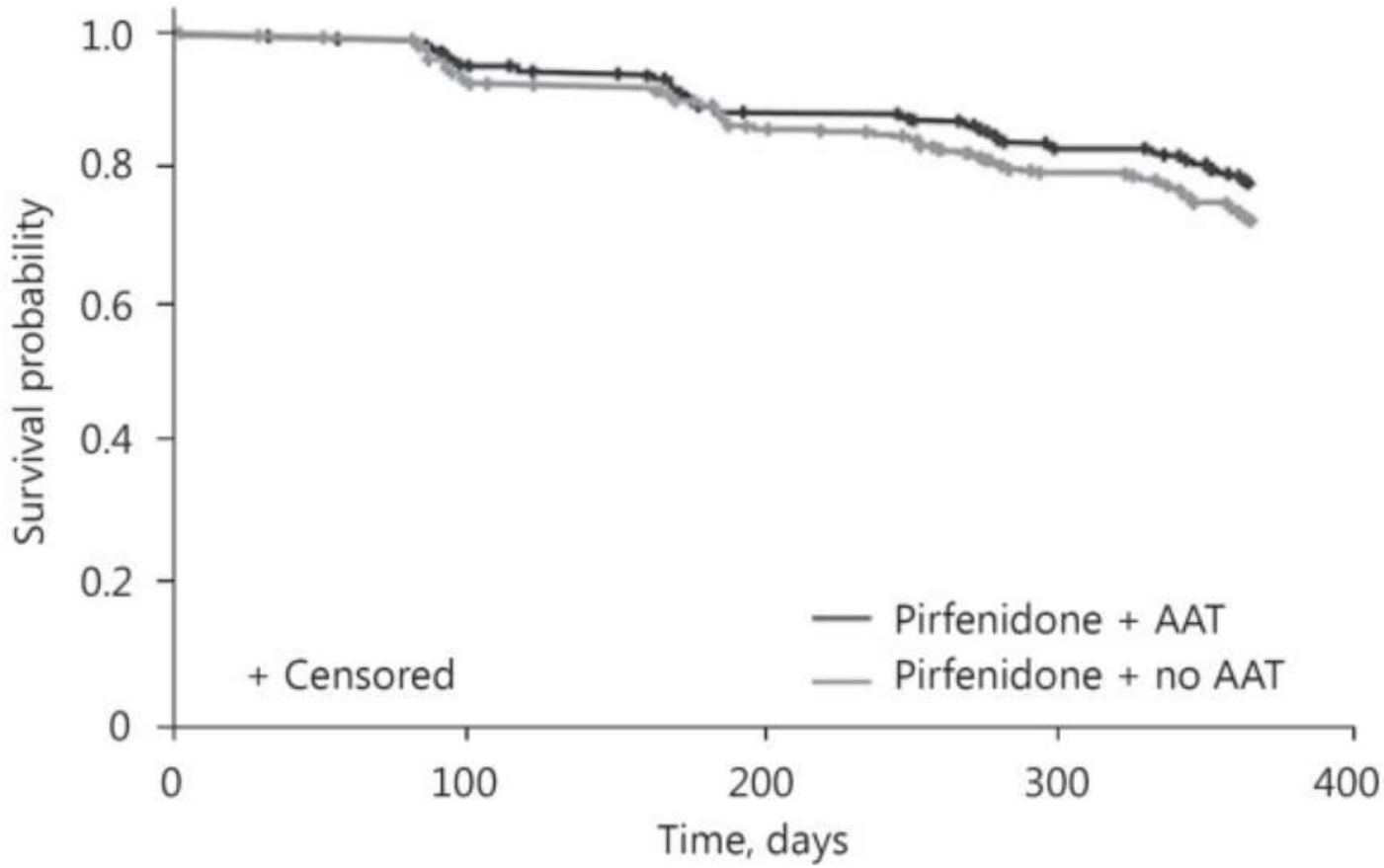
Evidence

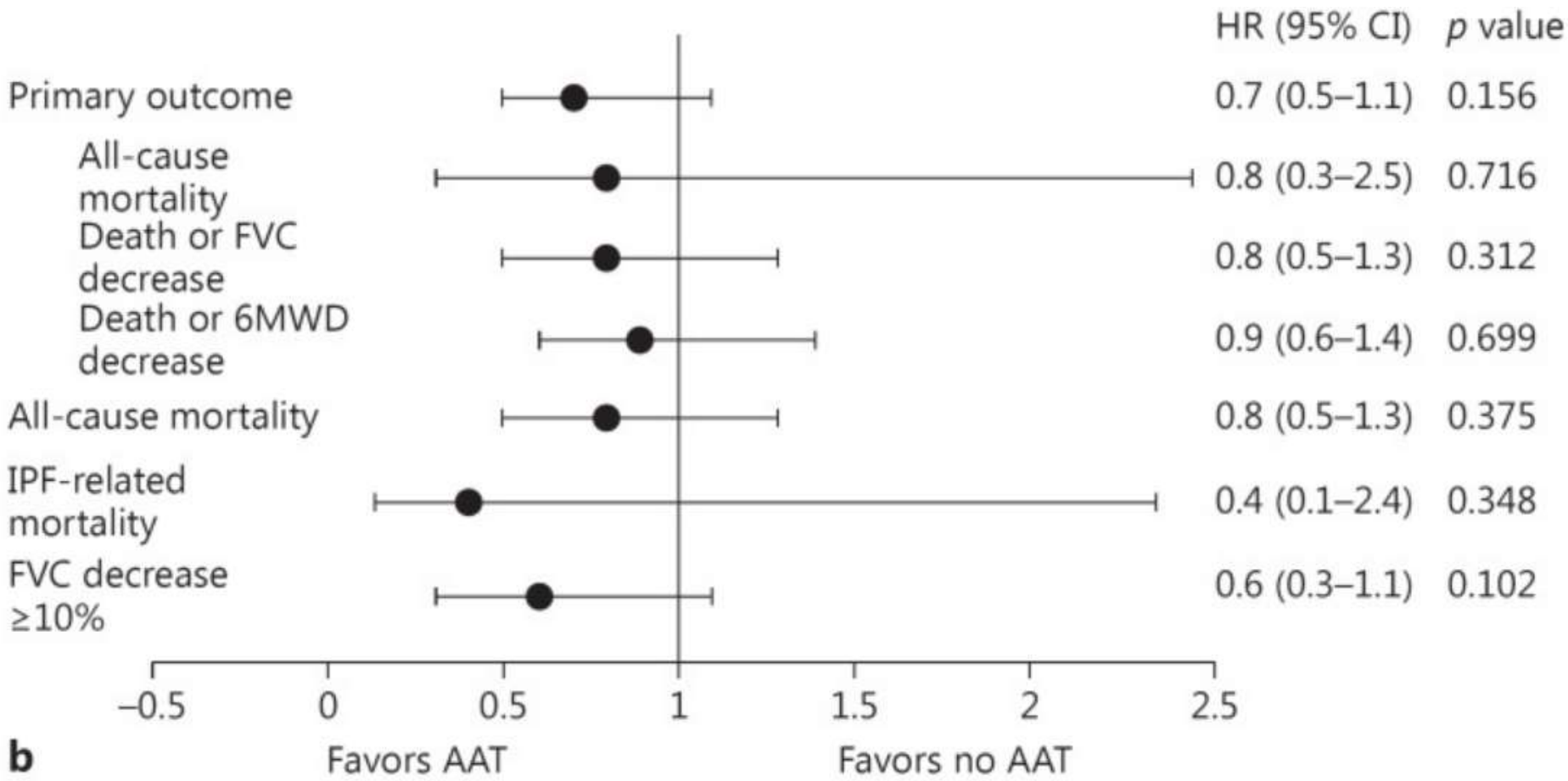
- *Post Hoc* analysis of CAPACITY and ASCEND trials
- A total of 623 patients were included in the study, of whom 273 (43.8%) received AAT and 350 (56.2%) received no AAT
- AAT users had similar mean changes in FVC from baseline to week 52 compared with non-AAT users
- Absolute and relative declines in FVC, a decrease in 6MWD of ≥50 m, and hospitalization rates after 52 weeks were similar between the two group

Keuter et al Respiration 2017 May; 93(6): 415–423.

Adverse events

- Patients who received AAT had significantly more severe GI-related AEs than those who did not (3.7 vs. 0.9%; $p = 0.015$)
- Overall, the incidence of infections was similar between the AAT and the non-AAT group (67.4 vs. 67.7%; $p = 0.934$).
- More severe pulmonary infections were observed in the AAT group than in the non-AAT group (3.7 vs. 1.1%; $p = 0.035$).


1.0
0.8
0.6
0.4
0.2
0
Survival probability
+ Censored
Pirfenidone + AAT
Pirfenidone + no AAT
0
100
200
300
400
Time, days

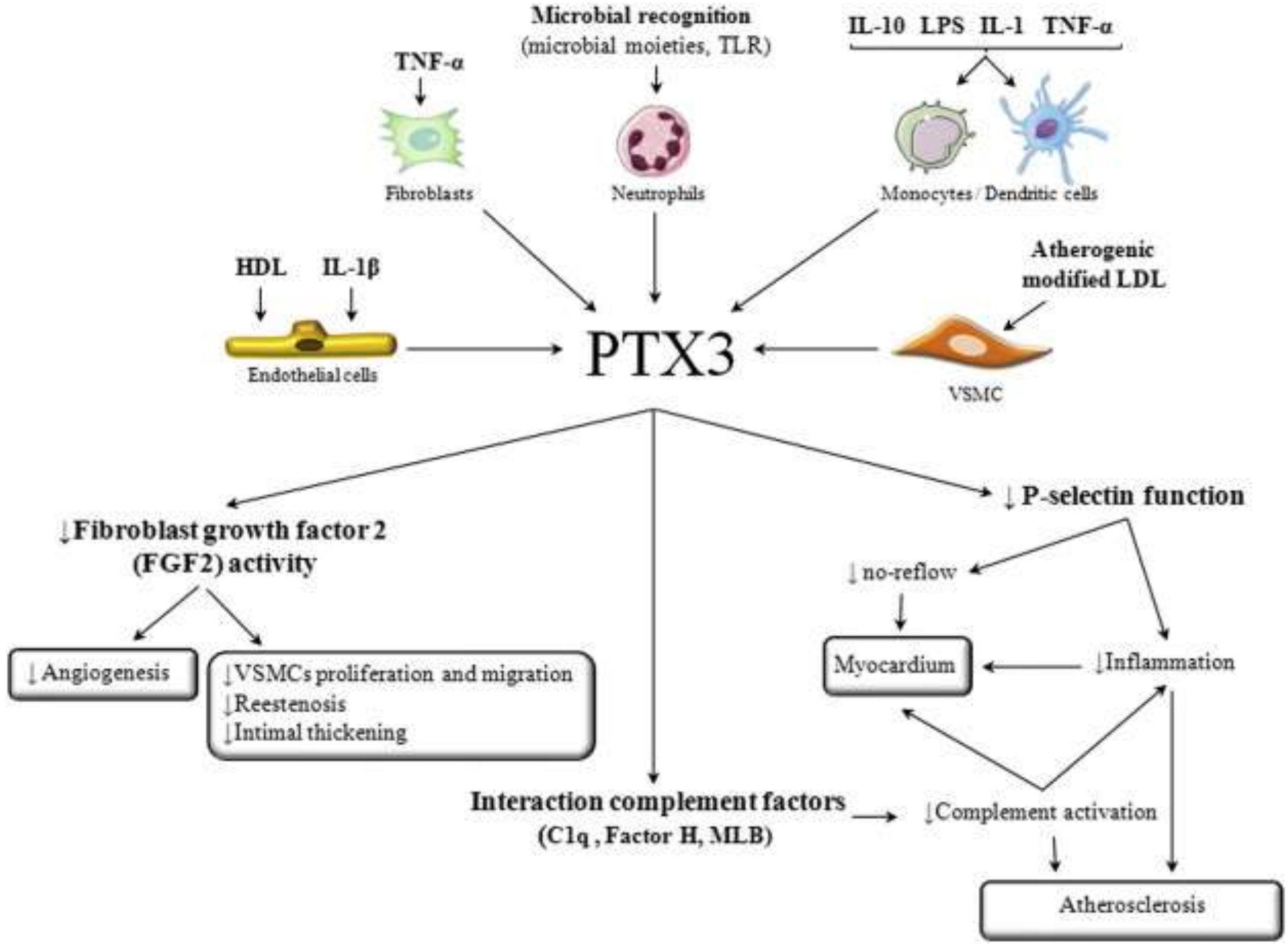

HR (95% CI)
p value
Primary outcome
0.7 (0.5–1.1)
0.156
All-cause mortality
0.8 (0.3–2.5)
0.716
Death or FVC decrease
0.8 (0.5–1.3)
0.312
Death or 6MWD decrease
0.9 (0.6–1.4)
0.699
All-cause mortality
0.8 (0.5–1.3)
0.375
IPF-related mortality
0.4 (0.1–2.4)
0.348
FVC decrease ≥10%
0.6 (0.3–1.1)
0.102
–0.5
0
0.5
1
1.5
2
2.5
Favors AAT
Favors no AAT

b

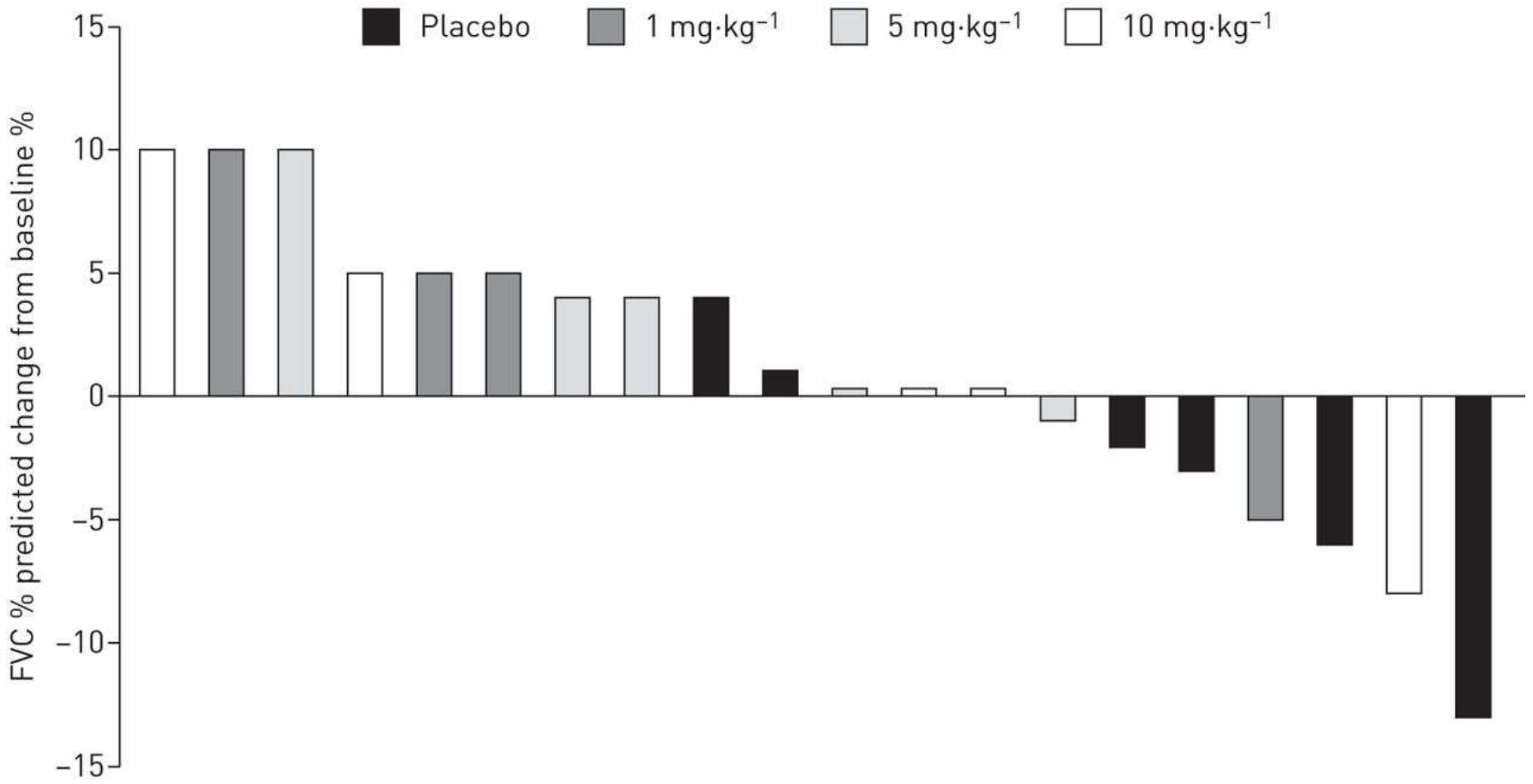
Emerging therapies

Recombinant Human Pentraxin 2

- PTX-2 has been shown to accumulate at sites of fibrosis in animal models
- PTX-2 inhibits differentiation of monocytes into pro-inflammatory and pro-fibrotic macrophages and fibrocytes
- This in turn promotes epithelial healing and resolution of inflammation and scarring.


Microbial recognition
(microbial moieties, TLR)
IL-10 LPS IL-1 TNF-α
TNF-α
Fibroblasts
Neutrophils
Monocytes / Dendritic cells
HDL
IL-1β
Atherogenic
modified LDL
Endothelial cells
PTX3
VSMC
↓Fibroblast growth factor 2
(FGF2) activity
↓ P-selectin function
↓ no-reflow
↓ Angiogenesis
↓VSMCs proliferation and migration
↓Reestenosis
↓Intimal thickening
Myocardium
↓Inflammation
Interaction complement factors
(C1q , Factor H, MLB)
↓Complement activation
Atherosclerosis

Phase II RCT: 21 patients



Blink et al Eur Respir J. 2016 Mar;47(3):889-97.

Anti- CTGF monoclonal antibody FG-3019

- FG-3019 is a fully human monoclonal antibody specific for CTGF.
- Pre-clinical studies suggest that FG-3019 penetrates into tissues to reduce effective tissue levels of CTGF resulting in reduction of pro-fibrotic factors
- In a mouse model of radiation-induced pulmonary fibrosis, administration of FG-3019 resulted in altered gene expression in the lungs, reversal of lung pathology, decrease in abnormal lung density, abrogation of fibrosis, improvement in lung function and prolonged survival

Evidence:

- Open-label, Single-arm, multicenter, phase IIa clinical trial
- 67 patients of IPF were randomized to receive either of the two doses of FG-3019.
- 15 $mg \cdot kg^{-1}$ (cohort 1) or 30 $mg \cdot kg^{-1}$ (cohort 2)
- Result showed: no significant AE, well tolerated, improvement on fibrosis scores on HRCT
- Decline in pulmonary function were equal in the two groups

Raghu et al Eur Respir J. 2016 May;47(5):1481-91

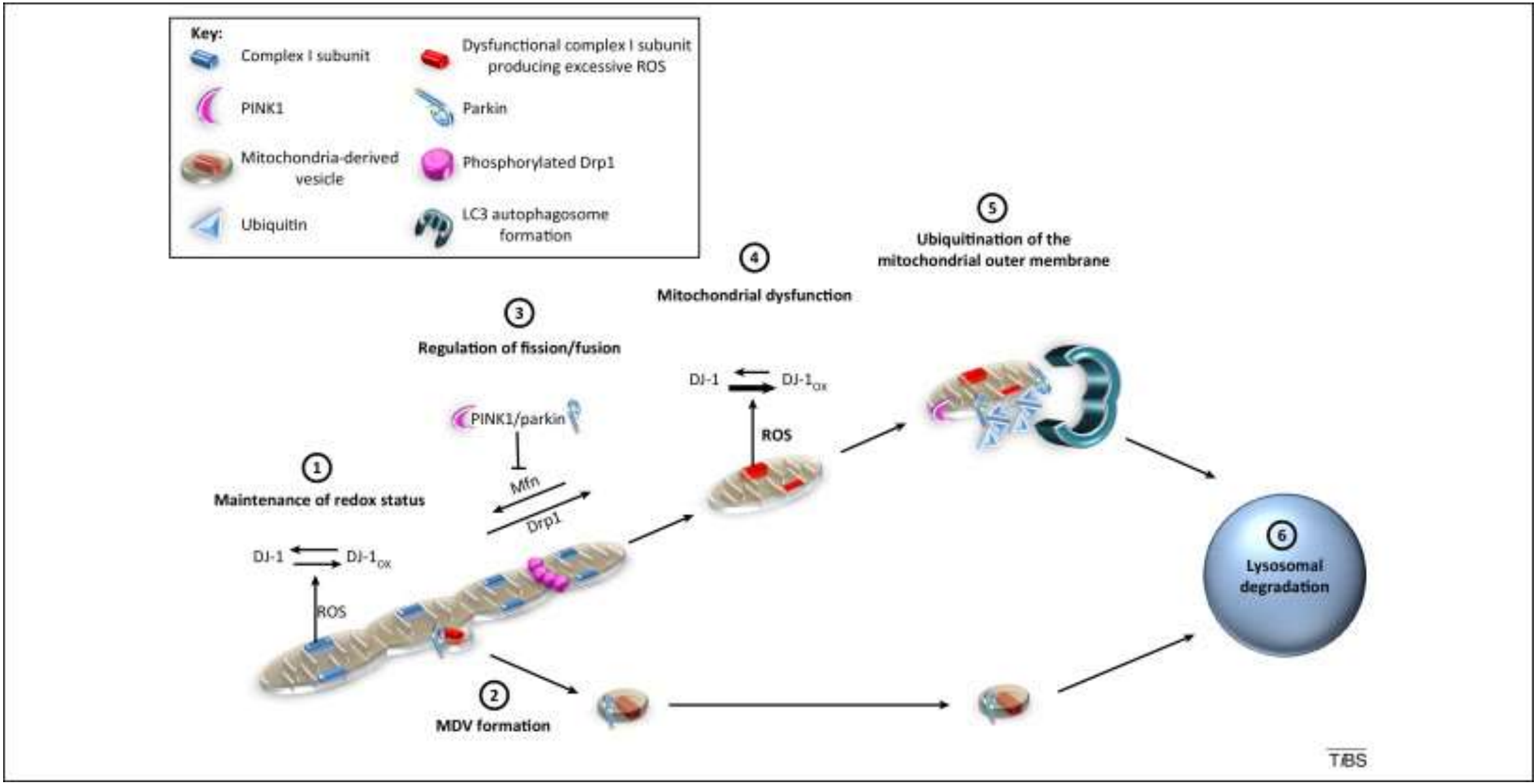
Mesenchymal stem cell

- MSC produce soluble factors such as HGF, fibronectin, periostin, and insulin-like growth factor-binding protein 7 that are implicated in epithelial repair.
- Recently results of Phase Ib study were published to confirm the safety of MSC in IPF
- No adverse events were noted win 8 IPF patients with no significant benefit in lung functions on incremental doses

Chambers et al Respirology. 2014 Oct;19(7):1013-8

Mitochondrial dysfunction

- In IPF lungs, dysfunctional mitochondria have been found in AEC2 and lung fibroblasts.
- Mitochondrial dysfunction contributes to DNA damage, senescence, SASP, telomere attrition, stem cell exhaustion, inflammation and other key age-related cell processes


Key:
Complex I subunit
Dysfunctional complex I subunit producing excessive ROS
PINK1
Parkin
Mitochondria-derived vesicle
Phosphorylated Drp1
Ubiquitin
LC3 autophagosome formation
1
Maintenance of redox status
2
MDV formation
3
Regulation of fission/fusion
4
Mitochondrial dysfunction
5
Ubiquitination of the mitochondrial outer membrane
6
Lysosomal degradation
PINK1/parkin
Mfn
Drp1
DJ-1
DJ-1ox
ROS
TiBS

Evidence

- MitoQ, a ubiquinone conjugated molecule, attenuates the expression of TGFβ1and NOX4 which are the two important mediators of myofibroblast differentiation
- Have found to decrease bleomycin induced lung fibrosis in rat models

Other mitochondrial agents

- Mice that are deficient in PINK1 are more susceptible for bleomycin induced lung fibrosis and increased secretion TGFβ1
- Kinetin almost completely restores the downstream pathways of PINK1 and have found to decrease fibrosis in mice models of BILI
- Similarly E3 ubiquitin ligase *Prakin* if knocked out in mice show enhanced myofibroblast trans-differentiation
- SRT3, NAD dependent deacetylase protects against ling fibrosis
- Hexaflurohonokiol induces expression of SRT3 and protect against lung fibrosis
- Similarly as a corollary Boosting NAD levels has been shown to be an effective approach to protect against BILI

Altered protein folding, trafficking

- Adaptive unfolded protein response has been found in AEC2 of IPF lungs secondary to alerted proteiostasis
- Multiple agents have been tried to overcome this particular mechanism
- mTOR inhibitors like Everolimus have been found to worsen the disease
- Trials to increase expression of chaperones by decreasing intracellular calcium are underway (Diltiazem and Verapamil)
- 4-PBA (4- phenyl butyric acid) has also been found to decrease ER stress, collagen synthesis, αSMA and myofibroblast differentiation

Malouf et al Respirology 16, 776–783(2011)

Epigenetics and miRNA

- Epigenetic modifications occur during ageing and other age related diseases like IPF
- Varied genes commonly found modified in IPF have the mechanism of action of altered expression based upon epigenetic like histone aceylation and miRNA
- *Vorinostat*- a pan histone deacetylase inhibitor has been shown to protect against paraquat induced lung fibrosis and induce apoptosis of myofibroblast by preventing the deacetylation of SMAD4
- Anti-fibrotic properties of Valproic acid are also being evaluated for in pre-clinical trials.

SASP: Senescence associated secretory phenotype

- Dual action protein which increases wound healing process by releasing growth factors but if present chronically induces fibrosis
- *Dasatinib* and *Quercetin* have been found to decrease senescence and SASP markers in AEC2 of bleomycin exposed mice
- *Navitoclax* is potent inhibitor of BCL2 and BCL X1 which are anti apoptotic proteins expressed by fibroblasts of IPF lungs. Mice models have demonstrated their efficacy against fibrosis
- GKT137831 is NOX1 and NOX 4 inhibitor which decreases reactive oxygen species and senescence markers in IPF fibroblasts
- Metformin, *Rapamycin, Omipalisib* and *Rupatadine* are being evaluated in pre-clinical trials for lung fibrosis through the mechanism of senescence prevention

Telomere attrition

- Telomere shortening has been associated senescence and IPF
- Estrogens and androgens have been long evaluated for lengthening of telomeres
- Trials with primary outcome of lengthening of telomeres in IPF are underway with Danazol and Nandrolone Decanoate.

LPA/Autotaxin inhibitors

- Autotaxin enzyme converts LPC into LPA
- LPA acts through G Protein coupled receptors
- LPA control migration of Fibroblasts, contraction and proliferation
- LPA and autotaxins have been found to be increased BAL fluids of IPF patients
- $LPAR^{1}$ inhibitors have been found to decrease Fibrosis in Mice models
- GLPG1690 (Galapagos): Inhibits Autotaxin and has been shown to have strong anti-fibrotic activity in BILI models
- FLORA trial in underway to assess its safety in humans

TGF β 1

- Most potent pro-fibrotic agent
- Fresolimumab is human monoclonal antibody to neutralize TGF β1
- Study for its use in IPF is completed but results have yet not published
- Receptor for TGFb1 α*v*β 6 integrin has also been targeted
- CWHM 12 is an inhibitor which is found to decrease fibrosis in multiple organs including lungs

- GSK3008348 (inhaled) and BG00011 (IV) are inhibitors of integrin receptors of TGF which have tested in pre clinical setting of BILI and found to decrease lung fibrosis
- BC1485 targets fibrosis inducing E3 ligase- PIAS4 pathway which promotes TGFβ signaling and reduces the severity of fibrosis in BILI mice model

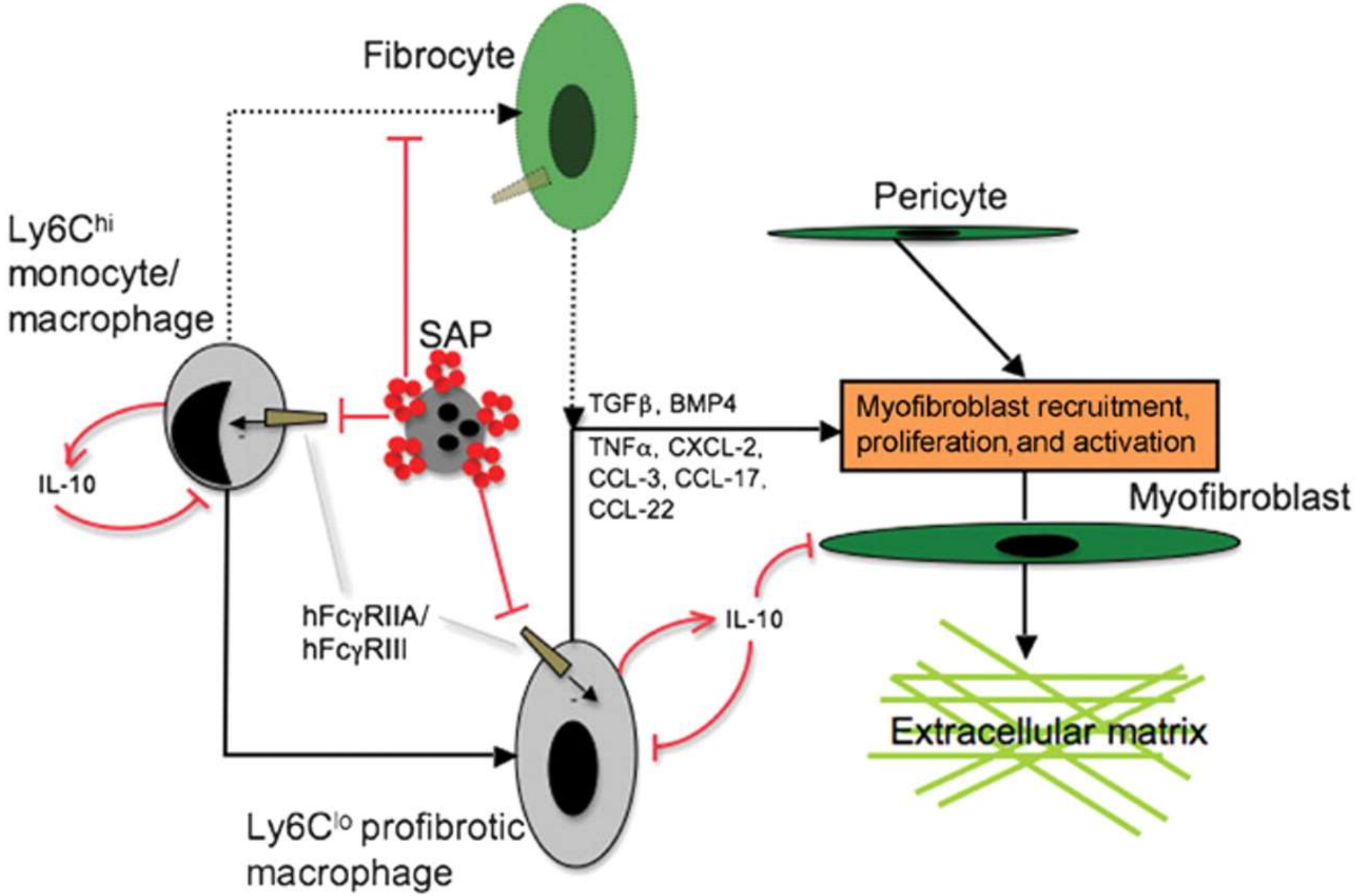
Growth factors

- FGF1 and KGF are potent anti fibrotic, mitogenic factors which if over expressed have been found to promote surfactant, AEC2 proliferation

Serum Amyloid P

- Serum Amyloid P (SAP) is a member of Pentraxin family of proteins
- SAP inhibits monocytes and macrophages
- It also decreases EMT and recruitment of Fibrocytes into lungs
- Anti-fibrotic activities of SAP has been shown to be mediated through F*cγ* Receptors
- Therapeutic administration of SPA has been shown to have protective effects of fibrosis in murine models

Murray et al PLoS One. 2010; 5(3): e9683.

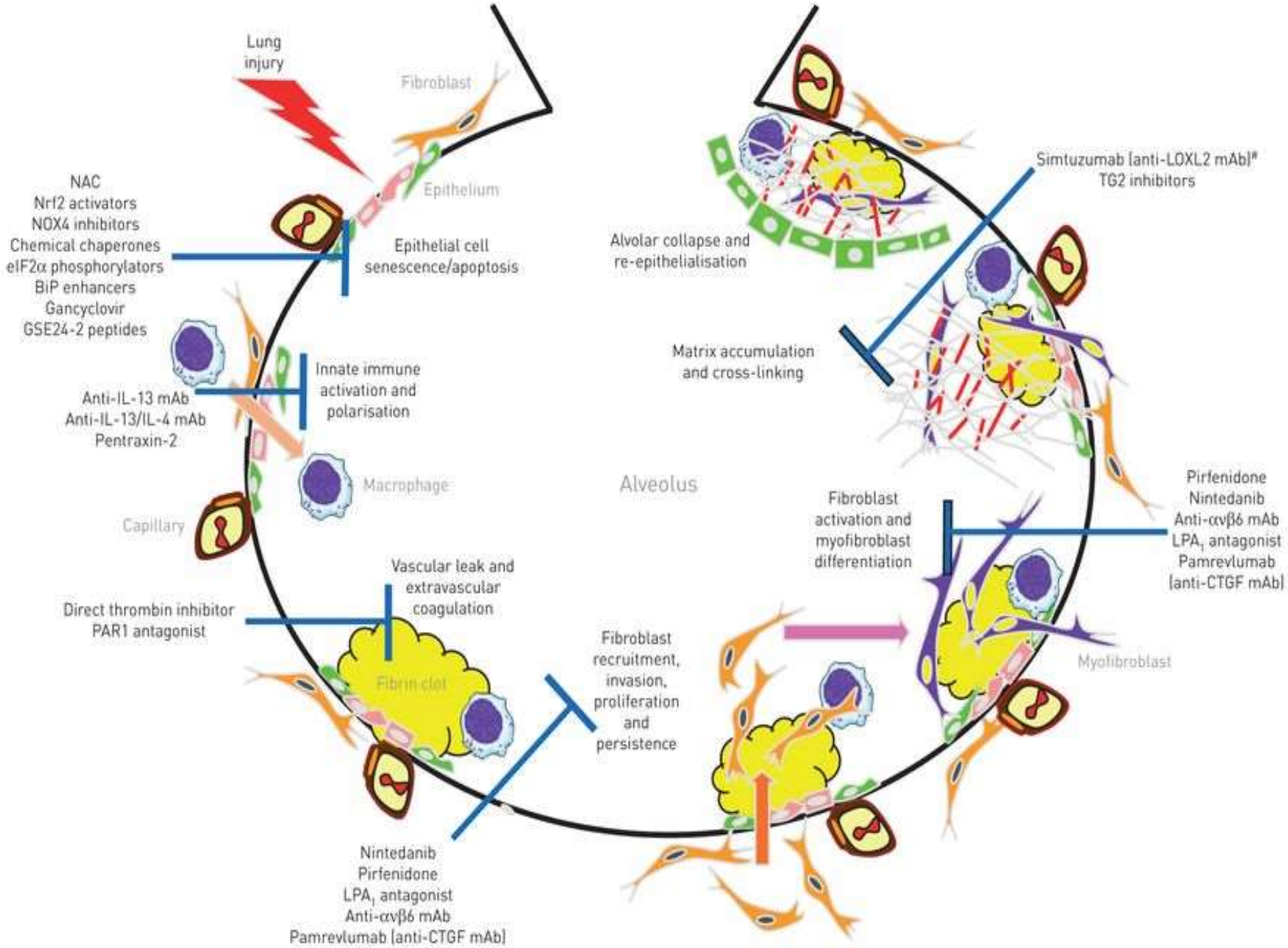

Fibrocyte
Ly6C^hi monocyte/ macrophage
SAP
Pericyte
TGFβ, BMP4
TNFα, CXCL-2, CCL-3, CCL-17, CCL-22
Myofibroblast recruitment, proliferation, and activation
Myofibroblast
IL-10
hFcγRIIA/ hFcγRIII
IL-10
Extracellular matrix
Ly6C^lo profibrotic macrophage

Other agents with RCTs

- **No results**: IFN γ, Etanercept, Bosentan and Macitentana, Sildenafil, Imatinib
- **Worsened disease activity**: Ambrisentan, Warfarin, Everolimus
- **On going safety studies:** Lebrikizumab (anti IL13), Tipelukast (Anti Leukotriene)

Markers of IPF- Summarizing

- Expression markers of AEC1, AEC2, airway cell, TGFβ1, WNT, p53 and PI3K are increased in IPF
- PDGF, TGFβ1, TNFα, endothelin, CTGF, Osteopontin, CXC chemokine ligand-12 are all expressed by AEC2 and promote profibrotic response
- Activated AEC2 inhibit angiogenesis, reflected by paucity of capillaries, also they activate the coagulation pathway that is involved in wound healing
- FXa induces αSMA expression promoting differentiation of fibroblasts into myofibroblast


Lung injury
Fibroblast
Epithelium
NAC
Nrf2 activators
NOX4 inhibitors
Chemical chaperones
eIF2α phosphorylators
BiP enhancers
Gancyclovir
GSE24-2 peptides
Epithelial cell senescence/apoptosis
Innate immune activation and polarisation
Anti-IL-13 mAb
Anti-IL-13/IL-4 mAb
Pentraxin-2
Macrophage
Capillary
Vascular leak and extravascular coagulation
Direct thrombin inhibitor
PAR1 antagonist
Fibrin clot
Fibroblast recruitment, invasion, proliferation and persistence
Nintedanib
Pirfenidone
LPA1 antagonist
Anti-αvβ6 mAb
Pamrevlumab (anti-CTGF mAb)
Alveolus
Alvolar collapse and re-epithelialisation
Simtuzumab (anti-LOXL2 mAb)#
TG2 inhibitors
Matrix accumulation and cross-linking
Fibroblast activation and myofibroblast differentiation
Pirfenidone
Nintedanib
Anti-αvβ6 mAb
LPA1 antagonist
Pamrevlumab (anti-CTGF mAb)
Myofibroblast