Fibrosis in DMD

Federica Montanaro, Ph.D.

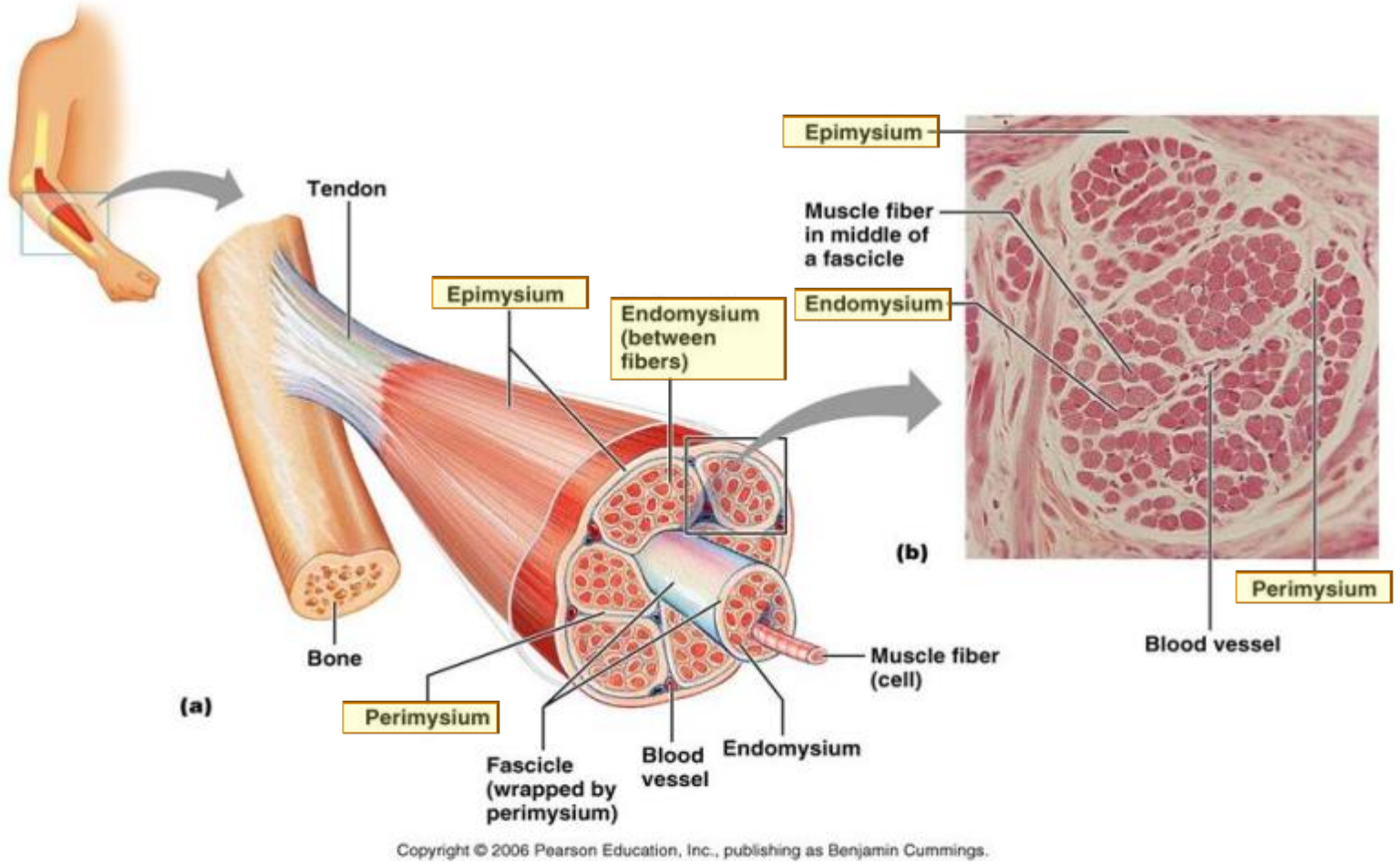
MVIMG# 7470: Fundamentals of Muscle Biology: Duchenne Muscular Dystrophy

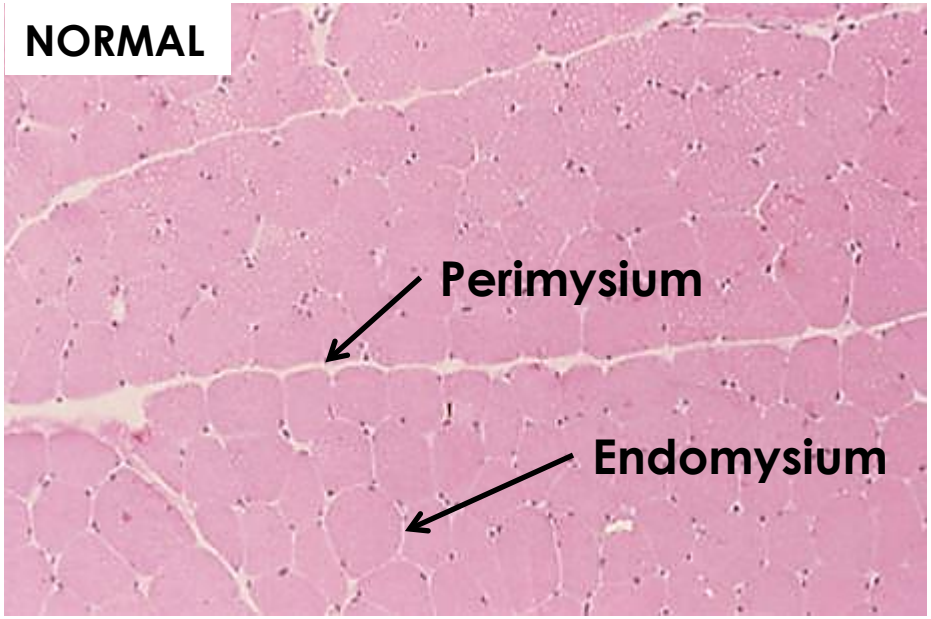
April 3, 2014

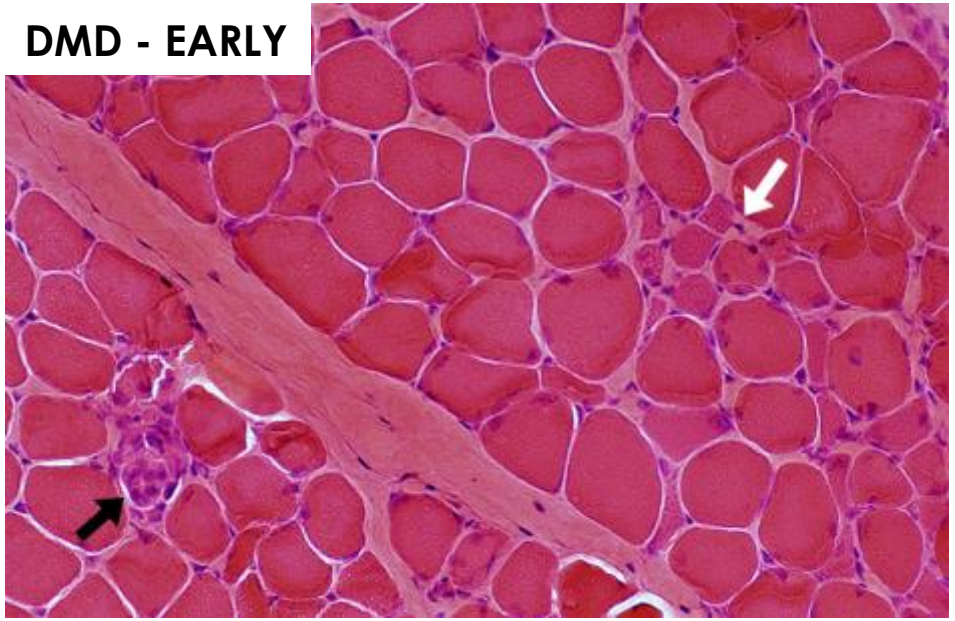
What is fibrosis?

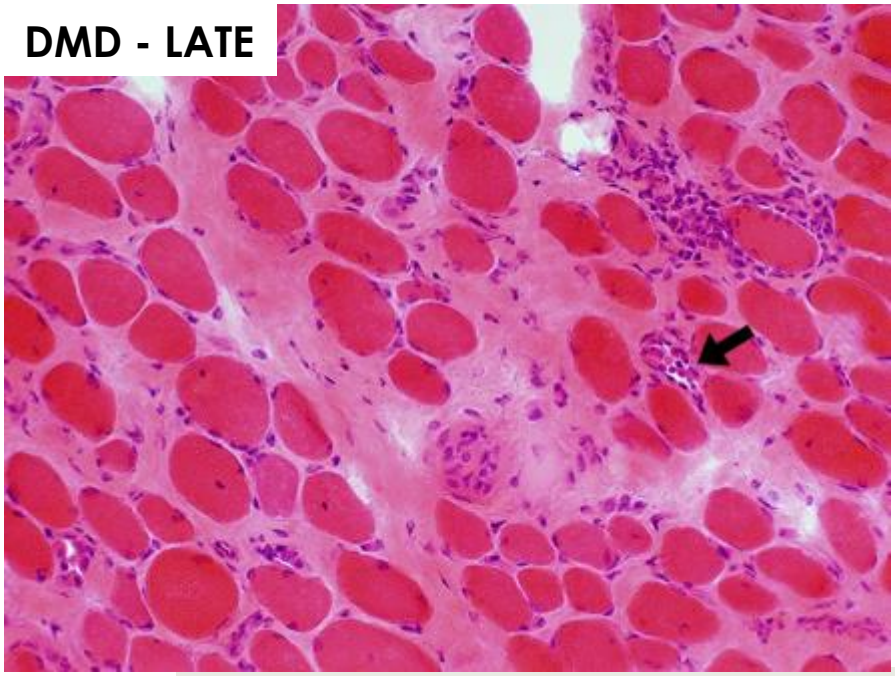
- Basic response of **any organ** that undergoes **repetitive** injury and inflammation.
- Characterized by the excessive deposition of extracellular matrix proteins (mainly collagens I and III, fibronectin) thus creating a scar.
- Leads to a disordered tissue structure, disruption of organ function, and ultimately organ failure.
- Major cause of mortality worldwide.
- No available FDA- or EMEA- approved anti-fibrotic therapies.

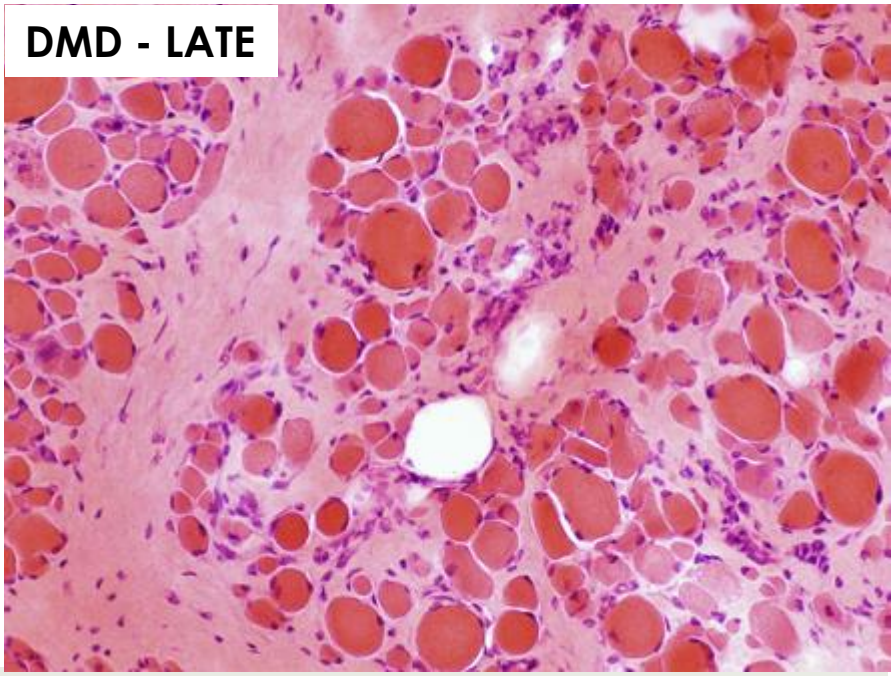
Impact on disease progression in DMD




NORMAL
Perimysium
Endomysium


DMD - EARLY


DMD - LATE


DMD - LATE

J Neuropathol Exp Neurol

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pp. 762–773

ORIGINAL ARTICLE

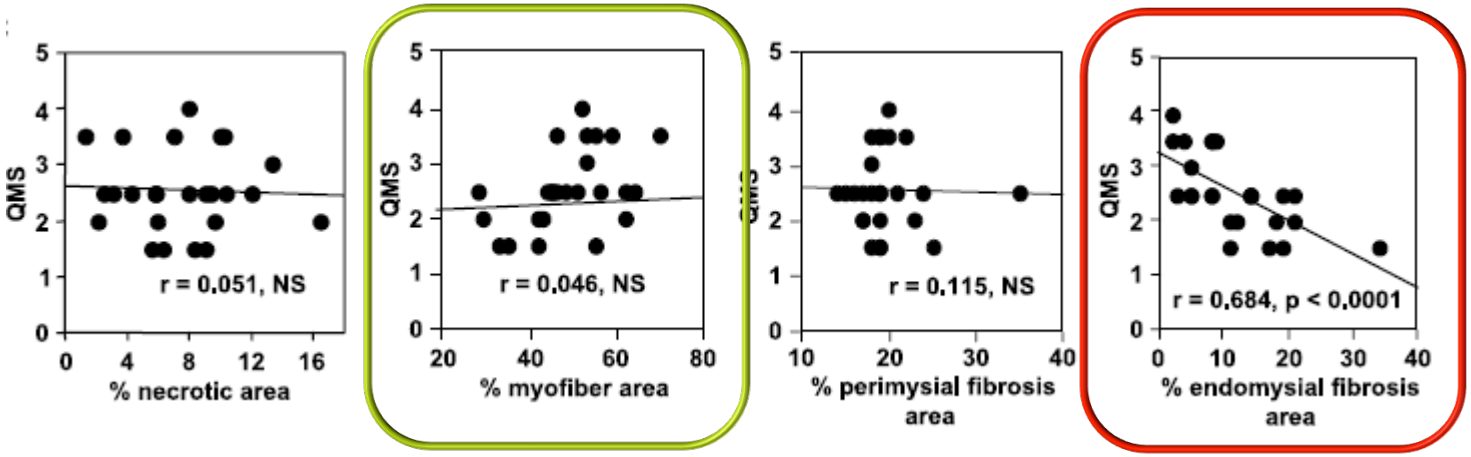
Endomysial Fibrosis in Duchenne Muscular Dystrophy: A Marker of Poor Outcome Associated With Macrophage Alternative Activation

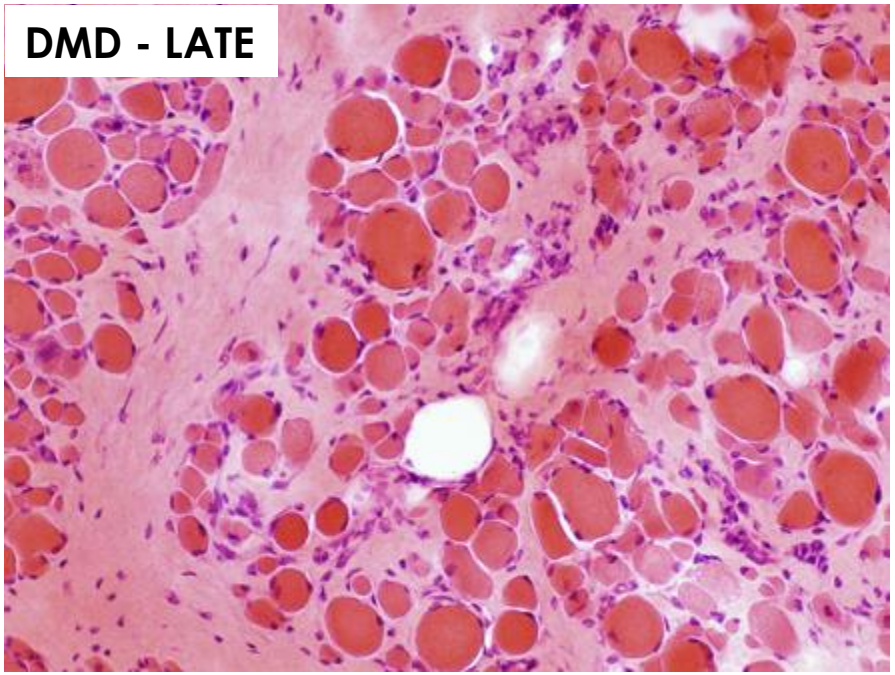
Isabelle Desguerre, MD, Michelle Mayer, MD, France Leturcq, PhD,
Jacques-Patrick Barbet, MD, PhD, Romain K. Gherardi, MD, and Christo Christov, MD

Endomysial fibrosis is the main histopathological parameter that correlates with poor motor outcome in DMD patients

Consequences of Endomysial Fibrosis

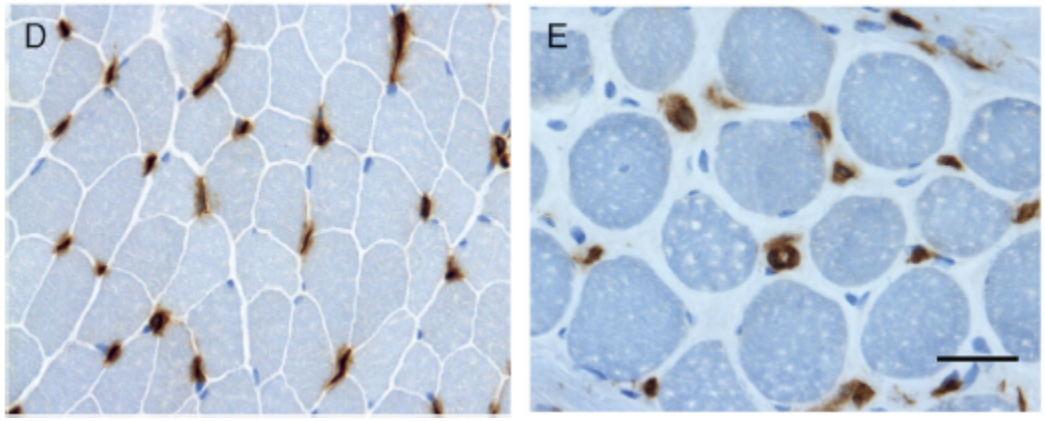
Quadriceps muscle strength





Consequences of Endomysial Fibrosis

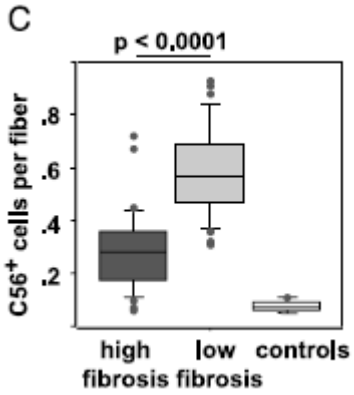
- Loss of tight association between muscle fibers and capillaries → *decreased oxygenation and nutrients*



CD31 staining (brown) of capillaries

Control **DMD**

- Decreased number of satellite cells → *impaired regeneration*



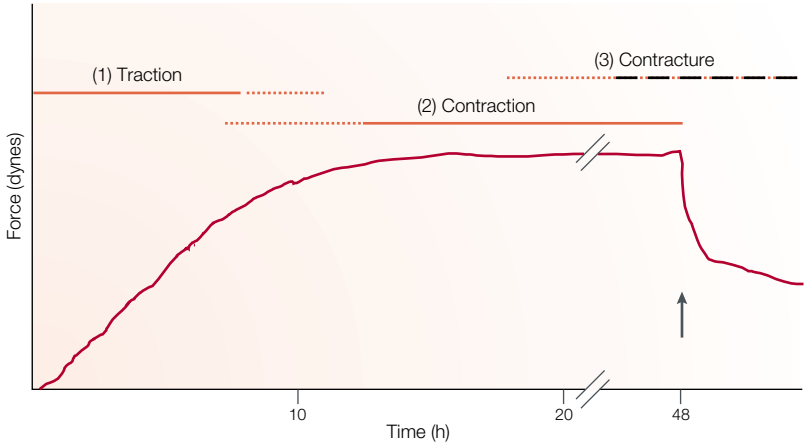
Consequences of Endomysial Fibrosis

- Tissue contracture
- Increased tissue stiffness

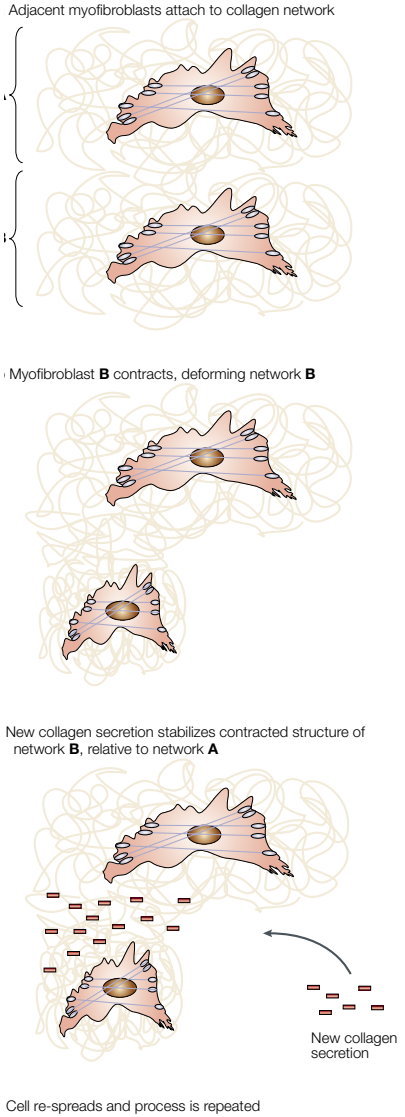
  → *inhibits the proliferation and differentiation of satellite cells*

  → *Enhances production of matrix proteins by fibrotic cells*

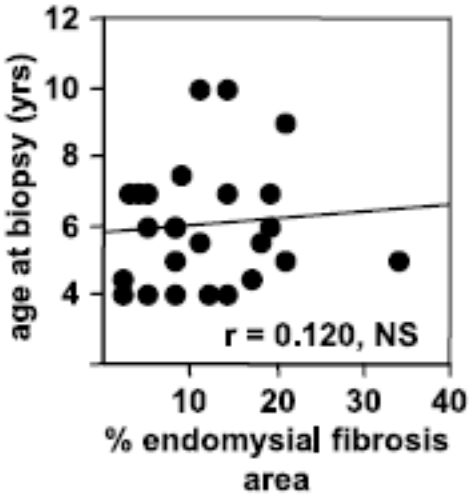
  → *Interferes with muscle contraction*



Tomasek et al., 2002, Nature Reviews 3: 349



Genetic modifiers of fibrosis



Variability in the rate of disease progression among patients, even if they have the same genetic mutation in the *DMD* gene or lack of dystrophin protein expression

Latent TGF-β–binding protein 4 modifies muscular dystrophy in mice

Ahlke Heydemann,[1] Ermelinda Ceco,[2] Jackie E. Lim,[3] Michele Hadhazy,[1] Pearl Ryder,[1] Jennifer L. Moran,[4] David R. Beier,[4] Abraham A. Palmer,[2] and Elizabeth M. McNally[1,2,3]

[1]Department of Medicine, Section of Cardiology, [2]Committee on Cell Physiology, and [3]Department of Human Genetics, University of Chicago, Chicago, Illinois, USA. [4]Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

The Journal of Clinical Investigation http://www.jci.org Volume 119 Number 12 December 2009

LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy

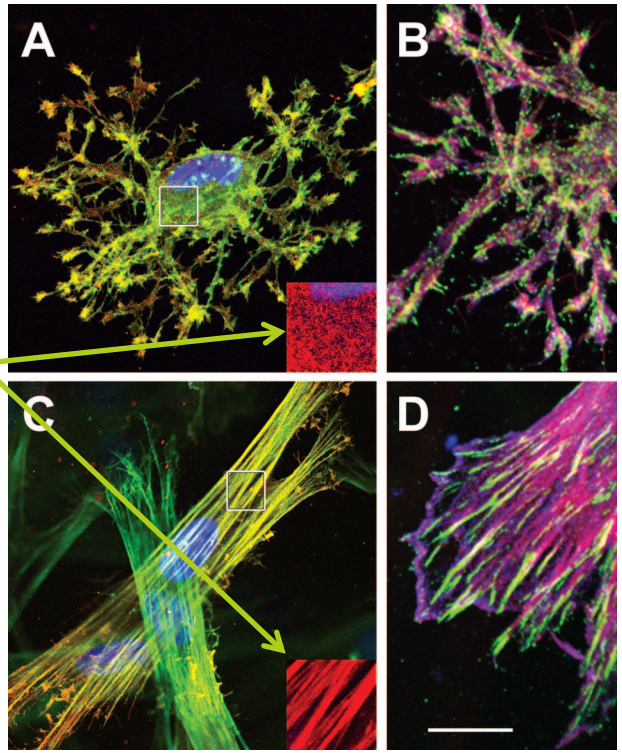
Kevin M. Flanigan, MD,[1,2,3] Ermelinda Ceco, BS,[4] Kay-Marie Lamar, BS,[4] Yuuki Kaminoh, BS,[1] Diane M. Dunn, BS,[5] Jerry R. Mendell, MD,[1,2,3] Wendy M. King, PT,[3] Alan Pestronk, MD,[6] Julaine M. Florence, DPT,[6] Katherine D. Mathews, MD,[7] Richard S. Finkel, MD,[8] Kathryn J. Swoboda, MD,[9] Eduard Gappmaier, PhD,[10] Michael T. Howard, PhD,[5] John W. Day, MD, PhD,[11] Craig McDonald, MD,[12] Elizabeth M. McNally, MD, PhD,[4] and Robert B. Weiss, PhD[5] for the United Dystrophinopathy Project

ANN NEUROL 2013;73:481–488

The Fibroblast

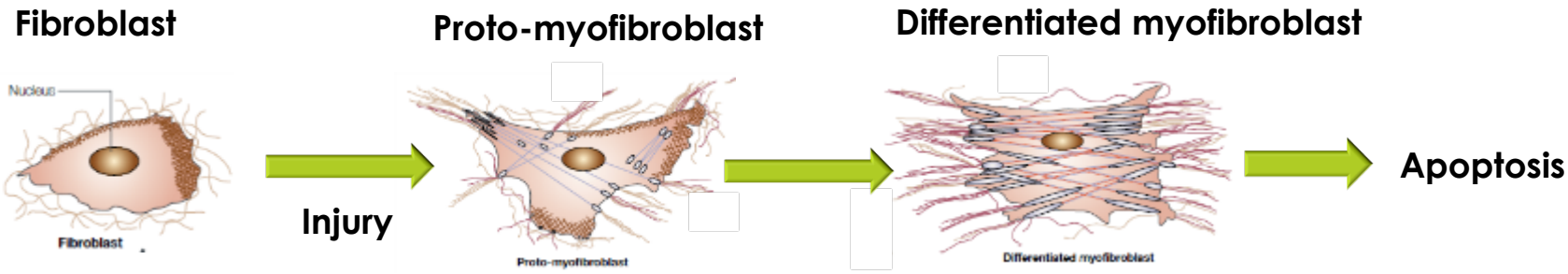
- Versatile in shape
- Versatile in gene expression
- Versatile in function
  - Developing muscle:
    - Promote slow muscle myogenesis
    - Fetal to adult switch
    - Myoblast fusion
  - Adult muscle
    - Regulation of satellite cell self-renewal and differentiation
    - Tissue integrity

Lung fibroblasts



Hinz et al, 2007, American Journal of Pathology, 170(6): 1807.

Fibroblast activation



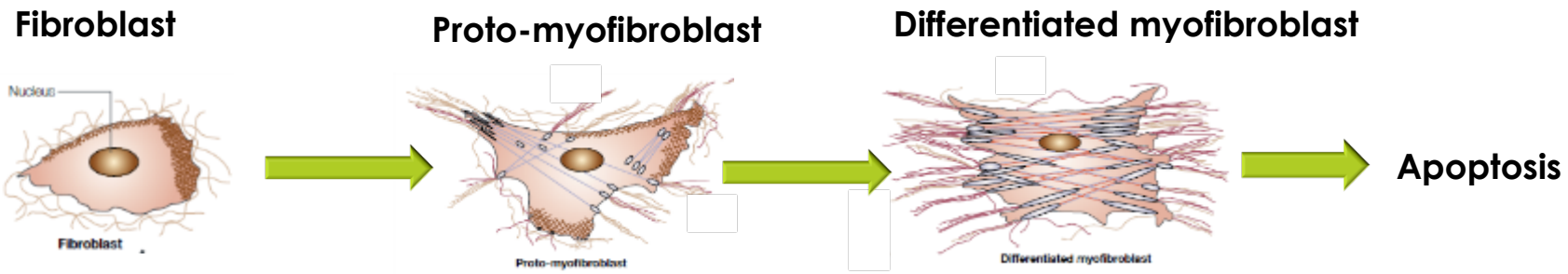
Phenotype	Tissue homeostasis	• Migratory cell • Matrix protein production, including specific forms of FN • Production of TGF-β	• Contractile cell • High matrix protein production • Production of TGF-β • Production of ROS

Myofibroblasts are the key pathogenic cells in all fibrotic diseases

Research has focused on identifying:

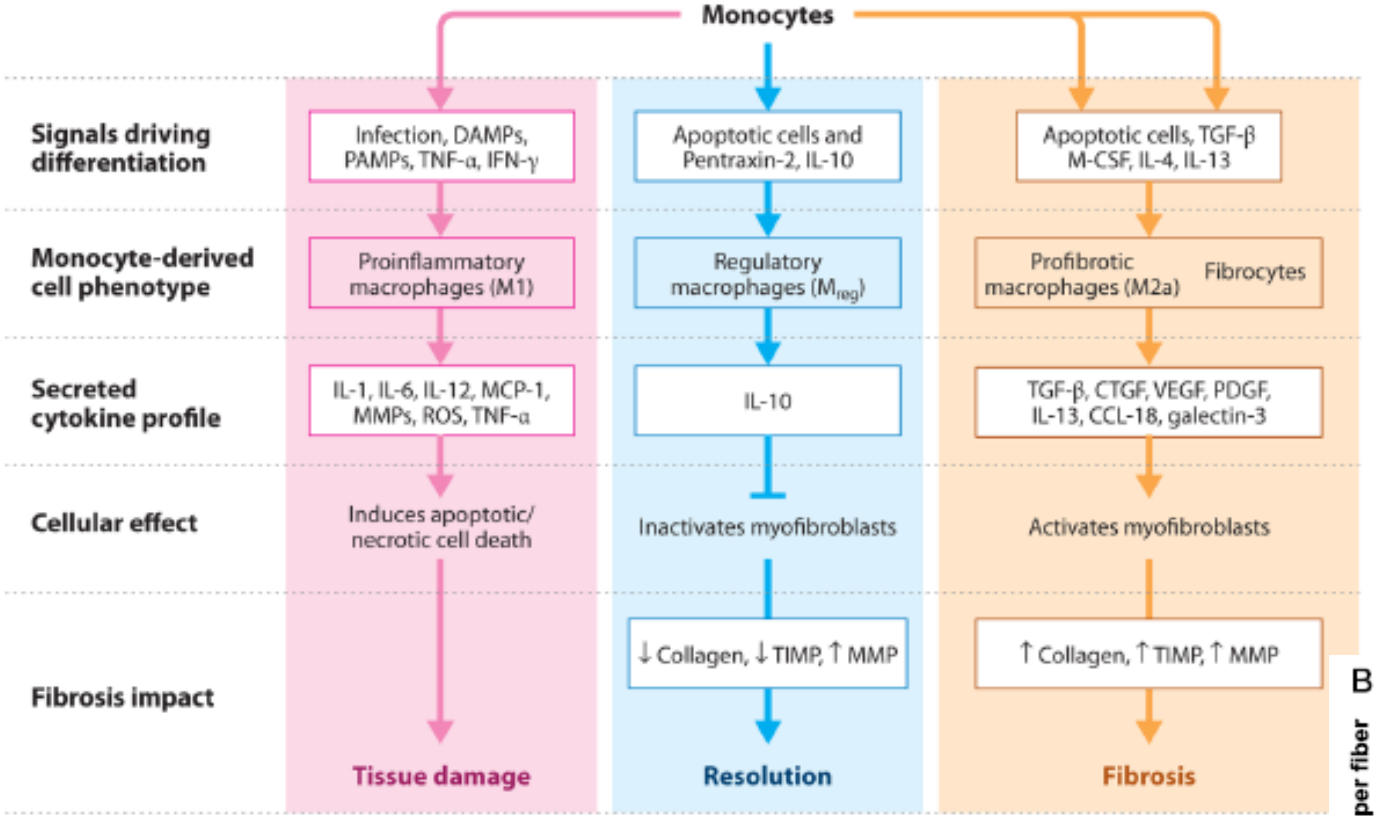
- the factors that activate myofibroblasts
- the mechanisms that contribute to myofibroblast apoptosis
- the cellular origins of myofibroblasts

Fibroblast activation

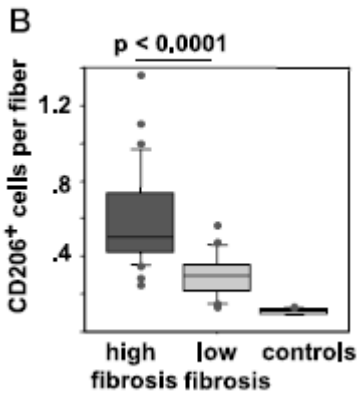


Stimulus	• Immune cell released cytokines and TGF-β • PDGF	• Stiff substrate • Specific FN isoforms • TGF-β	
Phenotype	Tissue homeostasis	• Migratory cell • Matrix protein production, including specific forms of FN • Production of TGF-β	• Contractile cell • High matrix protein production • Production of TGF-β • Production of ROS

Fibroblast activation – impact of immune cells



Duffield et al, 2013, Ann. Rev. Pathol., 8: 241.



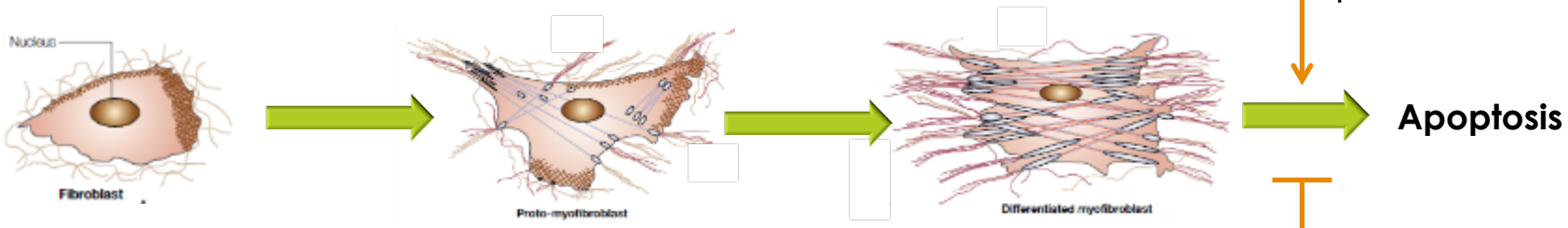
Desguerre et al, 2009, J. Neuropathol. Exp. Neurol., 68(7): 762.

Myofibroblast apoptosis

CCN1

- Synergizes with TNFα, Jun, NADPH oxidase 1, or p38 to induce senescence or apoptosis via p53 and Rb

Fibroblast → **Proto-myofibroblast** → **Differentiated myofibroblast** → **Apoptosis**



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TGF-β

- SMAD3/FAK-dependent pathway
- P38 MAPK/PI3 kinase/Akt dependent signaling

Cellular origins of fibroblasts/ myofibroblasts

- Circulating Fibrocytes
- Endothelial to mesenchymal transition
- Epithelial to mesenchymal transition
- Mesenchymal progenitors/fibroblast and adipocyte precursors
- Pericytes

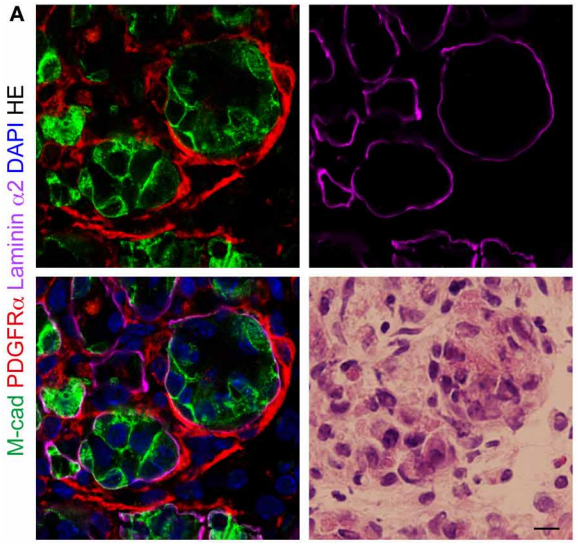
- Genetic fate mapping experiments in several organs, including skeletal muscle, brain, kidney, lung skin and liver indicate that **mesenchymal progenitors** and **pericytes** are the precursors of myofibroblasts.
- Many parallel genetic fate mapping studies show little or no evidence of direct differentiation of epithelial cells, endothelial cells, or circulating fibrocytes into myofibroblasts

Mesenchymal progenitors

- Location: interstitium
- Main markers: PDGFR-α, Sca-1, CD34
- Differentiation potential:
  - Fibroblasts
  - Adipocytes
  - Osteogenic
  - Chondrogenic

Acute muscle injury

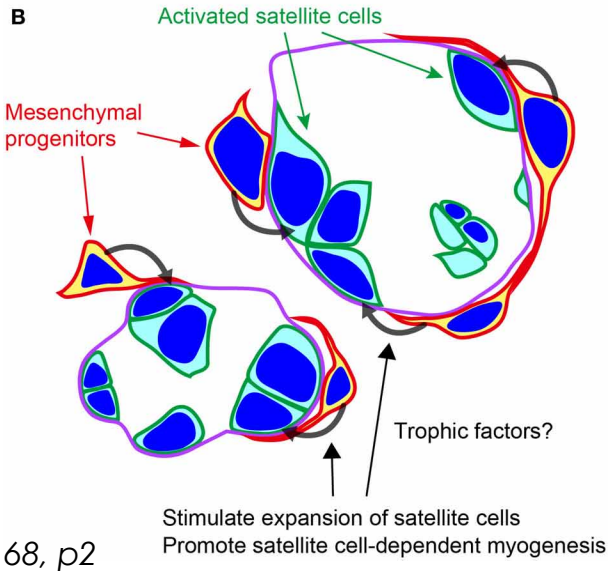
- Release trophic factors that support satellite cell expansion and myogenic differentiation
- Phagocytose dead cells and cellular debris



Type 2 Innate Signals Stimulate Fibro/Adipogenic Progenitors to Facilitate Muscle Regeneration

Jose E. Heredia,[1,10] Lata Mukundan,[1,10] Francis M. Chen,[1] Alisa A. Mueller,[6] Rahul C. Deo,[1,3,7,8] Richard M. Locksley,[3,4,5] Thomas A. Rando,[6,9] and Ajay Chawla[1,2,3,*]

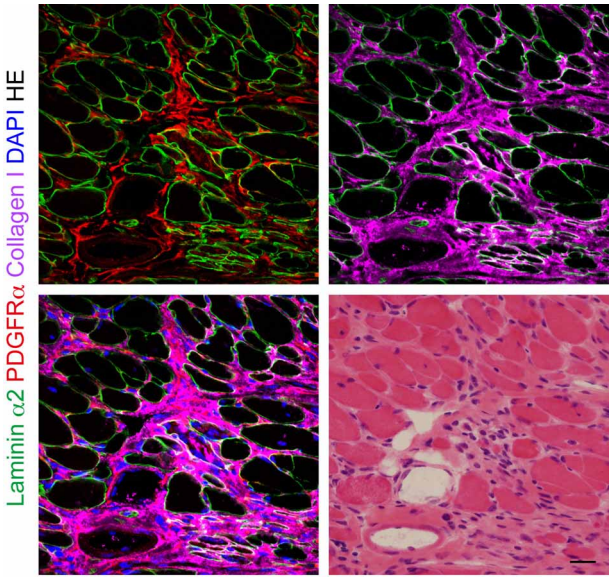
Cell *153*, 376–388, April 11, 2013



Uezumi et al., 2014, Frontiers in Physiology, Vol 5, Article 68, p2

Muscular Dystrophy

- Mesenchymal progenitors:
  - Produce collagens
  - Differentiate into fibroblasts and adipocytes

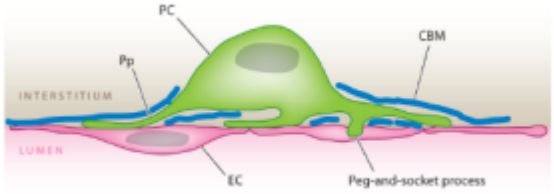


Uezumi et al., 2014, Frontiers in Physiology, Vol 5, Article 68, p2

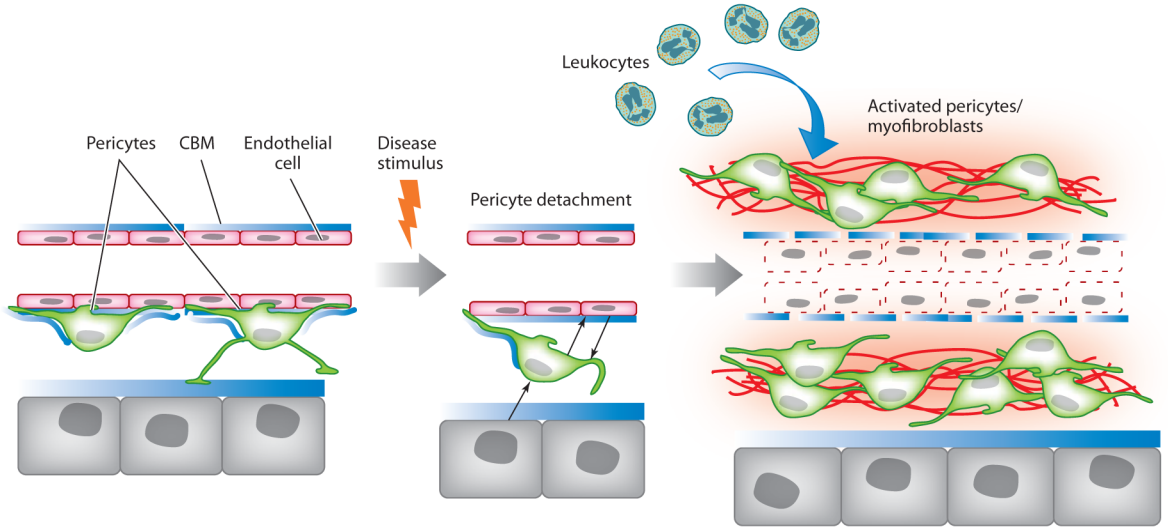
Pericytes

- Location: perivascular, around capillaries
- Main markers: PDGFR-β, NG2
- Activated by PDGF, VEGF, TGF-β
- Main function: Microvasculature homeostasis
- Differentiation potential:
  - Myogenic
  - Adipogenic
  - Osteogenic
  - Fibrogenic

  (Duloroy et al., 2012, Nat. Med., 18:1262)



Pericyte activation



Fibrosis

Compromised microvascular homeostasis

Duffield et al., 2013, Annu. Rev. Pathol., 8: 241

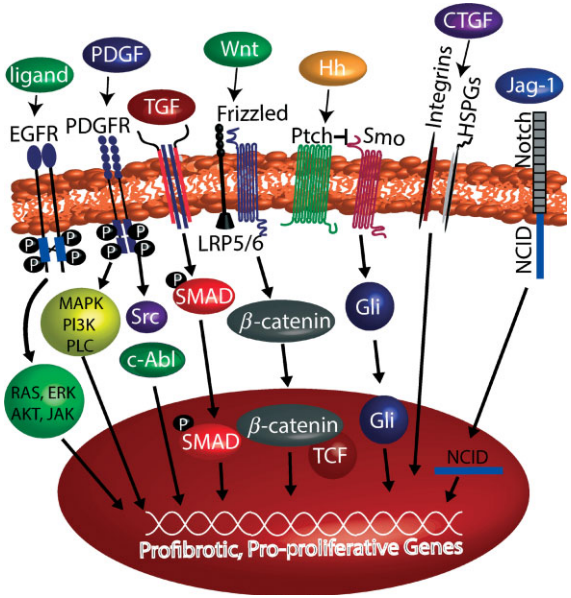
Summary

- Fibrosis is a major determinant of disease progression in DMD
- Replaces muscle tissue and impairs the function of residual muscle fibers
  - Inhibition of satellite cell proliferation
  - Impaired interactions with the microvasculature
  - Stiffens the matrix
- Tight relationship between fibrotic and immune cells
- Treatment targets:
  - Immune modulation
  - Inhibition of differentiation of fibroblast progenitors
  - Inhibition of fibroblast differentiation into myofibroblasts
  - Induction of apoptosis/senescence of myofibroblasts

Anti-fibrotic treatment targets in DMD

- Inflammation
  - Nfk-B inhibition (Flavocoxid [Phase 1], VBP15 [preclinical])
  - TNF-α inhibition (BKT-104, cV1q, LMP420, etanercept [pre-clinical])
- Pro-fibrotic pathways
  - TGF-β (ACE inhibitors, Myostatin inhibitors [MYO-029, ACE-031, Follistatin]
  - ROS (CoQ10 [Phase 2/3], Sunphenon Epigallocatechin-Gallate [Phase 2/3], Catena)
- Pro-regenerative pathways
  - IGF-1 [Phase 2]
  - Tissue vascularization (Tadalafil, Sildenafil, PDE inhibitors)

Anti-fibrotic treatments are a challenge



Kramann et al, 2013, Journal of Pathology, 231:273

Table 1. Therapeutics that are currently being tested or have been tested in fibrotic diseases (this list does not claim to be exhaustive)

Drug name	Company	Target/MOA	Indication	Phase/notes	Clinical Trials.gov identifier
Pirfenidone	Intermune	p38/TGFβ inhibitor	IPF	Approved in Europe and Asia, phase III in USA (ongoing)	NCT01366209
Fresolimumab	Sanofi	Anti-TGFβ monoclonal antibody	Diffuse systemic sclerosis	Phase I (recruiting)	NCT01284322
			FSGS	Phase II (recruiting)	NCT01665391
			IPF	Phase 1 (completed)	NCT00125385
LY2382770	Lilly	Anti-TGFβ monoclonal antibody	Diabetic kidney disease; diabetic nephropathy, diabetic glomerulosclerosis	Phase II (recruiting)	NCT01113801
STX-100	Biogen Idec	Anti-a_vb_6 monoclonal antibody	IPF	Phase II (recruiting)	NCT01371305
Macitentan	Actelion	Endothelin receptor antagonist ET-A and ET-B	IPF	Phase II (fail)	NCT00903331
Bosentan	Actelion	Endothelin receptor antagonist, ET-A and ET-B	IPF	Phase III (fail)	NCT00631475
			Digital ulcers in SSc patients	Approved in EU	NCT00077584 NCT00319696
			Interstital lung disease with SSc	Phase II/III (did not improve outcomes versus natural course)	NCT00319033
Ambrisentan	Gilead	Endothelin receptor antagonist selective for ET-A	IPF	Phase III (fail)	NCT00879229
RE-021	Retrophin	Selective endothelin type A receptor antagonist	FSGS	Phase II (not yet open)	NCT01613118
FG-3019	Fibrogen	Anti-CTGF	Liver fibrosis due to HBV	Phase II (ongoing)	NCT01217632
			IPF	Phase II (ongoing, with promising preliminary results)	NCT01262001
			Adolescents and adults with FSGS	Phase I (terminated)	NCT00782561
			Diabetic nephropathy	Phase II (terminated)	NCT00913393
			Locally advanced or metastatic pancreatic cancer	Phase I (ongoing)	NCT01181245
PF-06473871	Pfizer	Antisense CTGF	Hypertrophic skin scarring	Phase II (recruiting)	NCT01730339
RXI-109	RXi Pharmaceuticals	CTGF RNAi	Dermal scar prevention	Phase I (ongoing) Phase I (recruiting)	NCT01640912 NCT01780077
SAR156597	Sanofi	Bi-specific IL-4/IL-13 mAB	IPF	Phase I/II (recruiting)	NCT01529853
Tralokinumab	MedImmune	IL-13 inhibition	IPF	Phase II (recruiting)	NCT01629667
QAX576	Novartis	IL-13 inhibition	Pulmonary fibrosis secondary to SSc	Phase II -Terminated due to SAE	NCT00581997
			IPF	Phase II (terminated)	NCT01266135
Rilonacept	Regeneron	IL-1 trap	SSc	Phase I/II (recruiting)	NCT01538719
CNTO 888	Centocor	MCP-1(CCL2) inhibition	IPF	Phase II (completed)	NCT00786201
Etanercept	Pfizer/Amgen	TNF inhibition	IPF	Phase II (fail)	NCT00063869
Actimmune	Intermune	Human interferon-γ	IPF	Phase III (fail)	NCT00075998
Interferon-α lozenge	Amarillo Biosciences	Oral IFNα	IPF	Phase II (completed) Phase II (terminated)	NCT01442779 NCT00690885
PRM-151	Promedior	Recombinant pentraxin-2	IPF	Phase I (completed, improvements in FVC and 6MWT)	NCT01254409
			Scarring in trabeculectomy	Phase II (completed)	NCT01064817
Belimumab	GlaxoSmithKline	Anti-BAFF mAB	Membranous glomerulnephritis	Phase II (recruiting)	NCT01610492
Pomalidomide	Celgene	Multiple; anti angiogenic and immunomodulatory	IPF	Phase II (not yet recruiting)	NCT01135199
			SSc	Phase II (recruiting)	NCT01559129
IW001	United Therapeutics	Collagen V solution as immunomodulator	IPF	Phase I (completed)	NCT01199887