

**Do we need to know the mechanism of action:  
the pitfalls and rewards of targeting chitinases**

**Development of OATD-01: the First-in-Class  
Chitinase Inhibitor for Inflammatory and  
Fibrotic Diseases**

# The Low Hanging Fruits Are Gone.....

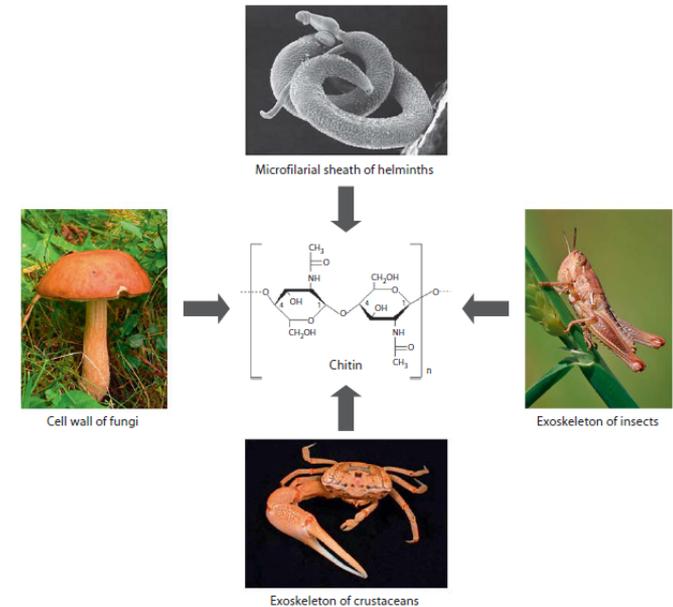


## Chitin and Chitinases:

- Chitinases hydrolyze chitin, the second most abundant polysaccharide in nature
- Chitin is straight chain polymer of N-acetylglucosamines linked by the 1-4  $\beta$ -glycosidic bonds
- Key structural element of the exoskeleton of many invertebrates and of cell walls in plants

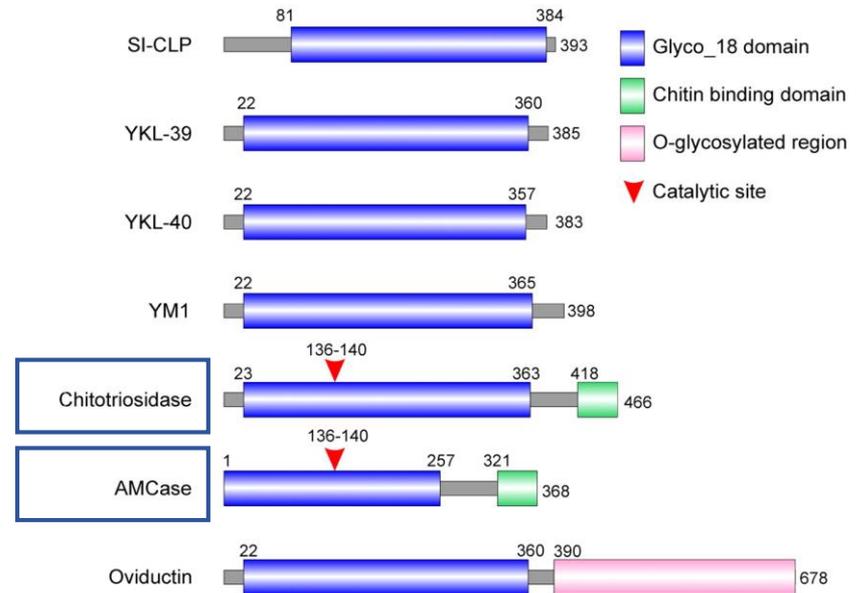
## Chitinases: Biological Functions

- Enable chitin containing organism to reshape permitting growth and development.
- Bacteria, insects and insects-eating animals use chitin as a nutrient source
- Protect plants and animals against chitin-containing organisms

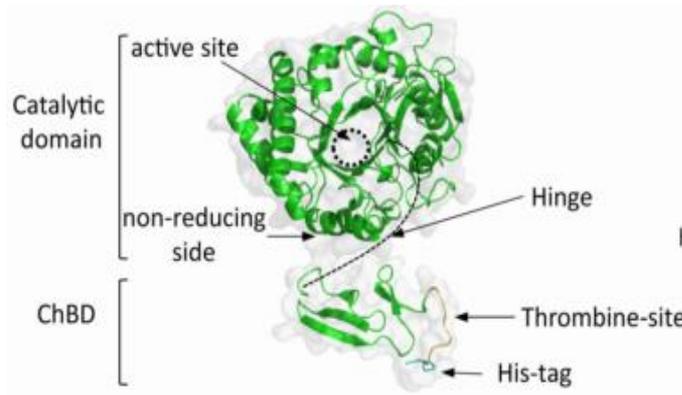


# Chitinase Family in Humans and Mouse

- Vertebrates do not synthesize chitin but express chitinases
- All members belong to the evolutionary conserved glycoside hydrolase 18 family (GH18)
- Two enzymatically active chitinases:
  - ❖ Acidic mammalian chitinase (AMCase)
  - ❖ Chitotriosidase (CHIT1)



## CHIT1



- CHIT1 is expressed mostly by activated macrophages, highly induced in multiple diseases
- AMCase is expressed mostly by epithelial cells
- Humans do not synthesize chitin. It was proposed that chitinases in humans protect against chitin-containing organisms
- However our exposure to chitin is limited and chitinases, particularly CHIT1, are highly upregulated in diseases not associated with chitin exposure and in organs and tissues not accessible to chitin-containing organisms.

Q: So if the function of chitinases in humans is not chitin hydrolysis, what is their mechanism of action and why are they upregulated in multiple diseases? How do they contribute to pathology?

A: We really don't know (but we are trying hard to understand it) but there is enough data to implicate chitinases in pathology of some diseases



## AMCase in asthma?

Acidic mammalian chitinase is **not** a critical target for allergic airway disease

AMCase **is** the crucial regulator of type 2 immune responses to inhaled house dust mite

Using AMCase deficient mice we show that AMCase was not required for the establishment of the type 2 inflammation in the lung in response to allergens and helminths.

...neutralizing AMCase activity.. resulted in a marked diminution of the IL-13-driven allergic inflammation...

..AMCase activity is largely dispensable in the development of the allergic airway reaction.

the overexpression or inhibition of AMCase exerts only a subtle impact on AAD.

absence of acidic mammalian chitinase during chronic fungal asthma results in a dramatic improvement in lung function

## Is it AMCase or is it CHIT1?

Although we did not detect CHIT1 protein in the airways...

Chitotriosidase is the primary active chitinase in the human lung

our findings that AMCase is the predominant chitinase in human BAL fluid...

Altogether data suggest that human AMCase has lost its chitinolytic activity by integration of nsSNPs during evolution...

AMCase protein was detectable in lavage fluid, AMCase transcripts in macrophages were consistent with an isoform lacking enzymatic activity

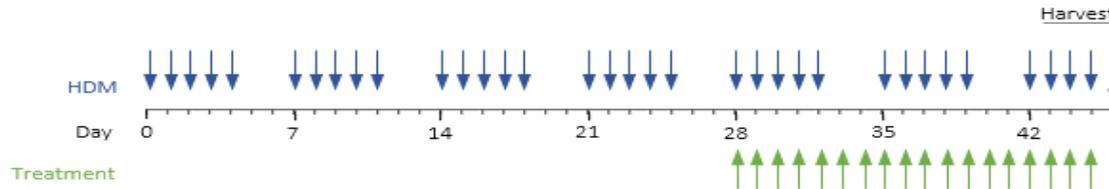
AMCase is a part of the non-redundant pathway for sustaining lung homeostasis, which is only apparent over time...

# OATD-01: First-in-Class Chitinase Inhibitor

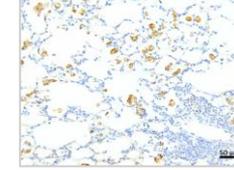
	hAMCase	hCHIT1	mAMCase	mCHIT1
OATD-01 IC <sub>50</sub> [nM]	9,2	23,4	7,8	27,5

- **OATD-01 is a highly potent, dual AMCase and CHIT1 small-molecule inhibitor with a nanomolar activity**
- **OATD-01 has a very favorable PK/PD profile in rodents**

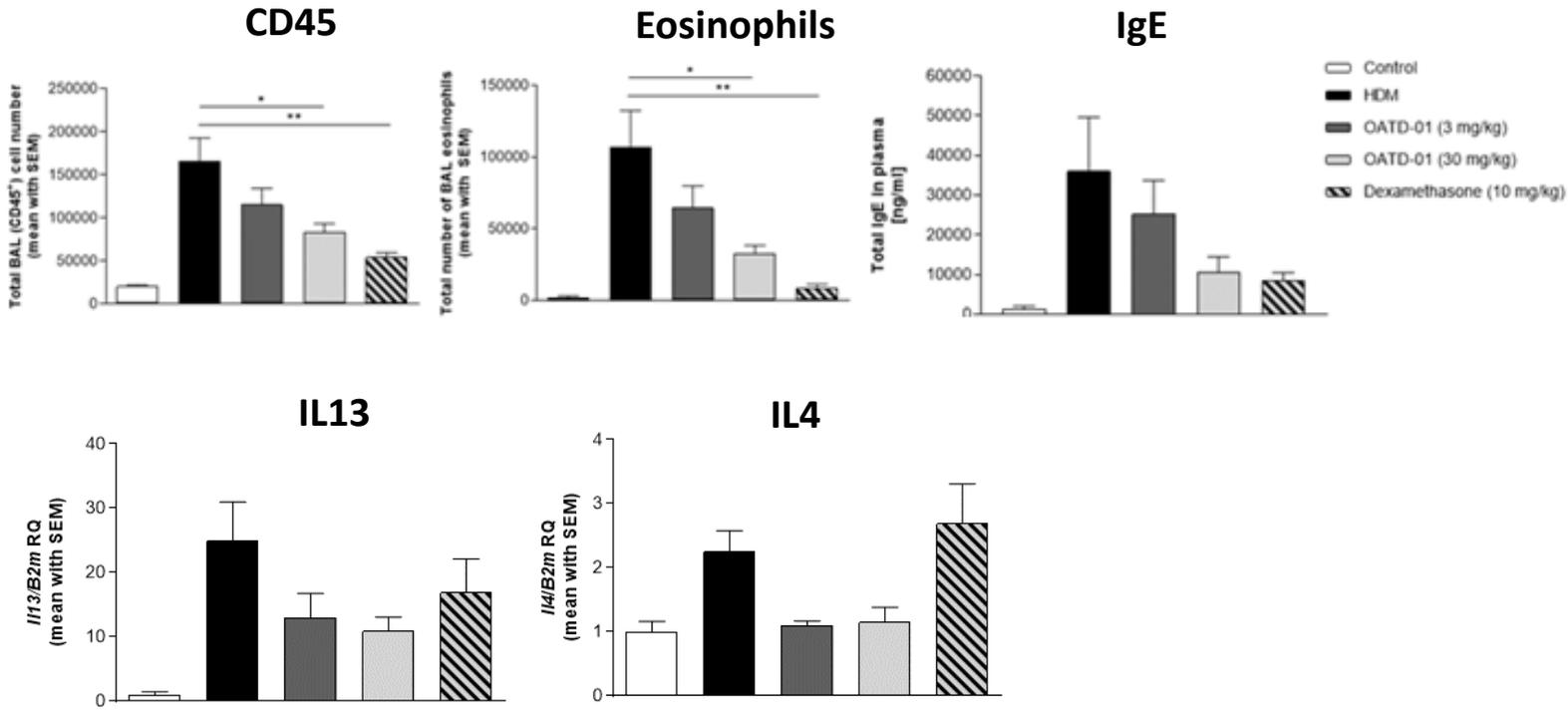
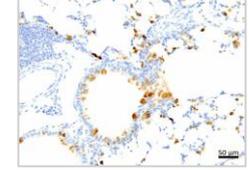
# Therapeutic Efficacy of OATD-01 in Chronic Asthma Model: Anti-inflammatory Activity



CHIT1 7w

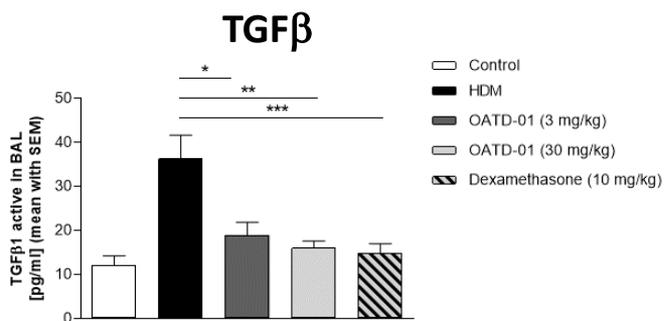
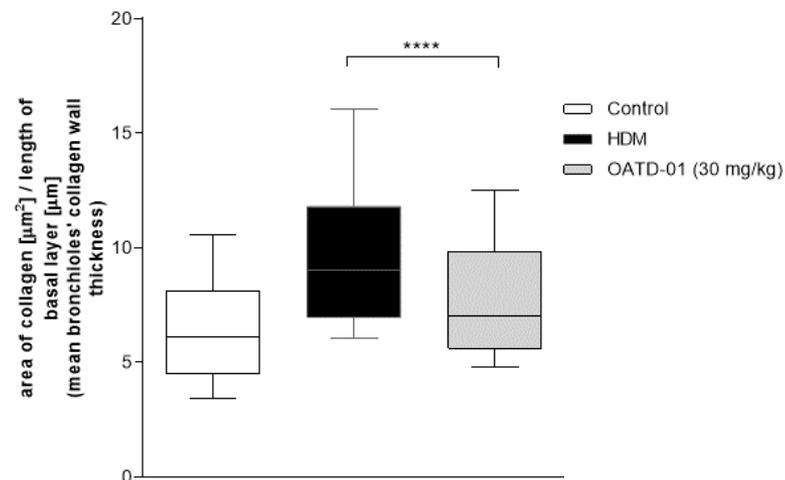
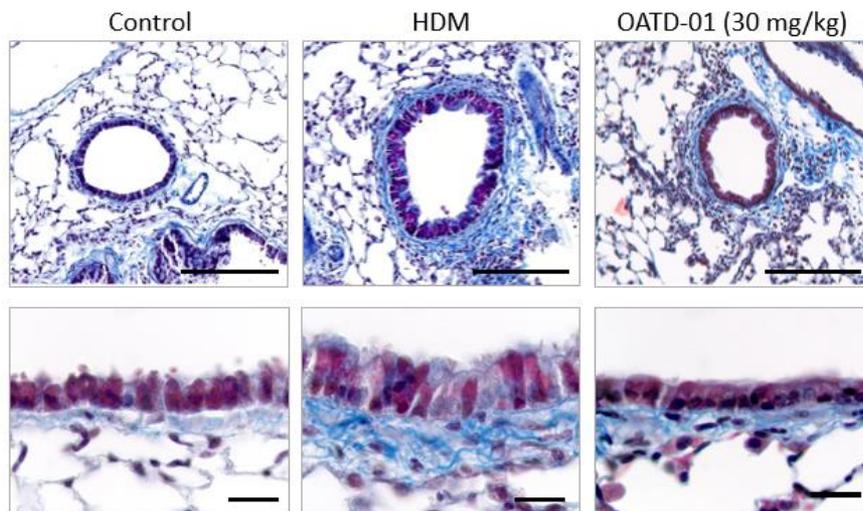


AMCase 7w



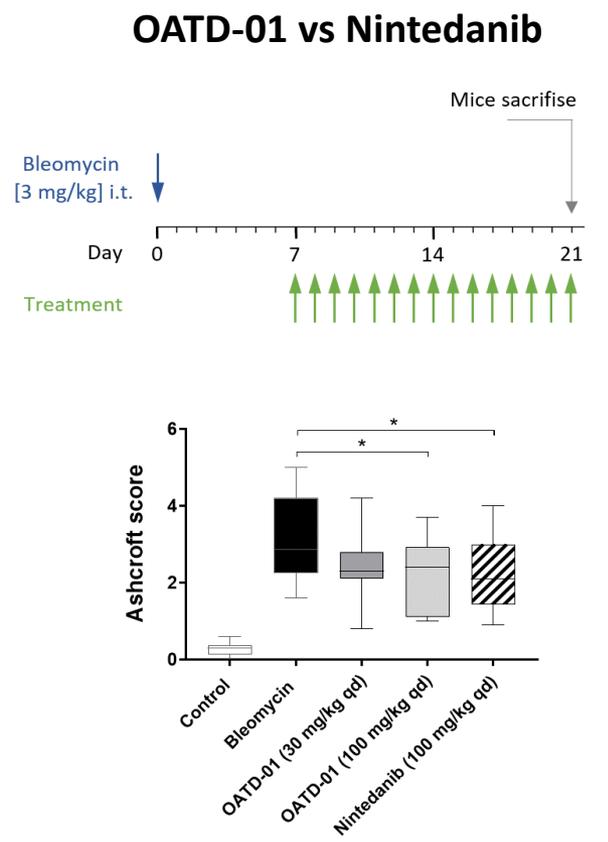
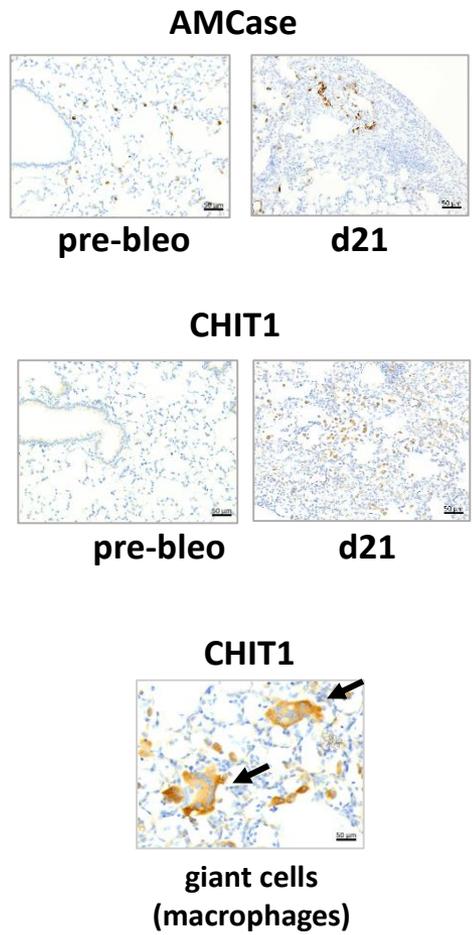
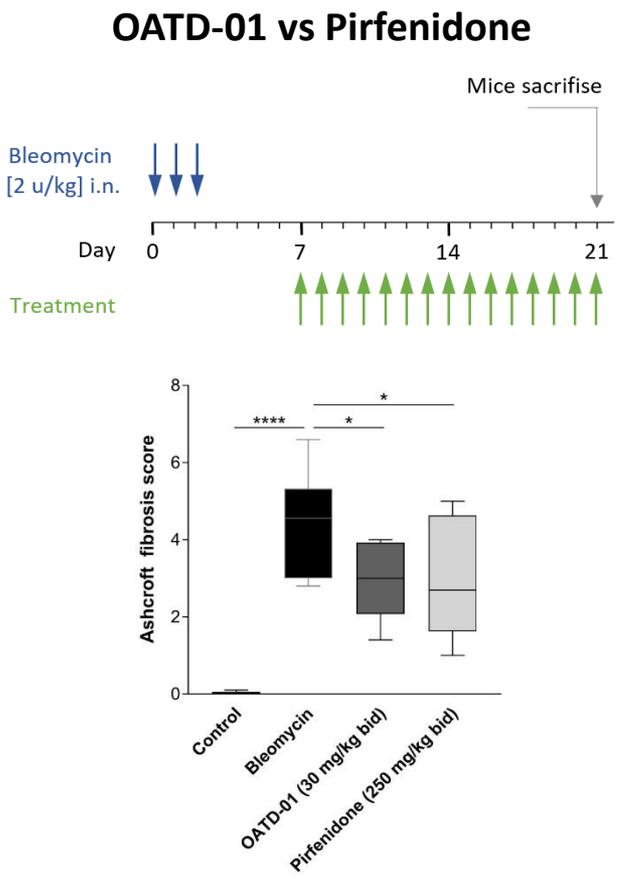
# Therapeutic Efficacy of OATD-01 in Chronic Asthma Model: Anti-fibrotic Activity

*Lung tissue remodeling: area of collagen around bronchioles and thickness of epithelium*



- OATD-01 demonstrated significant anti-remodeling activity in lungs in a therapeutic scheme of treatment

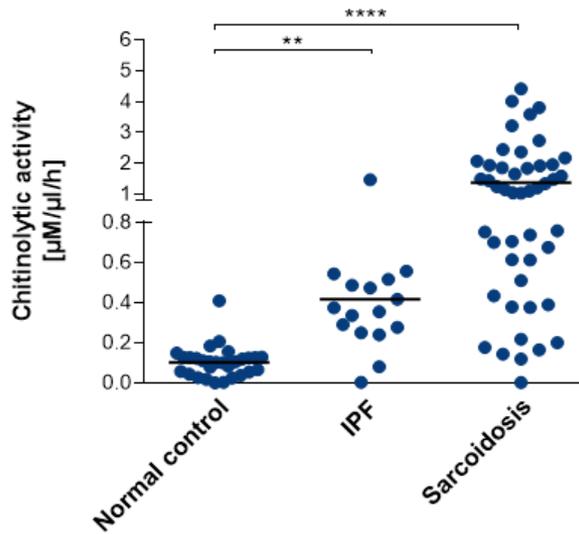
# Efficacy of OATD-01 in Bleomycin-Induced IPF Model is Comparable to Nintedanib and Pirfenidone



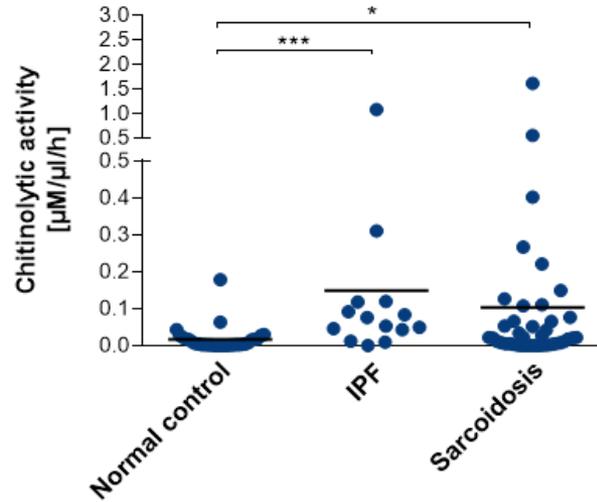
- **OATD-01 demonstrated anti-fibrotic efficacy comparable to nintedanib and pirfenidone, the only two approved drugs for IPF**

# CHIT1 Activity is Highly Elevated in Patients with ILDs

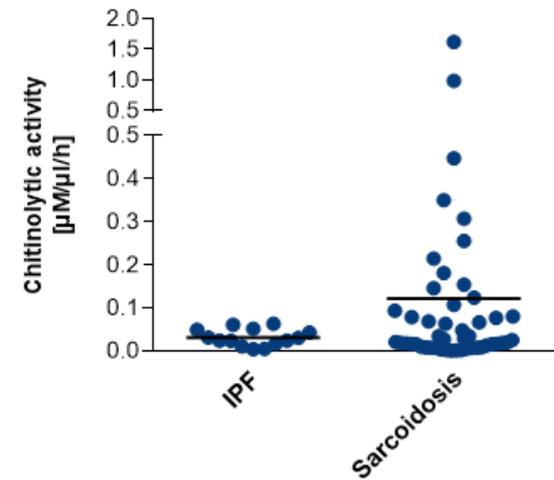
### Serum

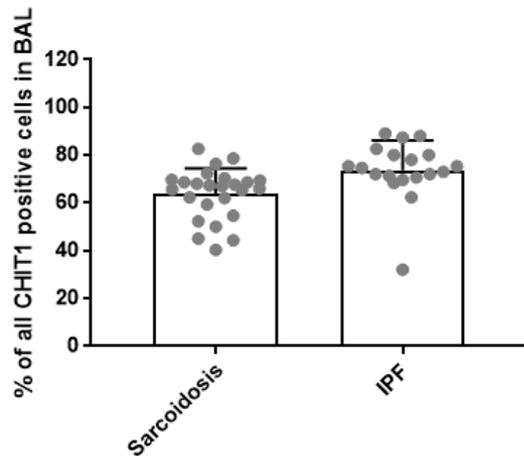
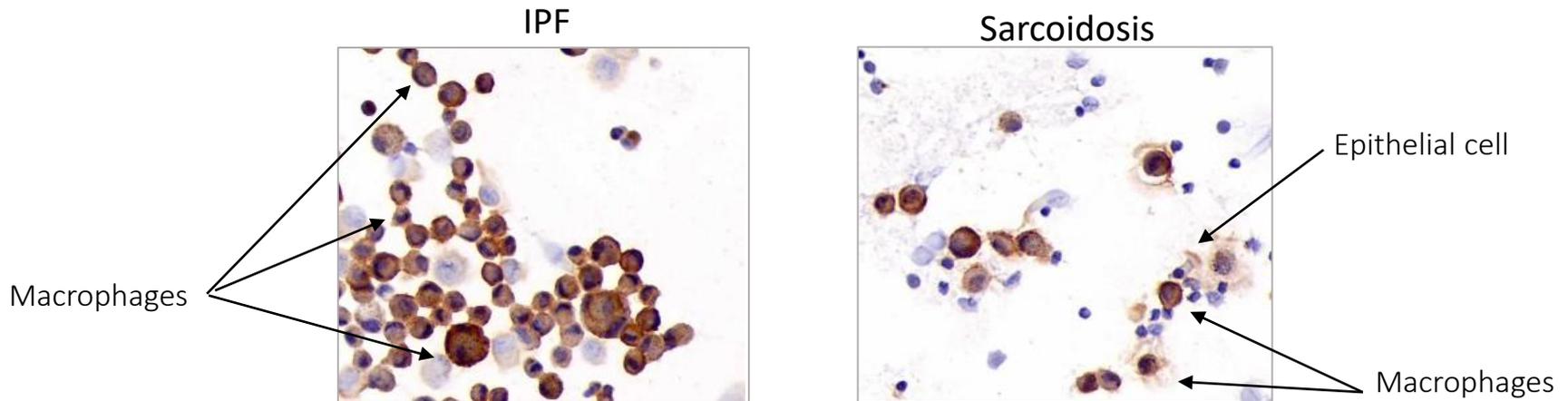


### Induced sputum



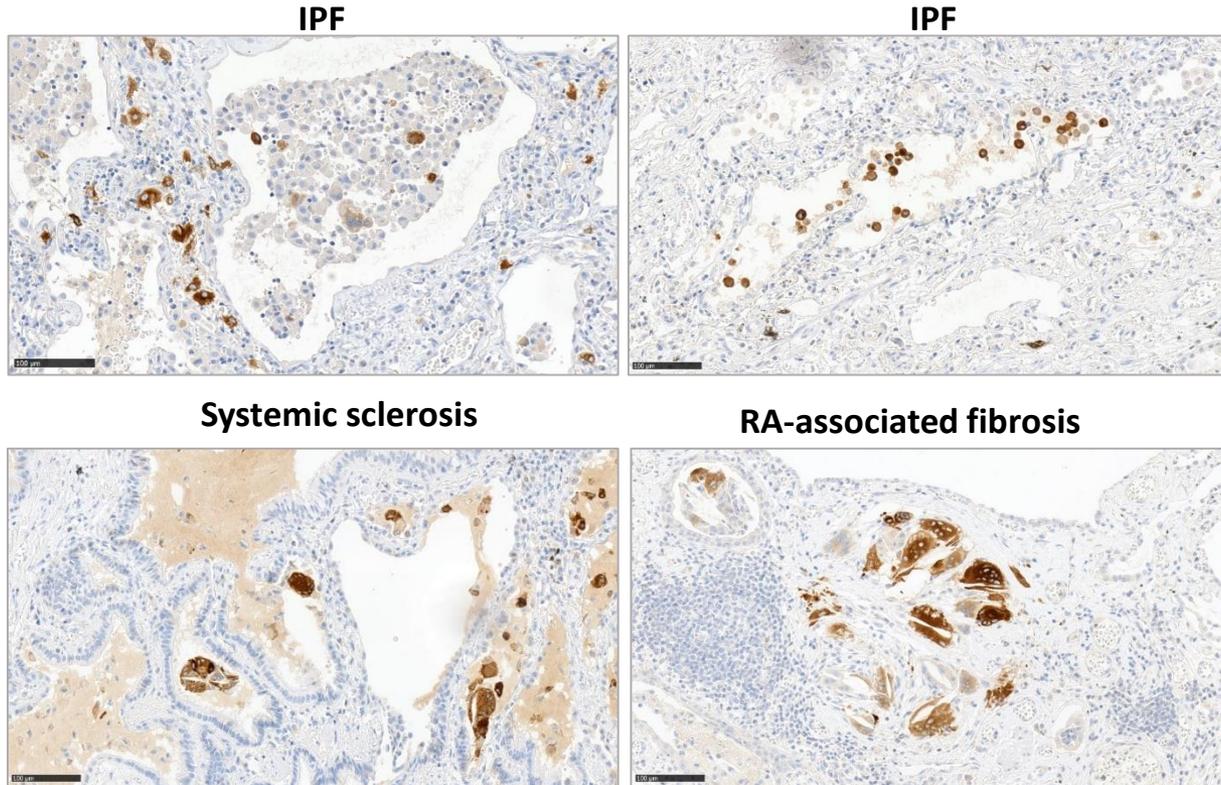
### BALf





Overall 65-75% of BALF cells expressed CHIT1. Cytological analysis confirmed that the main CHIT1-positive cell type in BAL are **macrophages** (80%), additionally **lymphocytes** stained positive (30-40%).

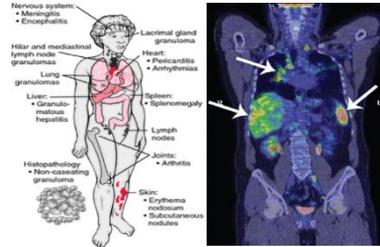
# CHIT1 is Highly Expressed in Lungs of Patient with ILDs



CHIT1 positive staining in alveolar macrophages

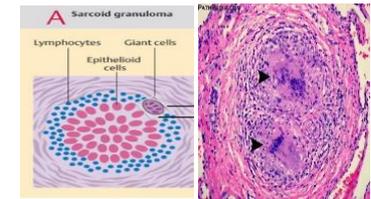
# Sarcoidosis: A Clinical Target for OATD-01

- Sarcoidosis is a systemic inflammatory disease characterized by formation of immune granulomas in various organs

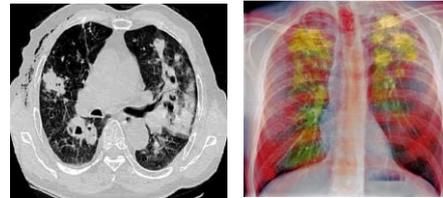


No drugs approved  
for sarcoidosis

- Granulomas are tight aggregates of immune cells consisting of centrally located, pathologically activated macrophages, epithelioid and giant cells, surrounded by T cells



- Over 90% of sarcoidosis patients develop pulmonary sarcoidosis with non-caseating granulomata in lungs



- Chronic, progressive and refractory disease can be debilitating

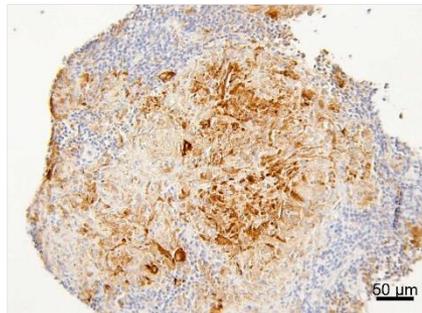


- CHIT1 is highly upregulated in serum and BALf of patients with sarcoidosis**
- CHIT1 levels significantly correlate with a stage, disease progression and clinical prognosis**
- CHIT1 levels correlate with a metabolic activity of granulomas**
- CHIT1 is considered the best marker of disease progression**

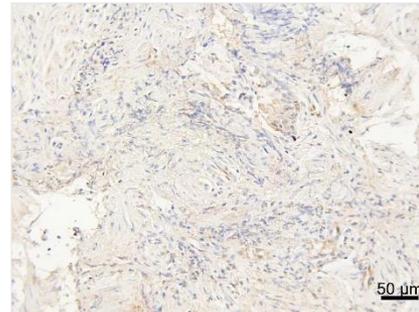
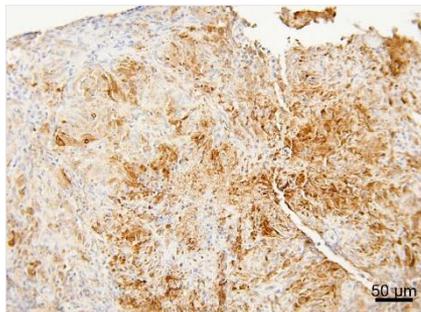
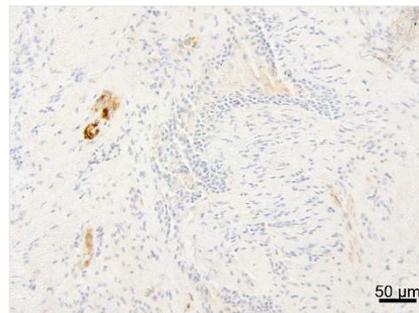
# CHIT1 is Highly Expressed in Granulomas of Patients with Sarcoidosis

## Lung sarcoidosis

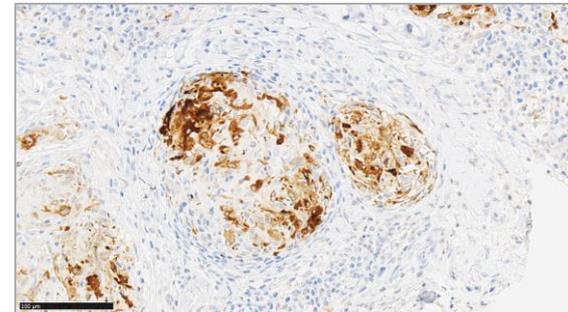
Biopsy 1. – diseased  
lungs with granulomas



Biopsy 2. – non-diseased  
lungs

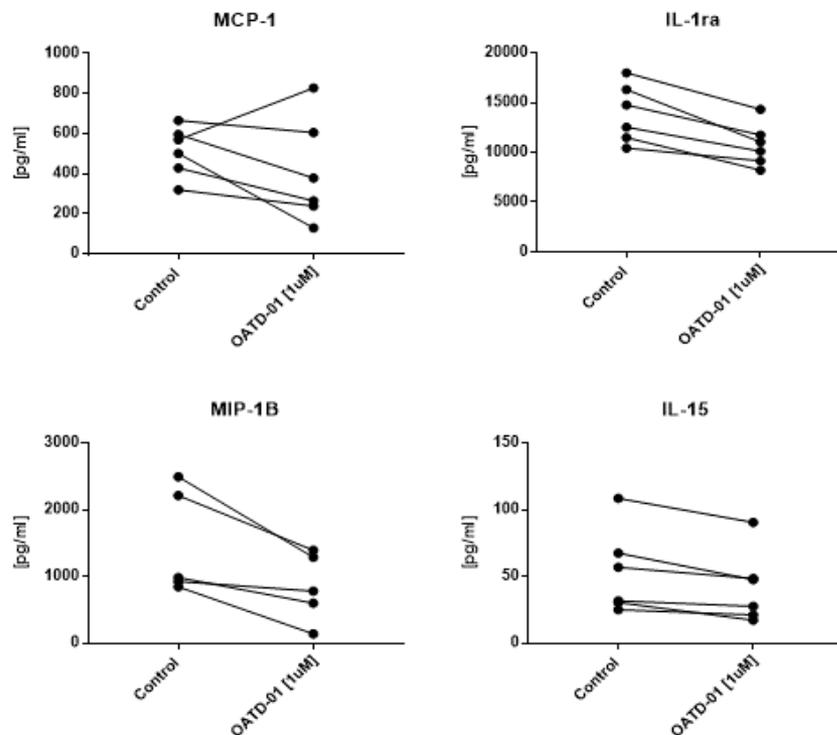


## Skin sarcoidosis

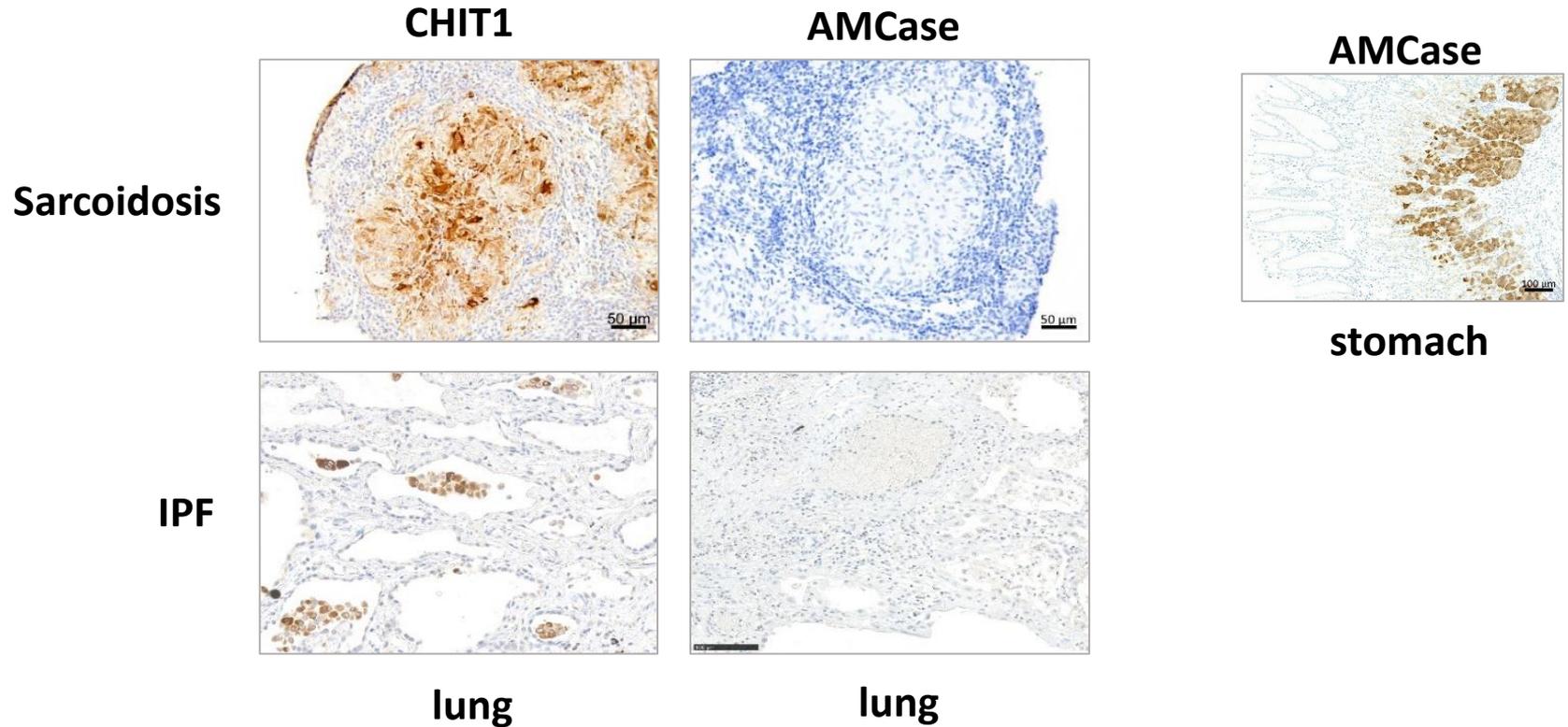


CHIT1 expression highest in pathologically activated macrophages, epithelioid and giant cells

# OATD-01 Inhibits Expression of Inflammatory Cytokines in Macrophages from BALf of Patients with Sarcoidosis

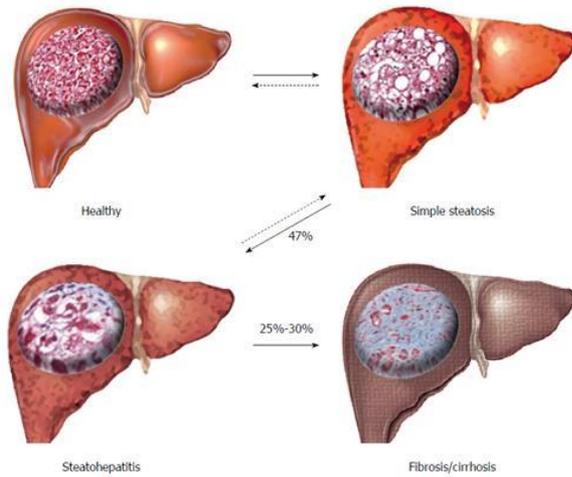


# CHIT1 but not AMCase is Expressed in Lungs of Patients with Sarcoidosis and IPF

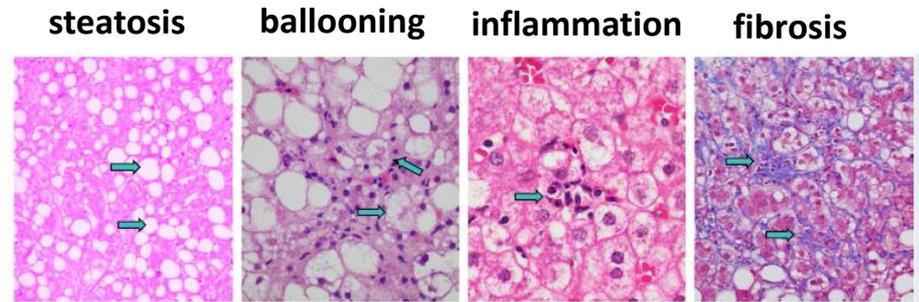


# Other Diseases with Highly Upregulated CHIT1 Expression

- Lysosomal storage diseases (Gaucher, Niemann-Pick, Fabry)
- Nonalcoholic steatohepatitis (NASH)
- Amyotrophic Lateral Sclerosis (ALS)
- Diabetic nephropathy
- Crohn's Disease
- Asthma

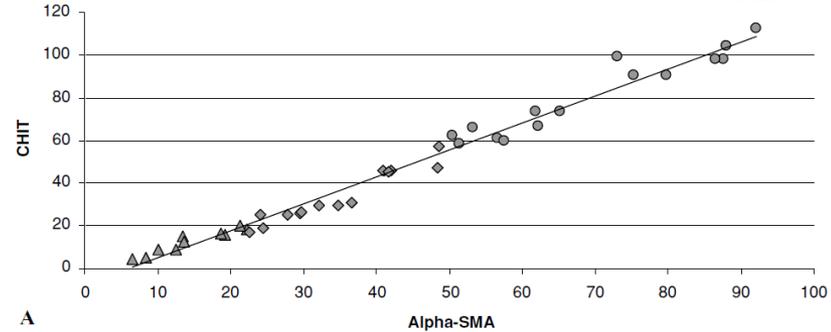
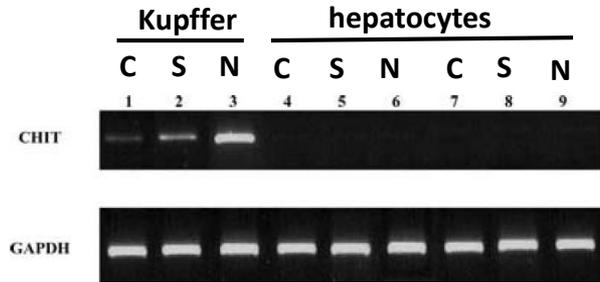


## NASH



- 30mln patients in US
- \$35bln market
- >25 Ph2/3 clinical trials

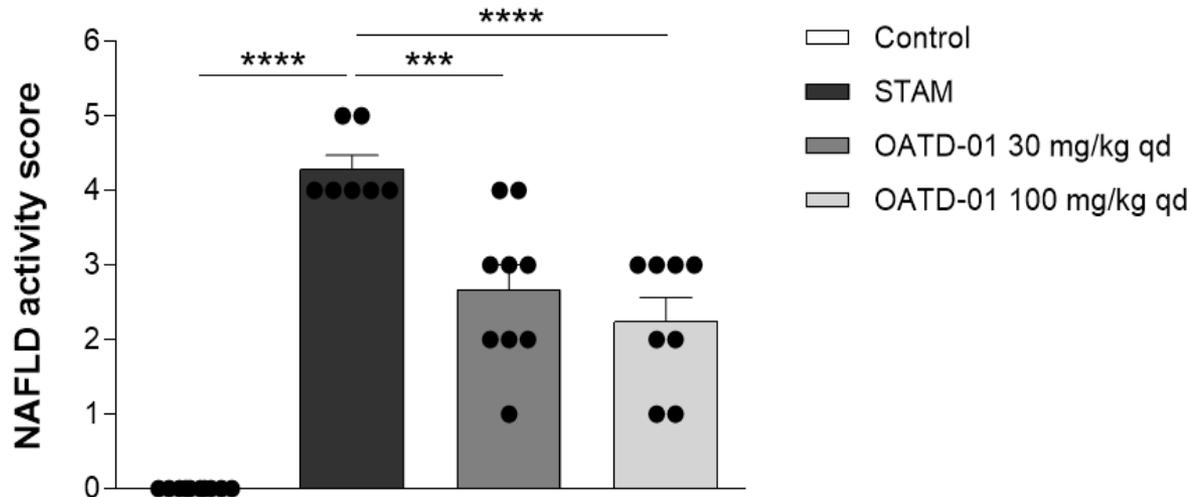
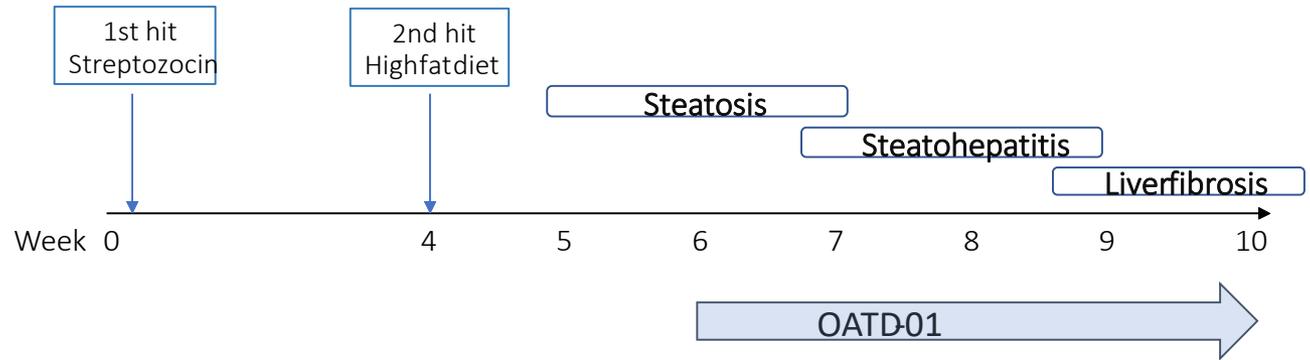
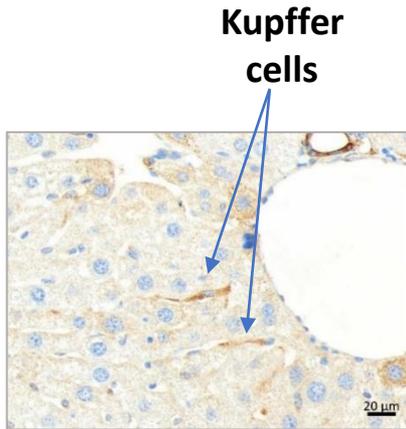
# CHIT1 Expression is Highly Upregulated in NASH Patients



- CHIT is expressed exclusively by Kupffer cells
- CHIT1 expression significantly upregulated in NASH vs steatosis
- CHIT1 significantly expression correlated with  $\alpha$ SMA levels
- CHIT1 levels significantly correlated with TNF $\alpha$  and lipid peroxidation

Malaguarnera et al. Am J Gastroenterol. 2006 Sep;101(9):2060-9

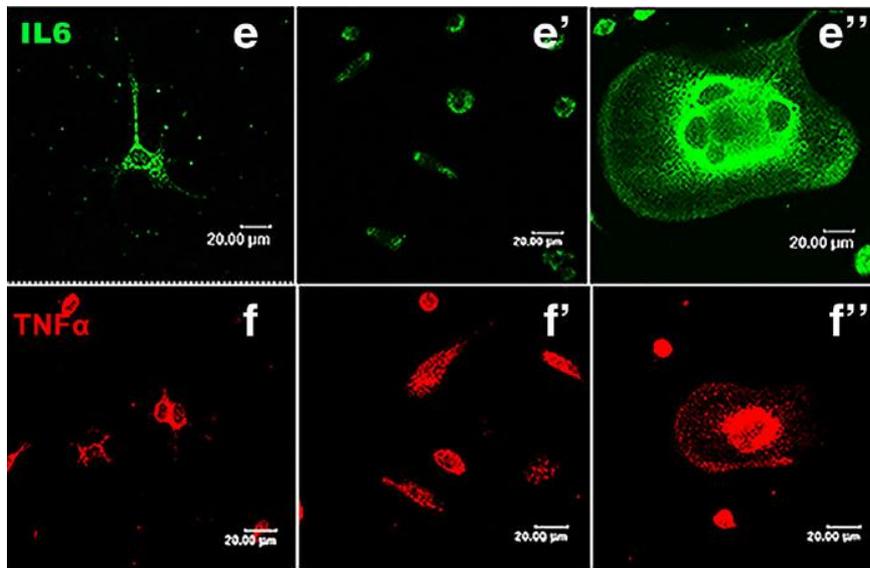
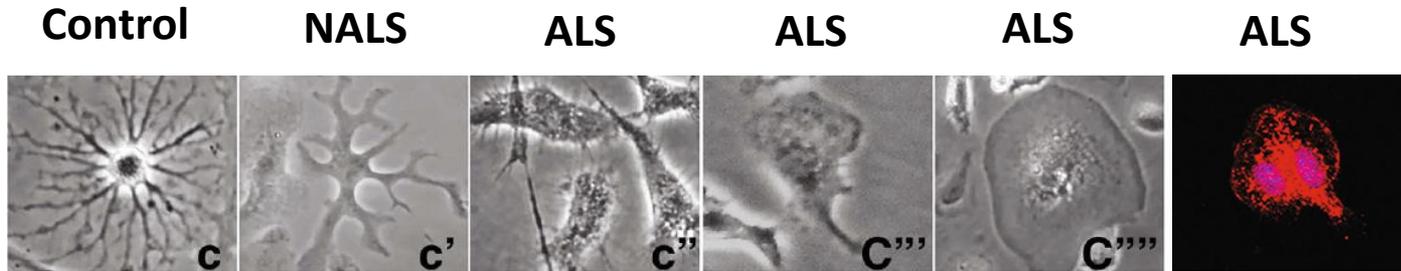
# Therapeutic Efficacy of OATD-01 in the STAM Model of NASH



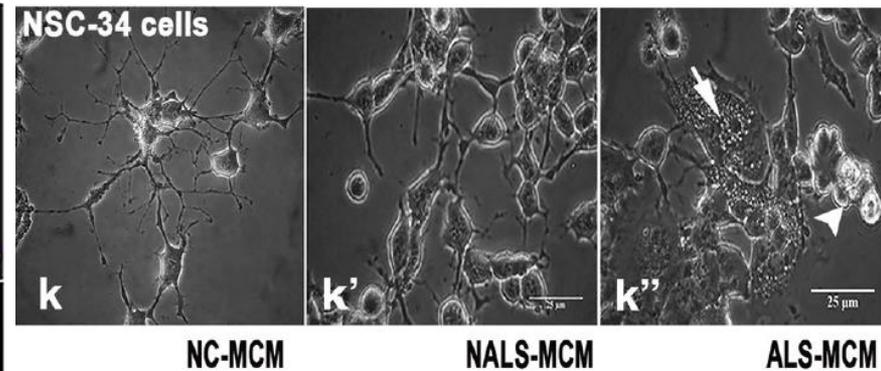
# CHIT1 Expression in CSF of ALS Patients: Summary

Study	Method	Samples	CHIT1	Comments
Vargese et al. Clin. Proteom. 2013	CSF: LC/MS (samples combined)	sALS vs controls	most upregulated (31) LC/MS: 10x ELISA: 17x Activity: 19x	Expr. in microglia (cell cultures)
Chen et al. BMC Neurol. 2016	CSF: ELISA	40 sALS vs 40 non-ALS neurol. disease	Strongly upregulated in sALS. Biomarker: 84% sensitivity	No ass. with rate of progression
Thompson et al. Ann. Neurol. 2018	CSF: uHPLC LC/MS	43 ALS 20 PD 12 ALS-mimics 25 healthy	773 proteins quant. CHIT1 most differentially abundant. Biomarker of ALS. Significantly ass. with progression rate and survival	CHIL1 and CHIL2 also biomarker of ALS. In combination with CHIT1 best predictor
Steinacker et al. JNNP 2017	CSF: ELISA	60 ALS 46 ALS-mimics 135 ND dis. 25 healthy	Upregulated in ALS: 87% sensitivity Slow prog: 2400-7700pg/ml Fast prog: 6000-40000pg/ml Healthy: 700-1600pg/ml Ass. with progression and severity	CHIT1 expr. in lateral and cortical CST. Colocalized with Iba-1+ microglia and CD68+ macrophages
Oeckl et al. Neurodegen. 2018	CSF: ELISA		Increased CHIT1 levels. Increased CHI3L1 levels	No CHIT1 increase in early stages

# CSF from ALS Patients Induces Inflammatory Neurotoxic Phenotype in Microglia

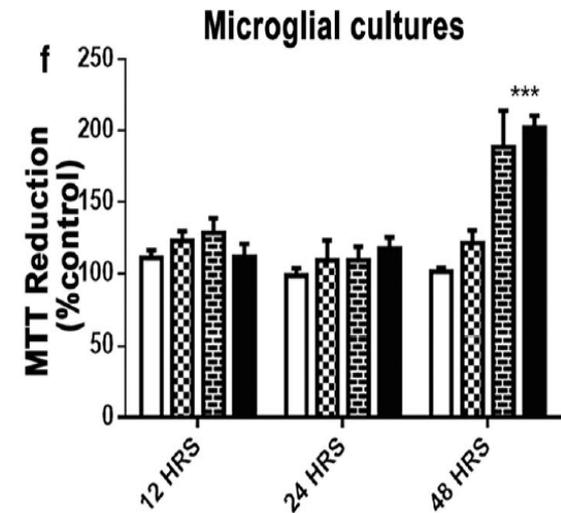
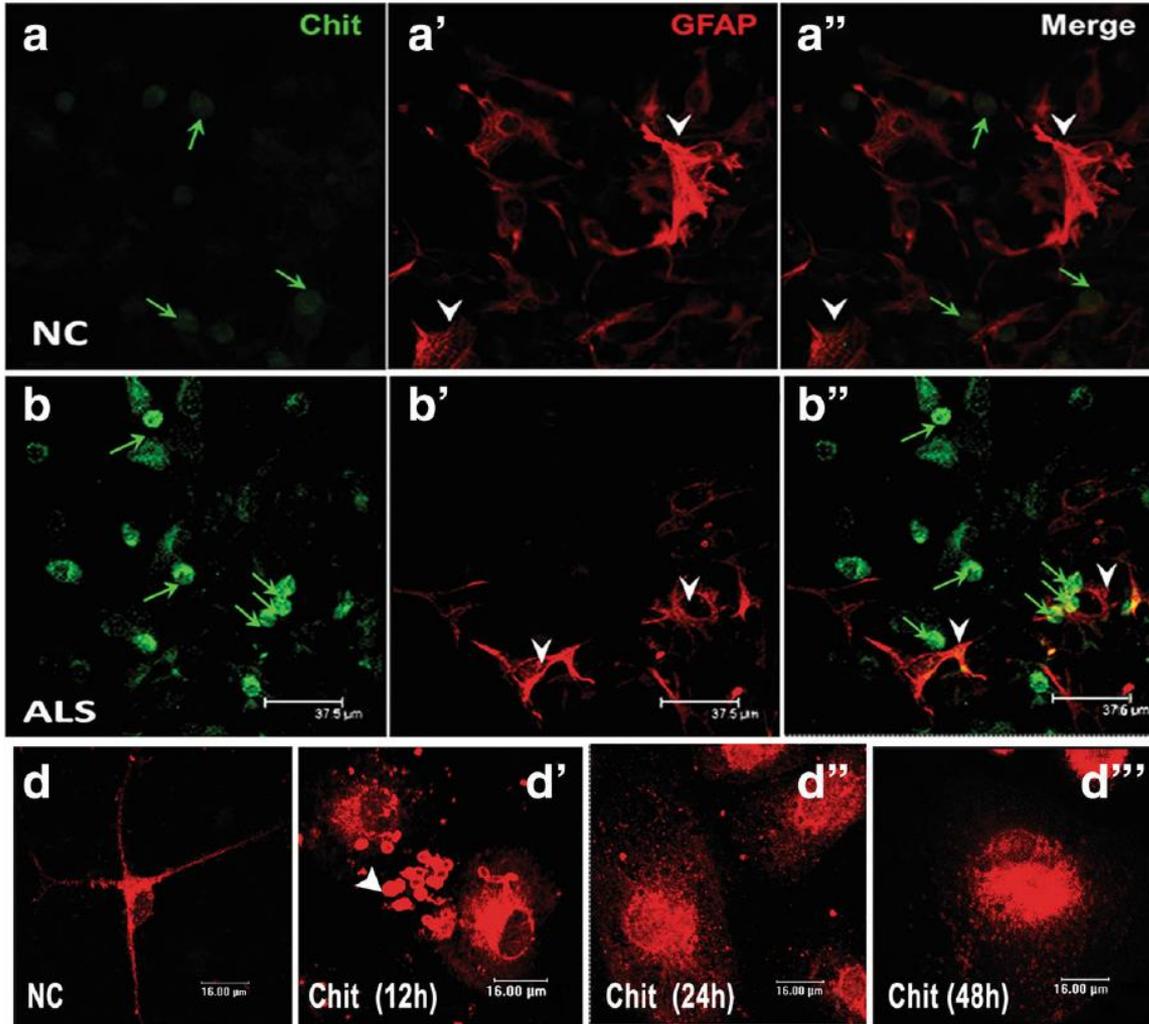


Control      NALS      ALS



Mishra *et al. Journal of Neuroinflammation* (2017) 14:251

# CHIT1 Induces Microglia Activation and Proliferation



Mishra et al. *Journal of Neuroinflammation* (2017) 14:251

# Do We Need to know the Mechanism of Action?

	Disease ass. expression	Clinical correlation	Disease: cell type expression	Preclinical validation
<b>IPF</b>	✓	✓ ✓	alveolar mΦ	✓
<b>Sarcoidosis</b>	✓	✓ ✓ ✓	epithelioid (mΦ) giant (mΦ) T cells	In progress
<b>NASH</b>	✓	✓ ✓	Kupffer (mΦ)	✓
<b>ALS</b>	✓	✓ ✓	microglia (mΦ)	✓
<b>Crohn's</b>	✓		macrophages (granuloma)	



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