GENOSCO

ATS 2023

Novel Small Molecule ROCK Inhibitor GNS-3595 Attenuates Idiopathic Pulmonary Fibrosis Progression in Preclinical Models

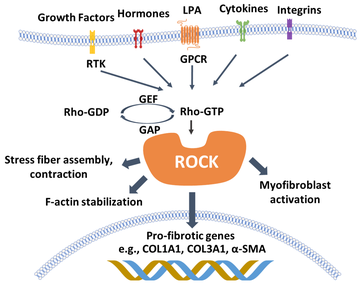
Soyoung Hwang, Wongil Lee, Miyong Yong, Sang-Ae Seung, William Devine, Bruce Diebold, Ravi Dashnamoorthy, Nicholas Ng, Vinay Sagar, Travis Clark, Youseung Shin, Jang Sik Choi, Jaekyoo Lee, Anu Gupta, Sewon Kim, Jong Sung Koh

Genosco Inc., Billerica, MA, United States

ROCK and Fibrosis

Idiopathic Pulmonary Fibrosis (IPF) is a chronic interstitial lung disease with progressive scarring of lung tissue and impaired oxygen transport with poor prognosis and limited therapeutic options. Currently, Nintedanib and Pirfenidone are FDA approved treatment options for IPF. Since existing drugs only block disease progression but do not completely cure IPF, novel pharmacological therapeutic interventions are needed.

IPF is caused by actin cytoskeleton re-organization mediated by epithelial cells, fibroblasts, endothelial cells, and macrophages, after tissue injury. In this process, the Rho-associated coiled coil forming protein kinase (ROCK) family, consisting of ROCK1 and ROCK2, is vital for actin filament assembly and actomyosin contraction. When activated by GTP-bound RhoA, ROCK phosphorylates multiple substrates which can impact cell morphology, adhesion, extracellular matrix remodeling, growth, migration, and apoptosis.



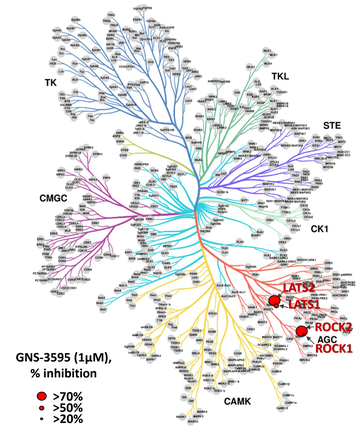
A deregulated wound healing process can result in chronic injury and organ fibrosis, and IPF patients have shown elevated RhoA/ROCK signaling activity. Profibrotic responses in lung and other organs are mediated by ROCK activation, suggesting that ROCK may play an important role in fibrotic diseases. In several disease models, ROCK inhibitors were found to be effective against fibrosis and even reversed existing fibrosis, indicating that ROCK inhibition is a promising therapy.

ROCK inhibitor, GNS-3595

In this study, we report the identification of a highly potent, selective, and orally active ROCK inhibitor GNS-3595 which demonstrates anti-fibrotic effect both *in vitro* and *in vivo*. GNS-3595 showed high potency against ROCK2 with an IC50 of 5.7 nM and ~80-fold selectivity over ROCK1 at 1 mM ATP. A cell-based target engagement assay showed that GNS-3595 inhibited pMLC activity in various cell-lines, and significantly down-regulated pro-fibrotic proteins such as collagen, fibronectin and α-SMA in IPF cells. When examined in the context of fibroblast-to-myofibroblast transition (FMT), a critical process in IPF diseases, GNS-3595 suppressed TGF-β-induced FMT in normal human lung fibroblast (NHLF) cells. GNS-3595 exhibited excellent oral bioavailability and drug-like properties. Furthermore, GNS-3595 suppressed pulmonary lung fibrosis in a bleomycin-induced *in vivo* mouse model, stabilized body weight loss and prevented fibrosis-induced lung weight gain. Therapeutic exposures with GNS-3595 at oral dose of 0.1, 0.3 or 1 mg/kg once daily showed a significant reduction in Ashcroft scores, collagen, and α-SMA in a dose-dependent manner. Our data suggest that GNS-3595 is a promising therapeutic agent against pulmonary fibrosis and has the potential to target other fibrotic diseases of similar underlying mechanisms.

GNS-3595 shows potent ROCK inhibition and superior selectivity

Kinase Profiling Under Physiological ATP Concentration



In Vitro Kinase Assay, IC50 (nM) [ATP 1mM]		
Compound	GNS-3595	KD025
ROCK I	493.3	NC
ROCK II	5.7	8984

(TR-FRET Assay, in house)

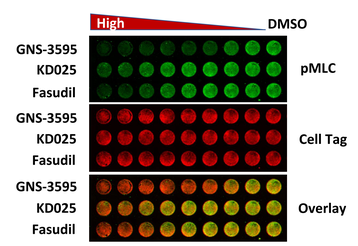
Residence Time (min)		
Compound	GNS-3595	KD025
ROCK I	45	NE
ROCK II	71	3.8

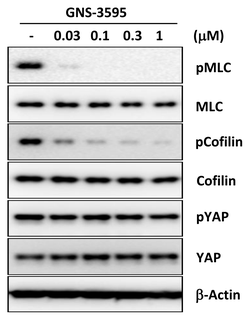
(Enzymlogic, CRO)

Kinase Target	1 μM GNS-3595, % inhibition at 1 mM ATP
LATS2	81.2
ROCK2	70.1
LATS1	55.1
ROCK1	28.2

- *GNS-3595 is a highly selective compound, causing strong inhibition in 2 kinases, LATS2 and ROCK2.*
- *It led to >70% inhibition of both LATS2 and ROCK2, >50% inhibition of LATS1, and >20% inhibition of ROCK1.*
- *Notably, results from cell-based assays demonstrated a stronger inhibitory effect against ROCK2 than against LATS2.*

GNS-3595 inhibits ROCK-dependent signaling pathway

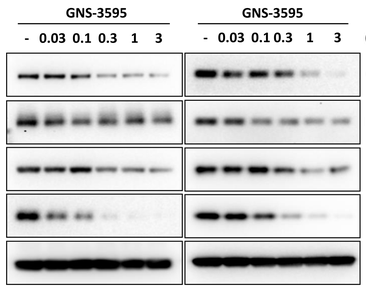


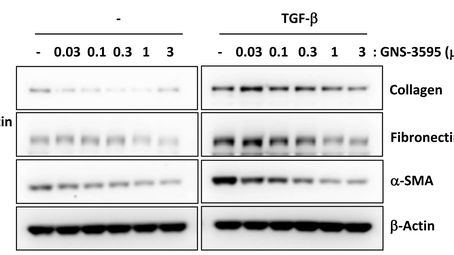


pMLC Inhibition Activity, IC50 (nM)			
Compound	GNS-3595	KD025	Fasudil
In Cell Western Assay	48	4472	1939

- *GNS-3595 strongly inhibits ROCK-dependent downstream pMLC level in A7r5 smooth muscle cells.*
- *GNS-3595 shows a stronger inhibitory effect on ROCK-dependent signaling pathway compared to known ROCK inhibitors.*

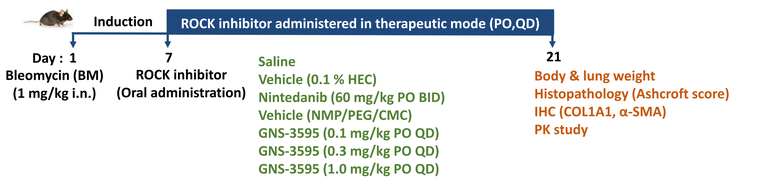
GNS-3595 inhibits profibrotic signaling pathway

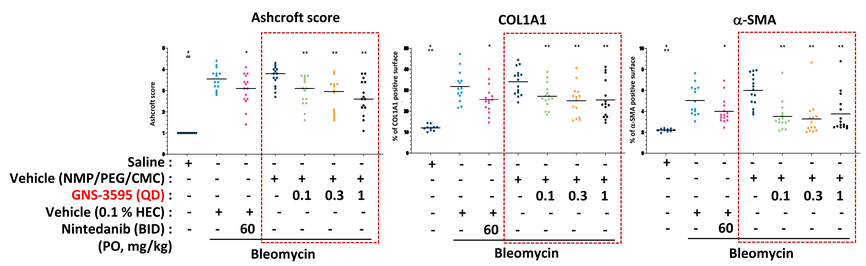


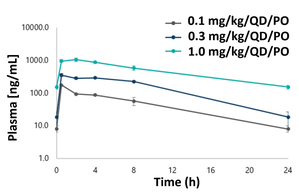


- *GNS-3595 suppresses profibrotic protein expression levels in IPF cells*
- *GNS-3595 inhibits TGF-β induced fibroblast-to-myofibroblast transition (FMT) in normal human lung fibroblast cells*

GNS-3595 shows good therapeutic efficacy in bleomycin-induced lung fibrosis model



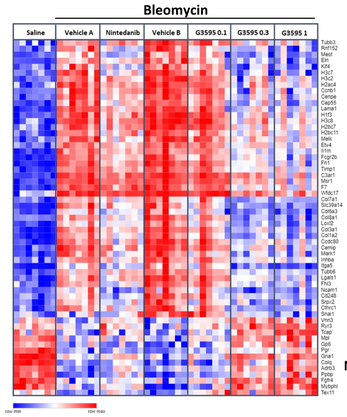


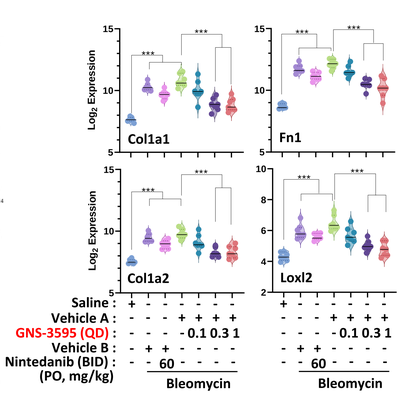


Plasma parameters	GNS-3595		
	0.1 mg/kg QD/PO	0.3 mg/kg QD/PO	1.0 mg/kg QD/PO
Cmax (ng/mL)	176	355	1,045
Tmax (h)	0.5	0.5	2
AUC(0-24h) (ng.h/mL)	1,104	3,487	11,690
t1/2(h)	5.76	4.82	8.03

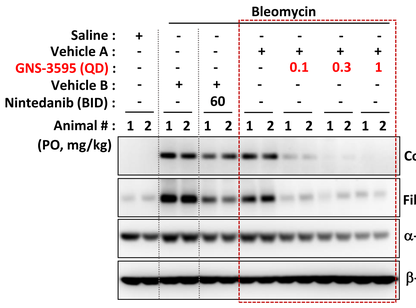
PK data from efficacy study in a 21-day mouse model of BM-induced IPF

Suppression of fibrosis-related gene expression by GNS-3595





- *BM-induced fibrosis model displayed changes in gene expression profile reflective of extracellular matrix reorganization, representing the disease-related phenotype.*
- *GNS-3595 largely reversed BM-induced changes in gene expression profile to that of normal mice, particularly for fibrosis-related genes and extracellular matrix reorganization genes.*
- *GNS-3595 showed a stronger effect than Nintedanib in reversing disease-related gene expression changes.*
- *In addition, GNS-3595 inhibited collagen, fibronectin and α-SMA protein expression level.*



Drug-like ADMET properties and PK profiles of GNS-3595

Study	Type (Unit)	Results	
Metabolic Stability	t1/2 (min)	Mouse	>58
		Rat	>60
		Dog	>60
		Human	>60
Human Recombinant CYP Inhibition	IC_{50} (μM)	1A2	>50
		2C9	13
		2C19	10
		2D6	>50
		3A4	8
Permeability	Caco-2 Cell (10^{-6}cm/sec)	A to B	16
		B to A	34
		Efflux Ratio	2

Species	Mouse		Rat		Dog	
Route	Oral	IV	Oral	IV	Oral	IV
Dose (mg/kg)	5	2	5	2	2	1
t1/2 (hr)	5.1	4.8	14.2	12.6	7.2	5.83
Cmax (ng/mL)	7920	6881	8483	6850	2113	5467
AUClast (h*ng/mL)	105669	45901	128461	59756	26711	23743
Vz (L/kg)		0.3		0.5		0.3
CL (mL/min/kg)		0.7		0.4		0.7
F (%)	92.6		86		56.3	

Conclusion

- GNS-3595 is a highly potent, selective ROCK inhibitor based on *in vitro* kinase assay and kinase profiling results.
- GNS-3595 showed anti-fibrotic activity and inhibited TGF-β-induced FMT.
- GNS-3595 is orally bioavailable and has excellent pharmacokinetics profiles.
- GNS-3595 reduced lung weight, Ashcroft score, collagen and α-SMA expression level in therapeutic murine bleomycin-induced lung fibrosis model.
- Whole transcriptome profiling of tissue samples from bleomycin-induced IPF mouse model treated with GNS-3595 demonstrated suppression of bleomycin-induced changes in extracellular matrix reorganization genes in response to GNS-3595. Hippo pathway target genes remained unaffected by GNS-3595, indicating that *in vivo* LATS inhibition may not occur.
- No cardiovascular toxicity was observed in GNS-3595-treated rats based on mean blood pressure.

References

1) Zhou et al, J Clin Invest. 2013, 2) Knipe et al, Am J Respir Cell Mol Biol. 2018, 3) Monaghan-Benson et al, Mol Biol Cell. 2018, 4) Knipe et al, Pharmacol Rev. 2015, 5) Hartmann et al. Front Pharmacol. 2015, 6) Lederer et al, N Engl J Med. 2018

Genosco Inc., Billerica, MA, United States, email: syhwang@genosco.com, www.genosco.com