The Anti-Fibrotic Effect of GB0139, a Small Molecule Galectin-3 Inhibitor, in Precision Cut Lung Slices from Idiopathic Pulmonary Fibrosis Tissue

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Introduction

GB0139 is an inhaled, small molecule inhibitor of galectin-3 (Gal-3) that is currently in phase 2b studies for IPF (NCT03832946). Gal-3 has been shown to be a key driver of lung fibrosis[1] and in this study the anti-fibrotic effect of GB0139 was investigated in idiopathic pulmonary fibrosis (IPF) precision cut lung slices (PCLuS).

Methods

Human fibrotic lung tissue was sourced ethically from human explants from three patients with IPF undergoing lung transplantation. All patients had their IPF diagnosis confirmed based on medical history and evaluation of their explanted lung tissue by a board-certified respiratory pathologist. Tissue was inflated with 2–3 % low boiling point agarose and allowed to set at 4°C. PCLuS were then cut at 400 µm on a vibrating microtome and cultured in DMEM media. PCLuS were rested for 48 h prior to treatment with inhibitors for 6 days with media replaced every 24 h. Soluble mediators released were measured daily via standard ELISAs. In addition, analysis of experimental proteomics data derived from MS testing on the PCLuS were completed.

Results

GB0139 caused a concentration-dependent reduction in Gal-3 in IPF PCLuS. This was associated with a reduction in markers of fibrosis (Co1α1, hyaluronic acid, TIMP-1, MMP-7) comparable to pirfenidone and nintedanib. A number of pathways were perturbed by down-regulated proteins that included transcriptional activity of SMAD2/SMAD3 and platelet activation, signalling and aggregation.

Conclusions

GB0139 demonstrates Gal-3 target engagement that results in an anti-fibrotic effect comparable to approved IPF therapies in PCLuS, at concentrations achieved in the lung when dosed in IPF patients[2]. In addition, GB0139 inhibited pathways in PCLuS that correlate with biomarker changes observed in IPF[2] and COVID19[3] clinical studies.

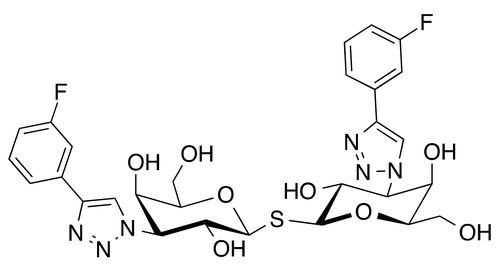
References

1. MacKinnon AC *et al. Am. J. Respir. Crit. Care Med.* 2012;185(5):537-546.
2. Hirani N *et al., Eur. Respir. J.*, 2021:57:2002559.
3. Gaughan EE et al., Am J Respir Crit Care Med 2022; 207(2):138-149.

Results

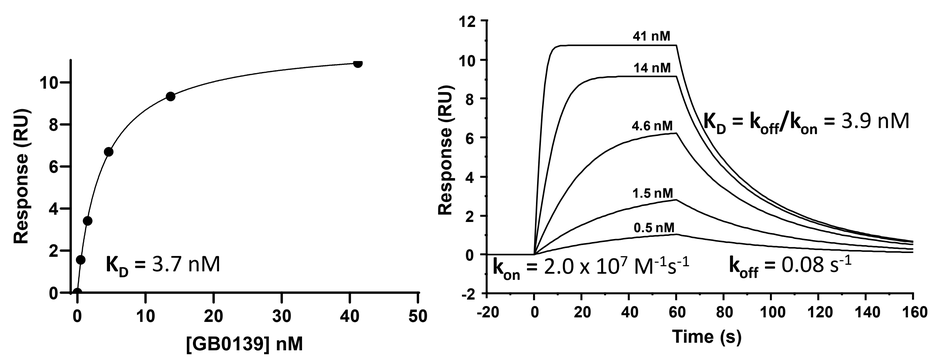
In vitro characterization of GB0139 (biochemical & cellular)

GB0139

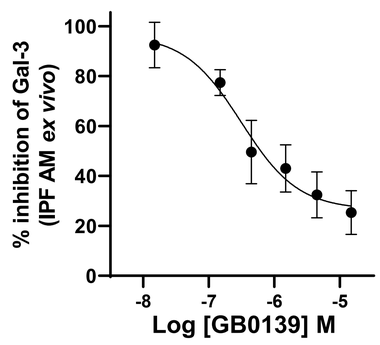


	Human ±SD (n)	Mouse ±SD (n)
FP K_D (nM)	2.1 ± 0.1 (4)	54 ± 17 (3)

GB0139 Gal-3 Affinity & Kinetics (SPR)



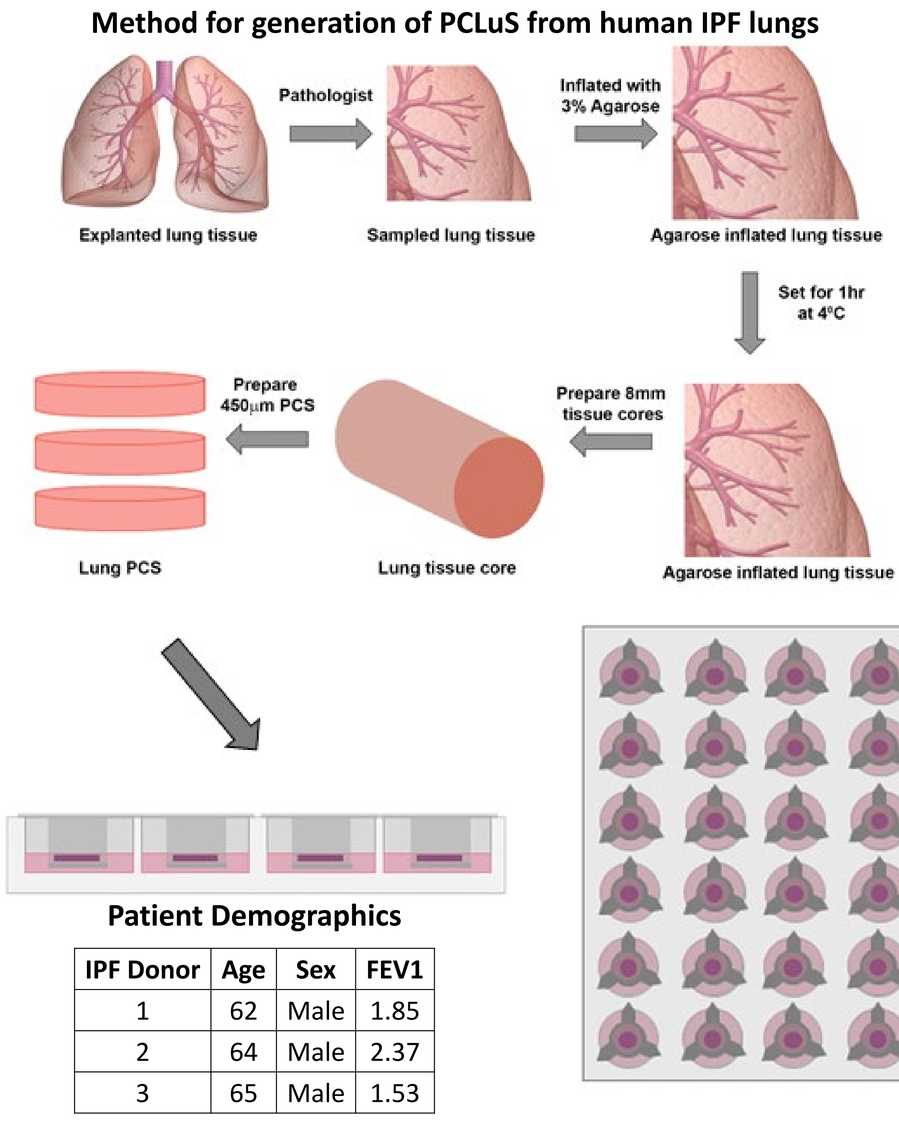
***Ex vivo* IPF BAL cells**



GB0139 demonstrates high affinity for Gal-3 & inhibits Gal-3 expression *ex vivo* on IPF BAL cells (IC_{50} = 361 ± 108 nM (mean ± SD, n=3 patients)).

Ex vivo precision cut IPF lung slice

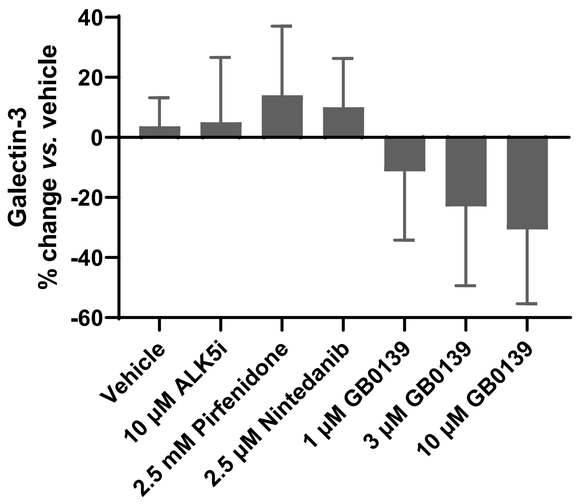
Method for generation of PCLuS from human IPF lungs



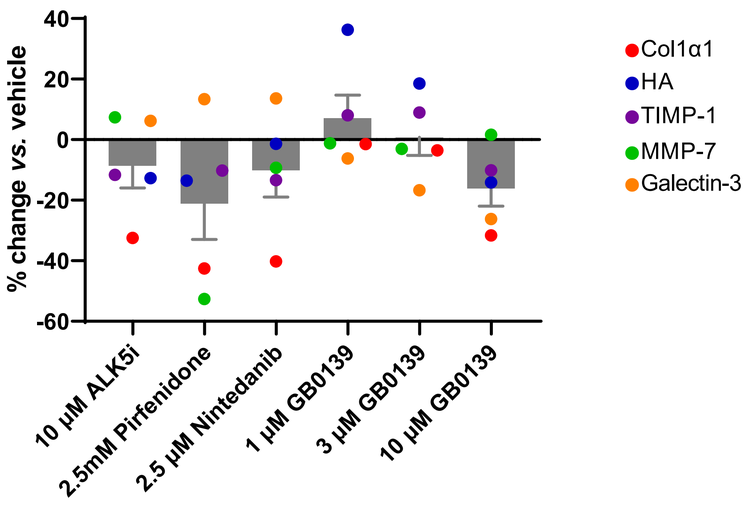
Patient Demographics

IPF Donor	Age	Sex	FEV1
1	62	Male	1.85
2	64	Male	2.37
3	65	Male	1.53

Secreted galectin-3 (Daily Average Change)

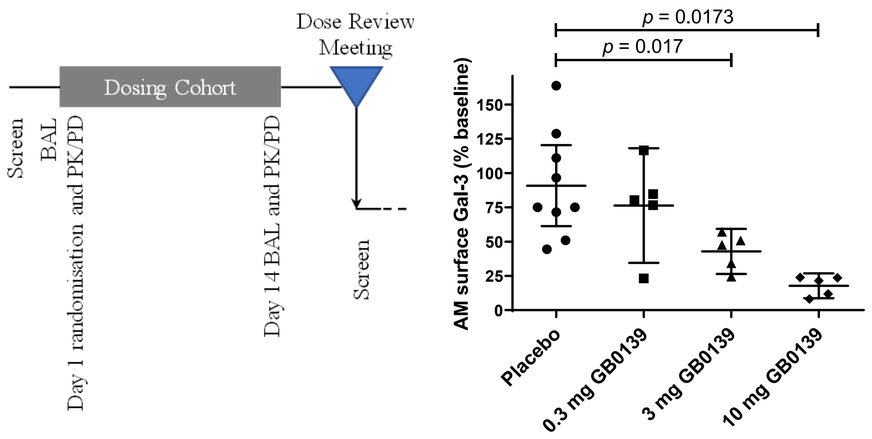


Secreted fibrotic markers (Daily Average Change)

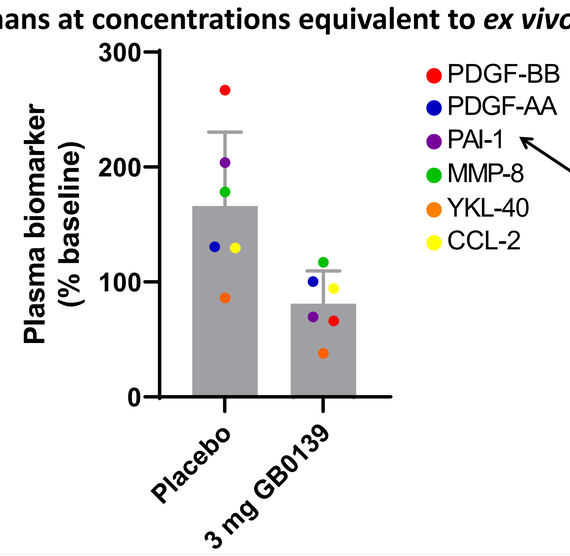


Translational pharmacology of GB0139 in IPF

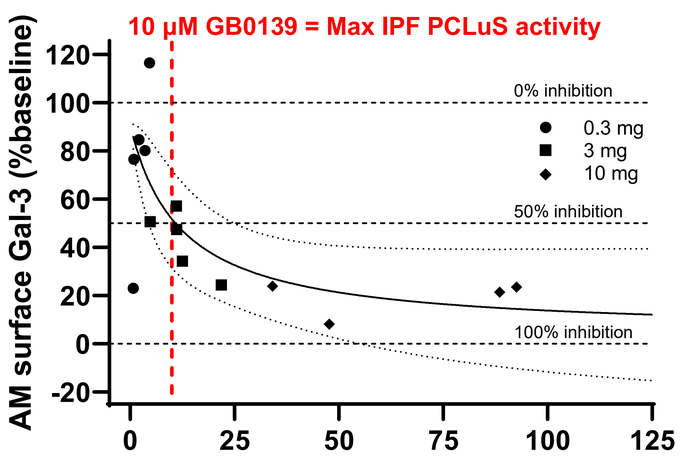
Phase Ib – GB0139 dose–dependently reduces galectin-3 in the lungs of IPF patients



Phase Ib – GB0139 inhibits IPF fibrotic markers in humans at concentrations equivalent to *ex vivo* PCLuS



Phase Ib – GB0139 *ex vivo* PCLuS *vs.* clinical target engagement in IPF patients



Pathways perturbed by proteins downregulated by 10 µM GB0139 across all IPF PCLuS patients

Pathway Identifier	Pathway name	#Entities found	#Entities total	Entities pValue	Submitted entities found
R-HSA-168249	Innate Immune System	21	1345	8.4E-06	CTSA;GRN;CD163;NHLRC3;AHSG;HEXB;CD180;RNASE6;GGH;TCIRG1;PLD3;VAV1;DOK3;IMPDH1;ATP6V0A2;MAN2B1;ACP2;PI3;LGMN
R-HSA-6798695	Neutrophil degranulation	12	480	1.2E-05	CTSA;GRN;CD163;DOK3;NHLRC3;AHSG;IMPDH1;HEXB;GGH;MAN2B1;ACP2;TCIRG1
R-HSA-168256	Immune System	27	2698	8.4E-04	CTSA;CD163;GRN;NHLRC3;HSPA5;AHSG;HEXB;CD180;RNASE6;GGH;TCIRG1;VAV1;PLD3;MT2A;DOK3;IMPDH1;INPP5D;MRC1;ATP6V0A2;ACP2;MAN2B1;PI3;LGMN
R-HSA-114608	Platelet degranulation	5	141	1.1E-03	CD163;HSPA5;AHSG;SERPINE1;ANXA5
R-HSA-76005	Response to elevated platelet cytosolic Ca2+	5	148	1.4E-03	CD163;HSPA5;AHSG;SERPINE1;ANXA5
R-HSA-9662834	CD163 mediating an anti-inflammatory response	2	14	2.8E-03	CD163
R-HSA-381183	ATF6 (ATF6-alpha) activates chaperone genes	2	15	3.1E-03	HSPA5
R-HSA-381033	ATF6 (ATF6-alpha) activates chaperones	2	17	4.0E-03	HSPA5
R-HSA-1483115	Hydrolysis of LPC	2	18	4.5E-03	PLA2G15;PLBD1
R-HSA-76002	Platelet activation, signaling and aggregation	6	293	5.5E-03	CD163;HSPA5;AHSG;SERPINE1;ANXA5;VAV1
R-HSA-9029569	NR1H3 & NR1H2 regulate gene expression linked to cholesterol tr	3	66	5.8E-03	PLTP
R-HSA-1483148	Synthesis of PG	2	21	6.0E-03	PLD3
R-HSA-1483257	Phospholipid metabolism	6	314	7.6E-03	PLA2G15;MTMR14;INPP5D;PLBD1;PLD3
R-HSA-8964058	HDL remodeling	2	24	7.8E-03	PLTP
R-HSA-381119	Unfolded Protein Response (UPR)	4	155	1.1E-02	HSPA5;WIPI1
R-HSA-9024446	NR1H2 and NR1H3-mediated signaling	3	85	1.2E-02	PLTP
R-HSA-1660662	Glycosphingolipid metabolism	3	86	1.2E-02	CTSA;HEXB;GBA
R-HSA-77387	Insulin receptor recycling	2	30	1.2E-02	ATP6V0A2;TCIRG1
R-HSA-381070	IRE1alpha activates chaperones	3	101	1.8E-02	HSPA5;WIPI1
R-HSA-174824	Plasma lipoprotein assembly, remodeling, and clearance	3	102	1.9E-02	NPC1;PLTP
R-HSA-917977	Transferrin endocytosis and recycling	2	39	2.0E-02	ATP6V0A2;TCIRG1
R-HSA-1855204	Synthesis of IP3 and IP4 in the cytosol	2	39	2.0E-02	INPP5D;PLD3
R-HSA-390471	Association of TriC/CCT with target proteins during biosynthesis	2	40	2.1E-02	GBA;LONP2
R-HSA-9768919	NPAS4 regulates expression of target genes	2	41	2.1E-02	GEM
R-HSA-3656248	Defective HEXB causes GM2G2	1	4	2.2E-02	HEXB
R-HSA-2206282	MPS IIIB - Sanfilippo syndrome B	1	4	2.2E-02	NAGLU
R-HSA-432720	Lysosome Vesicle Biogenesis	2	43	2.3E-02	M6PR;DNASE2
R-HSA-1368108	BMAL1:CLOCK,NPAS2 activates circadian gene expression	2	43	2.3E-02	SERPINE1
R-HSA-2173796	SMAD2/SMAD3:SMAD4 heterotrimer regulates transcription	2	44	2.4E-02	SERPINE1
R-HSA-5218920	VEGFR2 mediated vascular permeability	2	46	2.7E-02	STK4;VAV1
R-HSA-512988	Interleukin-3, Interleukin-5 and GM-CSF signaling	2	50	3.1E-02	INPP5D;VAV1
R-HSA-109582	Hemostasis	9	803	3.1E-02	GRN;CD163;HSPA5;AHSG;INPP5D;SERPINE1;ANXA5;ACP2;VAV1
R-HSA-1483206	Glycerophospholipid biosynthesis	4	221	3.3E-02	PLA2G15;PLBD1;PLD3
R-HSA-8963899	Plasma lipoprotein remodeling	2	56	3.8E-02	PLTP
R-HSA-9748787	Azathioprine ADME	2	58	4.1E-02	IMPDH1;VAV1
R-HSA-4341670	Defective NEU1 causes sialidosis	1	8	4.3E-02	CTSA
R-HSA-9634815	Transcriptional Regulation by NPAS4	2	60	4.3E-02	GEM
R-HSA-9639288	Amino acids regulate mTORC1	2	61	4.4E-02	TCIRG1;LAMTOR4
R-HSA-2173793	Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	2	64	4.8E-02	SERPINE1
R-HSA-2132295	MHC class II antigen presentation	3	149	4.9E-02	CTSA;ACP2;LGMN
R-HSA-1632852	Macroautophagy	3	150	4.9E-02	MTMR14;WIPI1;LAMTOR4

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