INVOLVEMENT OF TYPE 1 INVARIANT NATURAL KILLER T CELLS IN DRIVING LUNG FIBROSIS

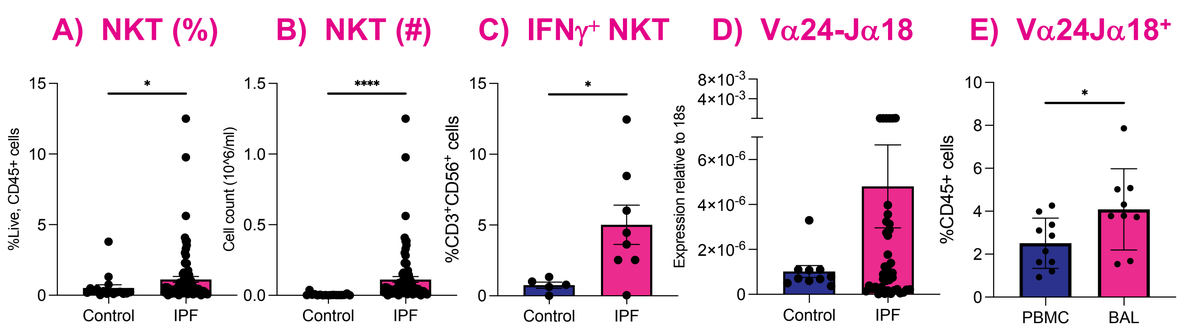
Emily Calamita[1], Wing Han Liu[1], Patricia P. Ogger[1], Christina Michalaki[1], Faye Murphy[2], Cormac McCarthy[2], Albert Agro[3], Toby M. Maher[4], Clare. M. Lloyd[1], Philip Molyneaux[1], Adam. J. Byrne[2], Vipin Kumar[3], Marc Hertz[3]

1. National Heart & Lung Institute, Imperial College London, London, United Kingdom; 2. School of Medicine & Conway Institute of Biomedical Science, University College Dublin, Dublin, Ireland; 3. GRI Bio, Inc. La Jolla, California, USA; 4. Keck School of Medicine of USC, Los Angeles, California, United States

Introduction

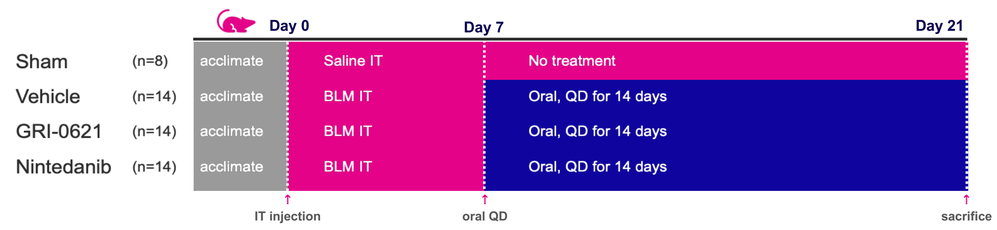
Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease characterized by excessive extracellular matrix deposition in lung parenchyma[1]. Understanding the mechanisms involved in IPF is critically needed to develop new treatments. We have investigated the role of innate-like Natural Killer T (NKT) cells in IPF patients and a novel iNKT inhibitor, GRI-0621, in a treatment model of pulmonary fibrosis. GRI-0621 is currently being evaluated in a Phase 2a proof-of-concept biomarker study in patients with IPF (NCT06331624).

iNKT cells are elevated in IPF patients



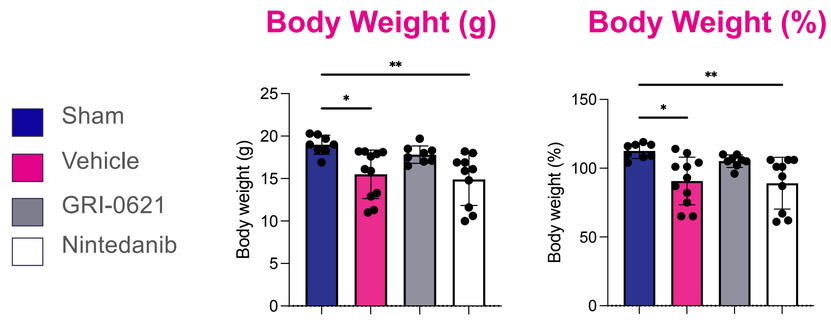
A) Percent and B) total number of CD45⁺ CD3⁺ CD56⁺ NKT-like cells in BAL of healthy (n=17) or IPF patients (n=86). C) IFN-γ⁺ NKT-like cells in BAL of healthy (n=5) or IPF patients (n=8). D) expression of Vα24-Jα18 in whole BAL pellets in healthy controls (n=10) or IPF patients (n=29). E) Vα24Jα18 iNKT cells as percent of CD45⁺ cells in PBMC and BAL fluid of IPF patients (n=10).. Data presented as mean ± SEM. * p<0.05, **** p<0.001.

Bleomycin pulmonary fibrosis model design



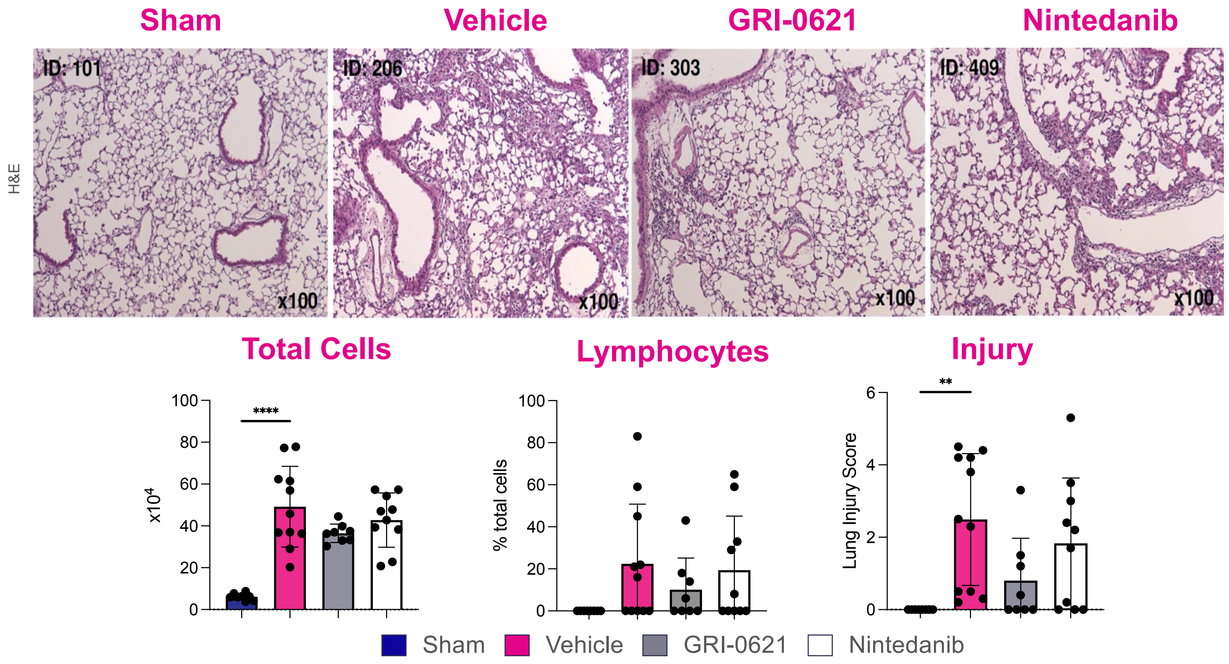
Pulmonary fibrosis induced on day 0 in C57B/L6 mice with intratracheal bleomycin (3.0 mg/kg). Vehicle (5% DMSO, 0.1% Tween 80 in PBS), GRI-0621 (1.0mg/kg), or nintedanib (100mg/kg) was administered for 14 days beginning on day 7. Studies conducted at SMC Laboratories (Tokyo, JP).

GRI-0621 treated animals maintain body weight



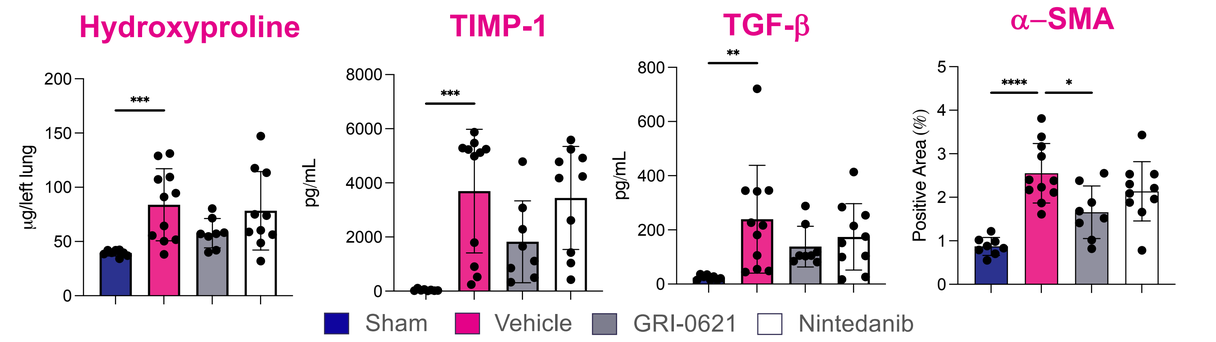
Body weight in grams or as a percent of Day 0 weight. Body weight was recorded daily and dosing volume was adjusted based on the latest body weight from the same day. Animals with >40% body weight loss were euthanized and samples were not collected. Data presented as mean ± SD. * p<0.05, ** p<0.01.

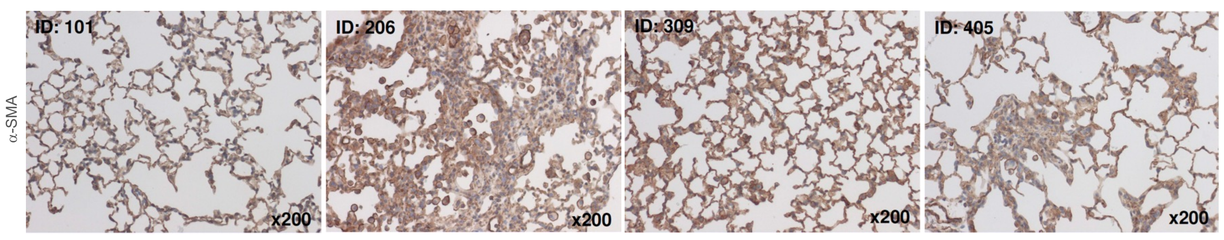
Reduced inflammation & lung injury in GRI-0621-treated animals



Total cell numbers are expressed as x 10^4 cells and lymphocytes as percent CD45⁺ cells. For quantitative analysis of lung injury, bright field images of H&E-stained sections were randomly captured including vessel at 100x magnification and inflammation in 10 field/mouse were evaluated according to the criteria for lung injury[2]. Data presented as mean ± SD. * p<0.05, ** p<0.01, *** p<0.005

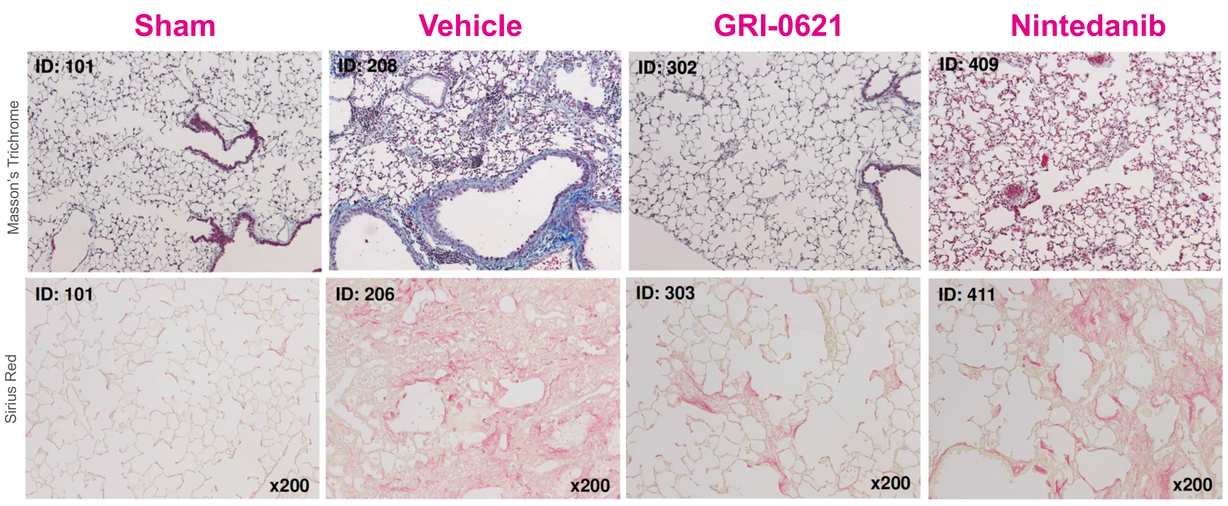
GRI-0621 treatment reduces collagen breakdown, pro-fibrotic mediators, & activation of myofibroblasts

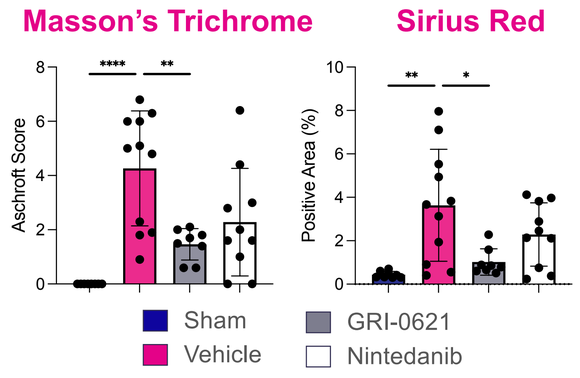




Lung hydroxyproline content from frozen left lung samples expressed as µg per left lung. TIMP-1 and TGF-β levels quantified using R&D Systems Quantikine ELISA kits. For quantitative analysis of α-SMA-positive area, bright field images of α-SMA-stained sections were captured around the central vein including vessel at 200x magnification and the positive areas in 5 field/section were measured using ImageJ software. Data presented as mean ± SD. * p<0.05, ** p<0.01, *** p<0.005

GRI-0621 treatment significantly reduces fibrosis





For quantitative analysis of lung fibrosis area, bright field images of Masson's Trichrome stained sections were randomly captured at 100x magnification and the subpleural regions in 20 field/mouse were evaluated according to the Ashcroft criteria for grading lung fibrosis[3]. For collagen deposition and analysis of fibrosis area, bright field images of Sirius Red-stained sections were captured at 200x magnification and the positive areas in 5 field/section were measured using ImageJ software. Data presented as mean ± SD. * p<0.05, ** p<0.01, *** p<0.005

Summary

- We have previously shown that iNKT cells are increased in patients with fibrotic disease[4,5], and here we show that iNKT cells are increased in number and proportion in the airways of IPF patients compared to healthy controls
- We have previously shown that inhibition of iNKT cell activity can prevent fibrosis and pathology in a prevention model of pulmonary fibrosis[5], and here we show potent inhibitor of iNKT cell activity, GRI-0621, is therapeutic in a treatment model of pulmonary fibrosis

Group	Body Weight	Left Lung	Post-Caval	Total Cells	Lung Injury	BALF TGF-β	BALF TIMP-1	α-SMA	Lung Hydroxy-proline	Ashcroft Score	Sirius Red
Sham	↑	n.s.	⇡	↑	↑	↑	↑	↑	↑	↑	↑
GRI-0621	⇡	⇣	n.s.	⇣	⇣	⇣	⇣	↓	⇣	↓	↓
Nintedanib	⇣	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	⇣	n.s.	⇣	n.s.

↑↓ significant change vs vehicle control (P <0.05) using Bonferroni Multiple Comparison Test
⇡⇣ trend or tendency vs vehicle control (P <0.1) using One-Sided T-Test

GRI-0621 administered during the fibrotic phase of the bleomycin model of pulmonary fibrosis improved a majority of inflammatory, fibrotic and pathological features in a standard treatment model of IPF

Acknowledgements

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