Inactivation of iNKT Cells After the Inflammatory Phase Leads to Significant Inhibition of Fibroblast Activation and Fibrosis in a Model of Pulmonary Fibrosis

M. Hertz, A. Agro, and V. Kumar

GRI Bio, Inc., San Diego, CA, United States

RATIONALE: Immune dysregulation plays a key role in the pathogenesis of IPF. Type 1 invariant Natural Killer T (iNKT) cells are innate-like T cells expressing both NK cell and T cell receptors that recognize lipid antigens and impact both adaptive and innate immunity. iNKT cells produce type 1 (IFN-γ), type 2 (IL-4, IL-13), and type 3 (IL-17) cytokines and can influence both TGF-β1-dependent and -independent fibrotic pathways[1]. We have recently reported that activated iNKT cells are increased in BAL from IPF lungs in comparison to control subjects. Inhibition of iNKT cells prophylactically with tazarotene, the active ingredient in GRI-0621, significantly inhibited both inflammation and fibrosis in bleomycin-induced lung injury[2]. Here we show iNKT inhibition after the inflammatory phase resolves inflammation and fibrosis in bleomycin-induced fibrosis.

METHODS: We have used biochemical, qPCR and immunohistochemistry analysis to investigate whether iNKT cell inactivation during the fibrotic phase resolves lung injury, fibroblast activation, and fibrosis in the murine bleomycin model of pulmonary fibrosis.

RESULTS: In a therapeutic regimen of the bleomycin-induced fibrosis model, orally administered GRI-0621 after completion of the inflammatory phase (day 7), significantly inhibits lung injury and several important fibrotic cellular and molecular drivers of lung disease, including fibroblast activation and fibrosis. GRI-0621 impacts key innate and adaptive cell activity, cytokine production, myofibroblast activation, and ECM deposition and fibrosis.

CONCLUSIONS: Collectively, our data indicate that oral administration of GRI-0621 has both anti-inflammatory and anti-fibrotic effects in pulmonary fibrosis. These data contribute to our understanding of the role of iNKT cell activity in driving pulmonary fibrosis and underpin the ongoing Phase 2a study examining the safety and tolerability of GRI-0621 and its effect on various biomarkers in IPF patients.

		Day 0 IT Injection	Day 7 Tx Oral QD	Day 21 Sacrifice
Sham	(n=16)	acclimate	Saline IT	No treatment
Vehicle (GRI)[1]	(n=32)	acclimate	BLM IT	Oral, QD for 14 days
GRI-0621	(n=32)	acclimate	BLM IT	Oral, QD for 14 days
Combination[2]	(n=32)	acclimate	BLM IT	Oral, QD for 14 days
Vehicle (Nint)[3]	(n=32)	acclimate	BLM IT	Oral, QD for 14 days
Nintedanib	(n=32)	acclimate	BLM IT	Oral, QD for 14 days
			Acute Injury & Inflammation	Active Fibrosis

Figure 1. IPF Model. Pulmonary fibrosis induced on day 0 in 8-week-old C57BL/6 mice with intratracheal bleomycin (3.0 mg/kg). Vehicle[1,3], GRI-0621 (1.0mg/kg), Combination[2] (GRI-0621 1.0mg/kg + nintedanib 100mg/kg) or nintedanib (100mg/kg) was administered for 14 days beginning on day 7. Studies conducted at SMC Laboratories (Tokyo, JP).

[1] Vehicle (GRI): 5% DMSO, 0.1% Tween 80 in PBS
[3] Vehicle (Nint): 1% methylcellulose

Previously, we have shown that GRI-0621 (tazarotene) inhibition of iNKT cells can prevent pulmonary inflammation, injury, and fibrosis in a bleomycin-induced model of pulmonary fibrosis[2]. To determine the effect of iNKT inhibition after the inflammatory phase, pulmonary fibrosis was induced on day 0 in C57BL/6 mice with a single intratracheal injection of bleomycin. Following the inflammatory phase, animals received daily administration of GRI-0621, nintedanib, a combination of GRI-0621 + nintedanib, or vehicle alone for 14 days starting on day 7 through day 21 (see Fig. 1). Results from two independent studies are presented in Figures 3 and 4.

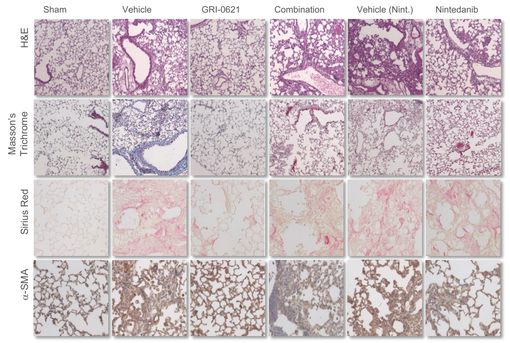

Figure 2. Histology. Right lung tissue was prefixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4 µm. H&E staining was performed with Lillie-Mayer's Hematoxylin (Muto Pure Chemicals Co, Ltd., JP) and eosin solution (Fujifilm Wako Pure Chemical Corp., JP). Masson's Trichrome staining was performed on deparaffinized, rehydrated, and re-fixed (Bouin's solution) with Weigert's iron Hematoxylin working solution (Fujifilm Wako Pure Chemical Corp., JP), Biebrich Scarlet Acid fuchsin solution (Sigma-Aldrich). Immunohistochemistry performed on dewaxed and rehydrated sections with anti-α-SMA antibody (Abcam).

Inflammation, lymphocyte and total cell counts in BALF, as well as lung injury, were reduced across treatment groups compared to vehicle controls. Expression of pro-inflammatory genes INF-γ, the NLRP3 inflammasome, and CCL3 (MIP-1α) were reduced in treated groups as determined by qPCR. GRI-0621 and combination-treated groups also showed a reduction in IL-13 (qPCR) and TGF-β1 (both qPCR (not shown) and ELISA) cytokines that can drive the differentiation of fibroblasts to activated myofibroblasts. Reduced myofibroblast activity and collagen deposition is further shown in hydroxyproline and significant inhibition of α-SMA, Mason's Trichrome, and Sirius Red staining in GRI-0621-treated groups.

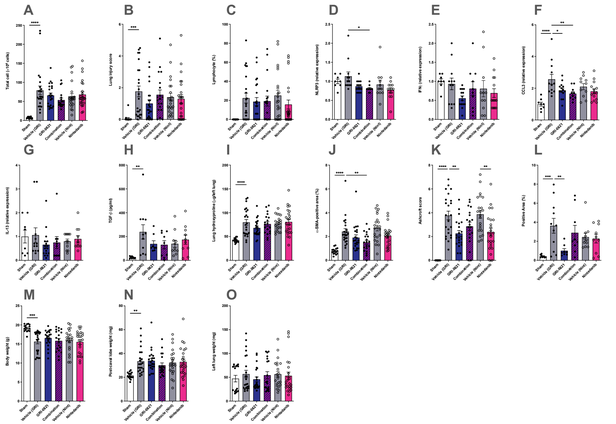

Figure 3. Summary data from two independent bleomycin-induced pulmonary fibrosis studies. A) total cells counted by hemocytometer; B) quantitative lung injury calculated from bright field images from 10 fields of each section (superior, middle, and inferior lobes) of H&E-stained sections; C) % lymphocytes determined from 100 µl cell suspension on slide; qPCR D-G) to determine NLRP3, IFN-γ, CCL3 (MIP-1α), and IL-13 gene expression from RNA extracted from lung tissue normalized to reference gene 36B4; H) TGF-β1 ELISA; I) lung hydroxyproline content quantified from left lung; J-L) α-SMA quantified from 5 fields of each section (superior, middle, and inferior lobes) of lung histology (Figure 2) from 5 fields of each section of α-SMA, Ashcroft score quantified from 20 fields of each section (superior, middle, and inferior lobes) of each individual animal from Masson's Trichrome staining, and Positive Area (%) from Sirius Red staining; M-O) body, post-caval lobe, and left lung weights measured on day 21.

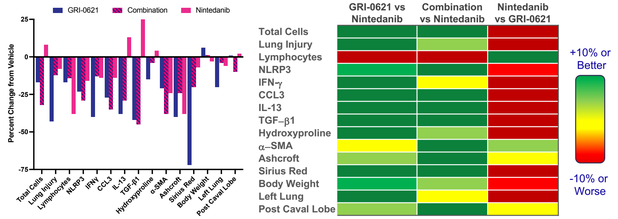

Figure 4. Summary data. Percent change from vehicle (left graph) and relative change (right table) versus nintedanib monotherapy (left and middle column) or versus GRI-0621 monotherapy (right column). Shaded cells represent a +10% or greater improvement (dark green) to -10% or greater reduction (dark red) in efficacy compared to monotherapy.

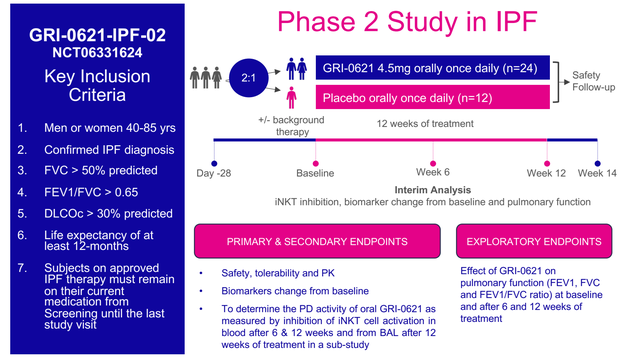

Figure 3. Schematic outline of GRI-0621-IPF-02 Phase 2a clinical study.

GRI-0621 is being assessed in a 12-week, double-blind, randomized, placebo-controlled study in patients with IPF (Fig. 3). The objective is to assess the safety and tolerability of GRI-0621 in the IPF patient population. In addition, the effect of GRI-0621 on a number biomarkers both from the blood and BAL will be evaluated. These include several biomarkers associated with disease progression, NKT cell and other immune cell numbers and activity, differential gene expression, as well as pulmonary function tests.

A pre-planned interim safety analysis demonstrated GRI-0621 to be safe and well tolerated in the first 12 patients evaluated after 2-weeks of treatment, consistent with the RARβγ receptor selectivity and toxicity profile observed in over 1,700 patients treated for up to 52-weeks with 4.5mg of tazarotene in earlier studies.

In summary, iNKT cells are found in increased number and activity in the BAL of patients with IPF as compared to age matched controls, and correlate with progressive IPF and are part of a proposed immune cell composite to identify progressive IPF patients at baseline[3]. GRI-0621 inhibition of iNKT cell activity is therapeutic in treatment models of pulmonary fibrosis and performs as well or better than the approved drug nintedanib.

1. Kumar V, Hertz M, Agro A, Byrne AJ. Type 1 invariant natural killer T cells in chronic inflammation and tissue fibrosis. Front Immunol 2023;14:1260503.
2. Calamita, E., W. H. Liu, P. P. Ogger, L. Griffin, C. Michalaki, F. Murphy, J. Worrell, C. McCarthy, A. Agro, M. Hertz, T. M. Maher, C. M. Lloyd, P. Molyneaux, V. Kumar, and A. J. Byrne. 2024. 'Type 1 Invariant Natural Killer T Cells Drive Lung Fibrosis', *Am J Respir Crit Care Med*, 210: 521-23.
3. Mendoza, N., et al., Blood Immunophenotypes of Idiopathic Pulmonary Fibrosis: Relationship with Disease Severity and Progression. Int J Mol Sci, 2023. 24(18).

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