Selective ROCK2 inhibitor ameliorates established dermal and pulmonary fibrosis in murine systemic sclerosis and shifts the balance between Th17 and Treg cells in vivo

W. CHEN[1], M. S. NYUYDZEFE[1], J. MORETTI[1], A. BOURG[1], E. STILLMAN[2], L. NGUYEN[2], C. PICHAVANT[2], M. GHOSH[2], S. D. WAKSAL[1] and A. ZANIN-ZHOROV[1]

[1]Graviton Biosciences B.V., Amsterdam, Netherlands 1017 CG

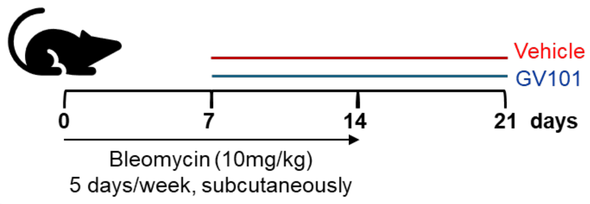
[2]Aragen Biosciences Inc., Morgan Hill, CA 95037 USA

INTRODUCTION

Systemic sclerosis (SSc) is a complex autoimmune disorder characterized by dysregulation of both innate and adaptive immunity as well as excessive fibrosis in skin and visceral organs leading to irreversible scarring and organ failure. Pulmonary complications, including fibrosis and hypertension, represent two principal causes of death in patients with SSc. Rho-associated coiled-coil-containing protein kinase 2 (ROCK2) regulates key pro-fibrotic pathways implicated in both pro-inflammatory reactions and altered extracellular matrix remodeling. We therefore hypothesized that targeting ROCK2 could impact inflammatory and fibrotic pathways and potentially improve SSc severity.

METHODS

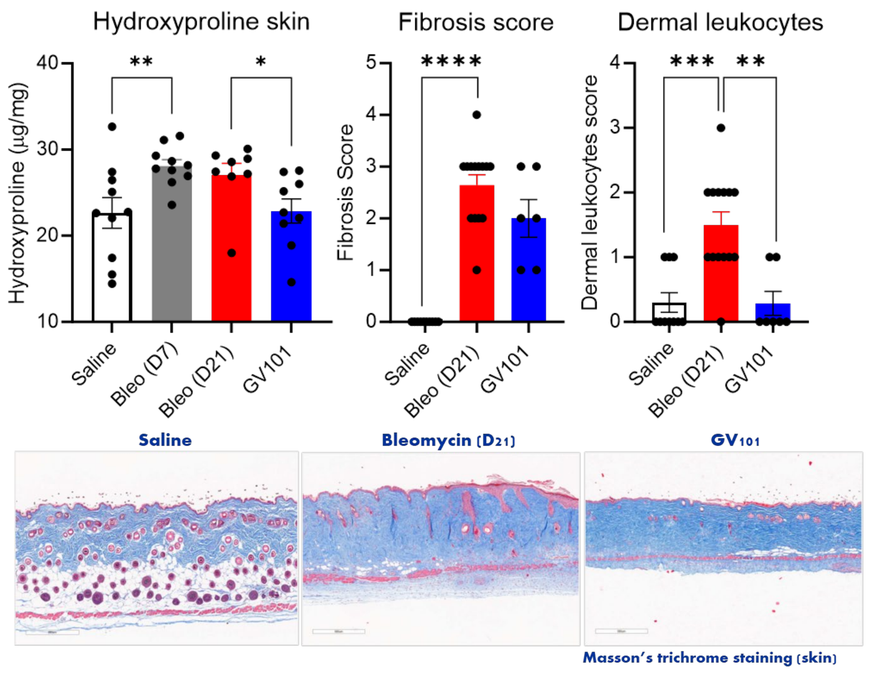
We used the irreversible systemic sclerosis model induced by repeated subcutaneous injections of bleomycin in mice and examined the efficacy of highly selective ROCK2 inhibitor on skin and lung fibrosis, as well as its impact on immune cell infiltration when administered orally starting on Day7.



	ROCK2 (IC50 nM)	ROCK1 (IC50 nM)
GV101	7.5±7.9	>30000

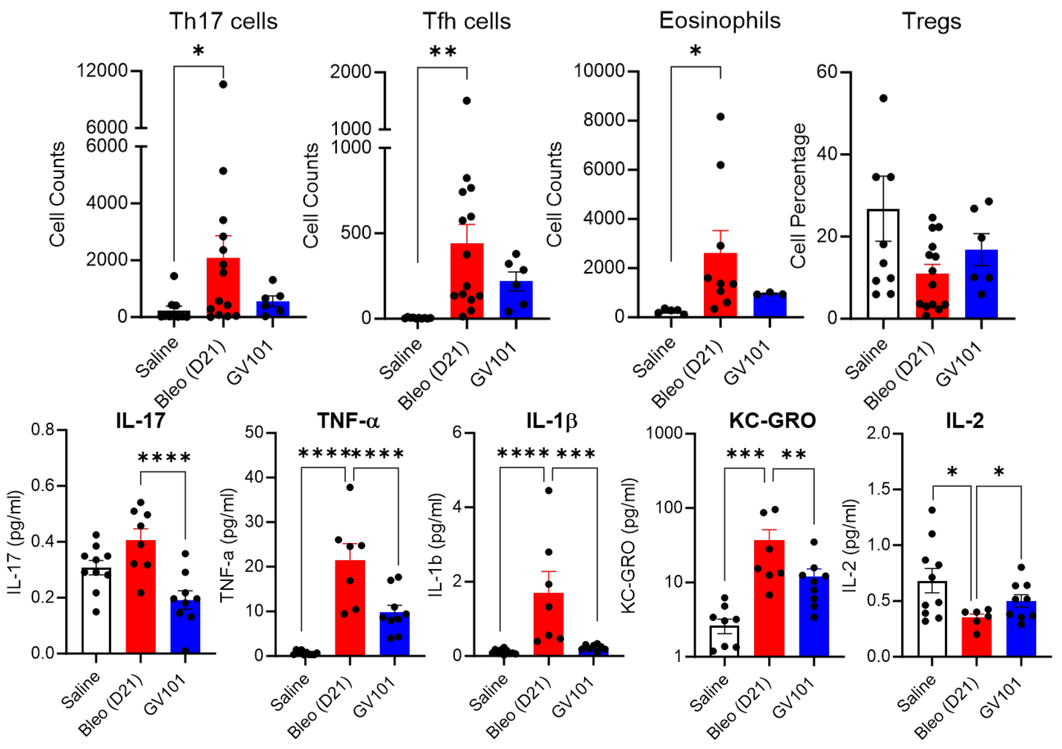
RESULTS

1. GV101 reduced skin collagen and leukocyte infiltration in SSc model



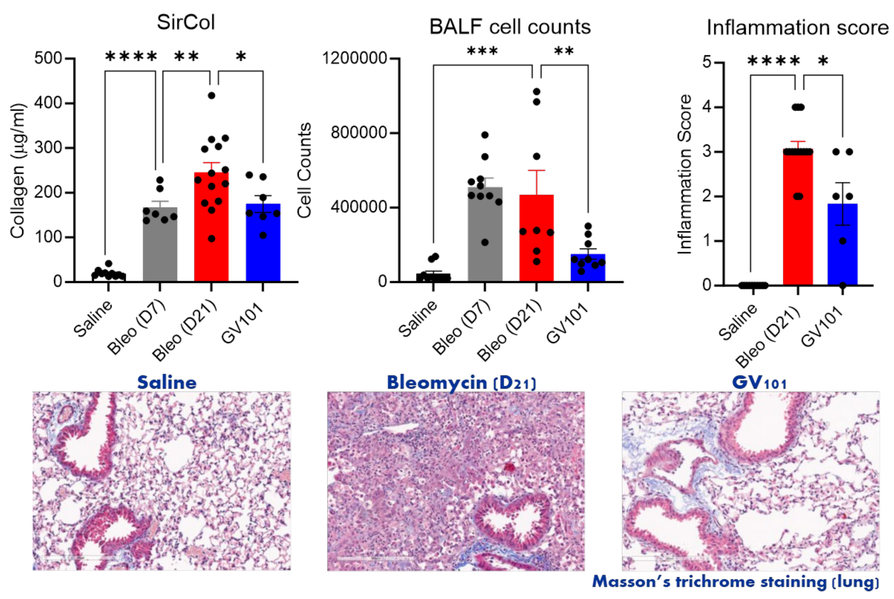
C57BL/6 mice were treated with vehicle or GV101 (30 mg/kg) by oral gavage on Day 7 after starting repeated bleomycin injections and continued for 14 days. Hydroxyproline assessment and histology were performed by using skin patches. Fibrosis and leukocyte infiltrates (inflammation) were scored in stained sections. Two-tailed t-test statistical analysis was performed: * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.001; **** p≤ 0.0001. Representative images of Masson's trichome staining are shown for each group.

4. Selective ROCK2 inhibition in SSc shifts the Th17/Treg balance in lung



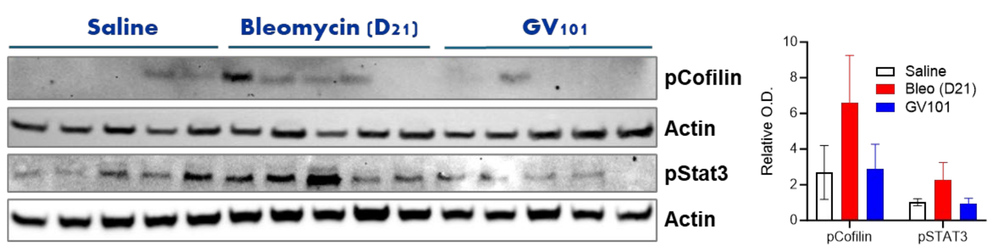
C57BL/6 mice were treated with vehicle or GV101 (30 mg/kg) by oral gavage on Day 7 after starting repeated bleomycin injections and continued for 14 days. Bronchoalveolar fluids (BALF) were evaluated for immune cell subsets by Flow Cytometry and cytokine levels by MSD-based ELISA. Two-tailed t-test statistical analysis was performed: * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.001; **** p≤ 0.0001.

2. GV101 treatment decreased pulmonary fibrosis and inflammation



C57BL/6 mice were treated with vehicle or GV101 (30 mg/kg) by oral gavage on Day 7 after starting repeated bleomycin injections and continued for 14 days. Bronchoalveolar fluids (BALF) were evaluated for cell counts and soluble collagen (SirCol). Histology was performed by using lung tissue and scored for inflammation. Two-tailed t-test statistical analysis was performed: * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.001; **** p≤ 0.0001. Representative images of Masson's trichome staining are shown for each group.

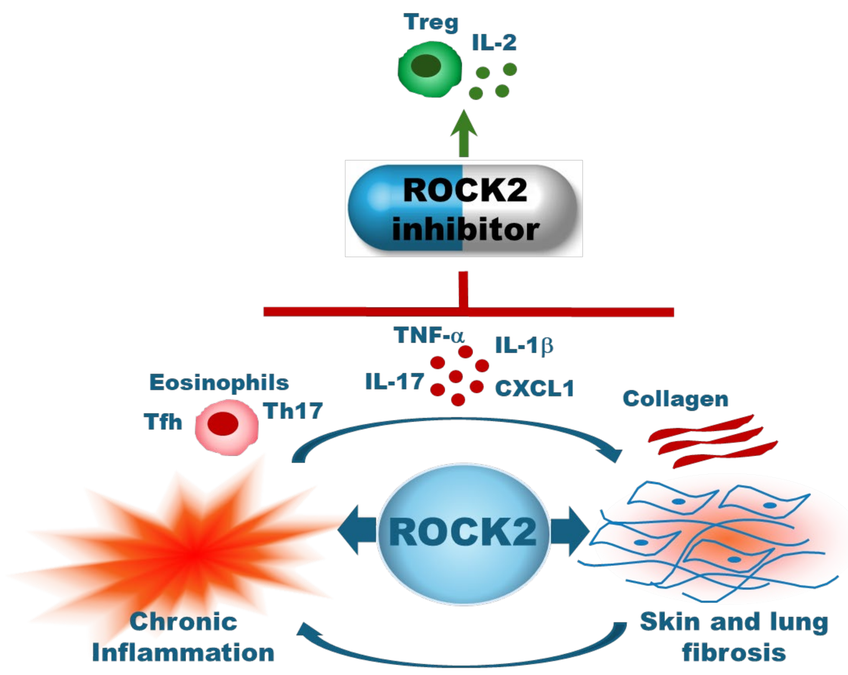
3. In vivo ROCK2 target engagement in spleen tissue



C57BL/6 mice were treated with vehicle or GV101 (30 mg/kg) by oral gavage on Day 7 after starting repeated bleomycin injections and continued for 14 days. Spleens were harvested on Day 21 and tissue lysates were prepared in RIPA buffer. The levels of pSTAT3 and pCofilin were determined by Western Blot (WB), normalized to Actin as a loading control and quantified. Graph represents Mean +/- SEM of 5 animals

CONCLUSIONS

- ✓ Selective ROCK2 inhibitor GV101 decreases both skin and lung collagen levels and inflammation in bleomycin-induced SSc model in mice
- ✓ The robust efficacy of GV101 in vivo involves concurrent down-regulation of pro-inflammatory cytokines including IL-17, TNF-α, IL-1β, KC-GRO (CXCL1) and immune cells, such as Th17, Tfh and Eosinophils, whereas Tregs and IL-2 were increased in lungs
- ✓ These data further demonstrate the ROCK2-specific mechanism of action defined by simultaneously targeting both fibrotic and inflammatory pathways and highlight the therapeutic potential of GV101 for Systemic Sclerosis



CONTACT INFORMATION

Alexandra.zanin-zhorov@gravitoncorp.com