Combined blockade of IL-1, IL-33 and IL-36 signaling by targeting IL1RAP ameliorates skin and lung fibrosis in preclinical models of systemic sclerosis

Sara Rattik[1], Caitriona Grönberg[1], Petter Skoog[1], David Liberg[1], Thuong Trinh-Minh[2,3], Jörg Distler[2,3]

[1] Cantargia AB, Ideon Gateway, Scheelevägen 27, 22363, Lund, Sweden, [2] Department of Rheumatology, University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany, [3] Hiller Research Center, University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Introduction

- CAN10 is a fully humanized, LALA-mutated IgG1 anti-IL1RAP antibody that blocks IL-1α, IL-1β, IL-33, IL-36α, IL-36β and IL-36γ signaling (Fig. 1)
- IL1RAP-mediated signaling results in activation of fibroblasts, endothelial cells and immune cells with production of pro-fibrotic mediators, upregulation of adhesion molecules and recruitment of inflammatory cells, all of which are events relevant for the pathogenesis of systemic sclerosis (SSc)
- IL1RAP-blockade has shown potent effects in preclinical models of several different diseases, such as myocarditis, peritonitis and psoriasis
- Here, we investigate the anti-fibrotic potential of IL1RAP blockade by CAN10 or its murine surrogate antibody, mCAN10, in translational and preclinical models of SSc to strengthen the scientific rationale for clinical development in SSc
- CAN10 is currently in Phase I clinical trial investigating safety, pharmacokinetics and pharmacodynamic effects in healthy individuals and subjects with mild to moderate psoriasis (NCT06143371)

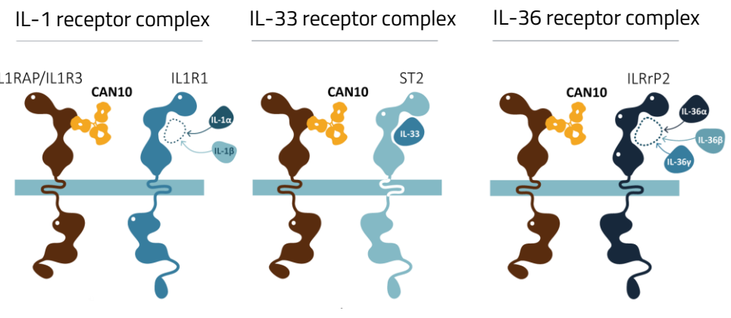

Fig. 1. CAN10 binds IL1RAP and blocks IL-1/33/36 signaling. CAN10 binds to IL1RAP and blocks association to the IL-1 receptor (IL1R1), the IL-33 receptor (ST2) and the IL-36 receptor (ILRrP2). This results in blockade of IL-1α, IL-1β, IL-33, IL-36α, IL-36β and IL-36γ signaling.

IL-1/-33/-36 and IL1RAP are higher in SSc patient skin

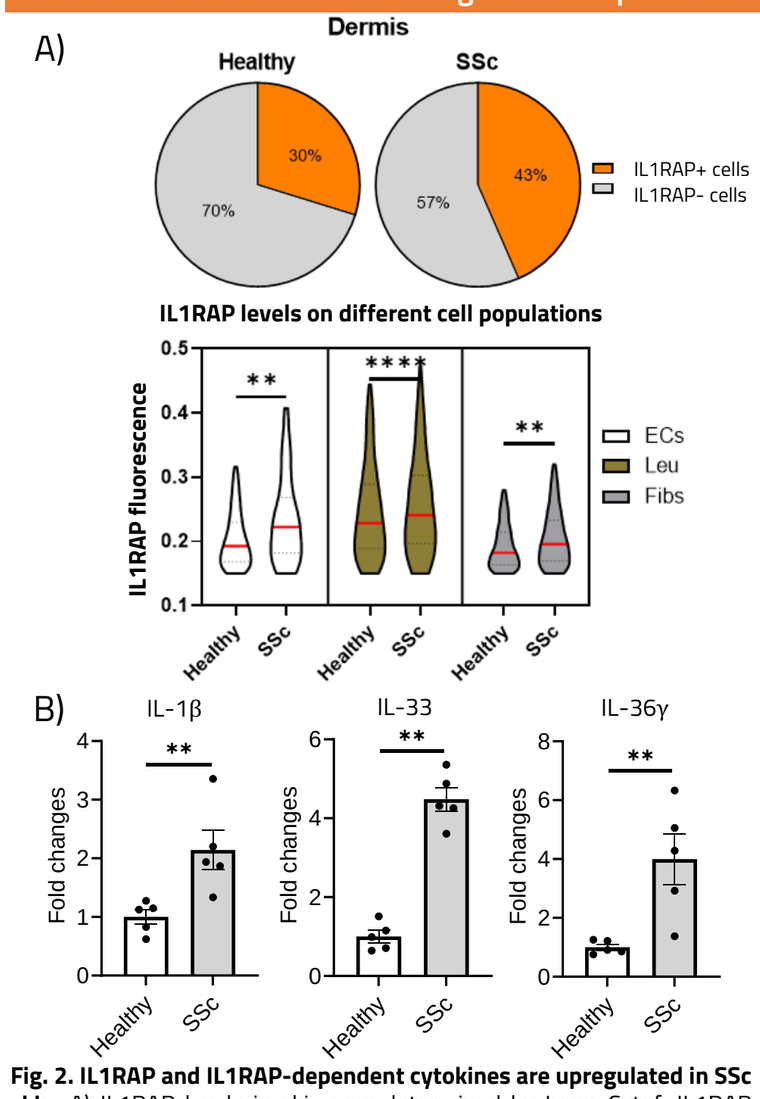

Fig. 2. IL1RAP and IL1RAP-dependent cytokines are upregulated in SSc skin. A) IL1RAP levels in skin was determined by ImageCytof. IL1RAP was shown to be expressed on endothelial cells (EC), leukocytes (Leu) and fibroblasts (Fibs), and present at higher levels in SSc patient skin compared to healthy. B) Staining of IL-1β, IL-33, and IL-36γ using immunofluorescence revealed higher levels in SSc patient skin compared to healthy skin.

IL1RAP signaling induces fibrosis in SSc fibroblasts

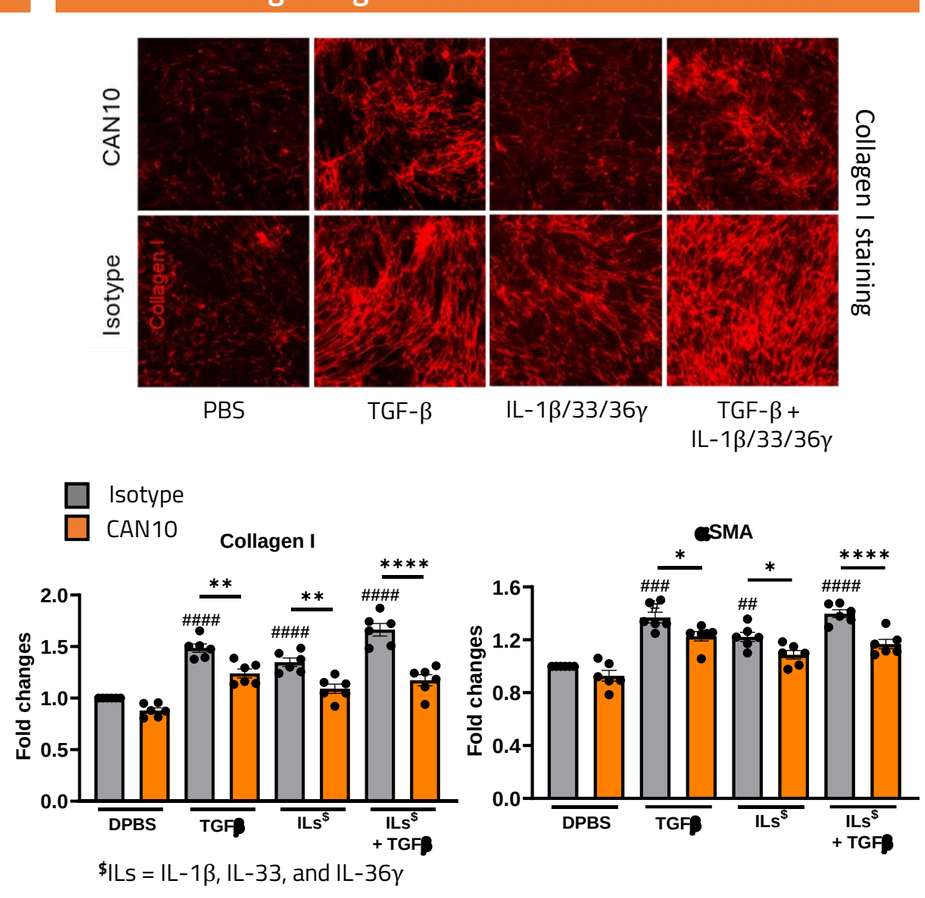

Fig. 3. CAN10 decreases the profibrotic effects induced by IL-1, IL-33 and IL-36 in SSc fibroblasts. Fibroblasts isolated from SSc patients were treated with CAN10 or isotype control and stimulated with TGFβ or a combination of IL-1β, IL-33, and IL-36γ (abbreviated as ILs) with or without TGFβ *in vitro*. The ILs induced deposition of type I collagen and upregulated the protein levels of αSMA, which could be blocked by CAN10. In addition, CAN10 reduced the profibrotic effects induced by TGFβ alone. # indicates differences of cytokine-stimulated fibroblasts compared to DPBS fibroblasts pretreated with isotype control. * indicates differences of fibroblasts treated with the CAN10 antibody compared to isotype control for each stimuli. # and * p< 0.05; ## and ** p< 0.01; ### and *** p< 0.001; #### and **** p< 0.0001

In vivo study design

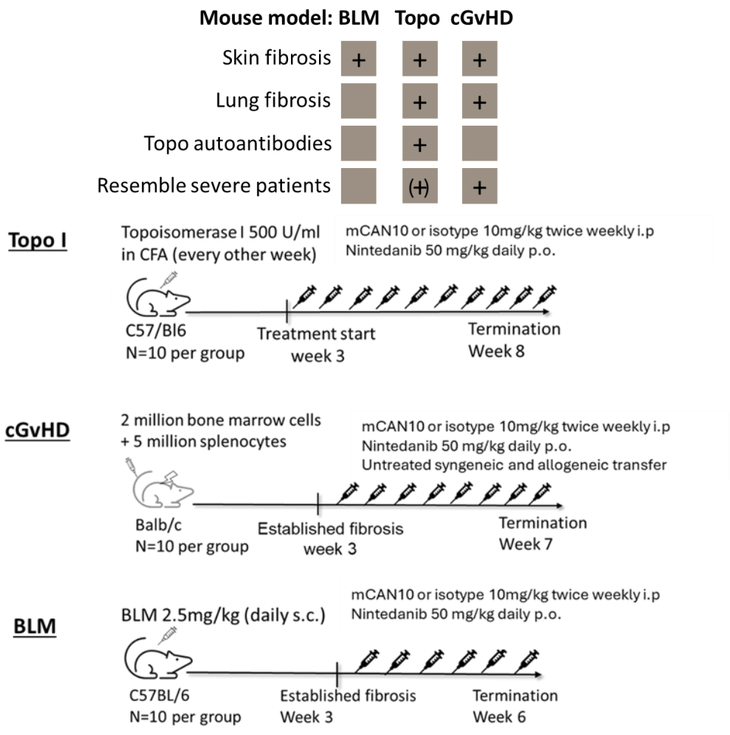

Fig. 4. Study design of the three SSc mouse model experiments. As SSc is a multifactorial disease, we employed 3 complimentary mouse models displaying different features of the disease. Daily subcutanous injections of bleomycin (BLM) induce skin fibrosis while immunization against topoisomerase I (Topo I) or allogeneic bone marrow transfer (chronic graft vs host disease, cGvHD) induce both skin and lung fibrosis. In all 3 models, twice-weekly treatments with mCAN10, a functional surrogate antibody of CAN10, or isotype control, initiated when fibrosis had been established, was used. Treatment with nintedanib (p.o.) was included as a positive control. Skin fibrosis was analysed by histology (dermal thickness) and by myofibroblast quantification (αSMA staining). Lung fibrosis was determined by Trichrome and Sirius Red staining and Ashcroft scoring.

Therapeutic IL1RAP blockade decreases lung and skin fibrosis in 3 models of SSc

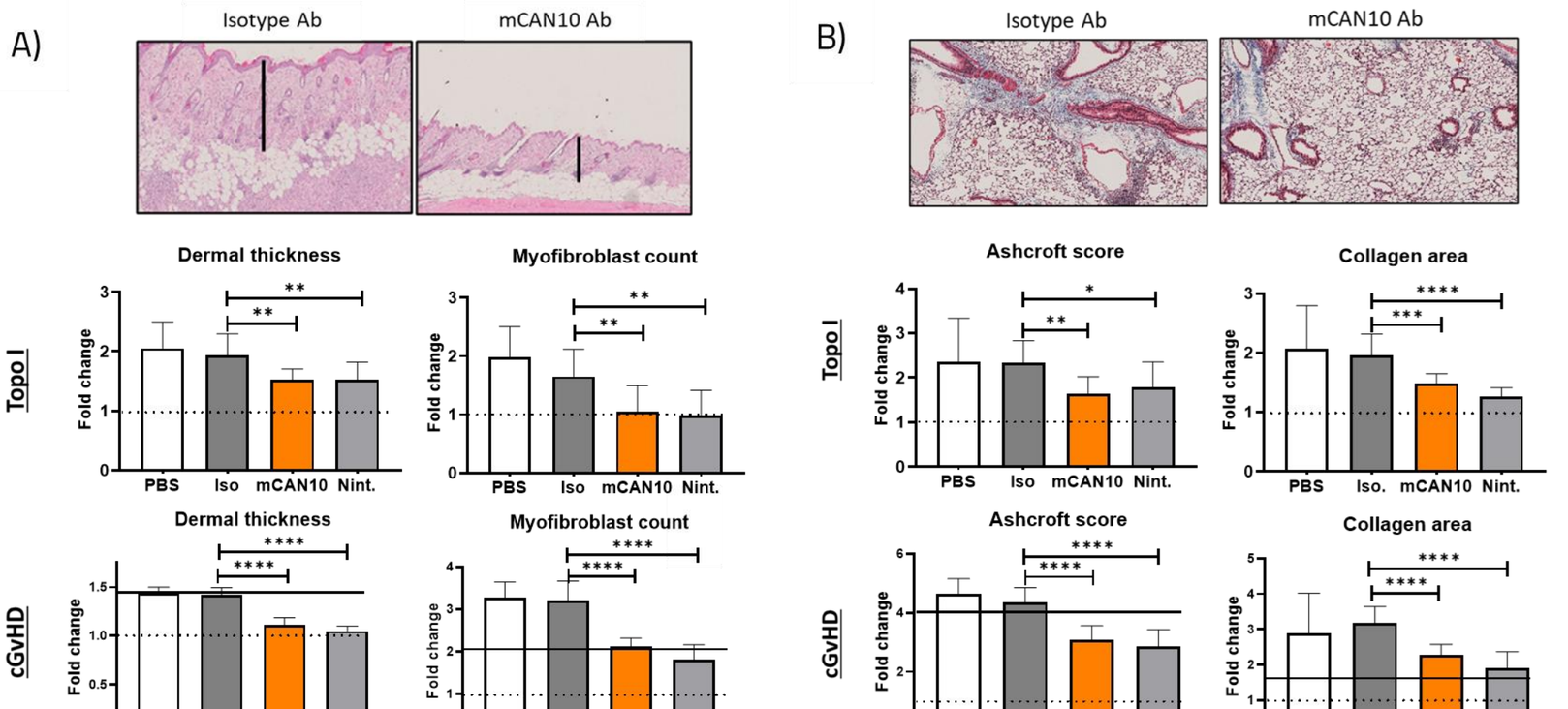

Fig. 5. Therapeutic IL1RAP blockade by mCAN10 reduces skin and lung fibrosis in three mouse models of SSc. Therapeutic treatment with the mCAN10 antibody reduced A) skin fibrosis in the Topo I, cGvHD and the BLM mouse models, and B) lung fibrosis in the Topo I and the cGvHD mouse models. The anti-fibrotic effects of mCAN10 were comparable to those of the positive control, daily high-dose nintedanib. Data is shown as fold change compared to the respective no fibrosis controls (no bleomycin, NaCl/CFA or syngeneic transplant, dotted lines) and compared to baseline fibrosis when applicable (line). * p< 0.05; ** p< 0.01; *** p< 0.001; **** p< 0.0001

IL1RAP blockade affects several key disease-related genes in SSc skin

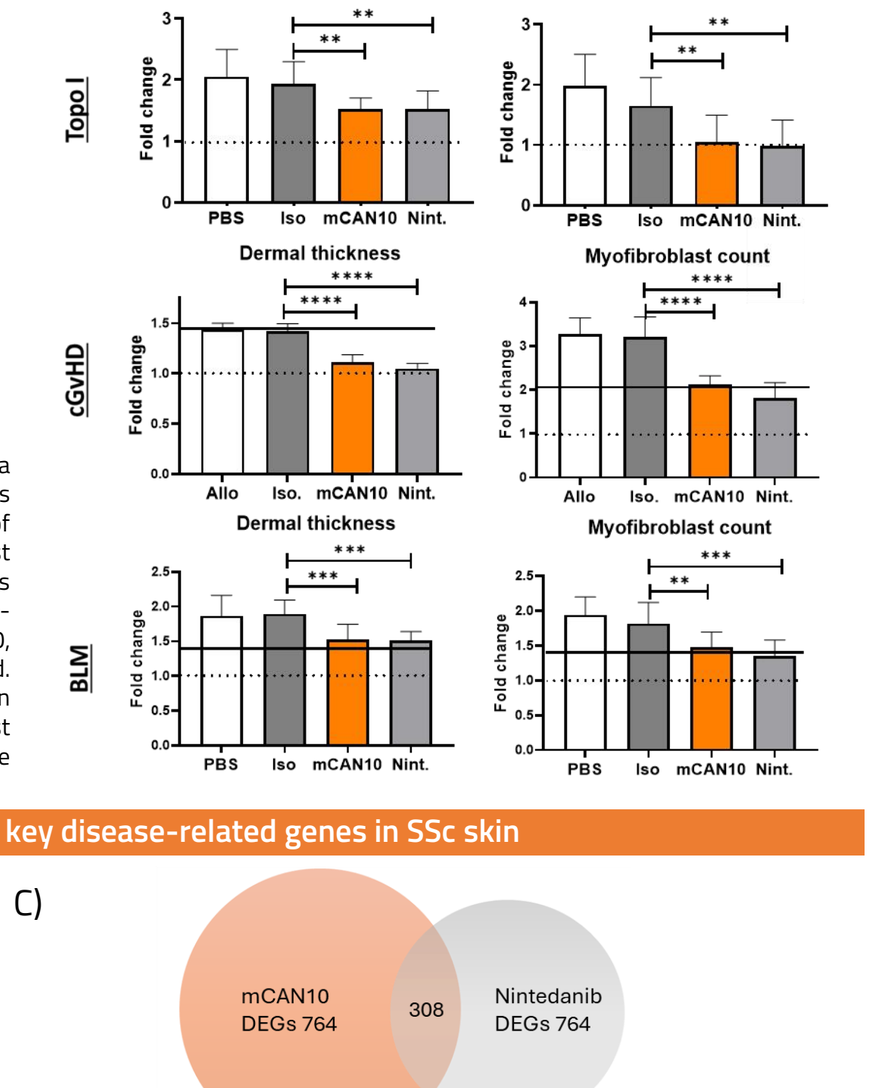

Fig. 6. IL1RAP blockade by mCAN10 normalize the expression of several SSc related genes. To identify downstream targets of IL1RAP, we performed RNASeq of skin samples from cGvHD mice receiving allogeneic transplantation (SSc mice) and treatment with mCAN10 or isotype control. A) mCAN10 treatment demonstrated 1539 DEGs in mouse SSc skin. To determine the relevance of the mCAN10 DEGs for the pathogenesis of human SSc, we assessed the overlap between mCAN10 DEGs, mouse SSc vs healthy DEGs (allogeneic vs. syngeneic transplant, mouse SSc-DEGs) and genes differentially expressed in SSc skin (SSc vs. healthy; GSE59787, human SSc-DEGs). We found an overlap of 273 genes between mouse SSc-DEGs and human SSc-DEGs. Inclusion of the mCAN10-DEGs yielded 167 overlapping genes between the three groups (61%). In addition, mCAN10 affects another 149 genes dysregulated in SSc patients, but not recapitulated in the mouse SSc vs healthy comparison. B) Expression levels of profibrotic mediators in allogenically-transplanted mice treated with mCAN10 or isotype control. mCAN10 specifically normalized the expression of a number of genes known to be involved in the pathogenesis of SSc. C) The number of overlapping DEGs in skin comparing mCAN10 and nintedanib treated SSc mice and D) a comparison plot illustrating the signature signaling pathways between nintedanib and mCAN10 treatments. The majority of mCAN10 and nintedanib-regulated genes are distinct and only partly overlapping, indicating that mCAN10 and nintedanib ameliorate fibrosis in experimental cGvHD through distinct molecular mechanisms.

Summary

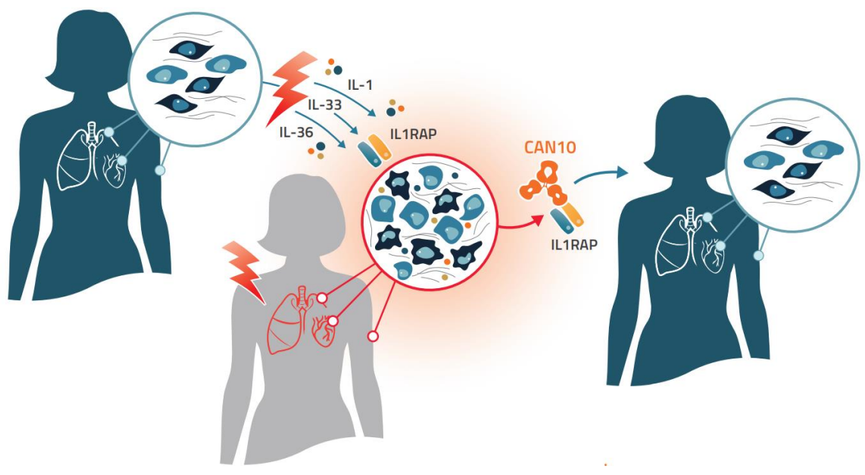

Fig. 7. CAN10 decreases both inflammation and fibrosis in several organs, holding potential as a broad therapeutic in SSc patients

- SSc patient skin biopsies show an upregulation of IL1RAP and IL1RAP-dependent cytokines (IL-1, IL-33 and IL-36)
- IL-1, IL-33 and IL-36 induce fibrotic activity in SSc patient skin fibroblasts, which can be reversed by the anti-IL1RAP antibody CAN10
- Therapeutic treatment with a CAN10 mouse surrogate (mCAN10) counteracts skin and lung inflammation as well as fibrosis in 3 mouse models of SSc
- IL1RAP-blockade in cGvHD mice normalizes the global gene expression profile in skin and affects 61% of the key SSc genes that are commonly dysregulated in human SSc skin and the cGvHD mouse model
- These studies highlight the potential of CAN10 to treat inflammatory and fibrotic diseases such as SSc
- FDA has granted Orphan Drug Designation to CAN10 for the treatment of systemic sclerosis and CAN10 is currently in phase I clinical development (NCT06143371)

hhu Heinrich Heine Universität Düsseldorf

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