Plasmacytoid dendritic cells (pDCs) are immune cells that express CD123, secrete IFN- α , and play a role in inflammation and disease pathogenesis observed in systemic sclerosis (SSc) patients^{1,2}. Therefore, depletion of pDCs or attenuation of pDC function may represent a novel approach to treating SSc patients.

Objectives: To assess the ability of SL-401 to deplete pDCs from healthy volunteers (HV) and SSc patients *ex vivo*.

Methods: Patients fulfilled the 2013 ACR/EULAR classification criteria for SSc³. PBMCs from either SSc patients or healthy volunteers (HV) were prepared using Ficoll-Paque density gradient from fresh blood. pDCs were isolated from PBMCs as previously described⁴ and used to further enrich additional PBMCs. pDC-enriched PBMCs (3%–6% pDCs) were cultured at 2×10^5 cells per well in the presence or absence of CpG-274 (0.5 mM) to activate pDCs and then incubated with SL-401 (0.01–100 ng/ml, 0.17 pM–1.7 nM) at 37°C, 5% CO₂, and 95% humidity. After 24 hours of culture, pDC survival was assessed by flow cytometry (CD14⁺, CD3⁺ BDCA4⁺ CD123⁺), and supernatants were collected for cytokine quantification by a multiplexed Luminex assay.

Results: CD123 expression levels on pDCs from HV and SSc donors were comparable, suggesting that targeting of pDCs in SSc can be modelled in HV. SL-401 was cytotoxic towards pDCs from both HV (n=5) and SSc donors (n=3) to a similar extent. The ED₅₀ of SL-401 against pDCs from HV and SSc was 4.3 and 3.3 ng/ml (74.5 and 57.2 pM), respectively (figure 1). No effect was observed on B or T cells across the SL-401 dose range tested. SL-401-mediated pDC depletion was further accompanied by a significant reduction in CpG-induced IFN- α secretion.

Conclusions: SL-401 is a novel CD123-targeted therapy capable of killing pDCs from both HVs and SSc patients. These data present a potentially novel approach of targeting pDCs in the treatment of SSc and warrant further investigation. A clinical trial is planned.

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FRI0399

RNAI-BASED IDENTIFICATION OF NOVEL DRUG TARGETS TO REDUCE ENDOTHELIAL TO MESENCHYMAL TRANSITION (ENDOMT) IN HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS: A TARGET DISCOVERY APPROACH FOR SCLERODERMA

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Background: Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterised by early vascular abnormalities and subsequent fibroblast activation and differentiation into myofibroblasts, leading to fibrosis.^{1,2} Recently, endothelial to mesenchymal transition (EndoMT), a complex biological process in which endothelial cells lose their specific markers and acquire a mesenchymal or myofibroblastic phenotype, was reported in SSc.³

Objectives: We developed a high-content screening assay with the aim to identify novel proteins which, upon inhibition, will reduce EndoMT in SSc.

Methods: Human dermal microvascular endothelial cells (HMVECs) were seeded in 384 well plates and transfected with a RNAi-based library targeting 866 genes. Cells were then triggered with either disease-related cytokines, such as transforming growth factor beta (TGF- β), or with serum from very early diagnosed, limited cutaneous and diffuse cutaneous SSc patients. Serum derived from healthy donors was used as a control. After 72 hours of triggering, changes in expression level of endothelial and mesenchymal markers were quantified by immunocytochemistry and high content imaging.

Results: We developed a high-throughput EndoMT assay that allows for monitoring of changes in endothelial and mesenchymal markers upon triggering with disease-related cytokines and serum derived from SSc patients. We used this assay to screen an RNAi-based library in HMVECs and identify more than 100 targets able to reduce EndoMT triggered by either TGF- β or SSc patient serum. Identified hits subsequently will be extensively validated in secondary biological assays, and the validated targets will represent excellent candidate drug targets.

Conclusions: This program constitutes a critical path of experiments that will enable the selection of only those targets which meet pre-determined target acceptance criteria. We have chosen to identify targets in a human primary cell system and employ patient's serum as trigger of EndoMT to be able to closely investigate the cellular processes promoting EndoMT in SSc pathogenesis. By using this high-throughput screening platform, we aim to find disease-modifying targets that will be used as an entry point for small molecule drug discovery.

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FRI0400

THE AP1 TRANSCRIPTION FACTOR CJUN AMPLIFIES HEDGEHOG-INDUCED FIBROBLAST ACTIVATION AND TISSUE FIBROSIS

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Background: The pathologic activation of fibroblasts is a key feature of fibrotic disorders such as Systemic Sclerosis (SSc). Deregulation of TGF β - and Hedgehog signalling has been shown to be critical for the persistent, uncontrolled activation of fibroblasts in SSc.^{1–3} However, the consequences of the concomitant upregulation of multiple profibrotic pathways are unknown and cross-talk between individual pathways in fibrotic diseases is currently poorly characterised. Mutual activation and amplification of profibrotic signals might be central for the persistent activation of fibroblasts.