mortality. Epigenetic changes might play important roles in mediating chronic fibroblast activation. Trimethylation of H3 at lysine residue K27 (H3K27me3) is a repressive epigenetic mark that was recently identified as an important negative regulator of fibroblast activation [1]. Jumonji domain containing protein 3 (JMJD3) mediates H3K27me3-demethylation. JMJD3 inhibitors are being tested as therapeutic strategies in malignant diseases.

Objectives: The aim of this study was to characterize the role of JMJD3 in fibrotic disease and to explore JMJD3 as a potential drug target in SSc.

Methods: Expression analyses of JMJD3 were performed using qPCR, IF and Western blot. siRNA mediated knockdown and the pharmacologic H3K27me3-demethylase inhibitor GSKJ4 were used to target JMJD3. *In vivo*, we analyzed the effects of GSKJ4 in bleomycin-induced dermal fibrosis and in Topoisomerase-I-induced (TopoI) fibrosis. H3K27me3 levels at the Fra2 promoter were analyzed by CHIP.

Results: We observed increased expression of JMJD3 in SSc skin compared to healthy controls. Fibroblast-specific overexpression of JMJD3 was also reflected in experimental fibrosis models. TGF β upregulated JMJD3. Inhibition of JMJD3 increased H3K27me3 *in vitro* and *in vivo*. Inhibition of JMJD3 reverted the activated fibroblast phenotype in SSc fibroblasts and decreased the expression of contractile fibers and of α -smooth muscle actin. In addition, JMJD3 inhibition reduced the basal and TGF β induced collagen secretion of SSc fibroblasts. JMJD3 regulated the TGF β induced expression of Fra2. GSKJ4 reverted the TGF β induced reduction of H3K27me3 at the Fra2 promoter. Moreover, the anti-fibrotic effects of JMJD3 inhibition were evened in Fra2 knockout fibroblasts. Overexpression of Fra2 in JMJD3-knockdown fibroblasts restored the profibrotic effect of JMJD3. *In vivo*, inhibition of JMJD3 ameliorated fibrosis in bleomycin- and TopoI- induced experimental fibrosis and reduced dermal thickening, hydroxyproline content and myofibroblast differentiation.

Conclusions: We present first evidence that JMJD3 contributes to the activated phenotype of SSc fibroblasts. TGF β upregulated JMJD3. Inhibition of JMJD3 prevented the aberrant activation of fibroblasts *in vitro* and ameliorated dermal fibrosis in several mouse models *in vivo*. The profibrotic effects of JMJD3 might be mediated by reducing the H3K27me3 at the Fra2 promoter and consecutive overexpression of Fra2.

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SAT0319 MULTIPARAMETRIC DETECTION OF AUTOANTIBODIES TO INVESTIGATE RELATIONSHIPS BETWEEN SEROLOGICAL AND CLINICAL SUBSETS OF SYSTEMIC SCLEROSIS PATIENTS

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Background: Systemic sclerosis (SSc) is a largely heterogeneous autoimmune disease, with patients exhibiting an extensive range of clinical presentations and various disease course. The most widely used classification divides SSc into two major subsets diffuse cutaneous (dcSSc) and limited (lcSSc) SSc by the extent and severity of skin fibrosis. However, not all patients fit into these subsets. This has created great interest to examine disease heterogeneity at the molecular level to uncover unrecognized SSc subtypes that may differ with regard to clinical manifestations, prognosis or therapy response.

Objectives: In large-scale "omics"-type autoantibody (AAB) profiling studies we have recently identified novel SSc-associated autoantigens. Here, we describe the development of a 20 marker multiplexed AAB assay to facilitate the discovery and validation of AAB-based patient subgroups.

Methods: A Luminex bead-based AAB assay was designed by combining 8 connective tissue disease (anti-centromere, anti-Scl70, U1-snRNP, SSB, Ro52, Ro60, Smb, anti-ribosomal P) antigens with 12 novel antigens (including BICD2, JMJD3/KDM6B, and PPP1R2). Novel AAB targets were previously detected in SSc patients with a p-value <0.05 (Mann-Whitney-U-test) and frequency >15%. AAB reactivity was analysed in 92 SSc patients (dcSSc: n=32, lcSSc: n=50, SSc overlap: n=9). The mean modified Rodnan skin score (mRSS), mean disease duration (month), and mean age (years) of the SSc cohort was 10.51, 162.5 and 56.94, respectively. To analyze the individual-level patient similarity of AAB reactivity, the total number of AABs reactive in each patient was calculated and referenced to the number of all available antigens in percent. Patient's demographics and clinical data were dichotomized into patients with mRSS higher or lower than the mean value. Hierarchical cluster analysis was performed to investigate the relationship between AAB patient signatures and clinical and demographic features.

Results: Based on their AAB reactivity pattern, the SSc sample cohort can be decomposed into four apparent clusters and additional fine-level clusters. LcSSc patients were spread over three clusters, each with clearly distinct AAB profile. Compared to dcSSc patients, lcSSc patients were more heterogeneous in their AAB profile. The percentage of lcSSc patients in clusters 1–3 was 70%, 90% and 53%, respectively. Patients in cluster 2 had an extended AAB repertoire that is

anti-centromere, KDM6B, SSA, BICD2 and PPP1R2. 63% of all patients in cluster 4 were dcSSc and anti-Scl70 positive, who were most afflicted of the disease. AAB signatures of patients were mapped against dichotomized demographic and clinical features. Moving from cluster 1 to 4 the number of patients with shorter disease duration, lower age, higher mRSS and higher frequency of lung involvement increased.

Conclusions: The multiplexed analysis of AABs in SSc enables defining an AAB reactivity score and patient clusters. This might support to subclassify SSc beyond lcSSc and dcSSc.

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SAT0320 SSC- IGG EFFECTS ARE MEDIATED THROUGH DISTINCT PATHWAYS IN THP-1 CELLS

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Background: Peripheral blood mononuclear cells (PBMCs) are thought to play a key role in the pathogenesis and progress of systemic scleroderma (SSc) with patients displaying distinct shifts in count, receptor expression profile and cytokine secretion patterns. SSc-IgG with elevated anti-AT₁R (angiotensin II type 1 receptor)/ET_AR (endothelin-1 type A receptor) -AAB (autoantibody) titers have been correlated to disease severity and progression¹. It remains poorly understood through which pathways SSc- IgG mediates its effects.

Objectives: In this study we sought to analyze the expression patterns of THP-1 cells (a monocytic cell line) after SSc-IgG application and their reversibility through application of numerous pharmacological inhibitors.

Methods: Transcription of IL-8- and CCL-18 in THP-1-cells after SSc-IgG and normal IgG stimulation was quantified by qPCR. Stimulations of THP-1 cells with total IgG of phenotypically different groups of SSc patients as well as ET-1 and AT-2 were carried out. In addition, stimulation with pharmacological inhibitors was conducted in a dose-dependent-manner. The results were quantified by IL-8- and CCL18-ELISA of the supernatants.

Results: Expression of IL-8 and CCL-18 is induced by SSc-IgG treatment in comparison to normal IgG which does not follow this trend. IL-8 secretion of THP-1 cells upon SSc-IgG stimulation is mediated through specific autoantibody effects and transduced through NF- κ B, ERK-, and AP-1 pathways. CCL-18 secretion of THP-1 cells upon SSc-IgG stimulation is not mediated through aforementioned pathways.

Conclusions: A stable cell culture system able to reproduce previous PBMC data on IL-8 and CCL-18 induction upon SSc-IgG-treatment could be established and insight was gained regarding key pathways which are involved in the transduction leading to IL-8 secretion. The effects of specific surface receptor expression profiles on transduction remain to be elucidated.

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SAT0321 MKP-1 AS A PROTECTIVE FACTOR AND NOVEL DRUG TARGET IN SCLERODERMA: MKP-1 DEFICIENT MICE DEVELOP MORE SEVERE DERMAL FIBROSIS IN A WIDELY USED EXPERIMENTAL MODEL OF SCLERODERMA

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Background: Scleroderma is a chronic connective tissue disease of unknown aetiology. In early stages, vascular injury and inflammation lead to fibrosis, resulting in irreversible damage in various organs. Inflammation is believed to be necessary in order to activate fibroblasts to over-produce extracellular matrix components. At the present, there is no effective standard treatment to reverse or slow down the progression of scleroderma but one of the feasible approaches is to target key inflammatory pathways that are involved in the pathogenesis of the disease.

MKP-1 (Mitogen-Activated Protein Kinase Phosphatase-1) is a nuclear phosphatase present in most cell types and tissues. Studies with MKP-1 deficient mice have undoubtedly shown that MKP-1 is an important regulator of innate and adaptive immune responses to limit and suppress inflammation (1) but its role in fibrosing diseases has not been studied.

Objectives: In the present study, we aimed to investigate the potential protective role of MKP-1 in the pathogenesis of scleroderma by using MKP-1 deficient mice and a widely studied experimental model of scleroderma.

Methods: We used bleomycin-induced dermal fibrosis in the mouse as an experimental model of scleroderma (2). Wild type (WT) and MKP-1 deficient mice were injected subcutaneously with bleomycin every other day for 28 days. Dermal

thickness and collagen accumulation were determined by histological analyses. The expression of several inflammatory and pro-fibrotic mediators were measured by quantitative RT-PCR.

Results: We found that bleomycin-induced dermal thickness and lipodystrophy were increased in MKP-1 deficient mice. Collagen accumulation in the dermis and mRNA expression of collagens 1A1 and 3A1 were enhanced in the skin from MKP-1 deficient mice as compared to the skin from WT animals. Affected skin from MKP-1 deficient mice presented increased expression of factors related to inflammation and fibrosis, namely IL-6, TGF- β 1, fibronectin-1 and YKL-40 as well as chemokines MCP-1, MIP-1 α and MIP-2.

Conclusions: This study demonstrates, for the first time, that MKP-1 deficient mice develop more severe bleomycin-induced dermal fibrosis than their WT counterparts, indicating that MKP-1 regulates the inflammatory and fibrotic processes typical for experimentally-induced scleroderma. These findings suggest that compounds which enhance expression/activity of MKP-1 have potential as novel drugs for the stage-specific modulation of the pathogenesis of scleroderma.

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SAT0322 ADAM-17 IS EXPRESSED IN THE INFLAMMATORY MYOPATHY, AND IS INVOLVED WITH INTERSTITIAL LUNG DISEASE (ILD)

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Background: A disintegrin and metalloprotease (ADAM) family is protease that is thought to have an important role in tissue destruction and inflammatory reaction. ADAMs are also involved in the amputation from the cell surface of inflammatory cytokines. ADAM-17 is one of the ADAM family, and is first described as the protease responsible for tumor necrosis factor (TNF)- α shedding. The implication of ADAM-17 substrates in immunoregulation has made this enzyme an efficient therapeutic target in the treatment of a number of pathological conditions including airway inflammation and arthritis.

Objectives: The function of ADAM-17 in myositis is unclear. Therefore, we clarify the expression of ADAM-17 in inflammatory myopathy and the role of inflammation in interstitial lung diseases (ILD).

Methods: The serum were collected from the patients who were diagnosed with inflammatory myopathy in Showa University Hospital from 2003 to 2015. Twenty-six patients were diagnosed with polymyositis (PM), 34 patients were diagnosed with dermatomyositis (DM), and 10 patients were diagnosed clinically amyopathic dermatomyositis (CADM). Clinical manifestations and clinical data were also collected. The levels of ADAM-17 in the serum samples were measured using enzyme-linked immunosorbent assay (ELISA). ADAM-17 expression was determined in muscle tissues from DM using immunohistological staining. To determine that the role of lung fibrosis in inflammatory myopathy with ILD, we used human lung fibroblasts (HLF). ADAM-17 expression on HLF was also demonstrated by immunohistological staining. ADAM-17 expression in interleukine (IL)-6 and IL-6 receptor (IL-6R) stimulated HLF was performed by ELISA.

Results: ADAM-17 in inflammatory myopathy was significantly higher than in healthy control (n=19) (mean $\pm$ SEM; 1048 $\pm$ 312 pg/ml and 361 $\pm$ 18 pg/ml, respectively, p<0.05). ADAM-17 in corticosteroid and/or immunosuppressant treatment patient serum was also significantly decreased compared with in pre treatment patient serum (1465 $\pm$ 562 pg/ml and 1059 $\pm$ 503 pg/ml, respectively, p<0.01). In addition, ADAM-17 in inflammatory myopathy with ILD patients (n=46) was significantly higher than in non-ILD patients (n=24) (1379 $\pm$ 454 pg/ml and 413 $\pm$ 226 pg/ml, respectively, p<0.05), while ADAM-15 did not have the differences between ILD and non-ILD group. Finally, we found the expression of ADAM-17 in muscle biopsy tissue. Hence, ADAM-17 on HLF was expressed by immunohistochemistry. ADAM-17 in IL-6 and IL-6R stimulated HLF was significantly higher compared with non-stimulated HLF (48 $\pm$ 6 pg/ml and 0 $\pm$ 0 pg/ml, respectively, p<0.05).

Conclusions: ADAM-17 is expressed in inflammatory myopathies especially with ILD and expressed on HLF, suggesting that ADAM-17 may play the role in lung fibrosis. ADAM-17 may be a potential target in inflammatory myopathies with ILD.

Disclosure of Interest: None declared

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SAT0323 THE ENDOTHELIAL-TO-MESENCHYMAL TRANSITION (ENDOMT) IN SCLERODERMA CAN BE PREVENTED BY THE USE OF DUAL ENDOTHELIN RECEPTOR ANTAGONISTS BOSENTAN AND MACITENTAN

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Background: Systemic sclerosis (SSc) is characterized by early vascular abnormalities and subsequent fibroblast activation and differentiation into myofibroblasts, leading to fibrosis. 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, has been reported in SSc.

Objectives: The goal of the study was to evaluate the potential of endothelin-1 (ET-1) dual receptor antagonists bosentan (BOS) and macitentan (MAC) to antagonize EndoMT *in vitro*.

Methods: 20 patients with limited SSc were enrolled and underwent double skin biopsy (affected and nonaffected skin). Fibroblasts and microvascular endothelial cells (MVECs) were isolated from biopsies. Mono- or coculture of MVECs (isolated from nonaffected skin) with fibroblasts (isolated from affected skin and stimulated with ET-1 and transforming growth factor beta [TGF- β]) were performed. In cocultures, the MVEC layer was left undisturbed or was preincubated with either BOS or MAC. After 48 h of coculture, MVECs were analyzed for their capillary formation ability and for messenger RNA and protein expression of different vascular (CD31, vascular endothelial growth factor-A [VEGF-A], VEGF-A165b) and profibrotic (alpha-smooth muscle actin [α -SMA], collagen type I [Col I], TGF- β) molecules.

Results: MVECs showed a reduced capillary formation ability when cocultured with SSc fibroblasts with respect to mono cultures. CD31 and VEGF-A resulted in downregulation, while VEGF-A165b, the antiangiogenic isoform, resulted in upregulation. At the same time, mesenchymal markers α -SMA, Col I, and TGF- β resulted in overexpression in MVECs. Capillary formation ability was restored when MVECs were preincubated with BOS or MAC, also reducing the expression of mesenchymal markers and restoring CD31 expression as well as the imbalance between VEGF-A and VEGF-A165b.

Conclusions: BOS and MAC seem able to antagonize EndoMT phenomenon in MVECs *in vitro*. Blocking EndoMT is important for two reasons: first, because capillary formation ability in MVECs can be restored; second, because the endothelium-derived fibrotic development in SSc can be counteracted.

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SAT0324 INCREASED FREQUENCIES OF CIRCULATING CXCL10-, CXCL8- AND CCL4-PRODUCING MONOCYTES AND SIGLEC-3-EXPRESSING MYELOID DENDRITIC CELLS IN SYSTEMIC SCLEROSIS PATIENTS

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Background: Systemic sclerosis (SSc) is an inflammatory and fibrotic disease characterized by vascular dysfunction, excessive extracellular matrix deposition and immune dysregulation. Recent observations suggest that monocytes and dendritic cells (DCs) might be involved in SSc; including cell recruitment, trafficking, activation and an enhanced pro-fibrotic phenotype. Hence these cells might be important contributors to the disease pathogenesis. However, detailed analysis of circulating monocytes and DCs in SSc in relationship to disease activity has not been performed so far.

Objectives: To investigate the *ex vivo* pro-inflammatory properties of classical and non-classical monocytes as well as myeloid dendritic cells (mDCs) in SSc patients in relationship to disease activity.

Methods: This study enrolled 43 SSc patients, 30 classified as limited cutaneous SSc (lcSSc) and 13 as diffuse cutaneous (dcSSc). The healthy control group (HC) included 20 age- and gender- matched individuals. The Spontaneous production of CXCL10, CCL4, CXCL4 and IL-6 was intracellularly evaluated in classical and non-classical monocytes and Siglec-3-expressing mDCs from peripheral blood using flow cytometry. In addition, the production of these cytokines was determined upon toll like receptor 4 (TLR4) plus Interferon- γ (IFN- γ) *in vitro* stimulation.

Results: The frequency of non-classical monocytes spontaneously producing CXCL10 was increased in both lcSSc and dcSSc subsets of SSc patients (p<0.05) and CCL4 was augmented in the dcSSc patient subset (p<0.05). The proportion of CCL4 producing- mDCs were also elevated in dcSSc patients (p<0.01) and the percentage of mDCs producing CXCL10 only in lcSSc patients (p<0.05 compared to HC, but p<0.01 comparing to dcSSc). Upon *in vitro* stimulation the frequency of non-classical monocytes expressing CXCL8 was