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**FRIDAY, 15 JUNE 2018**

## Systemic sclerosis, myositis and related syndromes – etiology, pathogenesis and animal models

### FRI0396 THE ATM KINASE AND PTEN, DRIVE MYOFIBROBLASTS DIFFERENTIATION BY ACTIVATING THE TGFB AUTOCRINE LOOP

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**Background:** Pulmonary fibrosis is a major cause of mortality in scleroderma (SSc) and Idiopathic Pulmonary Fibrosis (IPF). Fibrosis is driven by Inappropriate myofibroblast differentiation and persistence. Understanding this process, is vital for developing an effective treatment. Angiotensin II, is implicated in fibroblast activation in the heart and kidney, through interactions with growth factors (e.g. EGF and TGFβ).
**Objectives:** We examined the role of Angiotensin II in myofibroblast activation in the lung.
**Methods:** Lung fibroblasts were isolated from SSc, IPF, or control patient lungs (6 each). Fibroblasts were also cultured from PTEN null and wild-type mice. Protein expression after angiotensin II treatment (AngII) was investigated by western blotting. Myofibroblast differentiation and function was assayed through the contraction of 3D collagen gels and scratch migration assays. The signalling pathways involved were dissected using specific inhibitors: PI3-kinase/AKT (wortmannin, LY294002), TGFβ (1d11 neutralising antibody, SB431542 ALK5 inhibitor) Ataxia-Telangiectasia Mutated (ATM – Ku55933), AngII (Losartan).
**Results:** SSc and IPF lung fibroblasts showed increased AKT phosphorylation and suppressed PTEN expression ($p<0.05$). Their phenotype was more myofibroblast-like, with higher αSMA expression ($p<0.05$), increased collagen gel contraction (control; 207±14 vs SSc; 93±15 vs IPF 91±21, $p<0.05$), and enhanced migratory capacity ($p<0.05$). PTEN-null fibroblasts showed a similar phenotype. AngII treatment activated AKT, suppressed PTEN and induced myofibroblast differentiation in normal lung fibroblasts. In both AngII-treated and PTEN lung fibroblasts AKT activation required the ATM kinase. Inhibition of AKT either with PI3K or ATM inhibitor abrogated these effects. The increased expression of Myofibroblast-related genes after AngII treatment, was also blocked by inhibition of TGFβ with a neutralising antibody or an ALK5 inhibitor. AKT phosphorylation on the other hand was only partially blocked was partially blocked by TGFβ inhibition.
**Conclusions:** Our data demonstrate for the first time that AngII signals via the ATM kinase, which together with PTEN suppression are essential for the activation of AKT by AngII. AngII promotes myofibroblast differentiation, by stimulating the fibroblast TGFβ autocrine loop through AKT. Our data shows that activation of AKT through ATM and PTEN, may serve as the molecular link between pulmonary hypertension and lung fibrosis in fibrotic diseases.
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### FRI0397 PECULIAR EXPRESSION OF AUTOPHAGY BIOMARKERS IN NECROTIZING AUTOIMMUNE MYOPATHY MUSCLE

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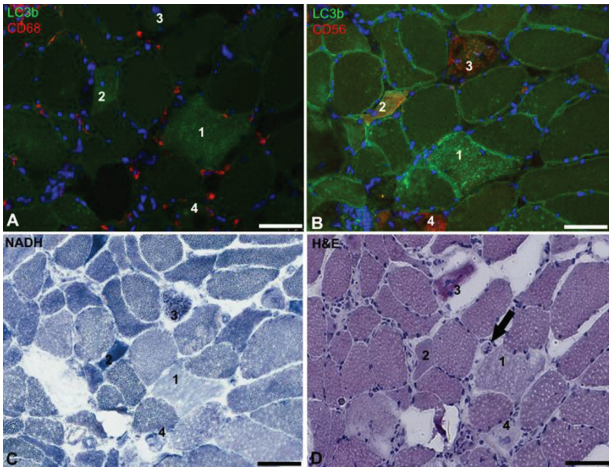
**Background:** Immune mediated necrotizing myopathy (IMNM) is a recently recognised pathology within the spectrum of idiopathic inflammatory myopathies (IIMs). Specific autoantibodies and the response to immunosuppressants aid to make the diagnosis and suggest immune-mediated pathogenesis, although histopathological features are not specific for IMNM. Autophagy and ubiquitin-proteasome system are two interacting systems by which dysfunctional cellular components are degraded in the cell. Their dysregulation, in sporadic Inclusion Body Myositis (sIBM), seems to be responsible for the protein aggregates. The autophagy dysfunction in IMNM was not widely investigated.
**Objectives:** To investigate autophagy marker expression, macrophages localization and accumulation of misfolded proteins in non-necrotic fibres of IMNM muscle in comparison with Dermatomyositis (DM), Polymyositis (PM) and sIBM.
**Methods:** Among 52 IIMs diagnosed from January 2015 to June 2017, we reviewed muscle biopsies and stored sera. Six subjects were included in the IMNM group, characterised by many necrotic muscle fibres, regenerating muscle fibres and no significant inflammation despite of numerous but scattered macrophages removing necrotic muscle fibres. Two patients had anti-signal recognition particle (SRP) autoantibodies, two patients anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the others tested negative for specific autoantibodies. All IMNM patients had a positive response to immunosuppressants. Muscle sections were immunolabelled with the following antigens: ubiquitin, autophagy markers LC3b, p62 (a receptor of autophagy), TDP-43 (a marker of ubiquitinated proteic inclusions), SMI31 and SMI310 (Phosphorylated Neurofilaments), CD31 (endothelial cell marker), C5b-9 (membrane attack complex), CD4 (T-helper lymphocytes), CD8 (T-suppressor lymphocytes), CD68 (macrophages), CD20 (B-lymphocytes), CD56 (NK lymphocytes and regenerating muscle fibres), MHC I, MHC II. Quantitative results were compared among IMNM (n=6), DM (n=4), sIBM (n=4), PM (n=5) and healthy controls (n=4).
**Results:** In IMNM, inflammation was mild compared with DM, PM, sIBM, and consisted in sporadic endomysial and/or perivascular cells CD68+. Skeletal muscle fibres (SMFs) containing LC3b+puncta were significantly higher in IMNM and IBMs than in DM or PM. In all IMNM, the greater proportion of LC3b+puncta was localised in CD56 +fibres (figure 1), instead, sIBM showed a high number of LC3b +puncta in vacuolated SMFs with low expression of CD56 +SMFs. As expected, P62 and SMI31 aggregates were significantly higher in sIBM than in the other IIMs, even if, also in IMNM, there were moderate p62 accumulations and a little proportion of SMFs stained by SMI31. Finally, in IMNM there was the highest number of ubiquitin +SMFs.




**Conclusions:** These findings suggest an involvement of cellular clearance systems in the pathophysiology of IMNM similarly to sIBM. Nevertheless, LC3b +puncta in regenerating fibres can be considered a peculiar biomarker in IMNM. Further studies of larger cohort of patients are needed to better define IMNM.
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### FRI0398 SL-401, A NOVEL TARGETED THERAPY DIRECTED TO THE INTERLEUKIN-3 RECEPTOR (CD123), KILLS PLASMACYTOID DENDRITIC CELLS FROM SYSTEMIC SCLEROSISPATIENTS

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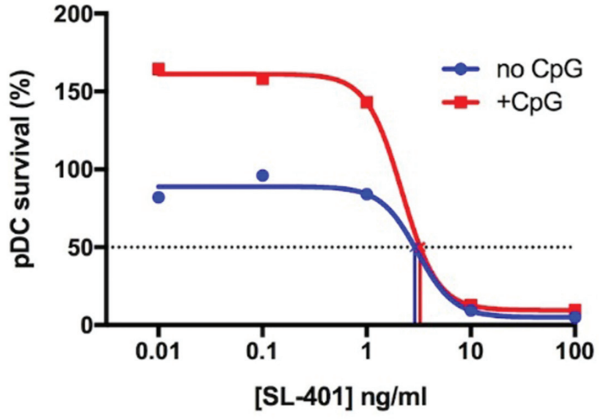
**Background:** SL-401 is a novel biologic targeted therapy directed to the interleukin-3 receptor (CD123). SL-401 is comprised of human IL-3 recombinantly fused to a truncated diphtheria toxin (DT) payload engineered such that IL-3 replaces the native DT receptor-binding domain. In this way, the IL-3 domain of SL-401 directs the cytotoxic DT payload to cells expressing CD123. Upon internalisation, SL–401 irreversibly inhibits protein synthesis and induces apoptosis of the target cell.

Plasmacytoid dendritic cells (pDCs) are immune cells that express CD123, secret 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 SSc[3]. 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 described[4] and used to further enrich additional PBMCs. pDC-enriched PBMCs (3%–6% pDCs) were cultured at $2 \times 10^5$ 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% $CO_2$, and 95% humidity. After 24 hour 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 $ED_{50}$ 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 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 TGFβ- 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.