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.

REFERENCES:

- [1] Ah Kioon MD, Tripodo C, Fernandez D, et al. Plasmacytoid dendritic cells promote systemic sclerosis with a key role for TLR8. *Sci Transl Med*. 2018 Jan 10;10(423).
- [2] van Bon L, Affandi AJ, Broen J, et al. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. *N Engl J Med*. 2014 Jan 30;370(5):433–43.
- [3] van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: An American College of Rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis*. 2013 Nov;72(11):1747–55.
- [4] Guiducci C, Ott G, Chan JH, et al. Properties regulating the nature of the plasmacytoid dendritic cell response to Toll-like receptor 9 activation. *J Exp Med*. 2006 Aug 7;203(8):1999–2008.

Disclosure of Interest: M.-D. Ah Kioon Grant/research support from: Stemline Therapeutics, Inc., R. Lindsay Shareholder of: Stemline Therapeutics, Inc., Employee of: Stemline Therapeutics, Inc., J. Chen Shareholder of: Stemline Therapeutics, Inc., Employee of: Stemline Therapeutics, Inc., J. Gordon: None declared, R. Spiera: None declared, F. Barrat Grant/research support from: Stemline Therapeutics, Inc., C. Brooks Shareholder of: Stemline Therapeutics, Inc., Employee of: Stemline Therapeutics, Inc.

DOI: 10.1136/annrheumdis-2018-eular.5657

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

C. Corallo¹, J. Benschop¹, M. Grimbergen¹, J. Crawford¹, J. Meuldijk¹, R. A. Janssen¹, N.E. Vandeghinste², N. Giordano³, M.A. Tessari¹. ¹Galapagos BV, Leiden, Netherlands; ²Galapagos NV, Mechelen, Belgium; ³Scleroderma Unit, Department of Medicine, Surgery and Neurosciences, University of Siena, Siena, Italy

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.

REFERENCES:

- [1] Denton CP. Advances in pathogenesis and treatment of systemic sclerosis. *Clin Med (Lond)* 2016;16:55–60.
- [2] Asano Y. Systemic Sclerosis. *J Dermatol* 2017. doi:10.1111/1346-8138.14153
- [3] Manetti M, Romano E, Rosa I, Guiducci S, Bellando-Randone S, De Paulis A, Ibbas-Manneschi L, Matucci-Cerinic M. Endothelial-to-mesenchymal transition contributes to endothelial dysfunction and dermal fibrosis in systemic sclerosis. *Ann Rheum Dis* 2017;76(5):924–934.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5406

FRI0400

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

C. Bergmann, B. Merlevede, C. Beyer, L. Hallenberger, A. Brandt, C. Dees, S. Pötter, A. Bozec, G. Schett, J. Distler. *Internal Medicine III-Rheumatology, University Clinic Erlangen-nuremberg, Erlangen, Germany*

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.

Objectives: The aim of this study is to characterise the crosstalk between AP1- and hedgehog signalling in fibrotic tissue disease in SSc.

Methods: Co-localization of cJUN and GLI2 in fibrotic skin was analysed by confocal microscopy and interaction was shown by Co-IP. cJUN/AP1 signalling and GLI2 signalling were inhibited *in vitro* and *in vivo* using the pharmacological inhibitors T5224 and GANT61. Hedgehog signalling was activated in mice by fibroblast-specific overexpression of constitutively active Smoothened (Smo^{ACT} mice).

Results: Expression profiling of all AP1 family members revealed most pronounced differences for cJUN in Smo^{ACT} mice. The expression of cJUN colocalized with the hedgehog transcription factor GLI2. Overexpression and colocalization of cJUN and GLI2 were also observed in fibroblasts in the skin of SSc patients. The number of GLI2- and cJUN double-positive fibroblasts was strongly increased in involved SSc skin compared to healthy controls and was particularly high in samples of SSc patients with diffuse and progressive disease. Based on the upregulation of both cJUN and GLI2 and their colocalization, we hypothesised that cJUN and GLI2 might interact with each other to amplify fibroblast activation and tissue fibrosis. Stimulation of resting fibroblasts with TGFβ induces both cJUN and GLI2 mRNA and protein in a time-dependent manner. cJUN and GLI2 are also induced upon stimulation with Sonic hedgehog (SHH), demonstrating that TGFβ and SHH are both capable to activate cJUN and GLI2-dependent transcription. This crosstalk occurs by direct interaction of cJUN and GLI2, which amplifies the transactivation potential of both transcription factors. Co-immunoprecipitation demonstrated that stimulation of fibroblasts with TGFβ or SHH induces direct interaction of cJUN and GLI2. Overexpression of cJUN and GLI2 resulted in activation in both AP1 and Hedgehog target genes. The central role of the crosstalk between cJUN and GLI2 for tissue fibrosis was further highlighted by the finding that hedgehog-induced fibrosis was strongly reduced by AP1 inhibition. Smo^{ACT} mice developed extensive skin fibrosis. Treatment with a cJUN/AP1 inhibitor T5224, however, strongly ameliorated hedgehog-induced fibrosis in Smo^{ACT} mice.

Conclusions: We demonstrate in the present study that the concomitant activation of AP1- and hedgehog signalling amplifies signalling through both cascades to promote excessive fibroblast activation and tissue fibrosis. This finding may open venues for combined inhibition of AP1- and hedgehog signalling for the treatment of fibrosis.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7374

FRI0401

THE TYROSINE PHOSPHATASE SHP2 CONTROLS TGFβ-INDUCED STAT3 SIGNALLING TO REGULATE FIBROBLAST ACTIVATION AND FIBROSIS

A. Zehender¹, J. Huang², A.-H. Györfi¹, A.-E. Matei¹, C. Dees¹, C. Beyer³, K. Gelse⁴, A. Ramming¹, C. Bergmann¹, W. Birchmeier⁵, O. Distler⁶, G. Schett¹, J. Distler¹. ¹Department of Rheumatology and Internal Medicine (Med3), Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen; ²Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg; ³Department of Internal Medicine 1; ⁴Unfallchirurgische Abteilung, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen; ⁵Max-Delbrück-Centrum für Molekulare Medizin, Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany; ⁶Rheumaklinik, University Hospital Zurich, Zurich, Switzerland

Background: SHP2, encoded by the PTPN11 gene, is a ubiquitously expressed non-receptor tyrosine phosphatase (PTP). Altered expression and activity levels of SHP2 have also been implicated into the pathogenesis of autoimmune diseases.

Objectives: In our study, we aim to characterise the role of SHP2 as a possible novel molecular checkpoint of transforming growth factor-β (TGFβ)-induced JAK2/STAT3 signalling in fibrotic diseases.

Methods: Mice carrying *Shp2^{fl/y} × Col1a2-CreER* were generated to specifically knockout *Shp2* in fibroblasts. SHP2 was inhibited using NSC-87877 inhibitor. The role of Shp2 was investigated in three different mouse models: Bleomycin- and TBRI^{CA} induced dermal fibrosis as well as TSK1 genetic model of skin fibrosis. The construct pCMV3-Flag-JAK2 was used for overexpression and for *in vitro* mutagenesis of human JAK2. Co-immunoprecipitation was performed to confirm the interaction between SHP2 and JAK2. STAT3 activation was analysed by reporter assay. Protein and mRNA expression levels were analysed by Western Blot and qPCR.

Results: Our data characterise SHP2 as an important regulator of TGFβ signalling in fibroblasts. Although SHP2 expression is decreased in systemic sclerosis (SSc), the moderate downregulation of SHP2 expression is not sufficient to counterbalance the hyper-activation of TGFβ signalling in SSc. However, *Shp2*-deficient murine fibroblasts are less responsive to TGFβ. An impaired response to the profibrotic effects of TGFβ was also observed in human SSc fibroblasts with

siRNA-mediated knockdown of *SHP2* and upon pharmacological inhibition of SHP2. We show that SHP2 can dephosphorylate JAK2 at the inhibitory Y570 site to promote TGFβ-dependent activation of JAK2 and its downstream mediator STAT3. In contrast, pharmacologic inhibition of SHP2, overexpression of a catalytically inactive SHP2 mutant or knockout of *SHP2* inhibits dephosphorylation of pJAK2^{Y570} preventing phosphorylation of JAK2 at the activation site Y1007/Y1008 and subsequent activation of STAT3 in TGFβ-stimulated fibroblasts and in experimental fibrosis. Fibroblasts overexpressing SHP2 are more susceptible to the profibrotic effects of TGFβ, whereas the stimulatory effects of TGFβ on myofibroblast differentiation and collagen release are reduced in *Shp2* knockout fibroblasts. In addition, fibroblast-specific inactivation of *Shp2* *in vivo* protected from experimental fibrosis induced by overexpression of a constitutively active TGFβ receptor type I, by bleomycin injection and ameliorated fibrosis in TSK1 mice.

Conclusions: The present study describes for the first time a role of SHP2 in the pathogenesis of SSc. SHP2 is required for the activation of JAK2 and STAT3 by TGFβ. Inactivation of SHP2 prevents fibroblast activation and tissue fibrosis, providing evidence that SHP2 may be a potential target for the treatment of fibrosis.

Disclosure of Interest: A. Zehender: None declared, J. Huang: None declared, A.-H. Györfi: None declared, A.-E. Matei: None declared, C. Dees: None declared, C. Beyer: None declared, K. Gelse: None declared, A. Ramming: None declared, C. Bergmann: None declared, W. Birchmeier: None declared, O. Distler: Grant/research support from: Actelion, Pfizer, Ergonex, BMS, Sanofi-Aventis, United BioSource Corporation, Roche/Genentech, Medac, Biovitrium, Boehringer Ingelheim, Novartis, 4D Science, Active Biotech, Bayer, Sinoxa, Serodapharm, EpiPharm, GSK, Pharmacyclics and Biogen, Consultant for: Actelion, Pfizer, Ergonex, BMS, Sanofi-Aventis, United BioSource Corporation, Roche/Genentech, Medac, Biovitrium, Boehringer Ingelheim, Novartis, 4D Science, Active Biotech, Bayer, Sinoxa, Serodapharm, EpiPharm, GSK, Pharmacyclics and Biogen, G. Schett: None declared, J. Distler: Shareholder of: 4D Science GmbH, Grant/research support from: Anamar, Active Biotech, Array Biopharma, BMS, Bayer Pharma, Boehringer Ingelheim, Celgene, GSK, Novartis, Sanofi-Aventis and UCB, Consultant for: Actelion, Active Biotech, Anamar, Bayer Pharma, Boehringer Ingelheim, Celgene, Galapagos, Inventiva, JB Therapeutics, Medac, Pfizer, RuiYi and UCB

DOI: 10.1136/annrheumdis-2018-eular.5312

FRI0402

MICRORNA-125B REGULATES APOPTOSIS IN SYSTEMIC SCLEROSIS

A. Kozlova¹, E. Pachera¹, B. Maurer¹, A. Jüngel¹, J.H. Distler², G. Kania¹, O. Distler¹. ¹Center of Experimental Rheumatology, Department of Rheumatology, University Hospital, Zurich, Switzerland; ²Department of Internal Medicine 3, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany

Background: The role of apoptosis in systemic sclerosis (SSc) is an emerging topic. MicroRNAs (miRs) are a class of small noncoding RNAs that regulate many important biological processes including apoptosis.

Objectives: To analyse the differential expression, regulation and pathophysiological role of miR-125b in systemic sclerosis.

Methods: MiR-125b expression was assessed by qPCR on RNA derived from cultured fibroblasts, whole skin biopsies and paraffin embedded dermis and epidermis from healthy controls (HC) and SSc patients. Fibroblasts were stimulated with pro-inflammatory and/or pro-fibrotic cytokines and with different epigenetic stimuli. Knockdown with anti-miR-125b (or scrambled controls) in HC fibroblasts was performed to identify downstream effects. RNA from healthy fibroblasts (n=4) after knockdown was proceeded to RNAseq using Illumina HiSeq2000. Bioinformatics analysis included differential gene expression and pathway analysis. Sequencing data were validated using qPCR (on HC and SSc fibroblasts). Apoptosis was assessed by Caspase-Glo 3/7 assay, Western Blot of cleaved caspase 3 and Annexin V live assay on cultured fibroblasts.

Results: MiR-125b was downregulated in SSc skin biopsies (n=10, median 47%, Q₁,35%, 69%; p<0.01) in comparison with HC skin (n=10). To localise its expression in the skin, we separately analysed miR-125b expression in dermis and epidermis of paraffin embedded skin where it was downregulated in both compartments (n=3 each). The expression of miR-125b in primary dermal fibroblasts was also downregulated (median 53%, Q₁,36%, 58%; n=10, p<0.01) compared to HC fibroblasts (n=10).

MiR-125b expression was not affected by major cytokines operative in SSc such as TGFβ or PDGF. However, TSA (histone deacetylase pan-inhibitor) downregulated miR-125b expression in a time- and dose-dependent manner (n=5, median 23%, Q₁,19%, 48%; p<0.01).

RNAseq identified >3500 differentially expressed genes with p<0.05. More than half of the differentially expressed genes with at least 15% change were predicted targets of miR-125b by TargetScan and MiRWalk, indicating successful functional