Shareholder of: Riptide Bioscience, Jesse Jaynes Shareholder of: Riptide Bioscience, James Stanway: None declared, Bahja Ahmed Abdi: None declared, Nikita Arumalla: None declared, Sarah Karrar: None declared, Sian Morris: None declared, Christopher Denton Grant/research support from: GlaxoSmithKline, Inventiva, CSF Behring, Consultant for: Roche-Genentech, Actelion, GlaxoSmithKline, Sanofi Aventis, Inventiva, CSL Behring, Boehringer Ingelheim, Bayer, David Abraham: None declared, Xu Shiwen: None declared, Richard Stratton: None declared

DOI: 10.1136/annrheumdis-2019-eular.4137

OP0183

EFFICACY AND SAFETY OF RIOCIQUAT IN PATIENTS WITH EARLY DIFFUSE CUTANEOUS SYSTEMIC SCLEROSIS AND INTERSTITIAL LUNG DISEASE (SSC-ILD): RESULTS FROM THE PHASE IIB RISE-SSC STUDY

Oliver Distler¹, Yannick Allanore², Christopher Denton³, Masataka Kuwana⁴, Marco Matucci-Cerinic⁵, Janet Pope⁶, Janette de Oliveria Pena⁷, Kaisa Laapas⁸, Zhen Yao⁹, Friederike Behnenburg¹⁰, Melanie Hemmrich¹⁰, Dinesh Khanna¹¹.
¹University Hospital Zurich, Zurich, Switzerland; ²Cochin Hospital, Paris, France; ³University College London, London, United Kingdom; ⁴Nippon Medical School, Tokyo, Japan; ⁵University of Florence, Florence, Italy; ⁶University of Western Ontario, Ontario, Canada; ⁷Bayer US LLC, Whippany, NJ, United States of America; ⁸StatFinn Oy, Espoo, Finland; ⁹Bayer Healthcare Co., Beijing, China; ¹⁰Bayer AG, Wuppertal, Germany; ¹¹University of Michigan, Ann Arbor, MI, United States of America

Background: Diffuse cutaneous systemic sclerosis (dcSSc) is an autoimmune connective tissue disease characterised by fibrosis and, in some patients, interstitial lung disease (ILD). Riociguat is a soluble guanylate cyclase (sGC) stimulator with vasoactive, anti-proliferative and anti-fibrotic effects. We hypothesised that riociguat may preserve lung function in patients with dcSSc.

Objectives: To evaluate the effect of riociguat on lung function in patients with early dcSSc from the multicentre, randomised, double-blind, placebo-controlled Phase IIB RISE-SSc study (NCT02283762).

Methods: Patients had dcSSc of duration ≤ 18 months, modified Rodnan skin score (mRSS) 10–22 units, forced vital capacity (FVC) $\geq 45\%$ predicted (%pred) and lung diffusion capacity for carbon monoxide (DLCO) $\geq 40\%$ pred at screening. Patients were randomised to riociguat (n=60) or placebo (n=61) in individually adjusted doses of 0.5 mg up to 2.5 mg tid. The primary endpoint was the change in mRSS from baseline to Week 52. Secondary endpoints included change in FVC%pred from baseline to Week 52. Effects on lung function (FVC%pred) were assessed in the overall population and in post-hoc analyses in patients with FVC %pred 50–75% or medical history of ILD at baseline. Between groups, differences were in least-squares [LS] means.

Results: Although the study did not meet its primary endpoint, there was a reduction in mRSS with riociguat vs placebo (–2.34; 95% CI: –4.99 to 0.30; p=0.08). There were no significant differences between treatment groups in the changes in FVC%pred (–0.20; 95% CI, –3.40 to +3.00; p=0.90) or DLCO%pred (2.01; 95% CI: –4.99 to 6.25; p=0.35) which included all patients irrespective of the presence of SSC-ILD. However, differences between riociguat and placebo were observed when patients with SSC-ILD were analysed: At baseline, 11/60 (18%) patients in the riociguat group and 12/61 (20%) in the placebo group had medical history suggesting ILD (SSc-ILD); 11/60 (18%), and 7/61 (11%), respectively, had restrictive lung disease (FVC%pred 50–75%). Results in subgroups were expressed as within-group mean change (SD). In patients with SSC-ILD, the decline in FVC%pred between baseline and Week 52 was smaller in the riociguat group (–2.7[4.0]) than the placebo group (–8.9[13.1]). The changes in DLCO%pred were –0.11 (0.22) and +0.87(0.71), respectively. In patients with restrictive lung disease, FVC %pred decreased by –8.7 (14.0) at Week 52 with placebo but was almost unchanged (+0.7[5.7]) with riociguat. The changes in DLCO%pred were +0.42 (0.85) and –0.27(0.39), respectively.

Patients with SSC-ILD receiving riociguat had lower incidences of adverse events and serious adverse events [82% (n=9) and 9% (n=1) respectively] compared with placebo [92% (n=11) and 25% (n=3) respectively] with no differences between treatment groups in the incidence of respiratory adverse events. No deaths occurred in the SSC-ILD subgroup, and no new safety signals were observed.

Conclusion: These results suggest that in an exploratory analysis, riociguat may be associated with preservation of lung function and good tolerability in patients with dcSSc and evidence of ILD on medical history.

Acknowledgement: Adelphi Communications Ltd, Bollington, UK provided medical writing support.

Disclosure of Interests: Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, AnaMar, Bayer, Boehringer Ingelheim, ChemomAb,

espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/had consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders, Yannick Allanore Grant/research support from: Inventiva, F Hoffman La-Roche, Sanofi, BMS, Pfizer, Consultant for: Actelion, Bayer, BMS, Boehringer, Roche, Sanofi, Christopher Denton Grant/research support from: GlaxoSmithKline, Inventiva, CSF Behring, Consultant for: Roche-Genentech, Actelion, GlaxoSmithKline, Sanofi Aventis, Inventiva, CSL Behring, Boehringer Ingelheim, Bayer, Masataka Kuwana Grant/research support from: Actelion, Consultant for: Chugai, Reata, GlaxoSmithKline, Bayer, Boehringer-Ingelheim, Corbus, CSL-Berling, Mochida, Speakers bureau: Actelion, Pfizer, Bayer, Nippon Shinyaku, Chugai, Marco Matucci-Cerinic Grant/research support from: Actelion, MSD, Pfizer, BMS, Chemomab, Sanipedia, Speakers bureau: Actelion, BMS; MSD; Janssen, Janet Pope Consultant for: Eli Lilly and Company, Janette de Oliveria Pena Employee of: Bayer, Kaisa Laapas Employee of: StatFinn Oy, partly insourced to Bayer, Zhen Yao Employee of: Bayer, Friederike Behnenburg Employee of: Bayer AG, Melanie Hemmrich Employee of: Bayer AG, Dinesh Khanna Shareholder of: Eicos Sciences, Inc, Grant/research support from: Bayer, BMS, Pfizer, Horizon, Consultant for: Actelion Acceleron, Arena, Bayer, BI, BMS, CSL Behring, Corbus, Cytori, GSK, Genentech/Roche, Galapagos, Employee of: Elcos Sciences, Inc

DOI: 10.1136/annrheumdis-2019-eular.6889

OP0184

PROFIBROTIC LNCRNA H19X: UNRAVELLING THE EFFECTS ON CHROMATIN REMODELING IN SYSTEMIC SCLEROSIS FIBROBLASTS

Elena Pachera¹, Shervin Assassi², Gloria Salazar², Mojca Frank-Bertoncelj¹, Jörg Distler³, Gabriela Kania¹, Oliver Distler¹. ¹Center of Experimental Rheumatology, University Hospital Zurich, Zurich, Switzerland; ²Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, United States of America; ³Department of Internal Medicine 3, University of Erlangen, Erlangen, Germany

Background: Long noncoding RNAs (lncRNAs) are noncoding transcripts involved in the regulation of gene expression in health and disease. lncRNAs function via different mechanisms either acting cis, regulating the expression of nearby genes, or in trans, modulating the expression of distant genes. We identified a novel TGF β regulated lncRNA, H19X, which was strongly upregulated in a wide variety of fibrotic disorders. Moreover, we were able to demonstrate that H19X is a key driver of myofibroblast formation and extracellular matrix (ECM) overproduction.

Objectives: To define the mode of action of H19X (cis or trans) and to assess H19X function on chromatin rearrangements that may drive myofibroblast differentiation and ECM production.

Methods: The function of H19X was investigated in SSC dermal fibroblasts (n=5) by knocking down H19X with locked nucleic acid oligonucleotides followed by TGF- β stimulation and microarray analysis with Illumina HT-12 arrays. In situ hybridization for H19X was performed using Stellaris FISH probes. Chromatin remodeling was investigated by Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq).

Results: As analyzed by microarray, none of the 16 genes belonging to the same genomic region as H19X locus was affected by its downregulation, indicating that in-cis regulation is not a major mechanism of H19X activity. Cellular localization can give a first indication about lncRNA trans function: FISH staining of SSC fibroblasts stimulated with TGF- β revealed tightly localized nuclear foci in TGF- β treated cells. H19X nuclear localization was confirmed by cell fractionation, where H19X expression was peaking in the nucleus of cells after 6h of treatment with TGF- β . We next hypothesized that H19X nuclear expression might induce chromatin rearrangements. We assessed whether chromatin changes caused by H19X knockdown might be correlated with changes in expression profile seen by microarray. Therefore, we analyzed the differences in the chromatin conformation flanking transcription start sites (TSSs) by ATAC-seq. A clear separation between the two experimental groups (scrambled vs H19X knockdown) was observed and 450 differentially accessible regions were identified (p<0.05, Log2FC>0.5).

After focusing on genes that displayed significant variations and Log2FC>0.5 for both the array and ATAC-seq analysis, eight candidate genes were found both upregulated by microarray and showed increased accessibility at their TSSs by ATAC-Seq. Similarly, three candidate genes were identified as being downregulated by microarray and showed decreased TSS accessibility by ATAC-Seq. These genes included histones HIST1H2BD and HIST1H1C, suggesting a direct regulation of chromatin organization as a mode of action for H19X. Moreover, the cell cycle progression inhibitors FABP3 and PTTG1 were pointing to a role of H19X in proliferation.

In addition, analysis for transcription factor motif enrichment in the differentially accessible peaks of the ATAC-Seq analysis revealed that motifs for TEAD2, RUNX family, and NFkB2 were less accessible, while motifs for JUN::FOS, NF2L2 and EHF were more accessible due to the chromatin changes. These results indicate that H19X could promote or repress the activity of these candidate transcription factors by changing chromatin conformation at the sites of their consensus sequence.

Conclusion: ATAC-Seq in combination with microarray analysis identified candidate mechanism for the TGF β regulated profibrotic effects of H19 X including direct effects on chromatin organization and on transcription factors associated with fibrotic pathways.

Disclosure of Interests: Elena Pachera: None declared, Shervin Assassi: None declared, Gloria Salazar: None declared, Mojca Frank-Bertonec: None declared, Jörg Distler: None declared, Gabriela Kania: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, AnaMar, Bayer, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/had consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders

DOI: 10.1136/annrheumdis-2019-eular.5938

OP0185

INFLAMMATION-DEPENDENT DECREASED EXPRESSION OF CD52 ON CIRCULATING CD14+ MONOCYTES FACILITATES ADHESION IN SYSTEMIC SCLEROSIS

Michal Rudnik¹, Mara Stellato¹, Przemyslaw Blyszczuk^{1,2}, Karin Klingel³, Jörg Henes⁴, Carol Feghali-Bostwick⁵, Oliver Distler¹, Gabriela Kania¹. ¹Center of Experimental Rheumatology, University Hospital Zurich, Department of Rheumatology, Zurich, Switzerland; ²Jagiellonian University Medical College, Department of Clinical Immunology, Krakow, Poland; ³University Hospital Tuebingen, Institute for Pathology and Neuropathology, Tuebingen, Germany; ⁴University Hospital Tuebingen, Department of Rheumatology, Tuebingen, Germany; ⁵Medical University of South Carolina, Division of Rheumatology and Immunology, Charleston, SC, United States of America

Background: During the course of systemic sclerosis (SSc), infiltration of inflammatory cells, including monocytes, into the organs is a major process leading to fatal fibrosis, remodelling and organ dysfunction.

CD52 protein is highly expressed on CD4⁺ T-cells and plays an important role in the modulation of T-cell receptor signalling. Nevertheless, the function of this protein on monocytes is not completely understood.

Objectives: We aimed to functionally investigate the role of circulating CD14⁺ monocytes in the course of SSc with a special focus on monocyte adhesion and the influence of CD52 expression.

Methods: Biopsies from the heart, lungs and skin of SSc patients (n=11, 7, 7 respectively) and healthy controls (HC) (n=10, 7, 9 respectively) were analysed by immunohistochemistry for the presence of CD14⁺ cells. PolyA RNA sequencing of CD14⁺ monocytes isolated from peripheral blood of lcSSc (n=5, age=54.4 $\pm$ 6.7), dcSSc patients (n=5, age=51.8 $\pm$ 7.2) and age- and sex-matched HC (n=5, age=50.8 $\pm$ 9.7) was performed using Illumina HiSeq 4000 platform. Differentially expressed genes were computed using DESeq2 algorithm. Gene ontology and pathway analysis were performed using Metacore software and ShinyApp. Expression of adhesion molecules was confirmed on the protein level using flow cytometry (HC n=8, SSc n=22). Adhesion of CD14⁺ monocytes to immobilized ICAM1 and TNF α -stimulated endothelial cells was checked using the 96-well plate adhesion assay (HC n=8, SSc n=22). CD52 regulation in CD14⁺ monocytes from HC (n=4) was analysed on mRNA level upon stimulation with different concentrations of LPS, IFN γ , IL-4 and IL-13. THP-1 cell lines with overexpression and silencing of CD52 were generated using lentiviral vectors, and further, their adhesion was investigated (n=4).

Results: Immunohistochemistry confirmed higher infiltration of CD14⁺ cells in the heart (p<0.01), lung (p<0.05), and skin (p<0.001) of SSc patients. We detected 1440 differentially expressed genes between dcSSc vs HC and 225 between lcSSc and HC CD14⁺ monocytes respectively (p $\leq$ 0.01; log₂ ratio $\geq$ 0.5). Pathway analysis revealed significant alterations in processes important for tissue infiltration, including adhesion. Flow cytometry analysis showed upregulation of adhesion molecules: integrin α_M (CD11b) (p<0.01) and integrin β_2 (CD18) (p<0.01). On the other hand, expression of CD52 was downregulated in SSc patients (p<0.05). SSc CD14⁺ monocytes exhibited increased adhesion both to ICAM1-coated plates (p<0.01) and to TNF α -stimulated endothelial cells (p<0.05). CD52 mRNA

was significantly increased in a dose-dependent manner after anti-inflammatory stimulation (IL-4, IL-13) and significantly decreased after inflammatory stimulation (LPS, IFN γ) (p<0.05). Overexpression of CD52 in THP-1 monocytes decreased adhesion to TNF α -stimulated endothelial cells (p<0.01). Accordingly, silencing of CD52 increased adhesion of THP-1 monocytes (p<0.01).

Conclusion: This is the first report pointing to an increased adhesion of peripheral blood CD14⁺ monocytes to ICAM1 and endothelial cells in SSc. Our results suggest the primary activation of monocytes in peripheral blood, which translates into higher organ infiltration in SSc patients. Finally yet importantly, we characterised a novel function of CD52 molecule on monocytes and its possible contribution during the course of the disease.

Disclosure of Interests: Michal Rudnik: None declared, Mara Stellato: None declared, Przemyslaw Blyszczuk: None declared, Karin Klingel: None declared, Jörg Henes: None declared, Carol Feghali-Bostwick: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, AnaMar, Bayer, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medac, MedImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Serodapharm and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/had consultancy relationship within the last 3 years with A. Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritides and related disorders, Gabriela Kania: None declared

DOI: 10.1136/annrheumdis-2019-eular.5952

OP0186

LIN-GP38+ STROMAL CELLS ARE KEY EFFECTOR CELLS IN MYOCARDIAL FIBROSIS AND DEFECTS OF THE CONDUCTION SYSTEM

Mara Stellato¹, Michal Rudnik¹, Florian Renoux¹, Przemyslaw Blyszczuk^{1,2}, Elena Osto³, Matthias Dewenter⁴, Petra Seebeck⁵, Elena Pachera¹, Oliver Distler¹, Gabriela Kania¹. ¹University Hospital Zurich, Department of Rheumatology, Zurich, Switzerland; ²Jagiellonian University Medical College, Krakow, Poland; ³Department of Clinical Immunology, Krakow, Poland; ⁴University Hospital Zurich, Institute of Clinical Chemistry, Zurich, Switzerland; ⁵University Hospital Heidelberg, Department of Molecular Cardiology and Epigenetics, Heidelberg, Germany; ⁶University of Zurich, Zurich integrative Rodent Physiology, Zurich, Switzerland

Background: Cardiac dysfunction is a significant cause of mortality in SSc due to inflammation, vasculopathy and fibrosis. In fibrotic conditions, the excessive number of fibroblasts, myofibroblasts and extracellular matrix in the myocardium may directly or indirectly affect the cardiac conduction system by different mechanisms. Myofibroblasts are the main players in cardiac fibrogenesis, but their origin in SSc remains unknown.

Objectives: To unravel the role of Fos-related antigen 2 (Fra2) in stromal cell activation, cardiac fibrosis and alteration of the conduction system.

Methods: Fra2 transgenic (tg) mice and Rag2^{-/-} Fra2 tg mice were studied. Hearts from Fra2 tg and WT mice were analysed by immunohistochemistry (IHC) and immunofluorescence (IF). Cardiac function was assessed by echocardiography, electrocardiogram (ECG) and telemetry. Myocardial stromal (Ter119 CD45 CD31⁻, hereafter Lin⁻) gp38⁺ stromal cells were sorted and cultured *in vitro*. The cellular phenotype was evaluated by qPCR, α -smooth muscle actin (α SMA) and stress fibre staining, secreted collagens and contraction assay.

Results: Echocardiography of 20-week-old Fra2 tg mice displayed increased ejection fraction (p=0.02) and fractional shortening (p=0.02) with decreased left ventricle end-diameter and end-volume in systole (p=0.006, p=0.008) and in diastole (p=0.007, p=0.008). ECG in conscious mice revealed lower heart rate (HR) (WT 741 $\pm$ 33 BPM, tg 644 $\pm$ 37 BPM, p<0.0001) and prolonged QRS, ST and QTc parameters (p=0.001, p=0.01, p=0.01), which may predispose to arrhythmias. Telemetry recording showed the presence of atrio-ventricular (AV) blocks that correlated with disease severity (R=0.9, p=0.02). The myocardium of Fra2 tg mice presented mild inflammation and fibrosis with higher expression of collagen (p=0.03, n=7) and periostin (p=0.001, n=7), increased number of α SMA⁺ myofibroblasts and expansion of stromal Lin⁻gp38⁺ cells compared to WT mice (1.4% $\pm$ 1.4, n=15 and Fra-2 tg: 6% $\pm$ 4.5, n=14, p=0.001). Fibrosis disrupts the conductivity of cardiac tissue. Therefore, we investigated whether these stromal cells might be responsible for fibrosis. The majority of Lin⁻gp38⁺ cells co-expressed α SMA, collagen, ADAM12 and periostin, indicating that myocardial Lin⁻gp38⁺ cells might acquire a myofibroblast-like phenotype. Moreover, gp38 expression correlated with collagen deposition (R=0.9, p<0.0001). The comparison between Lin⁻gp38⁺ cells isolated from WT and Fra2 tg showed that both α SMA total protein and α SMA fibres were increased in Fra2 cells. Importantly, α SMA fibres co-localized with stress fibres, resulting in a faster and stronger contraction capacity of Fra2 cells (p<0.0001, n=3). Proliferation of Fra2 cells was increased compared to WT (p=0.007, n=5), while apoptosis was unchanged (p=0.335, n=5). Interestingly,