We would like to thank the following generous financial support for the Workshop which is co-sponsored by the Scleroderma Foundation and Scleroderma Research Foundation

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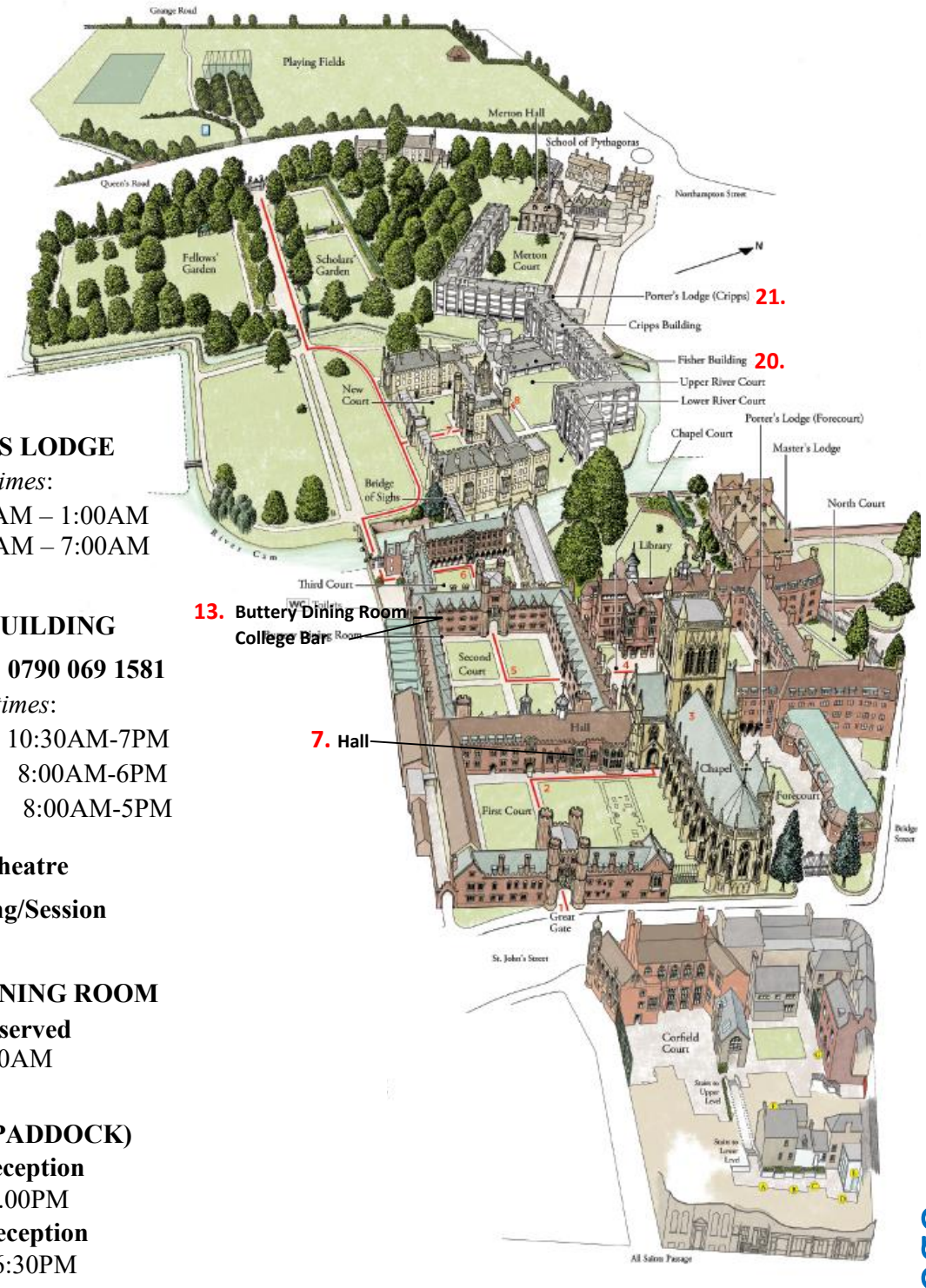


21. PORTER'S LODGE

Opening times:

Forecourt: 7:00AM – 1:00AM
Cripps: 1:00AM – 7:00AM

20. FISHER BUILDING

Conference Office: 0790 069 1581

Opening times:

Saturday	10:30AM-7PM
Sunday-Tuesday	8:00AM-6PM
Wednesday	8:00AM-5PM

Lecture Theatre

Poster Viewing/Session

13. BUTTERY DINING ROOM

Breakfast served
From 7.30AM

THE BACKS (PADDOCK)

Opening Reception
Saturday 7.00PM
Welcome Reception
Sunday 6:30PM

7. HALL

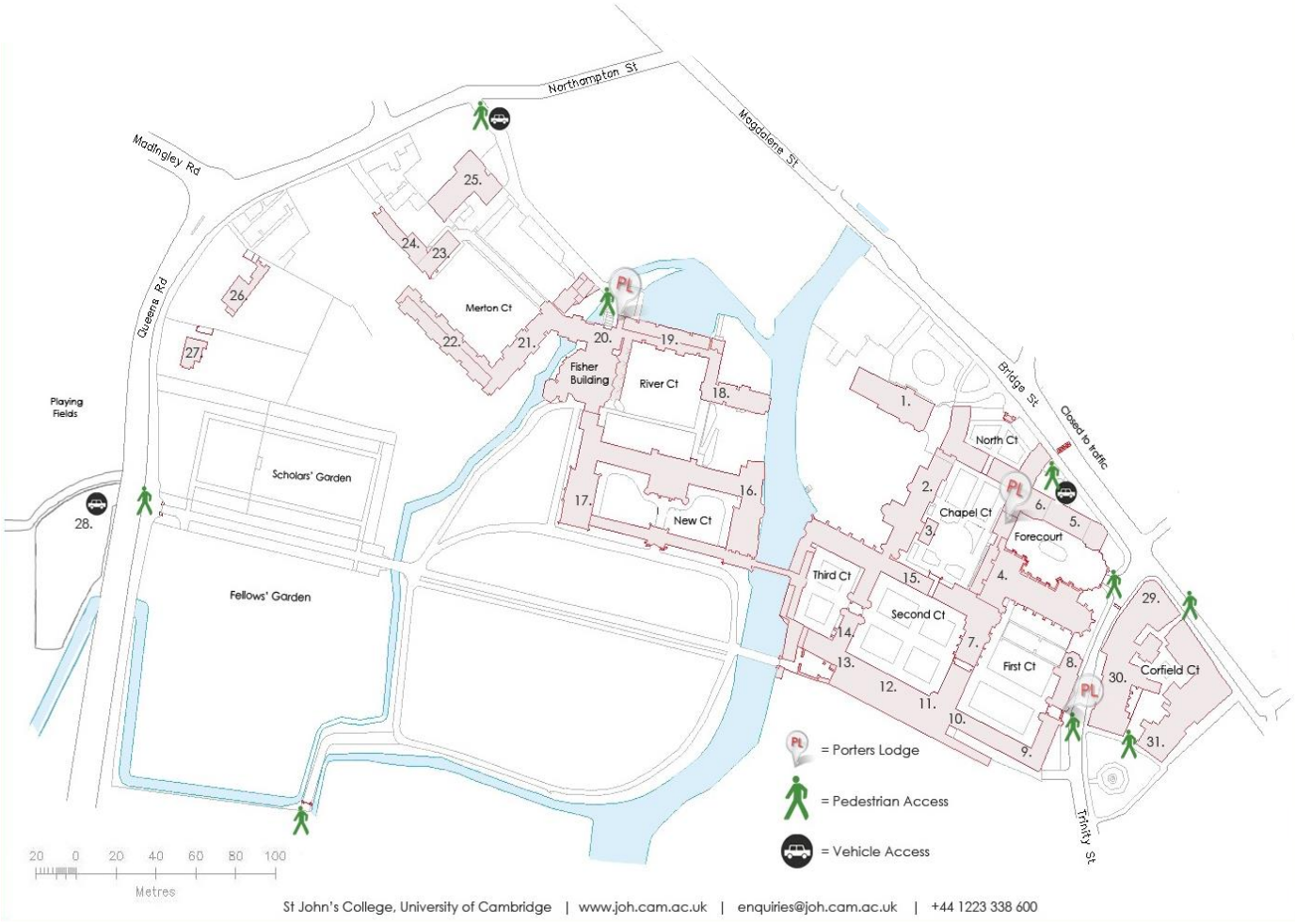
Lunch served
(See Programme for times)

Banquet
Tuesday 7.30PM

13. COLLEGE BAR

Coffee and bar snacks also available
Open 12.30 - 11.00PM
(Closing times may vary).

St. John's College



Main Site:

1. Master's Lodge
2. College Office
3. Library
4. Chapel
5. Lady Superintendent's Office
6. Computer Support Office
7. Hall
8. Old Music Room
9. ~~SBR~~
10. Parsons Room, Wordsworth Room
11. Catering and Conference Office
12. Wilberforce Room
13. Buttery
14. JCR
15. Development Office
16. Senior Guest Rooms
17. Bursary
18. 'A' staircase Cripps
19. 'B' & 'C' staircase Cripps
20. 'D' staircase Cripps
21. 'E' & 'F' staircase Cripps
22. 'G' & 'H' staircase Cripps
23. School of Pythagoras
24. Merton Hall
25. Maintenance Dept.
26. Merton House
27. Merton Cottage
28. Playing Fields car park
    Occasional use - for details contact the Domestic Bursar's office
29. Junior Guest Rooms
30. Old Divinity School
31. 1 All Saints Passage
    Teaching & Meeting Rooms

St. John's College

Advancing Scleroderma Research and Translational Medicine

SATURDAY 27 JULY — *Fisher Building*

10:30-18:00	REGISTRATION	Conference Office
18:00-18:45	**EDITH BUSCH FOUNDATION OPENING LECTURE**	
	Welcome and Introduction	Carol Black and Robert Lafyatis
	Resolution of inflammation: A new therapeutic frontier	Derek Gilroy Scientific Director- UCL
18:45-19:00	**EDITH BUSCH FOUNDATION** **Presentation: ' Lifetime Achievement Award'** **Professor Frank Wollheim – Lund University**	
	Introduction - Thomas Krieg Presentation on behalf of the Edith Busch Foundation: Ulrich Schanbacher and Martin Bäppler	
19:00	Opening Reception Sponsored by the Edith Busch Foundation	The Backs (Paddocks)

SUNDAY 28 JULY

8:00-18:00	Registration	
08:30	Welcome and Introduction	Carol Black and Robert Lafyatis
	SESSION 1 **Breakthrough 'Changing the Game' Keynotes** **Understanding Scleroderma Pathogenesis, Translational Biology and Clinical Trials** Chairs – Carol Black and Thomas Medsger	
08:40	Fundamental Bioscience	Carol Feghali-Bostwick (Charleston)
09:10	Translation and Clinical Trials	Dinesh Khanna (Michigan)
09:40	COMFORT BREAK	
	SESSION 2 **Advances in Platform Technologies: Genetics, Phenotyping and Omics** Chair – Maureen Mayes	
09:50	Introduction	Maureen Mayes (Houston)
10:00	Studying the genetic basis of autoimmune diseases	Javier Martin (Granada)
10:30	CyToF and deep cell phenotyping to study immune regulation	Shahram Kordasti (London)
11:00	Defining the architecture of autoimmune diseases at the single cell level	Adam Croft (Birmingham)
11:20	Discussion	
11:30	MORNING COFFEE	
	SESSION 3 **Wound Healing, Fibrosis and Regenerative Medicine** Chair – Armando Gabrielli	
11:50	Introduction	
12:00	Keynote: Fibrosis of the skin in scleroderma and wound repair	Michael Longaker (Stanford)
12:40	Fibroblast heterogeneity in development and healing	Emanuel Rognoni (London)
1:00	Discussion	
1:10	LUNCH	

Programme

SESSION 4 **Damage response and cytokine signaling** Chair – Jacques Behmoaras		
2:10	Introduction	Jacques Behmoaras (London)
2:20	The critical role of IL-11 in fibro-inflammatory diseases	Stuart Cook (London)
3:00	IL6 as a link between tissue damage and fibrosis	Simon Jones (Cardiff)
3:20	Myeloid signaling and type 2 immunity in fibrosis	Sabine Eming (Cologne)
3:40	Discussion	
3:50	AFTERNOON TEA	
SESSION 5 **Critical pathways in autoimmunity** Chair – Yannick Allanore		
4:20	Introduction	Yannick Allanore (Paris)
4:30	Checkpoints inhibitors in immune regulation	David Sansom (London)
5:00	T cells and complement	Claudia Kemper (Bethesda)
5:30	Defective B lymphocyte tolerance induction in systemic sclerosis pathogenesis	Rizgar Mageed (London)
6:00	Discussion	
6:20	END OF SESSION	
6:30	**~ WELCOME RECEPTION ~** **The Backs (Paddocks)**	
	~ POSTER VIEWING ~	

MONDAY 29 JULY

SESSION 6 **Young Investigators – Selected abstracts** Chairs – John Varga and Masataka Kuwana		
08:45	Introduction	
08:50	Silvia Bellando Randone	The very early diagnosis of systemic sclerosis (VEDOSS) project: predictors to develop definite disease from an international multicentre study
09:00	Andreea Bujor	Periostin as a novel marker of heart involvement in scleroderma
09:10	Kimberly Showalter	Histologic Features Correlate with the Modified Rodnan Skin Score, Serum Inflammatory Markers, and Patient Reported Outcomes in Patients with Early, Diffuse Cutaneous Systemic Sclerosis
09:20	Emily Mirizio	Defining the transcriptional profile of the skin in pediatric localized scleroderma (LS)
09:30	Victoria Flower	Assessment of the Repeatability and Convergent Validity with Dermal Collagen of High Frequency Ultrasound in Systemic Sclerosis
09:40	Benjamin Korman	TNF-alpha Drives Progressive Obliterative Pulmonary Vascular Disease and Represents a Novel Model of Scleroderma Associated Pulmonary Arterial Hypertension (SSc-PAH)
09:50	Diana Toledo	Molecular Analysis of a Skin Equivalent Tissue Culture Model of Systemic Sclerosis using RNA Sequencing, Epigenetic Assays, Histology, and Immunoassays
10:00	Blaž Burja	Metabolic Intermediate Alpha-Ketoglutarate Attenuates TGFβ-driven Profibrotic Responses of Dermal Fibroblasts
10:10	Rebecca Ross	BDCA2 Targeting of Human Plasmacytoid Dendritic Cells via CBS004 Reverts pDC-Dependent IFN Activation and Tissue Fibrosis in vitro and in vivo
10:20	Gianluca Moroncini	Generation of human PDGFRα-transgenic mouse: a novel experimental model of skin fibrosis

10:30	MORNING COFFEE	
SESSION 7 **Cancer associated fibroblast and the stromal microenvironment** Chair – Carol Feghali-Bostwick		
10:50	Introduction	
11:00	Microenvironment in cancer	Richard Hynes (Cambridge, MA)
11:30	The cell biology of the tumour microenvironment	Erik Sahai (London)
12:00	Discussion	
12:30	LUNCH	
SESSION 8 **Targeting the myofibroblasts and Metabolism** Chair – Oliver Distler		
1:50	Introduction	Oliver Distler (Zurich)
2:00	SSc myofibroblasts: evolving definitions, diversity, plasticity, targeting and clinical implications	John Varga (Chicago)
2:30	Targeting apoptosis of myofibroblasts in fibrosis	David Lagares (Boston)
3:00	Discussion	
3:30	AFTERNOON TEA	
SESSION 9 **Vascular biology: Remodeling, calcification and repair** Chair – Marco Matucci Cerinic		
3:50	Introduction	Marco Matucci Cerinic (Florence)
4:00	Control of vascular smooth muscle cell identity	Helle Jørgensen (Cambridge)
4:30	Mechanisms of vessel calcification	Cathy Shanahan (London)
5:00	Vascular remodeling	Maria Trojanowska (Boston)
5:30	Discussion	
6:00	END OF SESSION	
	COMFORT BREAK	
6:10	Marco Matucci Cerinic - **World Scleroderma Foundation** - **European Scleroderma Trials and Research Group - EUSTAR**	
6:30	**~ POSTER VIEWING ~**	

TUESDAY 30 JULY

SESSION 10 **Exploring lessons from other diseases and biomarkers** Chair – Luc Mouthon		
08:50	Introduction	Luc Mouthon (Paris)
09:00	Keynote: Hepatic Fibrogenesis	Massimo Pinzani (London)
09:40	Inflammatory myositis	Olivier Benveniste (Paris)
10:10	Synovial fibroblasts in arthritis	Trinidad Montero-Melendez (London)
10:40	Discussion	
11:00	MORNING COFFEE	
SESSION 11 **Scleroderma Clinical Trials Consortium** Chairs – Ariane Herrick and Lorinda Chung		
11:30-11.35	Introduction	
11:35-11:50	Selecting patients for clinical trials- opportunities, pitfalls, lessons learnt from recent clinical studies	Jaap van Laar (Utrecht)
11:50-12:00	Discussion	
12:00-12:15	Outcome measures in recent clinical trials – lessons learnt from recent RCTs	Robyn Domsic (Pittsburgh)
12:15-12:25	Discussion	
12:25-12:40	Development of a White Paper in systemic sclerosis	Dinesh Khanna (Ann Arbor) and Peter Merkel (Philadelphia)
12:40-12:55	Discussion	
12:55-1:00	Closing remarks	
1:00	LUNCH	
SESSION 12 **Translational Partnerships and Industrial Interactions** Chairs – Robert Lafyatis and Chris Denton		
This session provides a forum for our industrial partners to discuss and present aspects of academic-industry interactions		
Discovery partnerships with Industry		
2:00-2:10	Presentations by Industry Colleagues	Introduction
2:10-2:30	Lenabasum in the Treatment of Systemic Sclerosis	Barbara White M.D. – Chief Medical Officer Corbus Pharmaceuticals
2:30-2:50	Role of macrophages in the tumour extracellular matrix microenvironment	Henry Lopez - Executive Vice President Riptide Bioscience
2:50-3:10	Engineered human matrices to explore and enhance drug discovery in fibrosis and oncology	Giuseppe Mazza - Chief Executive Officer, ENGITIX
3:10-3:30	Discussion	
3:30	COMFORT BREAK	
SESSION 13 **Lessons from autologous Stem Cell Transplantation** Chair – Jaap van Laar		
4:00	Introduction	Jaap van Laar (Utrecht)
4:10	Keynote: Clinical impact of HSCT, hematopoietic stem cells, in SSc	Keith Sullivan (Durham)
4:40	Genetics and Profiling towards precision medicine	Michael Whitfield (Dartmouth)
5:00	Elucidating the Mechanisms and Future Directions	Alan Tyndall (Basel)
5:20	Discussion	

SESSION 14 **ECM and Mechanotransduction - revisited** Chair – Thomas Krieg		
5:40	Introduction	Thomas Krieg (Cologne)
5:50	The social networks of myofibroblasts	Boris Hinz (Toronto)
6:10	Matrix biomechanics and dynamics	Daniel Tschumperlin (Minnesota)
6:30	Discussion	
6:40	END OF SESSION	
6:40	**~ POSTER SESSION ~**	
7:30	**~ BANQUET ~** **Great Hall – St. John's College, Cambridge**	

WEDNESDAY 31 JULY

SESSION 15 **Advances in signal transduction pathways** Chair – Kristofer Rubin		
08:50	Introduction	Kristofer Rubin (Uppsala)
09:00	Control of TGFbeta signalling by the hippo pathway	Liliana Attisano (Toronto)
09:30	Targeting intracellular signaling in fibrosis	Jörg Distler (Erlangen)
10:00	Discussion	
10:20	MORNING COFFEE	
SESSION 16 **Macrophages, metabolism and mitophagy** Chair – Francesco Del Galdo		
10:50	Introduction	Francesco Del Galdo (Leeds)
11:00	Keynote: Mitochondrial function, metabolism and oxidative stress	Toren Finkel (Pittsburgh)
11:40	System genetics to study macrophage plasticity and metabolism inflammatory diseases	Jacques Behmoaras (London)
12:10	Discussion	
12:30	LUNCH	
END OF WORKSHOP		

Abstracts

Oral and Poster Presentations

SCTC Scholarships for Early Career Researchers

Silvia Bellando Randone	Italy	Abstract number 1
Andreea Bujor	USA	Abstract number 41
Kimberly Showalter	USA	Abstract number 56
Emily Mirizio	USA	Abstract number 54
Victoria Flower	UK	Abstract number 14

Scleroderma Workshop Awards

Benjamin Korman	USA	Abstract number 17
Diana Toledo	USA	Abstract number 16
Blaž Burja	Switzerland	Abstract number 61
Rebecca Ross	UK	Abstract number 25
Gianluca Moroncini	Italy	Abstract number 37

The Very Early Diagnosis of Systemic Sclerosis (VEDOSS) project: predictors to develop definite disease from an international multicentre study

S Bellando Randone[1], G Lepri[1], D Huscher[2], T Minier[3], S Guiducci[1,], C. Bruni[1], L Czirjak[3], M Cutolo[4], V Smith[5], J Avouac[6], D Furst[1,7], Y Allanore[6], O Distler[8], M Matucci Cerinic[1] & *VEDOSS co-workers**
University of Florence, Italy[1]; Charité – Universitaetsmedizin Berlin,Germany[2]; University of Pecs, Hungary[3]; University of Genova, Italy[4]; University of Ghent, Belgium[5]; Hopital Cochin, Paris, France[6];UCLA; USA[7]; University of Zurich,Switzerland[8]

Background: Early identification of patients is of key importance for the management and treatment of inflammatory rheumatic diseases.
The aim of the VEDOSS project is to determine through an at-risk population the predictive factors for the progression toward a definite systemic sclerosis (SSc).

Material and Methods: Patients with Raynaud phenomenon (RP), with or without anti-nuclear antibodies (ANA) were prospectively recruited. Fulfilling the 2013 classification criteria at baseline was an exclusion criterion. Patients with RP were recruited as controls. Patients had an annual assessment to determine organ involvement and severity. The endpoint was the fulfilment of the 2013 classification criteria. The time to fulfilling 2013 classification criteria was evaluated with Kaplan-Meier analysis, and predictors of evolution were determined by univariate and multivariate Cox regression.

Results: 735 patients with RP were recruited The sample is distributed as follows: i) *237* patients (143 with follow up) RP/ANA negative (ANA^-/pRP) as the control group, ii) *498 patients* (401 with follow up) RP/ANA positive (ANA^+/pRP)): 87 had puffy fingers (PF), 199 had anti-centromere antibodies (atb) positive, 45 had anti-topoisomerase-I atb positive and 182 had videocapillaroscopy (NVC) abnormalities at baseline. Out of 401 ANA^+/pRP patients, 7.4% within 1 year, 29.3% within 3 and 44.1% within 5 years satisfied the 2013 classification criteria. Out of the 143 ANA^-/pRP patients, none (0%) within 1 year, 4.6% within 3 years, and 4,6% within 5 years satisfied SSc criteria. After adjustment for age, the following baseline parameters were identified as independent predictors for progression into definite SSc: PF (OR=3.4 [2.0;5.6]), anti-centromere atb (OR=2.6 [1.6;4.1]) and anti-topoisomerase-1 atb (OR=3.1 [1.6;5.8]), and NVC abnormalities (OR=1.9 [1.3;2.9]) The presence of PF had a positive predictive value (PPV) of 79% and combination of PF+specific auto-antibodies showed 94% PPV to satisfy ACR/EULAR2013 criteria within 5 years (figure 1).

Conclusions: patients with very early SSc develop definite, classification criteria fulfilling SSc within 5 years of follow up. The VEDOSS study identified PF and SSc auto-antibodies at first visit as independent predictors of development of definite SSc. These data are of key importance for the risk stratification of patients with very early SSc in clinical practice and clinical studies.

1.Avouac J, et al. Ann Rheum Dis 2011;70:476

Figure 1: A matrix was built to show the PPV of various variables alone or in combination to predict subsequent progression of VEDOSS patients toward definite SSc after 5 years

	alone	Disease specic atb	scleroderma patten on NVC	puffy fingers
	PPV%	PPV%	PPV%	PPV%
Disease specific atb	70.2		82.2	94.1
scleroderma patten on NVC	75.0	82.2		77.8
puffy fingers	78.9	94.1	77.8	

Periostin as a novel marker of heart involvement in scleroderma

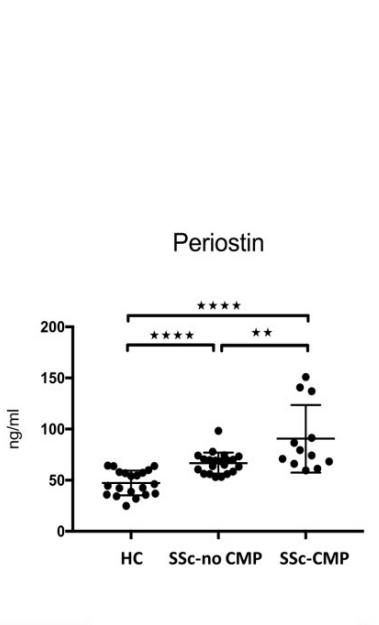
A.M. Bujor[1], F. El-Adili[1], A. Parvez[1] & F. Sam[2]
Boston University School of Medicine, Division of Rheumatology[1], Division of Cardiology[2], 72 E Concord St, Evans 50, Boston, MA, 02118, USA

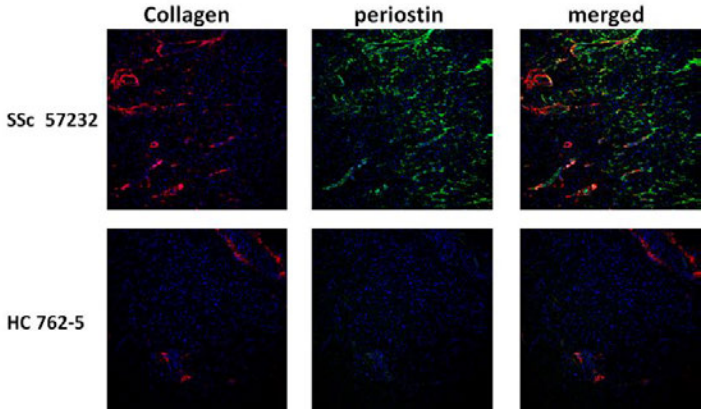
Background: Widespread myocardial fibrosis with diastolic dysfunction, a common SSc complication, is a strong predictor of death. Often subclinical, diastolic dysfunction is difficult to diagnose by conventional means, due to normal ejection fractions and minor elevations in BNP. To identify a new, more accurate marker to diagnose this SSc complication, we performed multiplex analysis of several markers known for their association with SSc or heart failure in the serum of patients with and without SSc cardiomyopathy (CMP).

Materials and methods: Serum samples from patients with and without SSc-CMP (pCWP >13, TPG<12, PVR <240, by RHC and LVEF >50%) and healthy, matched controls (HC), were analyzed for the levels of CXCL13, endothelin-1, Fas, FGF basic, IL-1 alpha, IL-18, IL-6, MMP-3, osteoprotegerin, pentraxin 3, periostin, renin, ST2, TNF-alpha, galectin-3, MMP-2, MMP-9 and MPO, using multiplex magnetic bead immunoassays. Quantitation was performed on a Luminex Magpix instrument with xPONENT 4.2 software and results were compared using one-way ANOVA. Periostin levels were confirmed by ELISA and checked in two additional patient groups: SSc-ILD and SSc-PAH, to assess for specificity. Paraffin embedded human heart tissue (National Disease Research Interchange, NDRI) from SSc patients and controls was stained for periostin (n=4 each) and co-stained for periostin and collagen type I (n=2 each) using immunofluorescence.

Results: Periostin, a matricellular protein recently shown to be a specific marker for cardiac myofibroblasts, was the only significantly increased circulating marker in SSc patients with isolated CMP compared with SSc patients without CMP. Additionally, no significant difference was found in the SSc-ILD and SSc-PAH groups when compared to SSc without internal organ involvement. Immunofluorescence staining of SSc hearts showed patchy periostin expression in all SSc patients, but not in controls. Furthermore, there was extensive periostin expression even in areas without collagen deposition, while all established fibrotic areas showed colocalization of collagen and periostin.

Conclusions: Periostin is elevated in SSc heart in vivo and circulating levels of periostin are increased in patients with SSc-CMP compared to SSc patients without heart involvement. These results suggest that periostin could be a potential marker of SSc-CMP.





Histologic Features Correlate with the Modified Rodnan Skin Score, Serum Inflammatory Markers, and Patient Reported Outcomes in Patients with Early, Diffuse Cutaneous Systemic Sclerosis

K. Showalter, C. Magro, D. Orange, Y. Zhang, P, Agius, J. Finik, R. Spiera, & J.K. Gordon.
Hospital for Special Surgery, Department of Medicine, Division of Rheumatology
535 E. 70th Street New York, N.Y. 10021, USA

Background: The association between systemic sclerosis (SSc) skin histology and clinical findings is not fully characterized. In two trials, we developed a scoring system to evaluate histologic change. The purpose of this study is to determine (1) reliability of histology scores and (2) if histologic features correlate with clinical findings.

Materials and methods: Skin biopsies were analyzed from Nilotinib and Belimumab trials. Our scoring approach assesses thickness, follicle count, infiltrate, collagen density, alpha-smooth muscle actin (aSMA) and CD34 staining, and global histologic severity. Blinded pathologists (CM, YZ) scored biopsies. Intraclass correlation coefficients (ICC) were calculated for reliability. Spearman correlations were used to correlate histology scores and clinical variables (local and total modified Rodnan skin score (MRSS), erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), 36-item Short Form Health Survey (SF-36), and physician global assessment (PGA)).

Results: 56 biopsies were analyzed from 26 SSc patients (median (IQR) disease duration 0.8 (0.54) years). Median (IQR) baseline MRSS was 25 (9). Reliability was excellent for follicle count and intra-rater CD34; good for thickness, inter-rater CD34, intra-rater collagen, and intra-rater global score; and moderate for infiltrate, intra-rater collagen, aSMA, and interrater global.

Histologic scores correlated moderately with MRSS, ESR, CRP, and SF-36 (Table 1). Post-treatment local MRSS correlated with thickness (r=0.674, p< 0.001), collagen density (r=0.497, p=0.04), global histologic severity (r=0.467, p=0.02), follicle count (r=-0.550, p=0.01), and CD34 staining (r=-0.653, p=0.001). Local MRSS did not correlate with infiltrate or PGA and weakly correlated with aSMA staining (r=0.337, p=0.01). In stratified analysis, collagen density correlated with local MRSS in patients with low but not high infiltrate score (r=0.578, p<0.001 vs. r=0.253, p=0.256). Total MRSS correlated with collagen density (r=0.425, p=0.001) and CD34 staining (r=-0.460, p< 0.001). Total MRSS and thickness correlated positively in patients with low infiltrate (r=0.326, p=0.05) but negatively in patients with high infiltrate (r=-0.456, p=0.03).

Conclusions: Our scores demonstrate moderate – excellent reliability. Histologic features correlate with MRSS, inflammatory markers, and patient reported outcomes. However, infiltrate does not correlate with MRSS and confounds the correlation of MRSS with collagen density and thickness. This supports further study of skin histology as an SSc outcome measure.

Table 1. Correlation between clinical variables and histologic features in diffuse systemic sclerosis

Clinical Feature	Significant* Spearman's Correlation Coefficients (r_s) for Histologic Features	p-value
Local modified Rodnan skin score	Collagen density (0.470)	<0.001
	CD34 staining intensity (-0.518)	<0.001
	Post-treatment thickness (0.674)	<0.001
	Post-treatment collagen density (0.497)	0.04
	Post-treatment global histologic severity (0.467)	0.02
	Post-treatment follicle count (-0.550)	0.01
	Post-treatment CD34 staining intensity (-0.653)	0.001
Total modified Rodnan skin score	Collagen density (0.425)	0.001
	CD34 staining intensity (-0.460)	<0.001
	Baseline infiltrate score (0.435)	0.03
	Post-treatment thickness (0.486)	0.02
	Post-treatment collagen density (0.425)	0.04
	Post-treatment CD34 staining intensity (-0.487)	0.02
C-reactive protein	Thickness (0.409)	0.01
	Post-treatment thickness (0.655)	0.002
	Post-treatment global histologic severity (0.475)	0.04
	Post-treatment CD34 staining intensity (-0.539)	0.02
Erythrocyte sedimentation rate	Follicle count (-0.430)	0.001
	Baseline follicle count (-0.410)	0.04
	Post-treatment thickness (0.526)	0.01
	Post-treatment CD34 staining intensity (-0.501)	0.01
36-Item Short Form Health Survey (SF-36)	Post-treatment global histologic severity (-0.567)	0.004

Legend: If no time point specified, reported data are from combined baseline and post-treatment samples.
*$p \leq 0.05$ and $r_s \geq 0.4$.

Defining the transcriptional profile of the skin in pediatric localized scleroderma (LS)

[1]Emily Mirizio, [2]Tracy Tabib, [3]Tao Sun, [1]Kaila Schollaert-Fitch, [3]Wei Chen, [2]Robert Lafyatis, [1]Kathryn S. Torok
[1]Pediatric Rheumatology, Univ of Pittsburgh Med Ctr, Pittsburgh, PA, USA
[2]Univ of Pittsburgh Med Ctr, Pittsburgh, PA, USA
[3]Research Computing Core at Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

Background: Scleroderma is an autoimmune disorder involving inflammatory driven fibrosis which encompasses systemic sclerosis (SSc) and localized scleroderma (LS). LS and SSc share histological characteristics, inflammatory, and fibrotic processes, but given divergent clinical phenotypes likely have unique pathways involving interaction between inflammation and fibrosis. Recent research indicates that T cells, macrophages, and related cytokines interact with fibroblasts to initiate an inflammatory phase followed by fibrosis. Identifying inflammatory cells expressing IFNγ-associated genes of interest and unique fibroblast populations will allow mechanistic studies of inflammatory-driven LS fibrosis, leading to more effective therapies.

Materials and Methods: Single cell RNA sequencing (scRNAseq) was performed on fresh and freshly frozen CryoStor® preserved skin (n=11 LS; 5 peds & 6 adult, n=10 healthy). Samples were collected under IRB #PRO11060222. Library preparation was done using a 10X Genomics® Chromium instrument and sequencing performed on Illumina NextSeq or HighSeq instruments. ScRNA-seq reads were examined for quality then transcripts were mapped to reference human genome GRCh38 and assigned to cells of origin using the Cell Ranger pipeline (10X Genomics®). R-language analyses using Seurat identified and visualized distinct cell populations by clustering methodologies.

Results: LS and healthy enzymatically digested skin cells clustered into 28 unique cell population groups. Inflammatory genes in LS populations were highly expressed in 3 main clusters: endothelial, lymphocyte/NK, and macrophage/DC. Sub-clustering showed high IFNγ signaling with CXCR3 and related chemokines (CXCL9 and CXCL10) being expressed from T cell and M1/M2 macrophages. Since chemokine expression from these inflammatory cells might stimulate fibroblasts and affect the degree of LS fibrosis, fibroblast populations were also studied. LS cells formed unique fibroblast clusters defined by COL1A1, SFRP2, and CXCL12 that expressed both inflammatory genes, like CXCL9 and 10, and fibrotic genes, like IGFBP5.

Conclusions: IFNγ associated gene transcripts including CXCR3 ligands (CXCL9 and CXCL10) are prevalent in macrophage, lymphocyte, and fibroblast populations in LS skin. The unique fibroblast subsets expressing these CXCR3 ligands, only found in LS skin, co-express reticular dermis fibroblast markers. Progression of inflammatory expression of these populations will be further investigated using advanced analysis techniques to determine the cellular trajectory and interaction of LS cells.

Assessment of the Repeatability and Convergent Validity with Dermal Collagen of High Frequency Ultrasound in Systemic Sclerosis

Victoria A. Flower[1,2], Shaney L. Barratt[3,4], Darren Hart[5], Amanda Mackenzie[2], Jacqueline Shipley[5], Stephen Ward[2], John D. Pauling[1,2]

1. Royal National Hospital for Rheumatic Diseases, Royal United Hospitals NHS Foundation Trusts, Department of Rheumatology, Bath BA1 1RL. United Kingdom.
2. University of Bath, Centre for Therapeutic Innovation & Department of Pharmacy and Pharmacology, Bath, BA2 7AY. United Kingdom.
3. North Bristol NHS Trust, Department of Respiratory Medicine, Bristol, BS10 5NB. United Kingdom.
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Background: There have been a number of recent negative clinical trials of SSc utilising the modified Rodnan Skin Score. High Frequency Ultrasound (HFUS) allows objective quantitative and qualitative assessment of dermal pathology and could be a useful surrogate measure of skin involvement in Systemic sclerosis (SSc). No previous studies have examined the convergent validity between HFUS features and dermal collagen in SSc. The repeatability of HFUS warrants further assessment before this method can be accepted in clinical practice. This study aims to assess the repeatability and validity of HFUS parameters with dermal collagen deposition in SSc.

Method: Fifty-three patients with SSc meeting ACR/EULAR 2013 criteria and 15 healthy controls (HC) underwent HFUS assessment of skin thickness (ST), echogenicity (as a reflection of cutaneous oedema) and Shear Wave Elastography (demonstrating skin stiffness, SWE) at the middle finger, hand, distal forearm and abdomen. Ten SSc patients and 10 HC underwent skin biopsies taken from the distal forearm. Dermal collagen was determined using Masson's Trichrome stain.

Results: Strong positive correlations were found between dermal collagen quantification and both ST (Spearman's rank correlation coefficient, $\rho = +0.697$, $p=0.025$) and SWE ($\rho = +0.709$, $p=0.022$) at the forearm in SSc, but not with echogenicity. Multiple linear regression analysis confirmed ST and SWE as significant predictors of localised skin collagen deposition in SSc ($R^2 = 0.876$). ST and SWE were highly reproducible across all 4 regions of interest for the combined cohort (SSc and HC combined) with Intra-class Correlation Coefficients (ICC) of 0.946-0.978 and 0.953-0.973 respectively. Echogenicity reproducibility was good, but weaker than ST and SWE (ICC 0.648-0.865).

Conclusions: We have demonstrated for the first time that ST and SWE on HFUS reflect collagen deposition in affected SSc skin. However, whilst low echogenicity is felt to reflect cutaneous oedema, increasing echogenicity does not accurately reflect fibrosis. HFUS parameters were highly reproducible although notably more so for ST and SWE than echogenicity. Our findings strongly support the use of ST and SWE in particular as a surrogate marker for skin fibrosis in clinical trials.

TNF-alpha Drives Progressive Obliterative Pulmonary Vascular Disease and Represents a Novel Model of Scleroderma Associated Pulmonary Arterial Hypertension (SSc-PAH)

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Background: Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary disease characterized by obliterative vasculopathy and vascular remodeling, right ventricular hypertrophy, and premature death. Systemic sclerosis associated PAH (SSc-PAH) occurs in 12-15% of SSc patients. We have recently shown that female TNF transgenic (TNF-Tg) mice die by 6-months from cardiopulmonary disease. Thus, we aimed to formally characterize this pathophysiology and assess its potential as a model of SSc-PAH.

Methods: Histologic analysis and immunofluorescent (IF) staining was performed on female TNF-Tg (3647 line) and wild type (WT) mice to characterize the pulmonary vascular and right ventricular pathology. Mice ($n \geq 4$) underwent: right heart catheterization, or barium-perfused micro-CT, or gas chromatography. Lungs/hearts from TNF-Tg/WT bone marrow chimeric mice, and anti-TNF vs. placebo treated TNF-Tg mice were assessed ($n \geq 3$). RNA sequencing was performed on lung tissue, and bioinformatics analysis was performed to compare TNF-Tg mouse lungs to human SSc-PAH lungs.

Results: TNF-Tg mice display progressive pulmonary vasculopathy beginning at 3 months manifested by vascular collagen deposition, enlarged pulmonary arteries, attenuation of distal arterioles, and vascular occlusion which closely resembles SSc-PAH histologically. By 4 months, TNF-Tg mice display right ventricular hypertrophy, with right ventricular systolic pressures of 83.7±10.3 vs. 25.7±0.4 mmHg. Barium perfused μCT analysis confirmed pruning of the vascular tree, and reduced gas exchange was seen in TNF-Tg mice. IF staining revealed increased aSMA staining, which corresponded to proliferation (Ki-67+), and loss of vWF+ vessels over time. There was an increase in $aSMA^{+}vWF^{+}$ cells, implicating endothelial-mesenchymal transition. Bone marrow chimera experiments revealed that mesenchymal cells, and not bone-marrow derived cells, are necessary to drive this process. Anti-TNF therapy halted the progression of SSc-PAH-like disease. Human SSc-PAH lungs displayed increased TNF-□ staining, and human microarray data demonstrate a prominent TNF–α signature that distinguishes PAH from control. Comparison of gene expression between TNF-Tg and SSc-PAH lungs showed significant differential gene expression overlap with enrichment in pathways including angiogenesis, Notch signaling, apoptosis, and VEGF signaling.

Conclusions: The TNF-Tg mouse represents a novel model of SSc-PAH, recapitulates most key features of the disease, and can serve as a valuable tool to test potential SSc-PAH therapeutics.

Molecular Analysis of a Skin Equivalent Tissue Culture Model of Systemic Sclerosis using RNA Sequencing, Epigenetic Assays, Histology, and Immunoassays

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Background: The molecular mechanisms of systemic sclerosis (SSc) have been difficult to study outside of patient samples. Mouse models lack key features of the disease, and fibroblast cultures show inconsistent results. We have developed an innovative skin-like tissue of SSc, self-assembled skin equivalents (sSE), where we study fibroblast behavior in a 3D microenvironment with epithelial-dermal crosstalk. Here we investigate SSc sSE tissues and show that they are molecularly like SSc skin biopsies.

Methods: Isolated fibroblasts from SSc patient skin (SScDF) and normal skin (NDF) are seeded into transwell chambers +/- monocytes. SSc patient plasma and healthy control (HC) plasma are added into the media during the polarization period. Keratinocytes are seeded at three weeks for epithelialization, and tissues are harvested after five weeks followed by IHC, RNA-sequencing, DNA methylation, ATAC-sequencing, and ELISA.

Results: We created 89 samples of sSE from one SScDF line and one NDF line. H&E staining of SScDF sSE showed increased dermal thickness compared to NDF. Differential expression of SScDF and NDF tissues with autologous plasma and monocytes showed increased expression in SScDF of: inflammatory/immune response, myeloid-mediated immunity/activation, leukocyte differentiation. The upregulated pathways in NDF sSE showed typical processes involved in epithelial proliferation and cell growth. Differential expression of SScDF sSE +/- monocytes found that tissues with monocytes had increased immune response, immune cell proliferation/activation, and macrophage migration. In samples without monocytes, collagen processes were upregulated, but the strong immune signal was missing. IL-6 and IL-13 increased in SScDF supernatant during macrophage polarization. Also, gene expression in SScDF sSE showed molecular similarity to human SSc patient skin biopsies and NDF clustered with HC skin biopsies. Lastly, 3D tissues and 2D monolayer fibroblast cultures have distinct gene expression, DNA methylation patterns, and chromatin accessibility.

Conclusions: There is a hierarchy of drivers in the creation of 3D tissues, with the fibroblast origin being the biggest modifier of disease morphology. The addition of monocytes is the next biggest factor in developing the immune response. These 3D tissues consistently replicate the molecular pathways found in SSc skin and allow for a controlled model of SSc to manipulate and test drug responses.

Metabolic Intermediate Alpha-Ketoglutarate Attenuates TGFβ-driven Profibrotic Responses of Dermal Fibroblasts

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Background: Metabolic perturbations are emerging as drivers of fibroblast activation in fibrosis. Our aim was to identify metabolic alterations in fibroblasts that could associate with fibrotic tissue process. Additionally, we studied how key metabolic intermediates, like dimethyl alpha-ketoglutarate (αKG) influence the profibrotic responses in dermal fibroblasts (DF).

Methods We analyzed publicly accessible transcriptomic data (GSE40839**)** from lung fibroblasts of healthy controls (HC) and patients with systemic sclerosis (SSc), using metabolic KEGG gene sets and STRING protein networks for gene set enrichment analysis (GSEA). Human DF from HC (n=3-7) and SSc patients (n=4-8) were treated with TGFβ and/or αKG (6 mM). Gene expression was analyzed by qPCR. Protein amounts were measured with Western blot. Apoptosis was assayed with flow cytometry (Annexin V assay). Contractile properties of DF were assessed by gel contraction assay. Significance ($p<0.05$) was determined by one sample t test or ANOVA with Tukey's correction.

Results Bioinformatic analysis of GSE40839 dataset showed enrichment of glycolysis pathway ($p = 0.0007$) and altered expression of genes from tricarboxylic acid (TCA) cycle (IDH2, ACLY) and oxidative phosphorylation (ATP6V0B, ATP5G1) in lung fibroblasts of SSc patients. In line with this, TGFβ-treated DF significantly upregulated mRNA expression of the core components of glycolysis (*GLUT1, PGK1, PGAM1, ENO, LDHA*) and the TCA cycle (*SUCLA, MDH)*. Additionally, the mRNA expression of *HIF1α*, an inducer of metabolic reprogramming, was enhanced (Fig. 1, $p=0.0001$) whereas the mRNA expression of *PGC1α*, the regulator of mitochondrial biogenesis and cellular energy metabolism, was decreased in TGFβ-stimulated DF (Fig. 1, $p<0.0001$). αKG reversed the TGFβ-driven upregulation of *HIF1α* (Fig. 1, $p=0.06$) and significantly repressed the TGFβ-driven profibrotic responses of DF, including the secretion of fibronectin ($p=0.047$) and the production of *αSMA* mRNA ($p=0.07$) and protein ($p=0.02$). αKG reduced the contractile capacity of TGFβ-stimulated DF ($p=0.003$), while had no effect on apoptosis.

Conclusion TGFβ perturbed the gene expression of key metabolic regulators in DF. Meanwhile, modulating cell metabolism with αKG, the key TCA cycle intermediate, attenuates the TGFβ-driven profibrotic responses of DF. This suggests an intimate crosstalk between metabolic and fibrotic pathways in skin. Targeting perturbed metabolism could offer novel anti-fibrotic strategies in SSc.

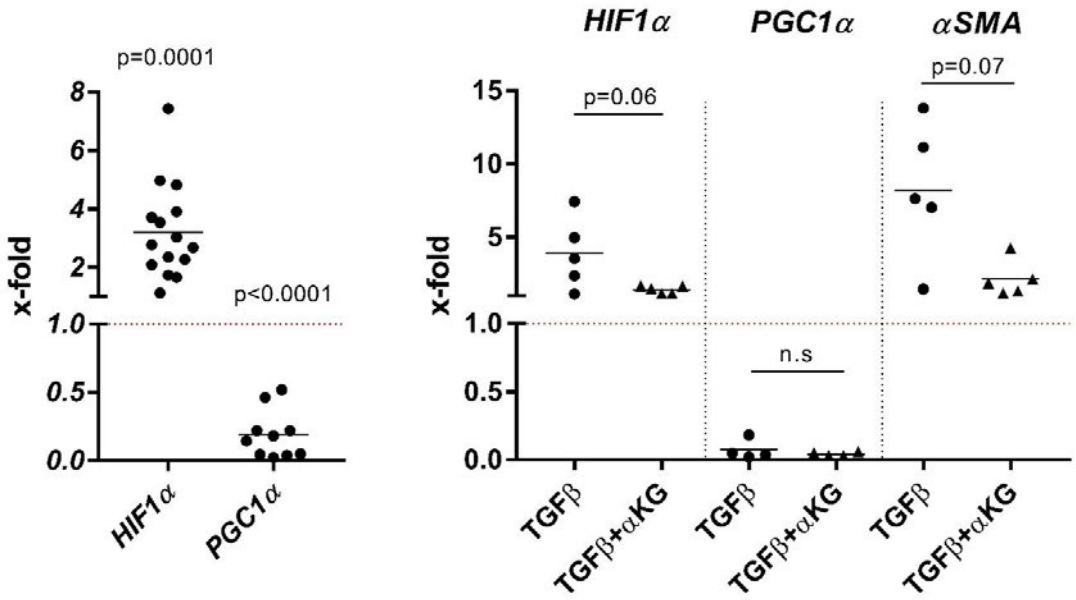

Figure 1. Gene expression analysis of *HIF1α*, *PGC1α* and *αSMA* in dermal fibroblasts stimulated with TGFβ in the presence or absence of alpha-ketoglutarate (αKG). The results are shown as x-fold change of normalized gene expression vs. untreated cells (set to 1, as denoted with red line).

BDCA2 Targeting of Human Plasmacytoid Dendritic Cells via CBS004 Reverts pDC-Dependent IFN Activation and Tissue Fibrosis in vitro and in vivo

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Background: Human plasmacytoid dendritic cells (pDCs) have been implicated in the pathogenesis of Scleroderma (SSc) through their ability to infiltrate the skin and secrete interferons (IFN). Blood dendritic cell antigen 2 (BDCA-2) is a pDC-type II C-type lectin that potently inhibits IFN secretion. Here we determined the effects of CBS004, a novel monoclonal antibody against BDCA-2, on IFN activation and tissue fibrosis in vitro and in vivo.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from SSc patients and healthy volunteers (HV) and IFNα secretion evaluated by ELISA. Full transcriptome of pDCs was analysed by RNA-sequencing. Xenotransplant models; NOD/SCID mice were injected in the tail vein with 25x10^{4}human pDCs, 12 h after topical application of Imiquimod for the inflammation model, and along bleomycin skin injections in the fibrotic model. CBS004 was delivered by intraperitoneal (IP) injection at 5 mg/kg. Harvested skin was analysed by FACS (pDC infiltration), by real-time PCR using a mouse type-I IFN response RT-qPCR array (Qiagen) and by histology (dermal thickness).

Results: PBMCs from SSc patients spontaneously produced higher levels (4.8x) of IFNα compared to HV. ODN stimulation of SSc PBMCs induced >30-fold increase in IFN-I secretion, which was completely abrogated by treatment with CBS004. RNA-seq analysis of human pDCs stimulated with ODN revealed 168 Differentially Expressed Genes mapping to IFN, JAK/STAT, IL-6, and NF-kB pathways. Pre-treatment with CBS004 induced an expression profile similar to non-stimulated pDCs. In the xenotransplant model, tail vein injection of pDC resulted in detection of human pDCs in the skin (0.3%) and >2-fold upregulation of 15 mouse type-I IFN response genes (e.g. Cxcl10, Ifit1-3, Mx1-2) compared to imiquimod treatment alone. Mice receiving IP injection of CBS004 had a 3-fold reduction in infiltrating pDCs (0.1%) and significantly repressed the skin type-I IFN response induced by pDC. In the bleomycin model, pDC injection significantly increased dermal thickness (30%), which was completely prevented by CBS004 treatment.

Conclusion: BDCA-2 targeting with CBS004 mAb blocks TLR-dependent pDC driven IFN activation in Scleroderma. Further, we show that BDCA2 targeting with CBS004 can revert human pDC driven tissue inflammation and fibrosis in two distinct human pDC xenotransplant mouse models.

Generation of human PDGFRα-transgenic mouse: a novel experimental model of skin fibrosis

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Background: Platelet Derived Growth Factor (PDGF) Receptor α (PDGFRα) is a target of the autoimmune response in scleroderma (SSc). Both total serum IgG (SSc-IgG) and anti-PDGFRα antibodies cloned from memory B cells of SSc patients (SSc-Mabs) [Moroncini G. et al., Arthritis & Rheumatology 2015] demonstrated the ability to increase collagen gene transcription in healthy donor skin fibroblasts and to induce fibrosis *ex vivo,* in skin grafts in SCID mice [Luchetti M. et al., Arthritis & Rheumatology 2016]. In order to replicate these findings *in vivo*, we generated human PDGFRα-transgenic mice.

Materials and methods: Full length human PDGFRα cDNA was knocked-in into the ubiquitously expressed Rosa26 locus on mouse chromosome 6. Correctly targeted C57BL/6 ES cell clones were selected for blastocyst microinjection, followed by chimera production. F2 heterozygous C57BL/6-hPDGFRα transgenic mice were used to establish the colony. Twelve weeks-old male mice were injected into the back skin at days 0, 3, 6 and 9, either with 0.02 mg/ml of SSc-Mabs (VH_{PAM}-V_κ16F4 or VH_{PAM}-V_κ13B8), or with 2 mg/ml of SSc-IgG or IgG purified from serum of healthy donors (HD-IgG). Vehicle only injection control was included. Age- and sex- matched C57BL/6 wild type mice were used as controls. Animals were sacrificed at day 14. Human PDGFRα transgene expression and collagen amount were assessed in explanted skin tissue.

Results: Transgenic mice were phenotypically normal, fertile, and did not display any apparent pathological features. Human PDGFRα mRNA and protein were detectable in the skin of all examined transgenic mice. Intradermal injection of stimulatory human SSc-Mab VH_{PAM}-V_κ16F4 or SSc-IgG resulted in dermal thickening and increased collagen deposition, whereas non-stimulatory human SSc-Mab VH_{PAM}-V_κ13B8 or HD-IgG did not induce any significant skin tissue alterations compared to vehicle control. C57BL/6 wild type mice did not show any significant skin tissue changes with any antibodies.

Conclusions: We generated a novel humanized mouse model of skin fibrosis based on the concomitant expression of human PDGFRα and injection of stimulatory anti-PDGFRα antibodies. This model may be useful to identify new therapeutic strategies for SSc and for their preclinical validation.

1. The Very Early DiagnOsis of Systemic Sclerosis (VEDOSS) project: predictors to develop definite disease from an international multicentre study

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Background: Early identification of patients is of key importance for the management and treatment of inflammatory rheumatic diseases.
The aim of the VEDOSS project is to determine through an at-risk population the predictive factors for the progression toward a definite systemic sclerosis (SSc).

Material and Methods: Patients with Raynaud phenomenon (RP), with or without anti-nuclear antibodies (ANA)were prospectively recruited. Fulfilling the 2013 classification criteria at baseline was an exclusion criterion. Patients with RP were recruited as controls. Patients had an annual assessment to determine organ involvement and severity. The endpoint was the fulfilment of the 2013 classification criteria. The time to fulfilling 2013 classification criteria was evaluated with Kaplan-Meier analysis, and predictors of evolution were determined by univariate and multivariate Cox regression.

Results: 735 patients with RP were recruited The sample is distributed as follows: i) *237* patients (143 with follow up) RP/ANA negative (ANA^-/pRP) as the control group, ii) *498 patients* (401 with follow up) RP/ANA positive (ANA^+/pRP)): 87 had puffy fingers (PF), 199 had anti-centromere antibodies (atb) positive, 45 had anti-topoisomerase-I atb positive and 182 had videocapillaroscopy (NVC) abnormalities at baseline. Out of 401 ANA^+/pRP patients, 7.4% within 1 year, 29.3% within 3 and 44.1% within 5 years satisfied the 2013 classification criteria. Out of the 143 ANA^-/pRP patients, none (0%) within 1 year, 4.6% within 3 years, and 4,6% within 5 years satisfied SSc criteria. After adjustment for age, the following baseline parameters were identified as independent predictors for progression into definite SSc: PF (OR=3.4 [2.0;5.6]), anti-centromere atb (OR=2.6 [1.6;4.1]) and anti-topoisomerase-1 atb (OR=3.1 [1.6;5.8]), and NVC abnormalities (OR=1.9 [1.3;2.9]) The presence of PF had a positive predictive value (PPV) of 79% and combination of PF+specific auto-antibodies showed 94% PPV to satisfy ACR/EULAR2013 criteria within 5 years (figure 1).

Conclusions: patients with very early SSc develop definite, classification criteria fulfilling SSc within 5 years of follow up. The VEDOSS study identified PF and SSc auto-antibodies at first visit as independent predictors of development of definite SSc. These data are of key importance for the risk stratification of patients with very early SSc in clinical practice and clinical studies.

1.Avouac J, et al. Ann Rheum Dis 2011;70:476

Figure 1: A matrix was built to show the PPV of various variables alone or in combination to predict subsequent progression of VEDOSS patients toward definite SSc after 5 years

	alone	Disease specic atb	scleroderma patten on NVC	puffy fingers
	PPV%	PPV%	PPV%	PPV%
Disease specific atb	70.2		82.2	94.1
scleroderma patten on NVC	75.0	82.2		77.8
puffy fingers	78.9	94.1	77.8	

2. CXCR4 antagonist AMD3100 enhances the beneficial effect of MSC injection on bleomycin-induced organ fibrosis

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Background: Mesenchymal stem cell (MSC) injection has shown promise in treating scleroderma (SSc). MSCs from mice or humans with fibrotic disease exhibit enhanced myofibroblast differentiation and inhibited adipocyte differentiation. Because the aberrant differentiation of MSCs can be suppressed by chemokine receptor antagonists, we reasoned that the CXCR4 antagonist AMD3100 might enhance the beneficial effects of MSC injection.

Methods: MSCs were derived from adipose tissue from bleomycin-treated mice. MSC treatment and AMD3100 treatment were combined either by pretreating MSCs with AMD3100 prior to injection or by injecting mice both with MSCs and AMD3100. Beneficial effects were read out in terms of lung/skin histology, and immunohistochemistry (IHC) for FABP4 (adipocyte marker), HSP47 (fibrosis marker), α-smooth muscle actin (ASMA) in the tissue.

Results: Bleomycin treatment thickens the dermis and thins the intradermal adipose layer. MSC treatment suppresses these effects. These beneficial effects on the skin are enhanced slightly by pretreatment of the MSCs with AMD3100 or by combined MSC and AMD3100 treatment in vivo; however, the effect of combined treatment on the adipose layer may be due simply to the effect of AMD3100 alone. Similarly, while MSC treatment has a marked beneficial effect on FABP4 and HSP47 IHC, these effects are not further enhanced by either method of AMD3100 delivery.

The pathological effects of bleomycin on lung morphology and expression of fibrosis markers are also suppressed by MSC injection. Pretreatment of the MSCs with AMD3100 provides no additional improvement of Ashcroft score, although it does further suppress the pathological effect of bleomycin on fibrosis marker expression. Combined treatment in vivo with MSCs and AMD3100 provides a markedly enhanced beneficial effect on fibrosis compared to either treatment alone.

Conclusions: Pretreating MSCs with AMD3100 increases some of their beneficial effects on organ fibrosis. However, the most striking beneficial effects on skin and particularly lung fibrosis are observed in mice injected with both MSCs and AMD3100 in vivo.

3. After 24 months observation period patient reported outcomes improved significantly in the juvenile scleroderma inceptions cohort

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Background: Juvenile systemic sclerosis (jSSc) is an orphan disease. Currently there is nearly no data published about the course of jSSc patients. We report our date from Juvenile Scleroderma Inception cohort (JSSIC) with a follow up of 24 months.

Materials and methods: The JSSIC is a prospective multicenter registry of patients with jSSc, who fulfill the adult classification criteria, and presented the first non-Raynaud symptom before 16 years of age and were younger then 18 years at the time of inclusion. Patients, who were followed at least 24 months in the JSSIC, were evaluated.

Results: 52 patients were included. 77 % of them were female and 77% had diffuse subtype. Mean disease duration at time of inclusion was 3.2 years. Mean age of at Raynaud's onset was 8.8 years and the first non-Raynaud's symptom 9.4 years. 85% received DMARDs at the time of inclusion and 96 % after 24 months. 88% of the patients were ANA positive, 35% anti-Scl70 positive and 3% anticentromere positive. The mean modified skin score decreased from 14.3 to 12.6. The frequency of Raynaud´s stayed around 87%. The frequency of the nailfold capillary changes increased from 56% to 63%, but the frequency of active ulcerations stayed stable around 21%. The number of patients with FVC <80 % decreased from 39 % to 34% (p=0.734). The number of patients with pulmonary hypertension assessed by ultrasound increased from 4% to 8% (p=0.652). No patient developed systemic hypertension or renal crisis. Gastrointestinal involvement decreased from 33% to 29% (p=0.829). Number of joints with decreased range increased from 46% to 63% (p=0.076). Total muscle weakness decreased from 8 % to 3% (p=0.237) and elevated CK from 22% to 9% (p=0.033) too.
Several PROs improved significantly like Patient global disease activity (VAS 0-100) (p=0.002), patient global disease damage (VAS 0-100) (p=0.02) and patient Raynaud activity VAS 0-100) (p=0.009) as physician global disease activity (VAS 0-100) (p=0.021) and physician global disease damage (P=0.01).

Conclusions: Over the 24 months observation period patient and physician related outcomes improved significantly. Regarding organ involvement there was an increase in patients of pulmonary hypertension and joint contractures.
Supported by the "Joachim Herz Stiftung"

4. Is there a Difference in Presentation of female and male Patients with juvenile systemic Scleroderma?

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Background: Juvenile systemic scleroderma (jSSc) is an orphan disease with a prevalence of 3 in 1 000 000 children. There are limited data published regarding the differences in clinical presentation of male and female patients with jSSc. The Juvenile Systemic Scleroderma Inception Cohort (JSSIC) is a multinational cohort with a prospective standardized assessment of the patients. The data regarding the difference in clinical characteristics at time of inclusion in the cohort are presented.

Materials and methods: The JSSIC is a prospective multicentre registry of patients with jSSc, who fulfil the adult classification criteria, and presented the first non-Raynaud symptoms before 16 years old and were younger than 18 years old at the time of inclusion in the cohort. Patient characteristics at time of inclusion in the cohort were evaluated.

Results: As of 15th of December 2018 120 patients are included in JSSIC. The great majority are female (80%). There were more female patients with CK elevation (29% vs 22%) and more female patients with Gottron papulae (25% vs 12%). The mean modified skin score was higher in males (18.6 vs 13.9).
Sclerodactyly was more frequent in males (90% vs 76%). Active ulceration was present in 33% of males compared to 14% of females (p=0.026). FVC<80% occurred more often in males with 47% compared with 24% in females (p=0.018). Pulmonary hypertension was more common in females with 7% compared to 4% in males. Urine sediment changes were more common in males (8% vs 4%). Gastrointestinal involvement was more common in females (37% vs 29%).
Contractures occurred more often in males with 62% compared with 46% in females. Tendon friction rub was observed in 21% of males and 3% of females (p=0.001). Physician global scores of disease activity and damage were higher in males with 48 for both assessments compared to 36 and 30 in females.

Conclusions: Male patients with jSSc have a higher severity of disease, as it has been reported in adults.
Supported by the "Joachim Herz Stiftung"

5. Update from the juvenile scleroderma Inception Cohort

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Background: Juvenile systemic scleroderma (jSSc) is an orphan disease. There are limited data regarding the clinical presentation of jSSc. The Juvenile Systemic Scleroderma Inception Cohort (JSSIC) is a multinational registry that prospectively collects information about jSSc patients.

Materials and methods: Patients were included in the JSSIC if they fulfilled the adult classification criteria, if they presented the first non-Raynaud symptom before 16 years old and if they were younger than 18 years of age at the time of inclusion. Patients' characteristics at time of inclusion were evaluated.

Results: Currently, the cohort includes 120 patients, being 89% Caucasian and 80% female. The majority had diffuse subtype (74%) and 18% had overlap features. The mean age of onset of Raynaud phenomenon was 9.7 years in the diffuse subtype (djSSc) and 10.7 years in the limited subtype (ljSSc). The mean age of non-Raynaud's symptoms was 10.0 years in the djSSc and 11.4 years in the ljSSc (p=0.041). Mean disease duration at time of inclusion was 3.4 years in the djSSc and 2.4 years in the ljSSc group. ANA positivity was 88% in both groups. Mean Modified Rodnan skin score was 17.5 in the djSSc and 7.3 in the ljSSc (p=0.002). Significant differences were found between the groups regarding Gottron papulae (djSSc 29% vs ljSSc 6%),)(p=0.011), physician global disease activity (VAS 0-100) (djSSc 41vs ljSSc 30) (p=0.020), physician global disease damage (VAS 0-100) (djSSc 37 vs ljSSc 18) (p=0.001) history of ulceration (djSSc 57% vs ljSSc 30%, respectively) (p=0.004). Pulmonary hypertension occurred around 7% in both groups. No systemic hypertension or renal crisis was reported. No significant difference between the groups was found regarding distribution of anti-Scl70, anticentromere antibodies, FVC <80%, gastrointestinal involvement, tendon friction rub and patient judgment of disease activity and damage

Conclusions: In this large cohort of jSSc patients there were surprisingly not many significant differences between djSSc and ljSSc. According to the physician global scores the djSSc patients have a significantly more severe disease.

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6. Characterization of the anti-centromere antibody response in systemic sclerosis patients suggests a broad and active B cell response that is associated with progression towards systemic sclerosis.

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Background: Although some studies suggest a possible association between clinical characteristics and isotypes of anticentromere antibodies (ACA) in patients with systemic sclerosis (SSc), characteristics of ACA have not been described thoroughly in SSc and little is known about the underlying auto-reactive B cell responses.

Methods: ACA IgG, IgA and IgM levels were measured in serum samples of 167 ACA IgG+ SSc patients. Patients were divided in a SSc (fulfilling ACR 2013 criteria, n=132) and a very early SSc group (fulfilling VEDOSS criteria, n=35). Differences in isotype expression and levels between the groups were evaluated. Additionally, PBMCs from ACA IgG+ SSc patients (and ATA IgG+ SSc and healthy donors (HD) as control) were cultured either in the presence of CD40L expressing fibroblasts, IL-21 and BAFF or without stimulation. Levels of ACA IgG, IgA and IgM were measured after one week of culture using ELISA.

Results: ACA IgG+ SSc patients displayed a broad isotype usage with 75% being ACA IgA+ and 68% being ACA IgM+ in serum. Patients within the SSc group showed higher ACA IgG levels and a higher percentage of ACA IgM positivity compared to the VEDOSS patients. ACA IgG, IgA and IgM could be measured in ACA SSc PBMC culture medium following stimulation, but not in ATA SSc and HD, indicating the presence of circulating ACA B cells of all three isotypes. In cultures that yielded sufficient Ig production, ACA IgG was detectable in 7/7 ACA SSc patients, ACA IgA in 3/5 and ACA IgM in 2/5. Furthermore, ACA IgG production was also detected in the absence of stimulation in 5/9 patients, suggesting the presence of ACA-producing plasmablasts in the circulation.

Conclusions: The higher ACA IgG levels and presence of ACA IgM in SSc patients compared to VEDOSS patients indicates that the ACA response in SSc is more pronounced showing signs of ongoing activity. Additionally, ACA IgG production by unstimulated PBMCs points towards continuous differentiation of memory cells into antibody secreting cells. These data provide insight into the ACA B cell response and its potential involvement in disease-relevant pathogenetic processes.

7. Stem Cell Transplant in Autoimmune Diseases: Development of Effective Targeted Conditioning through Antibody Drug Conjugates (ADC)

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Background: Autologous hematopoietic stem cell transplant (autoHSCT) is a highly effective treatment for multiple autoimmune diseases. AutoHSCT can induce long-term remission with 70-80% progression-free survival in patients with relapsed/refractory and secondary progressive multiple sclerosis. Likewise, patients with systemic scleroderma achieved 74% event-free survival at 72 months following autoHSCT, which was superior to cyclophosphamide treatment alone (Sullivan, 2018).

Current regimens for patient preparation, or conditioning, prior to autoHSCT are non-selective and toxic, which limit the use of this curative procedure due to morbidities and mortality. To address this, we developed an ADC targeting CD45, which is expressed on the surface of cells in the blood and immune system, to selectively deplete recipient cells safely without the use of chemotherapy or radiation.

Results: The CD45-ADC was engineered to deliver a payload to deplete target cells and to have a fast half-life, allowing the ADC to clear prior to transplant. Humanized mice treated with the CD45-ADC had >95% depletion of stem and immune cells following a single dose. We treated primates with the cross-reactive CD45-ADC to evaluate cell ablation in immune-competent animals. Dose-dependent decreases in target cells were observed with >95% depletion of HSC and >85% depletion of T cells after a single dose. Depletion was target- and payload- dependent, as the unconjugated antibody and a non-targeted-ADC had no effect. The ADC was well-tolerated at effective doses and was rapidly cleared, providing appropriate pharmacokinetics for transplant.

To evaluate this strategy in models of autoimmune disease, we created an anti-mouse CD45-ADC that achieved full myeloablation with a single dose in mice. Following CD45-ADC administration, transplantation of stem cells enabled full donor chimerism. We are testing this CD45-ADC in the murine experimental autoimmune encephalomyelitis (EAE) and sclerodermatous Graft-vs-Host-Disease (scGVHD) models. In EAE, conditioning with radiation followed by congenic transplant resulted in transient amelioration of disease, and further studies are in progress that utilize the targeted CD45-ADC approach.

Conclusions: Targeted depletion of cells with a single dose of CD45-ADC is an effective and well-tolerated approach for conditioning prior to autoHSCT in preclinical models of autoimmune disease. This may reduce conditioning-related morbidity and mortality and thereby increase the number of autoimmune patients eligible for transplant. This targeted approach is significantly differentiated from current chemotherapy and radiation regimens.

8. Secretion of TGFβ1 by fibroblasts and macrophages is executed by secretory autophagy

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Background: TGFβ1 is a pleiotropic cytokine with cell type-specific effects modulating growth, survival and differentiation. It potently induces tissue fibrosis and plays key roles in inflammation and tumorigenesis.
TGFβ1 is synthesized as small latent complex (SLC), consisting of a LAP (latency-associated peptide) prodomain and the mature growth factor. SLC is tethered to latent TGFβ binding protein (LTBP) to form the large latent complex (LLC). Only this complex is efficiently released to the extracellular space where it is sequestered in the extracellular matrix (ECM), from which the growth factor is liberated in order to reach its receptors and initiate signaling. ECM deposition by fibroblasts crucially depends on autocrine TGFβ1 signaling. While signaling from different TGFβ receptors and extracellular activation of TGFβ1 are well understood, information on its intracellular trafficking and secretion is sparse but has important biological and clinical implications. Here, we identified regulated secretion that is coupled to autophagy as the molecular pathway for TGFβ1 release from human and murine fibroblasts and macrophages.

Results & Methods: We found LAP-TGFβ1 to co-localize with GRASP55, involved in selecting cargo for trafficking in specialized Golgi-derived vesicles (IF). These were also positive for the autophagosomal marker LC3B (WB), and moreover, TEM analysis detected LAP-TGFβ1 in autophagosomes. Of note, abrogating autophagosome formation by ablating or silencing (KO or siRNA) ATG5, ATG7 or Beclin-1 or chemical inhibition (3-methyladenine) of autophagy effectively blocked TGFβ1 secretion (ELISA) in murine and human fibroblasts and macrophages, underscoring the crucial importance of autophagosome formation for TGFβ1 secretion. TGFβ1 containing 'secretory autophagosomes' are transported to the plasma membrane in a mechanism depending on RAB8A. It is unclear at present how TGFβ1 is released from its carriers to bind to ECM structures, and whether the other TGFβ isoforms or members of the large family of TGFβ proteins are also released by secretory autophagy.

Conclusion. The regulated secretion of this potent cytokine through the unconventional autophagy-dependent mechanism adds another level to control TGFβ1 bioavailability and may be targeted by specific inhibitors.

9. Serum biomarkers in SSc-ILD: **association with presence, severity and prognosis**

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Background: Interstitial lung disease (ILD) is the main cause of death in systemic sclerosis (SSc). The progression of SSc associated ILD (SSc-ILD) is highly variable, and markers predictive of severe or progressive ILD are needed to identify patients at risk.

Materials and Methods: Serum levels of 15 biomarkers were measured by Luminex assay and ELISA, as appropriate, in 189 SSc patients. Genotyping of rs2015085 in *CCL18* in was carried out using a TaqMan assay in 174 patients. Statistical analysis was performed using STATA12.

Results: CCL18 and MMP-7 levels were significantly higher in patients with ILD (median: 61,886 pg/ml and 1,385 pg/ml, respectively) compared to patients without ILD (48,486 pg/ml, p=0.0049 and 1,155 pg/ml, p=0.046, respectively), and periostin levels were significantly lower in patients with ILD than without (84,620 pg/ml compared to 105,096 pg/ml, p=0.027). Serum levels of CCL18 (p=0.038), MMP-7 (p=0.0069), CXCL12 (p=0.016), and MMP-12 (p=0.049) were all significantly higher in patients with extensive, rather than limited, lung involvement according to the Goh et al staging (Goh et al. Am.J.Respir.Crit.Care.Med. 2008 177:1248-1254), while periostin levels were significantly lower in extensive compared to limited lung disease (p=0.025). Higher concentrations of CCL18 (p=0.001) and IL-10 (p=0.018) were associated with mortality. The CCL18 association remained significant following adjustment for disease severity. Neopterin was associated with time to decline in DLCO ≥15% (p=0.042).

Conclusions: Our results suggest that CCL18, MMP-7, CXCL12, MMP-12, periostin, and neopterin may be effective biomarkers for predicting severity and/or progression of lung involvement in SSc.

10. Verification of genetic associations with Scleroderma associated Interstitial Lung Disease

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Background: Although genetic associations with scleroderma (SSc) as a whole are clearly established, very little is known on genetic susceptibility to SSc-associated interstitial lung disease (SSc-ILD) specifically. A number of common gene variants have been associated with SSc-ILD, but most have not been replicated in separate populations.

Materials and Methods: We genotyped 4 SNPs in *IRF5,* and one in each of *STAT4*, *CD226,* and *IRAK1,* in 633 Caucasian patients with SSc, of whom 379 had ILD. The control population (n=503) comprised individuals of European descent from the 1000 Genomes project. Statistical analysis was performed using Unphased v 3.1 and STATA12.

Results: Three of the *IRF5* SNPs and the *STAT4* rs7574865 were significantly associated with SSc compared to controls: rs2004640 (p=0.0013), rs4728142 (p=0.019), rs10488631 (p=0.0025) and *STAT4* rs7574865 (p=0.00013). Two SNPs in *IRF5* showed a significant difference between patients with SSc-ILD and controls; rs2004640 (p=0.01), and rs10488631 (p=0.028). Three SNPs in *IRF5* showed a significant difference between controls and patients without ILD, rs4728142 (p=0.036), rs10488631 (p=0.0023), and rs2004640 (p=0.0042), as did *STAT4* rs7574865 ($p=4.2x10^{-7}$). A significant difference between SSc with and without ILD was only observed for *STAT4* rs7574865, which was less frequent in patients with ILD (MAF 0.27 compared to 0.36, p=0.00093). An association between time to decline in FVC by ≥10% was seen for *IRF5* rs10488631 (p=0.007), and for *CD226* rs763361 (p=0.029).

Conclusions: In conclusion, of the seven tested SNPs, *STAT4* rs7574865 was protective against ILD. *IRF5* and *CD226* variants may be associated with progressive SSc-ILD and will need to be further tested.

11. APPA: A novel pharmaceutical in systemic sclerosis treatment

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Background: Systemic sclerosis (SSc) is an autoimmune idiopathic connective tissue disease that results in fibrosis of the skin and lungs. The disease is driven by activation of fibroblasts to myofibroblasts resulting in secretion of excessive extracellular matrix. APPA is a novel pharmaceutical compound comprised of Apocynin and Paenol and are plant metabolites that have been used in traditional Chinese medicine for centuries. This compound has been used in animal models of osteoarthritis where it has anti-inflammatory effects. Its effects in systemic sclerosis are unknown. The aim of this work was to examine the anti-inflammatory and fibrotic effects of APPA in SSc dermal fibroblasts.

Materials and Methods: Normal dermal fibroblasts were treated with the oxidant hydrogen peroxide alone or with APPA and levels of the pro-inflammatory cytokine Interleukin-6 were measured by a standard commercial ELISA. Dermal fibroblasts were treated with the pro-fibrotic TGF-β1 molecule 10ng/ml with or without 240μM of APPA and after 24 hours incubation the levels of collagen1, Nrf-2, Sirtuin3 and alpha tubulin as a load control by western blotting were measured. SSc dermal fibroblasts were measured for Nrf-2 levels by qPCR and also the nrf-2 target gene HO-1.

Results: In SSc dermal fibroblasts the levels of the antioxidant nrf-2 and its target gene were statistically significantly reduced compared to healthy controls cells. In fibroblasts derived from healthy donors treated with hydrogen peroxide a significant increase in IL-6 was observed that was reduced by over 50% by APPA. Treatment with TGF-β1 led to increased collagen that was reduced by the incubation of APPA that was also associated with an increase in nrf-2 content and the mitochondrial HDAC Sirtuin3.

Conclusions: APPA can reduce collagen in dermal fibroblasts that is associated with increased activation of nrf-2 and could involve the mitochondrial protein Sirt3. APPA may be a therapeutic in SSc for which currently no treatment exists.

12. Monocytes/macrophages may contribute to the pathogenic process of systemic sclerosis via downregulation of Interferon regulatory factor 8

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Background: Recent important observations suggest that monocytes/macrophages play important roles in the pathogenic process of systemic sclerosis (SSc). Interferon regulatory factor 8 (IRF8), a member of IRF family, is a transcriptional regulator that plays essential roles in the differentiation and function of monocytes and macrophages. We hypothesized that IRF8 may be involved in the fibrotic process of SSc by regulating phenotypes of monocytes/macrophages.

Materials and Methods: IRF8 levels in circulating monocytes from 33 of SSc patients (diffuse cutaneous SSc (dcSSc); n=13, limited cutaneous SSc (lcSSc); n=20) and 15 of healthy controls were first determined by quantitative RT-PCR. IRF8 was next silenced in circulating monocytes by RNA interference, and they were cultivated and differentiated into macrophages. Cell surface markers, cytokine/chemokine profiles, and expression levels of extracellular matrix (ECM) were assessed by flow cytometry, quantitative RT-PCR, and bead-based immunoassay. Finally, skin fibrosis was assessed in myeloid cell-specific IRF8 conditional knockout mice (Cre(LysM)IRF8(flox/flox) mice).

Results: IRF8 levels in circulating monocytes from dcSSc patients were significantly lower than that from healthy controls and from lcSSc patients. Its level was negatively correlated with modified Rodnan total skin thickness score. Cultivated macrophages derived from IRF8-silenced monocytes exhibit M2 phenotype. Furthermore, mRNA expression levels of pro-fibrotic cytokines and ECMs were significantly upregulated in these macrophages than that from control-derived macrophages, and the same trend was observed in protein level. Finally, skin fibrosis was not observed naturally in this mice but mRNA levels of ECM tended to be naturally upregulated. Bleomycin-induced skin fibrosis was significantly aggravated in Cre(LysM)IRF8(flox/flox) mice compared to that in control mice.

Conclusion: In conclusion, IRF8 was significantly downregulated in circulating monocytes from dcSSc patients and its pro-fibrotic phenotype was observed *in vitro* and *in vivo* experiments. Altered regulation of IRF8 in monocytes/macrophages may be involved in the fibrotic process of SSc.

13. Validation of the Swedish version of PROMIS-29v2 and FACIT-DYSPNOE index in patients with systemic sclerosis

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Background: Patient reported outcomes measures gain increasing interest to study disease activity and quality of life. Our study aimed to evaluate the internal-consistency, reliability and construct validity of the Swedish versions of FACIT-Dyspnea and PROMIS-29 instruments in patients with systemic sclerosis (SSc).

Materials and Methods: Forty-nine consecutive SSc patients with available clinical and laboratory data answered paper-based translated questionaires of FACIT-Dyspnea and PROMIS-29 and were compared to RAND 36, MRC Dyspnea score, Scleroderma Health Assessment Questionnaire (SHAQ) and medical data. The SHAQ score was obtained by pooling the HAQ Disability index and five SSc specific VAS scales.

Results: The study group (86% female, 73% lcSSc) had a mean (SD) disease duration of 11 (9.3) years, HAQ-DI of 0.5 (0.6) and modified Medsger Severity Score of 5.4 (2.2). PROMIS-29 showed strong and moderate spearman's correlations to equal RAND-36 domains ($|r| = 0.67$ *to* $– 0.82$, $p < 0.001$). PROMIS-29 domains physical functioning, pain interference and ability to participate in social roles and activities were strongly correlated to SHAQ score ($|r| = -0.71$ *to* -0.75, $p < 0.001$). FACIT-Dyspnea and Functional limitation had strong correlations to SHAQ Score and RAND-36 domain physical functioning ($|r| = 0.75$ *to* -0.85, $p < 0.001$). Floor effects were present in FACIT Dyspnea and FACIT Functional limitations and in three PROMIS-29 domains. Ceiling effects were detected in two PROMIS-29 domains. Test re-test reliability was acceptable for both instruments but revealed some weaknesses in 2 PROMIS-29 domains.

Conclusions: The Swedish version of FACIT-Dyspnea and PROMIS-29 largely meets the requirements for reliability and construct validity towards legacy measures in patients with SSc.

14. Assessment of the Repeatability and Convergent Validity with Dermal Collagen of High Frequency Ultrasound in Systemic Sclerosis

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Background: There have been a number of recent negative clinical trials of SSc utilising the modified Rodnan Skin Score. High Frequency Ultrasound (HFUS) allows objective quantitative and qualitative assessment of dermal pathology and could be a useful surrogate measure of skin involvement in Systemic sclerosis (SSc). No previous studies have examined the convergent validity between HFUS features and dermal collagen in SSc. The repeatability of HFUS warrants further assessment before this method can be accepted in clinical practice. This study aims to assess the repeatability and validity of HFUS parameters with dermal collagen deposition in SSc.

Method: Fifty-three patients with SSc meeting ACR/EULAR 2013 criteria and 15 healthy controls (HC) underwent HFUS assessment of skin thickness (ST), echogenicity (as a reflection of cutaneous oedema) and Shear Wave Elastography (demonstrating skin stiffness, SWE) at the middle finger, hand, distal forearm and abdomen. Ten SSc patients and 10 HC underwent skin biopsies taken from the distal forearm. Dermal collagen was determined using Masson's Trichrome stain.

Results: Strong positive correlations were found between dermal collagen quantification and both ST (Spearman's rank correlation coefficient, $\rho = +0.697$, $p=0.025$) and SWE ($\rho = +0.709$, $p=0.022$) at the forearm in SSc, but not with echogenicity. Multiple linear regression analysis confirmed ST and SWE as significant predictors of localised skin collagen deposition in SSc ($R^2 = 0.876$). ST and SWE were highly reproducible across all 4 regions of interest for the combined cohort (SSc and HC combined) with Intra-class Correlation Coefficients (ICC) of 0.946-0.978 and 0.953-0.973 respectively. Echogenicity reproducibility was good, but weaker than ST and SWE (ICC 0.648-0.865).

Conclusions: We have demonstrated for the first time that ST and SWE on HFUS reflect collagen deposition in affected SSc skin. However, whilst low echogenicity is felt to reflect cutaneous oedema, increasing echogenicity does not accurately reflect fibrosis. HFUS parameters were highly reproducible although notably more so for ST and SWE than echogenicity. Our findings strongly support the use of ST and SWE in particular as a surrogate marker for skin fibrosis in clinical trials.

15. Anti-angiogenic VEGF-A_{165}b is Associated with Systemic Sclerosis Peripheral Vasculopathy

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Background/Aims: The anti-angiogenic isoform of Vascular Endothelial Growth Factor-A (VEGF-A_{165}b) has been implicated in Systemic sclerosis (SSc) vasculopathy. High frequency ultrasound (HFUS) is a novel approach to assessing digital perfusion. We report on the relationship between plasma VEGF-A_{165}b and peripheral microvascular perfusion using HFUS in SSc.

Methods: Fifty-one patients fulfilling 2013 ACR/EULAR criteria for SSc and fifteen healthy controls (HC) underwent HFUS Doppler assessment of microvascular flow at the distal middle finger, from which a Vascularity Index was calculated. Plasma VEGF-A_{165}b levels were assessed using ELISA. Ongoing administration of vasodilator and disease modifying therapies were permitted.

Results: Plasma VEGF-A_{165}b was detectable in 16/51 (31%) of SSc with a peak level of >4000pg/mL. In contrast, only 3/15 (20%) healthy controls had plasma VEGF-A_{165}b greater than the lower limit of detection by ELISA, with a maximum plasma level of 46pg/mL. Median levels were not significantly different between groups. When VEGF-A_{165}b was detectable, it was associated with significantly reduced Vascularity Index in SSc compared to both SSc with undetectable VEGF-A_{165}b ($p=0.038$) and HC ($p<0.005$). In contrast, HC showed no difference in the Vascularity Index irrespective of VEGF-A_{165}b. Additionally, the Vascularity Index correlated with VEGF-A_{165}b in SSc (Spearman's $\rho = -0.289$, $p=0.039$) but not HC. The Vascularity Index was reduced in SSc even in those with undetectable VEGF-A_{165}b compared to HC (both with detectable ($p=0.047$) and undetectable (non-significant) VEGF-A_{165}b).

Conclusions: Increased levels of VEGF-A_{165}b are associated with reduced digital vascularity in SSc. Low levels of VEGF-A_{165}b are sometimes detected in HC but are not associated with reduced digital perfusion. Peripheral vascular compromise in SSc is evident even in the absence of detectable VEGF-A_{165}b. Further longitudinal studies are needed to investigate the role of VEGF-A_{165}b in determining microvascular flow and the impact of disease duration and intervention on VEGF-A_{165}b and digital perfusion over time.

16. Molecular Analysis of a Skin Equivalent Tissue Culture Model of Systemic Sclerosis using RNA Sequencing, Epigenetic Assays, Histology, and Immunoassays

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Background: The molecular mechanisms of systemic sclerosis (SSc) have been difficult to study outside of patient samples. Mouse models lack key features of the disease, and fibroblast cultures show inconsistent results. We have developed an innovative skin-like tissue of SSc, self-assembled skin equivalents (sSE), where we study fibroblast behavior in a 3D microenvironment with epithelial-dermal crosstalk. Here we investigate SSc sSE tissues and show that they are molecularly like SSc skin biopsies.

Methods: Isolated fibroblasts from SSc patient skin (SScDF) and normal skin (NDF) are seeded into transwell chambers +/- monocytes. SSc patient plasma and healthy control (HC) plasma are added into the media during the polarization period. Keratinocytes are seeded at three weeks for epithelialization, and tissues are harvested after five weeks followed by IHC, RNA-sequencing, DNA methylation, ATAC-sequencing, and ELISA.

Results: We created 89 samples of sSE from one SScDF line and one NDF line. H&E staining of SScDF sSE showed increased dermal thickness compared to NDF. Differential expression of SScDF and NDF tissues with autologous plasma and monocytes showed increased expression in SScDF of: inflammatory/immune response, myeloid-mediated immunity/activation, leukocyte differentiation. The upregulated pathways in NDF sSE showed typical processes involved in epithelial proliferation and cell growth. Differential expression of SScDF sSE +/- monocytes found that tissues with monocytes had increased immune response, immune cell proliferation/activation, and macrophage migration. In samples without monocytes, collagen processes were upregulated, but the strong immune signal was missing. IL-6 and IL-13 increased in SScDF supernatant during macrophage polarization. Also, gene expression in SScDF sSE showed molecular similarity to human SSc patient skin biopsies and NDF clustered with HC skin biopsies. Lastly, 3D tissues and 2D monolayer fibroblast cultures have distinct gene expression, DNA methylation patterns, and chromatin accessibility.

Conclusions: There is a hierarchy of drivers in the creation of 3D tissues, with the fibroblast origin being the biggest modifier of disease morphology. The addition of monocytes is the next biggest factor in developing the immune response. These 3D tissues consistently replicate the molecular pathways found in SSc skin and allow for a controlled model of SSc to manipulate and test drug responses.

17. TNF-alpha Drives Progressive Obliterative Pulmonary Vascular Disease and Represents a Novel Model of Scleroderma Associated Pulmonary Arterial Hypertension (SSc-PAH)

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Background: Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary disease characterized by obliterative vasculopathy and vascular remodeling, right ventricular hypertrophy, and premature death. Systemic sclerosis associated PAH (SSc-PAH) occurs in 12-15% of SSc patients. We have recently shown that female TNF transgenic (TNF-Tg) mice die by 6-months from cardiopulmonary disease**.** Thus, we aimed to formally characterize this pathophysiology and assess its potential as a model of SSc-PAH.

Methods: Histologic analysis and immunofluorescent (IF) staining was performed on female TNF-Tg (3647 line) and wild type (WT) mice to characterize the pulmonary vascular and right ventricular pathology. Mice ($n \geq 4$) underwent: right heart catheterization, or barium-perfused micro-CT, or gas chromatography. Lungs/hearts from TNF-Tg/WT bone marrow chimeric mice, and anti-TNF vs. placebo treated TNF-Tg mice were assessed ($n \geq 3$). RNA sequencing was performed on lung tissue, and bioinformatics analysis was performed to compare TNF-Tg mouse lungs to human SSc-PAH lungs.

Results: TNF-Tg mice display progressive pulmonary vasculopathy beginning at 3 months manifested by vascular collagen deposition, enlarged pulmonary arteries, attenuation of distal arterioles, and vascular occlusion which closely resembles SSc-PAH histologically. By 4 months, TNF-Tg mice display right ventricular hypertrophy, with right ventricular systolic pressures of 83.7±10.3 vs. 25.7±0.4 mmHg. Barium perfused μCT analysis confirmed pruning of the vascular tree, and reduced gas exchange was seen in TNF-Tg mice. IF staining revealed increased aSMA staining, which corresponded to proliferation (Ki-67+), and loss of vWF+ vessels over time. There was an increase in $aSMA^{+}vWF^{+}$ cells, implicating endothelial-mesenchymal transition. Bone marrow chimera experiments revealed that mesenchymal cells, and not bone-marrow derived cells, are necessary to drive this process. Anti-TNF therapy halted the progression of SSc-PAH-like disease. Human SSc-PAH lungs displayed increased TNF-□ staining, and human microarray data demonstrate a prominent TNF-α signature that distinguishes PAH from control. Comparison of gene expression between TNF-Tg and SSc-PAH lungs showed significant differential gene expression overlap with enrichment in pathways including angiogenesis, Notch signaling, apoptosis, and VEGF signaling.

Conclusions: The TNF-Tg mouse represents a novel model of SSc-PAH, recapitulates most key features of the disease, and can serve as a valuable tool to test potential SSc-PAH therapeutics.

18. Systemic sclerosis microenvironment provides a protective niche for tissue-resident B cells during B cell depletion therapy with anti-CD20 antibody

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Background: B cell depletion therapy with anti-CD20 antibody rituximab has emerged as a potential treatment for systemic sclerosis (SSc). Rituximab, which has been used to treat B cell malignancies and some autoimmune diseases, efficiently depletes circulating B cells. Tissue-resident B cells, on the other hand, have been shown to be more difficult to deplete, posing a therapeutic challenge. Resistance of tissue-resident B cells to rituximab has been associated with the inflammatory microenvironment, where B cell survival is promoted by various cytokines and chemokines. Among these survival factors, the following two have received much attention: CXCL12, a chemokine essential for the B cell niche in bone marrow, and B cell activating factor (BAFF), a cytokine promoting the survival of autoreactive B cells. In this study, we investigated the role of microenvironment on tissue-resident B cells during B cell depletion therapy in SSc.

Materials and methods: Bleomycin (BLM)-induced SSc model mice were treated with anti-CD20 antibody. AMD 3100, an antagonist of the CXCL12 receptor CXCR4, was used to block CXCL12. Anti-BAFF antibody was used to block BAFF. B cell depletion was assessed by flow cytometry in bone marrow, spleen, lymph nodes, lungs, and peripheral blood. Skin and lung fibrosis was evaluated histopathologically. CXCL12 and BAFF expression was quantified by qRT-PCR and immunofluorescence staining.

Results: While anti-CD20 antibody efficiently depleted circulating B cells and attenuated skin and lung fibrosis, a fraction of $CD20^+$ B cells persisted in spleen, lymph nodes, and lungs of BLM-induced SSc model mice, contrasting with efficient depletion of tissue-resident B cells in control mice. Residual B cells showed increased expression of CXCR4. CXCL12 and BAFF expression was increased in skin and lungs of BLM-induced SSc model mice. Co-administration of anti-CD20 antibody with AMD3100 or anti-BAFF antibody enhanced the depletion of tissue-resident B cells. Moreover, these combination therapies achieved greater attenuation of fibrosis in BLM-induced SSc model mice compared with anti-CD20 monotherapy.

Conclusions: SSc microenvironment provides a protective niche for tissue-resident B cells against anti-CD20 antibody. B cell survival factors like CXCL12 and BAFF are potential therapeutic targets to enhance the efficacy of B cell depletion therapy in SSc.

19. The DNA Methylation Landscape of Fibrotic and Systemic Sclerosis/SSc-Like Macrophage Cell Populations

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Background: Macrophages are myeloid-derived innate immune cells with multiple activation states. Classically-activated/M1 macrophages initiate immune responses and inflammation, while alternatively-activated/M2 macrophages mediate immune resolution, ECM deposition, and fibrosis, and play a key role in systemic sclerosis (SSc) pathogenesis. DNA methylation is used to infer cell-type proportions in a sample, since different cell types harbor distinct methylation profiles. DNA methylation patterns for macrophage subtypes, including SSc-like/fibrotic macrophages, have not been characterized to enable deconvolution of macrophage populations in SSc tissue samples.

Methods: Isolated monocytes from healthy, Caucasian females between the ages of 40 and 60y, matching SSc-demographics, were differentiated *in vitro* for one week in: M-CSF (M2), GM-CSF (M1), IL-6/TGF-β1/IL-6+TGF-β1 (SSc-like/fibrotic), IFNγ+LPS (M1-like), GM-CSF+IL4 (DC), untreated (naïve). Flow cytometry was performed on a subset of differentiated macrophages. DNA was extracted, bisulfite converted, and hybridized to the Illumina MethylationEPIC array (n=48 samples). Data was processed and normalized using Noob. RNA-/ATAC-sequencing was used for follow-up studies.

Results: SSc-like/fibrotic differentiated macrophages expressed surface markers characteristic of M2 polarization (high CD163, high CD206, low CD80). After data processing, 742,824 probes were used to identify the top differentially methylated CpG sites between the different macrophage states. Cell-type deconvolution using DNA methylation data confirmed the high proportion of monocyte-lineage cells, and the low proportion of other cell types, as expected. Pair-wise analyses were performed between each differentiation state. The top 100 CpG sites per cell subtype comparison (1,500 total) were hierarchically-clustered, and SSc-like/fibrotic macrophages were more similar to M2 macrophages. M1 macrophages and DCs formed a unique cluster distinct from other cell subtypes. Hypomethylation changes specific to SSc-like/fibrotic macrophages relative to M1s and M2s included: *RUNX3, ITGAM, ICAM3, CD38, IGF2R*. *FOXP1* transcription factor, which is required for monocyte differentiation, was significantly hypomethylated in SSc-like/fibrotic and M2s compared to M1s. *FOXP1* expression in SSc skin was significantly increased compared to healthy control skin and had increased chromatin accessibility in SSc samples compared to healthy controls surrounding *FOXP1*.

Conclusions: We have identified a methylation library of 1,500 CpGs for multiple macrophage subtypes. SSc-like/fibrotic macrophages were more similar in methylation patterns to alternatively-activated/M2 macrophages, which was validated using flow cytometry.

20. Identification of Distinct Pro-fibrotic Monocyte and Macrophage Subsets in Systemic Sclerosis

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Background: Chronic inflammation may modulate the balance of classical, intermediate and non-classical monocyte (NCM) subsets (defined by CD14/CD16 expression). Monocytes are heterogeneous and subsets can be further delineated by examining expression of other cellular markers such as CD163, a haemoglobin-scavenging receptor associated with resolving inflammation and fibrosis. Systemic sclerosis (SSc) is a prototype for studying immune regulation of fibrosis. We aim to characterise monocyte/macrophage subsets and function in SSc.

Materials and methods: Blood samples from healthy (HC) and SSc donors were collected for serum, cell isolation. Leukocytes were stained with CD14, CD16 and CD163 antibodies for FACS. Monocytes isolated by negative selection and Ficoll separation of blood were cultured for 7-days into macrophages. Soluble CD163 (sCD163) levels were measured by ELISA. Macrophage supernatants were applied to scratch-wounded healthy skin fibroblast monolayers and fibroblast-populated collagen gels, to assess their effect on fibroblast migration and contraction, respectively. Mann-Whitney U tests, 2-tailed unpaired t-tests, Spearman's Rank and linear regression were used for statistical analysis.

Results: In our SSc cohort we found more circulating $CD163^{+}CD14^{lo}CD16^{hi}$ NCMs (n=10, $6.02\pm0.8x10^{3}$cells/mL), than in HCs (n=9, $2.69\pm1.0x10^{3}$cells/mL), p=0.026. Accordingly, we observed higher levels of sCD163 in SSc (n=42, 682ng/mL) than in HC sera (n=32, 587ng/mL), Mann-Whitney U=413, p=0.010. Higher levels of CD163/total protein were detected in supernatants of SSc macrophages (n=27, $9.96x10^{-3}$) compared to those of HCs (n=13, $7.76x10^{-3}$), Mann-Whitney U=77, p=0.005. There were no associations between sera or supernatant sCD163 concentrations and clinical parameters (e.g. disease duration, mRSS and pulmonary fibrosis).

SSc macrophage media-treated fibroblasts migrated faster (n=2, rate-of-closure 2.9% wound-area/hour, R^2=0.90) into the wound than HC media-treated fibroblasts (n=2, rate-of-closure 1.7% wound-area/hour, R^2=0.82), p=0.017. SSc macrophage supernatant promoted fibroblast contraction as indicated by the lower weight of fibroblast-populated collagen gels cultured in SSc macrophage supernatant (n=5, 110.7±8.6mg) compared to those in HC supernatant (n=2, 153.6±6.8mg), p=0.035.

Conclusions: Our data suggests that the increased frequency of systemic $CD163^{+}$ NCMs corresponds with upregulation of sCD163 in SSc. Furthermore, the paracrine macrophage-fibroblast crosstalk supports a pro-fibrotic micro-environment. Thus, modulating both the systemic and local effects of non-classical monocytes mediated by CD163 may have therapeutic relevance in targeting SSc fibrosis.

21. Pathogenic effects of immune complexes containing systemic sclerosis specific autoantibodies antibodies in endothelial cells

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Background: In systemic sclerosis (SSc), autoantibodies provide the most reliable tool to predict disease subset and the pattern of organ involvement. Consistently with their diagnostic and prognostic role, immune complexes (ICs) containing scleroderma-specific autoantibodies were shown to elicit pro-inflammatory and pro-fibrotic effects in healthy skin fibroblasts, thus contributing to SSc aetiopathogenesis. The objective of this study was to investigate the pathogenicity of SSc-ICs in endothelial cells (ECs).

Materials and methods: Human umbilical vein ECs (HUVECs) were isolated from umbilical vein, fibroblasts were isolated from skin biopsies obtained from healthy controls (NHS); cells were cultured in adequate conditions. ICs were purified from sera of SSc patients bearing different autoantibody specificities (antibodies against centromeres, DNA topoisomerase I, RNA polymerase and Th/To), patients with systemic lupus erythematosus (SLE), primary anti-phospholipid syndrome (PAPS) or NHS using polyethylen glycol precipitation. Cell cultures were incubated with pathologic and control ICs and TLR3 [Poly(I:C)] and TLR4 (LPS) agonists. Several parameters were assessed: mRNA levels of endothelin-1 (ET-1) and type I interferons (IFN-α/IFN-β) were investigated by real-Time PCR; ICAM-1 expression was evaluated by cell-ELISA and the secretion of IL-6, IL-8, tumour growth factor (TGF)-β1 and Pro-CollagenIα1 in culture supernatants was measured by commercial ELISA kits. The involvement of intracellular signalling pathways culminating with the activation of p38MAPK was assessed. Furthermore, skin fibroblasts from NHS were stimulated with supernatants from HUVEC incubated with scleroderma and control ICs. The mRNA levels of collagen (col)Iα1 and matrix metalloproteinases (MMP)-1 and the secretion of TGF-□1 were evaluated in fibroblasts. Statistical analysis was performed comparing ICs from SSc and disease controls to NHS-ICs.

Results: Stimulation of HUVECs with scleroderma and control ICs resulted in the modulation of study mediators, as detailed in **Table 1**. When skin fibroblasts from NHS were stimulated with supernatants from HUVECs incubated with scleroderma and control ICs, we observed a significant modulation of mediators (**Table 2**).

Conclusions: These data provide the first demonstration of the pathogenicity of ICs from scleroderma patients with different autoantibody specificities in ECs. According to our preliminary data, the activation of ECs induced by SSc-ICs might ultimately lead to a pro-fibrotic phenotype in healthy skin fibroblasts.

Table 1. Modulation of study mediators in HUVECs

	ICAM-1	IL-6	IL-8	TGF-β1	ColIIIα1	ET-1	IFN-α	IFN-β	P38MAPK
Poly(I:C)	***	***	***	NA	NS	NA	NS	NS	***
LPS	***	***	***	NA	NS	NA	NS	NS	***
ATA-IC	NS	*	***	*	NS	**	NS	NS	***
ACA-IC	**	*	***	*	NS	NS	NS	NS	***
ARA-IC	NS	**	***	*	NS	NS	NS	NS	***
anti-Th/T0-IC	***	**	NS	*	NS	**	NS	NS	NS
PAPS-IC	**	NS	NS	NA	NS	***	NS	NS	NS
SLE-IC	NS	NS	**	NA	NS	NS	NS	NS	**
NHS-IC	NS	NS	NS	NS	NS	NS	NS	NS	NS

LPS: lipopolysaccharide; ATA: antibodies against DNA topoisomerase I; ACA: antibodies against centromeric proteins; ARA: antibodies against RNA polymerase III; anti-Th/T0: antibodies against Th/T0; SLE: systemic lupus erythematosus; PAPS: primary anti-phospholipid syndrome; NHS: normal healthy subjects; IL: interleukin; TGF: tumour growth factor; col: collagen; ET: endothelin; IFN: interferon.
*= p<0.05; **= p<0.001, ***=p<0.0001; NS: not significant; NA: not available.

Table 2. Modulation of study mediators in fibroblasts stimulated with supernatants from HUVECs treated with SSc-ICs and NHS-ICs

	TGF-β1	ColIIα1	MMP-1
TGF-β	NA	***	NS
ATA-IC	*	**	NS
ACA-IC	***	**	*
ARA-IC	NS	NS	*
anti-Th/T0-IC	***	***	*
NHS-IC	NS	NS	NS

TGF: tumour growth factor; ATA: antibodies against DNA topoisomerase I; ACA: antibodies against centromeric proteins; ARA: antibodies against RNA polymerase III; anti-Th/T0: antibodies against Th/T0; NHS: normal healthy subjects; col: collagen; MMP: matrix metalloproteinase.

*= $p<0.05$; **= $p<0.001$, ***=$p<0.0001$; NS: not significant;

22. CCL24 -A novel target playing a significant role in Systemic Sclerosis (SSc)

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Background: CCL24 (C-C chemokine ligand 24) is a chemokine that promotes pro-inflammatory and pro-fibrotic activities through its chemokine receptor, CCR3. Previous studies showed that both CCL24 and CCR3 are involved in lung and skin inflammation and fibrosis.
Aim: to assess the expression of CCL24 in SSc and to evaluate the possible pathogenic implications of the CCL24/CCR3 axis in these patients.

Materials and methods: CCL24 serum levels in healthy volunteers, diffuse and limited SSc was measured by a commercial Elisa kit. Affected skin forearm biopsies from early diffuse cutaneous SSc patients were co-stained for CCL24/CD31 and CCR3/αSMA. A bleomycin (BLM)-induced dermal fibrosis murine model was used to compare SSc related phenotypes between WT and CCL24 KO mice.

Results: in all 37 SSc patients serum levels of CCL24 were significantly increased (1072±146 and 816±94 in diffuse and limited SSc, respectively) in comparison to controls (262±32, $p<0.0001$ U-test). In diffuse SSc skin, immunostaining showed a significant upregulation of CCL24 (8-fold increase of cells, $p<0.001$) and CCR3 (6-fold increase of intensity, $p<0.001$) when compared to control skin. In the skin, CCL24-positive mononuclear cells, cells were captured during extravasation through vessel wall and migration into the dermis and the perivascular area. A significant CCR3 expression was also detected in SSc dermal spindle-shaped fibroblasts, microvascular endothelial cells and epidermal keratinocytes. Double immunostaining with α-smooth muscle actin (α-SMA) confirmed the strong expression of CCR3 in profibrotic myofibroblasts.
In the dermal BLM fibrotic model, CCL24 KO mice showed a significantly reduced response in tested disease parameters, compared to WT mice. In the KO population, the dermal thickness reduced significantly as well as the infiltration of immune cells into the BAL fluid were attenuated. This was further supported by a significant reduction of α-SMA expression in the skin lesions of CCL24 KO mice compared to WT mice (no elevation and 4-fold elevation, respectively).

Conclusion: in SSc, CCL24 circulating levels are increased and are upregulated in involved skin. Moreover, CCL24 seems crucial for disease development in the murine model of dermal fibrosis. These observations suggest that CCL24 may have a role in SSc pathophysiology and be a potential therapeutic target.

Our data were recently accepted for publication, reference:
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23. Long non-coding RNA HOTAIR induces myofibroblast activation in Systemic Sclerosis through EZH2 dependent de-repression of NOTCH signalling pathway activation.

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Background/purpose: Fibroblasts explanted from affected tissues in Systemic Sclerosis (SSc), conserve their pro-fibrotic phenotype characterised by increased secretion of collagens and other extracellular matrix proteins and comprise a large proportion of □-Smooth Muscle Actin (□-SMA) positive myofibroblasts. Long non-coding RNAs (lncRNAs) within the HOX loci have been described as master epigenetic regulators within the connective tissue. Specifically, HOTAIR has been shown to drive the specific phenotype of the hands and feet which are also the first body regions affected by SSc. Here we aimed to unravel the mechanisms responsible for the epigenetically stable activation of SSc fibroblasts.

Materials and Methods: Full thickness skin biopsies were surgically obtained from the forearms of twelve adult patients with SSc of recent onset. Fibroblasts were isolated and cultured in monolayers and protein and RNA extracted from the fibroblast cultures. HOTAIR was expressed in healthy dermal fibroblasts by lentiviral induction employing a vector containing the specific sequence. Gamma secretase inhibitors RO4929097 and DAPT were employed to block Notch signalling in SSc fibroblasts. EZH2 was blocked in SSc fibroblasts with the specific inhibitor GSK126.

Results: HOTAIR expression was increased in SSc patients' skin (100 fold) and in SSc explanted fibroblasts (5 fold). These results were highly significant ($p<0.001$ for both). In vitro, we demonstrated that lentiviral induced stable overexpression of HOTAIR in healthy dermal fibroblasts led to their profibrotic activation. We further showed that HOTAIR-induced profibrotic activation was due to EZH2 dependent spread of H3k27me3 methylation marker, as demonstrated by complete inhibition by treatment with the GSK126. HOTAIR and EZH2 cooperate to enhance Notch1 expression which is important for the profibrotic phenotype. Consistent with these findings, treatment of the HOTAIR expressing cells with two different types of gamma secretase inhibitors known to block NOTCH activation, suppressed collagen production by 50% and normalised a-SMA expression to control levels.

Conclusion: Here we show that the epigenetically stable activation of SSc dermal fibroblasts is due to HOTAIR led EZH2 dependent de-repression of NOTCH signalling. The results of these studies identify a new venue to modulate fibroblasts biology which could inform clinical research to resolve chronic fibrosis and re-establish tissue homeostasis in SSc.

24. Targeting the Alternatively Active M2 Macrophages in Fibrotic Lesions Prevents and Even Reverses Established Lesions in Animal Models

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Background: We have been studying peptides which target alternatively activated profibrotic M2 macrophages and repolarizes them to a M1 non-fibrotic phenotype. Detailed studies show that these peptides target a unique domain in CD206, a surface receptor 50 fold more abundant on the M2 macrophages, and induces signaling via toll receptors which causes them to change their phenotype as also occurs in bacterial infections.

Method: These peptides were studied for any beneficial effect against macrophages from patients suffering scleroderma. Also, they were assessed for efficacy administered during mouse models of bleomycin induced skin and lung fibrosis.

Results: It was found that about half of the patients have an active signature in peripheral blood macrophages. In the cultures with active macrophages, the peptides significantly reduce the active signature and also inhibit pro-fibrotic cross talk with scleroderma fibroblasts. Interestingly, in quiescent macrophage cultures there was little or no effect. When administered to animals treated with bleomycin they prevent skin and lung fibrosis. Peptides are effective delivered either systemically or in the case of lung also by aerosol. A significant reduction in lung fibrosis was observed in treating established fibrosis. Detailed analyses show a reduction in TGFβ and smooth muscle actin as well as CD206 positive cells in the lung. These peptides bind to albumin and have a half-life of some 90 minutes delivered SC. However, since they repolarize the macrophages secreting the factors driving the lesions, their activity is more prolonged. No toxicity is observed even at much higher doses.

Conclusion: Many studies have shown the abundance of M2 macrophages in various fibrotic disorders including IPF and scleroderma. Targeting the M2 macrophages producing factors which promote fibrosis could have a significant clinical benefit.

25. BDCA2 Targeting of Human Plasmacytoid Dendritic Cells via CBS004 Reverts pDC-Dependent IFN Activation and Tissue Fibrosis in vitro and in vivo

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Background: Human plasmacytoid dendritic cells (pDCs) have been implicated in the pathogenesis of Scleroderma (SSc) through their ability to infiltrate the skin and secrete interferons (IFN). Blood dendritic cell antigen 2 (BDCA-2) is a pDC-type II C-type lectin that potently inhibits IFN secretion. Here we determined the effects of CBS004, a novel monoclonal antibody against BDCA-2, on IFN activation and tissue fibrosis in vitro and in vivo.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from SSc patients and healthy volunteers (HV) and IFNα secretion evaluated by ELISA. Full transcriptome of pDCs was analysed by RNA-sequencing. Xenotransplant models; NOD/SCID mice were injected in the tail vein with 25x10^{4}human pDCs, 12 h after topical application of Imiquimod for the inflammation model, and along bleomycin skin injections in the fibrotic model. CBS004 was delivered by intraperitoneal (IP) injection at 5 mg/kg. Harvested skin was analysed by FACS (pDC infiltration), by real-time PCR using a mouse type-I IFN response RT-qPCR array (Qiagen) and by histology (dermal thickness).

Results: PBMCs from SSc patients spontaneously produced higher levels (4.8x) of IFNα compared to HV. ODN stimulation of SSc PBMCs induced >30-fold increase in IFN-I secretion, which was completely abrogated by treatment with CBS004. RNA-seq analysis of human pDCs stimulated with ODN revealed 168 Differentially Expressed Genes mapping to IFN, JAK/STAT, IL-6, and NF-kB pathways. Pre-treatment with CBS004 induced an expression profile similar to non-stimulated pDCs. In the xenotransplant model, tail vein injection of pDC resulted in detection of human pDCs in the skin (0.3%) and >2-fold upregulation of 15 mouse type-I IFN response genes (e.g. Cxcl10, Ifit1-3, Mx1-2) compared to imiquimod treatment alone. Mice receiving IP injection of CBS004 had a 3-fold reduction in infiltrating pDCs (0.1%) and significantly repressed the skin type-I IFN response induced by pDC. In the bleomycin model, pDC injection significantly increased dermal thickness (30%), which was completely prevented by CBS004 treatment.

Conclusion: BDCA-2 targeting with CBS004 mAb blocks TLR-dependent pDC driven IFN activation in Scleroderma. Further, we show that BDCA2 targeting with CBS004 can revert human pDC driven tissue inflammation and fibrosis in two distinct human pDC xenotransplant mouse models.

26. Ultrasound Detection of Calcinosis and Correlation with Ulnar Artery Occlusion in Patients with Systemic Sclerosis

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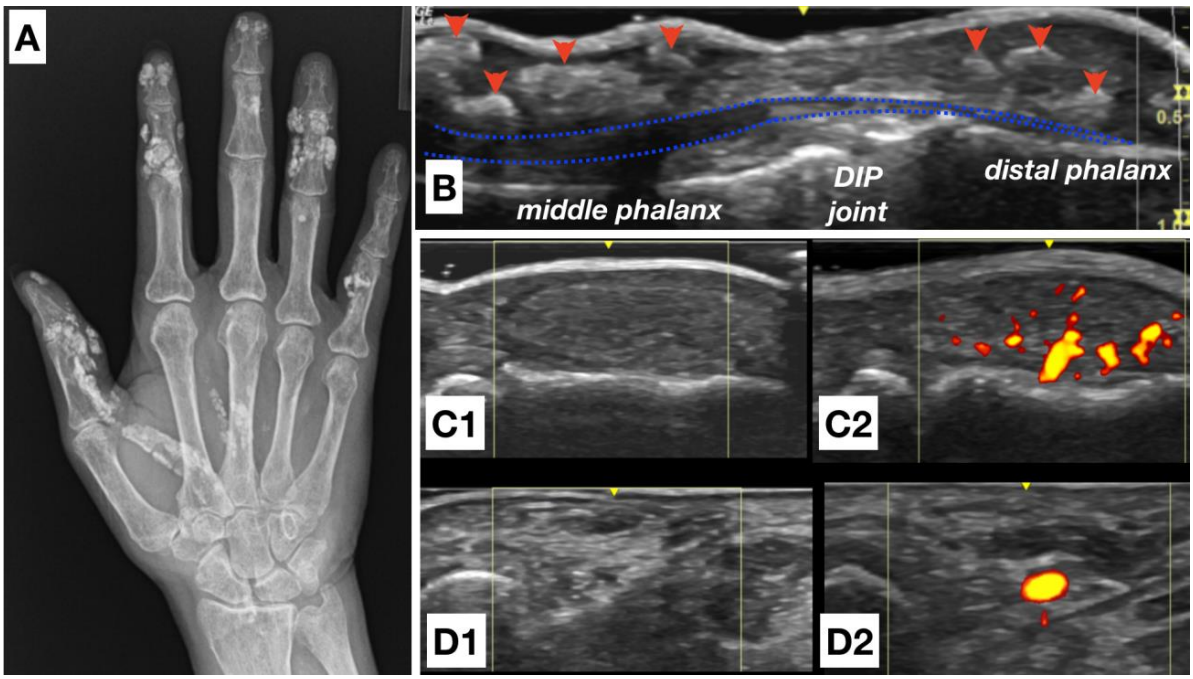
Background: Calcinosis cutis in systemic sclerosis (SSc) is characterized by calcium deposition in skin and subcutaneous tissues. While calcinosis pathophysiology is poorly understood, vascular ischemia may play a role. Ultrasound (US) is well-suited to vascular and soft tissue interrogation. We evaluated the ability of US to detect calcinosis in the hands/wrists of SSc patients versus X-ray (XR) identified calcinosis, and evaluated the relationship between US markers of pathologic perfusion and presence of calcinosis.

Materials and methods: SSc patients meeting 2013 ACR/EULAR classification criteria were prospectively evaluated for calcinosis in the hands/wrists by XR and US. US was performed by submerging hands/wrists in a water bath. Presence of calcinosis was recorded for 44 zones based on published methods, and sensitivity and specificity for calcinosis detection by US versus XR was determined. Bilateral US vascular measurements of ulnar artery occlusion (UAO) and finger pulp blood flow (FPBF) in the 3rd-4th digits were obtained. For each hand, associations between markers of pathologic blood flow (UAO, FPBF, and a composite severity score of UAO and FPBF) and calcinosis were assessed using generalized estimating equations.

Results: Of 43 SSc patients (19 diffuse, 24 limited), 17 (39.5%) had XR evidence of calcinosis (10 bilateral, 7 unilateral). US identified 13 patients with calcinosis (9 bilateral, 4 unilateral). Of 748 zones evaluated, XR identified 72 (9.6%) with calcinosis while US showed 76 (10.2%). Sensitivity and specificity for US vs. XR-identified calcinosis was 61% and 95% by zone, 78% and 98% by hand, and 76% and 100% by patient, respectively. UAO appeared in 13/43 (30%) and 12/43 (28%) of left and right hands, and FPBF was absent in ≥1 digit of the left and right hands in 21 (49%) and 19 (44%), respectively. UAO was strongly associated with XR-identified calcinosis (OR 8.08, 95% CI 2.45-26.60, p< 0.001), whereas FPBF and the composite severity score were not.

Conclusions: Ultrasound detection and quantification of calcinosis was sensitive and highly specific versus X-ray. UAO, a marker of decreased peripheral perfusion, was associated with XR-identified calcinosis, providing further support for the role of vascular disease in the pathogenesis of calcinosis in SSc.

Figure 1. Ultrasound and X-ray findings in systemic sclerosis



Systemic sclerosis patients with (A) X-ray findings of calcinosis, (B) ultrasound identified calcinosis [red arrowheads] in the longitudinal palmar aspect of the 2nd finger [blue outline = flexor tendon], (C1-C2) absent and normal FPBF respectively, and (D1-D2) UAO and no UAO, respectively. UAO = ulnar artery occlusion, FPBF = finger pulp blood flow, DIP = distal interphalangeal

Table 1. Sensitivity and specificity of ultrasonography for X-ray identified calcinosis

	Sensitivity	Specificity	TP	FP	TN	FN
By Zone	61%	95%	44	32	644	28
By Hand	78%	98%	21	1	58	6
By Patient	76%	100%	13	0	26	4

TP = true positive, FP = false positive, TN = true negative, FN = false negative

Table 2. US and X-ray features and associations between US markers of pathologic blood flow and calcinosis

	Imaging Features	
	Left Hand	**Right Hand**
	n =43	**n =43**
UAO, n (%)	13 (30)	12 (28)
FPBF absent*, n (%)	21 (49)	19 (44)
Calcinosis by US, n (%)	11 (26)	11 (26)
Calcinosis by X-ray, n (%)	15 (35)	12 (28)
	Associations with Calcinosis	
	OR (95% CI)	***p***
UAO	8.08 (2.45, 26.60)	0.0006
FPBF	2.66 (0.83, 8.52)	0.0994
FPBF + UAO severity score	3.23 (0.96, 10.92)	0.0593

US = ultrasound, OR = odds ratio by generalized estimating equations. UAO = ulnar artery occlusion, FPBF = finger pulp blood flow. *FPBF absent if one or both measurements per hand were absent.

27. Safety and Efficacy of Lenabasum at 21 Months in an Open-Label Extension of a Phase 2 Study in Diffuse Cutaneous Systemic Sclerosis (dcSSc) Subjects

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Background: Lenabasum is a non-immunosuppressive, selective CB2 agonist that limits inflammation and fibrosis in animal models of SSc. Lenabasum was safety and well-tolerated and improved efficacy outcomes in dcSSc subjects in the double-blinded, randomized, placebo-controlled Part A of Phase 2 trial JBT101-SSc-001 (NCT02465437).

Materials and methods: Subjects who completed Part A were eligible to receive lenabasum 20 mg BID in an open-label extension (OLE) that assessed safety and efficacy at 4 weeks, then every 8 weeks.

Results: Thirty-six/38 (95%) eligible subjects enrolled in the OLE and as of March 2019, 29/36 (81%) patients completed ≥ Week 92. Reasons for subject discontinuation included: withdrawal of consent (N=4), AEs unrelated to lenabasum (N=2; tendonitis and scleroderma renal crisis), and other reasons (N=1). Thirty-five (97%) subjects experienced ≥1 AE, with 249 total AEs. Seven (19%) subjects had ≥ 1 AE considered related to lenabasum. Three (8%) had AEs judged to be probably or definitely related to lenabasum: mild fatigue (N=1); moderate skin ulcer and lymph node pain (N=1); and mild disturbance in attention and lethargy and moderate feeling abnormal (N=1). Most subjects experienced AEs that were mild (n=6, 17%) to moderate (n=23, 64%) in maximum severity. Six (17%) had severe AEs and 1 (3%) had a life-threating AE of renal crisis associated with exposure to high-dose steroids. AEs in ≥ 10% of subjects were: upper respiratory tract infection (n=11, 31%); skin ulcer, urinary tract infection, and arthralgia (each n=6, 17%); and diarrhea, nasopharyngitis, and cough (each n=4, 11%). Dizziness and fatigue occurred in 3 (8%) subjects each. Multiple efficacy outcomes showed stable improvement from about 1 year in the OLE or continued improvement in some cases. Median ACR CRISS score was 0.96 and mean (SE) change in mRSS was –10.3 (1.4) at Week 92.

Conclusions: Lenabasum continues to be safe and well-tolerated to date in the OLE of Phase 2 trial JBT-101-SSc-001, with no serious AEs or study discontinuations related to lenabasum. Improvement in efficacy outcomes have been durable. Background therapy, natural history of the disease, and open-label dosing limit what efficacy can be definitely contributed to lenabasum

28. Performance of American College of Rheumatology (ACR) Combined Response Index in Diffuse Cutaneous Systemic Sclerosis (CRISS) Score in a Phase 2 Study of Lenabasum Patients with Diffuse Cutaneous Systemic Sclerosis (dcSSc)

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Background: ACR CRISS score is calculated from change in 5 clinically relevant core items of mRSS, HAQ-DI, Patient Global Assessment (PtGA), Physician Global Assessment (MDGA), and FVC % predicted, using a weighted exponential formula. The score was provisionally approved by ACR as a primary efficacy outcome for 12-month trials in dcSSc, noting the score had not been validated using external data. ACR CRISS score was pre-specified as primary efficacy outcome in Phase 2 study JBT101-SSc-001 (NCT02465437) of lenabasum in dcSSc. The hypothesis was that data from JBT101-SSc-001 would provide initial external validation of ACR CRISS score.

Materials and methods: JBT101-SSc-001 (NCT02465437) included 4-month, double-blinded, randomized, placebo-controlled Part A and an open-label extension (OLE). Baseline, 4-month and 12-month data were analyzed for Spearman's correlations between pairs of: core items at baseline; change in core items; and ACR CRISS score and change in each core item. Median ACR CRISS scores were determined in subjects with different levels of improvement in patient-reported HAQ-DI and PtGA.

Results: Core items at baseline and change in core items at 4 and 12 months were not redundant, defined as correlations < 0.80. The strongest correlations at baseline were between PtGA and HAQ-DI, HAQ-DI and MDGA, and PTGA and MDGA ($r \geq 0.60$, $p \leq 0.0001$). Correlations between ACR CRISS and change in each core item were all statistically significant, $p \leq 0.05$ at both 4 and 12 months and greatest for ACR CRISS and change in mRSS ($p < 0.0001$). Median ACR CRISS scores increased with increasing levels of improvement in HAQ-DI and PtGA. For example, for improvements in HAQ-DI at 12 months, of no improvement, and improvement at least - 0.125, -0.250, and -0.375 points, median ACR CRISS scores were 0.02, 0.39, 0.82, and 0.97, respectively.

Conclusions: These analyses provide preliminary validation of ACR CRISS score, showing core items and change in core items were not redundant, each core item was reflected in the score, and clinically important improvement in outcomes that reflect how the patient feels or functions (HAQ-DI and PtGA) were reflected in higher ACR CRISS scores. Additional validation in other trials is warranted.

29. Fli1 and ERG regulate lymphangiogenesis in systemic sclerosis

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Background: SSc is characterized by autoimmunity, blood vessel vasculopathy, and fibrosis causing damage in multiple organ systems. Recent studies have also revealed significant rarefaction of lymphatic endothelium in SSc patients, but the underlying mechanism is unknown. The aim of this study was to address the molecular and cellular mechanisms responsible for the impairment of the lymphatic system in SSc.

Materials and methods: Lymphatic endothelial cells (LEC) (Podoplanin and Ve-cadherin positive population) were isolated from human foreskin and used in all in vitro experiments.

Cutaneous wound healing experiments were performed using 6-10 weeks old *Fli1* $^{flox/flox}$; *Cdh5*-Cre$^{ERt2+/-}$ mice and control littermates. Wounds were evaluated by immunochemistry after epithelization.

Results: Immunostaining analysis revealed that protein expression of Fli1 and ERG was significantly decreased in both blood and lymphatic vessels in SSs skin. In order to investigate the functional significance of Fli1 and ERG deficiency in LEC, transcriptome analysis on LEC transfected with scrambled, Fli1, ERG, or Fli1+ERG siRNAs was performed. The combination of siFli1 and siERG affected the largest group of genes (4166 with $p<0.05$ and 653 with $p<0.001$). Furthermore, Fli1 and ERG formed protein-protein complexes in LEC consistent with the coordinated regulation of target genes. The gene expression analysis strongly suggested that ERG and Fli1 regulate the key genes involved in lymphatic vessel specification. Prox1, LYVE1, and FLT4 were among the genes that were significantly downregulated at the mRNA and protein levels by siERG/siFli1 RNA treated LEC. In functional assays, the ability of tube formation significantly decreased in LEC treated with double siRNA. Wound healing experiments demonstrated delayed wound epithelization and fewer blood and lymphatic vessels in the wound areas in Fli1 mutant mice.

Conclusions: This study demonstrates that transcription factors Fli1 and ERG are novel regulators of lymphatic cell function and adds to the previous findings on the role of Fli1 and ERG in blood endothelium. In SSc patients, deficiency of Fli1 and ERG is a shared pathological feature between blood and lymphatic endothelial cells, which may contribute to the impaired angiogenesis and lymphangiogenesis in those patients. Targeting Fli1/ERG deficiency may provide a major therapeutic advance for patients with SSc.

30. Proteomic and transcriptomic analysis of human eosinophilic fasciitis fibroblasts

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6Genomic core facility of Paris Descartes University, Paris, France

Background: Eosinophilic fasciitis (EF) is a rare scleroderma-like disorder with less than 200 cases reported. Due to the rarity of the disease, data regarding its pathophysiology are lacking. Herein we aimed at studyingthe transcriptome and the proteome of EF fibroblasts.

Materials and methods: Skin fibroblasts from EF patients (n=4), systemic sclerosis (SSc) patients (n=4), and healthy controls (HC) (n=4), were cultured and harvested for transcriptomic and proteomic analysis. Transcriptomic and proteomic analyses were performed next generation sequencing and using label-free quantification, respectfully. Functional analyses were performed using Ingenuity Pathway Analysis and Panther softwares.

Results: Five hundred and nine genes involved in 14 biological processes (BP) and 199 pathways were differentially expressed between EF and control groups. Among them, the circadian clock system (CCS) (p=1.70 10-9), the plasminogen-activating cascade (PAC) (p=3.82 10-5), the FGF signaling pathway (p=1.15 10-4), and the integrin signalling pathway (p=1.33 10-3) were overrepresented. When comparing EF to HC genes, Panther showed overrepresented down-regulated pathway including CCS (p=1.71 10-12), and overrepresented up-regulated pathways such as flavin biosynthesis, (p=9.22 10-4), PAC (p=1.19 10-6), de novo purine biosynthesis (p=1.63 10-3), FGF signalling pathway (p=1.47 10-3) and integrin signalling pathway (p=9.14 10-4). When comparing EF to SSc genes Panther showed overrepresented down-regulated pathway such as CCS (p=2.51 10-4). Two hundred and twenty four proteins involved in 10 BP and 126 pathways were differentially expressed between EF and control groups. When comparing EF to HC proteins, Panther showed specific overrepresented up-regulated biological processes such as vesicle-mediated transport (p=4.66 10-5) and intracellular protein transport (p=1.24 10-4), and overrepresented up-regulated pathways such as 5-hydroxytryptamine degradation (5-HTD) (p=1.56 10-4) and overrepresented downregulated pathways such as heterotrimeric G-protein signalling pathways-rod outer segment phototransduction (HTGSP) (p=1.24 10-4). When comparing EF to SSc proteins, Panther showed specific overrepresented down-regulated biological processes such as 5-HTD (p=4.48 10-4), and overrepresented down-regulated pathways such as HTGSP (p=1.98 10-4), and ubiquitin proteasome pathway (p =1.05 10-3).

Conclusions: This work described the transcriptome and proteome of EF fibroblasts and highlighted significant specificities of EF fibroblasts when compared to HC and SSc.

KEYWORDS: proteomic; transcriptomic; eosinophilic fasciitis; systemic sclerosis; fibroblasts.

31. Altered network of antibodies against G protein-coupled receptors favours immune cell trafficking to the lungs in patients with systemic sclerosis

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Background: We have recently identified a physiological network of autoantibodies directed against various G protein-coupled receptors (GPCR), growth factors, and growth factor receptors, which is affected by age and sex (Cabral-Marques, Nature Communications 2018). Based on disturbed tolerance to autoantigens, systemic autoimmune diseases could have more exaggerated changes in this ab network. Therefore, we asked for changes of the ab network in SSc.

Methods: ELISAs were applied to measure the respective ab levels directed to 17 GPCR, four growth factor receptors, two signalling molecules, and eight growth factors in 84 SSc patients versus 200 healthy donors. Spearman's rank correlation coefficients, hierarchical cluster analyses, network mapping and target interactions using STRING and gene ontology (GO) analyses were performed as well as transwell migration assays. In addition, animal experiments were performed to identify the in vivo function of anti-ETAR ab.

Results: In comparison to healthy individuals, we have identified distinct and novel correlations between autoantibodies in SSc. Here, antibodies against the AT1R correlated with ab against the epithelial growth factor receptor, the vascular endothelial growth factor A (VEGFA), as well as with the muscarinic receptor 3. In addition, strong correlations were found between the hepatocyte growth factor receptor and the placental growth factor, the epithelial grown factor receptor and the VEGFA as well as between the endothelin receptor type-B and the complement receptor C3AR1. Hierarchical cluster analysis revealed specific clusters different from healthy donors. Network mapping and GO analysis of ab targets displayed multiple associations and a central role of the endothelin receptor type-A (ETAR) in cell migration and chemoattraction. Indeed, anti-ETAR ab were (in an ab-level-dependent manner) chemo attractive for immune cells such as lymphocytes and neutrophils expressing ETAR. Immunization with ETAR induced high anti-ETAR ab levels as well as lung inflammation.
Conclusion: In this unbiased approach, the role of ab against AT1R and ETAR in SSc, as previously suggested, has been supported. Increased anti-ETAR ab could play a role to guide immune cells into the lungs, which express ETAR

32. Antibodies against the angiotensin receptor type-1 (AT1R) contribute to lung and skin inflammation present in patients with systemic sclerosis

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Background: Based on increased levels, associations and correlations with clinical features in SSc patients, their capacity to predict mortality and vascular complications in SSc, as well as by several in vitro experiments using IgG from SSc patients showing induction of cytokines, chemokines, and collagen-1, antibodies against the angiotensin receptor type-1 (AT1R) are suggested to contribute to the disease pathogenesis in SSc. However; this hypothesis has not been proven so far.

Methods: We have immunized female C56/Bl6 mice with membrane extracts (ME) from cells overexpressing the human AT1R versus ME without AT1R overexpression. ELISAs were preformed to analyse the anti-hAT1R ab levels. The function of the ab were detected by in vitro experiments. Histology and immunohistology of the lungs and skins have been performed. In addition, we have transferred IgG antibodies into the skin of C56/Bl6 mice and have analysed skin and lung sections.

Results: Upon hAT1R immunization, female C56/Bl 6 mice developed high anti-AT1R ab levels not present in mice treated with the control antigen. The presence of high anti-hAT1R ab levels were accompanied by perivascular inflammations of the skin as well as increased skin thickness in comparison to the mice injected by empty ME. In the lung, both parenchymal as well as perivascular infiltrations were identified not present in the mice immunized with the control antigen. The induced antibodies were functional as shown by increased expression of IL-8 in monocytic ThP1 cell lines. Transfer of IgG fractions containing high levels of anti-hAT1R ab induced lung and skin infiltration, which was different from mice upon transfer of IgG without AT1R enrichment or by using an isotype control.

Conclusion: Immunization of mice with the human AT1R induced high anti-hAT1R ab levels as well as inflammations in the skin and lung, which usually express AT1R. In this model, vasculopathy was detected only marginally. The data indicate a role of anti-AT1R ab in tissue inflammations present in most SSc patients. Therefore, therapies targeting ab-receptor interaction, could provide a novel tool in the therapy of SSc.

33. Fibrosis is not just fibrosis - Co-stimulation of fibrosis with inflammatory and vascular growth factors induce different extracellular matrix profile.

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Background: Systemic sclerosis (SSc) is a disease with fibrosis as the main hallmark. The fibrosis is continuously stimulated by immune activation (eg. IL-6) and vascular damage (eg. PDGF). Growth factors as TGF-β and PDGF are believed to be drivers of SSc. The objective was to investigate co-stimulation of multiple growth factors on heathy dermal fibroblasts (DF) to understand the pathogenesis of SSc.

Methods: Primary healthy human DF were grown in DMEM containing 0.4% FCS, FICOLL and ascorbic acid for 14 days. The cells were stimulated with or without (w/o) TGF-β and/or PDGF, interleukin 6 (IL-6; together with soluble IL-6 receptor). Type I, III and VI collagen formation (PRO-C1, PRO-C3, PRO-C6) and fibronectin (FBN-C) were evaluated by ELISAs.

Results: Stimulation with TGF-β primary induced PRO-C1 from day 3, with a 4-fold change (FC) detected on day 10 compared to w/o ($P<0.0001$). Single stimulation with PDGF induced PRO-C3 from day 6, with an 8.3-FC by day 10 ($P<0.0001$) and PRO-C6 from day 3 with a 6.0-FC by day 10 ($P=0.0006$). Visual examination showed type I and III collagen attachment to the bottom of the wells.

Stimulation with IL-6 reduced FBN-C levels with a 0.3-FC compared to w/o, whereas TGF-β increased this with 2.2-FC. A combination of both cytokines resulted in a 4.1-FC increase in FBN-C level ($P<0.0001$). By histology IL-6 showed to activate the STAT3 pathway.

Co-stimulation of TGF-β and PDGF induced PRO-C1 with a 1.7-FC and a 5.7-FC compared to TGF-β and PDGF, respectively. Co-stimulation further induced FBN-C with a 4-FC and a 13-FC compared to TGF-β and PDGF, respectively.

Conclusion: In this study, we show that co-stimulation of fibrosis inducer together with inflammation or vascular damage appear to have a synergistic effect on extracellular matrix production and mimics SSc. Further studies will determine if this model can capture the complex etiology behind SSc and be useful as a preclinical model.

34. The fibrotic index of type III and VI collagen follows the modified Rodnan skin score: A pilot study

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Background: Tissue balance of skin is altered in systemic sclerosis (SSc), with the balance shifted towards tissue accumulation. Biomarkers have previously been used to monitor effects of therapies in rheumatological diseases. Serological biomarkers assessing tissue formation and degradation separately may be used to quantify the tissue balance.

Methods: Forty-three patients fulfilling the 2013 ACR/EULAR criteria for SSc (limited cutaneous [lcSSc, n= 20] and diffuse cutaneous SSc [dcSSc, n=23]) and 10 healthy controls (HC) were included (recruited at Lund University, approval number Dnr 590/2008).

Biomarkers of type III, IV and VI collagen formation (PRO-C3, PRO-C4, PRO-C6) and degradation (C3M, C4M, C6M) were measured in serum samples by validated ELISAs. The fibrotic index of the individual collagens (FICol) were examined (formation divided by degradation).

Results: The FICol of type III and VI collagen (FICol3 and FICol6) were significantly increased in dcSSc compared to lcSSc (FICol3: 1.4 vs 0.8, P=0.0001; FICol6: 1.2 vs 0.9, P=0.03). The FICol of type IV collagen (FICol4) was not different between the groups but were 1.5 times higher than HC (HC: 6.9, lcSSc 10.4, dcSSc: 10.5).

Both FICol3 and FICol6 correlated with modified Rodnan skin score with rho's of 0.59 (P<0.0001) and 0.35 (P=0.04). Furthermore, FICol3 showed a tendency to follow modified Rodnan skin score over time.

Conclusion: This study shows the importance of looking at both collagen formation and degradation. The collagen turnover was altered in lcSSc and dcSSc compared to HC. In addition, dcSSc presented with a more skewed fibrotic index towards collagen formation. Examining collagen turnover could be beneficial in following patients' fibrosis development and possibly identifying disease progression.

35. Adeno-associated virus type 5 targets Platelet-derived growth factor receptor alfa and stimulatory antibodies in the lung and in the peripheral blood of patients with Systemic sclerosis

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Background: SSc patients contain stimulating antibodies targeting Platelet-derived growth factor receptor alpha (PDGFRα), which represents the main entry site for the adeno-associated virus type 5 (AAV5). We investigated the presence of AAV5 DNA in the lung and peripheral blood of SSc patients, and its relationship with anti-PDGFRα stimulating antibodies.

Methods: *In silico* molecular docking was performed to predict the binding between the three-dimensional structures of monomeric human PDGFRα and AAV5 capsid monomeric subunit. A surface plasmon resonance (SPR) assay was performed to validate *in vitro* the *in silico* prediction of the possible molecular complex between AAV5 and PDGFRα. The PDGFRα KO (by CRISP-CAS9 technology) human alveolar basal epithelial cell line A549, was used to assess the role of PDGFRα for AAV5 transduction. Bronchoalveolar lavage of 66 SSc patients and 77 controls affected by conditions other than SSc was analyzed by PCR for the presence of AAV5 DNA. The presence of the virus in the lung was also assessed by in situ hybridization, immunohistochemistry and confocal microscopy to demonstrate colocalization of PDGFRα and AAV5. Molecular docking, SPR, and immunoprecipitation studies, were performed to demonstrate the binding of anti-PDGFRα antibodies to AAV5.

Results: AAV5 *in silico* interacts with the extracellular domains of PDGFRα and SPR assay showed that the AVV5 capsid monomer binds PDGFRα with high affinity. Deletion of PDGFRα by CRISP-CAS9 in A549 cells inhibits significantly the transduction efficiency. AAV5 genomic sequences were found in 71.2% of SSc patients and in 28.5% of controls ($p<0.0001$). AAV5 was present in alveolar epithelial cells by immunohistochemistry and in situ hybridization and co-localized with PDGFRα by confocal microscopy. Both total SSc-IgG and human monoclonal anti-PDGFRα antibodies (Moroncini et al 2015) immunoprecipitated PDGFRα and AAV5 capsid from infected cells. Specific PDGFRα and AAV5 peptides recognize the monoclonal anti-PDGFRα antibodies.

Conclusions: The present study demonstrates that AAV5 is present in the lung and blood in a significant fraction of SSc patients, and recognizes stimulatory anti-PDGFRα antibodies. AAV5 contributes to the pathogenesis of systemic sclerosis by eliciting adaptive immunity targeting the PDGFRα complex.

36. PDGFRα and NADPH oxidase HLA-I trans-spliced peptides are targeted by SSc autoantibodies

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Background: Scleroderma stimulatory IgG autoantibodies (SSc-IgG) recognize specific sequences of the Platelet-derived growth factor receptor α (PDGFRα) and activate collagen gene expression via reactive oxygen species (ROS) intracellular production by NADPH oxidase in the lipid rafts of the plasma membrane. We investigated whether: i. NADPH forms a molecular complex with PDGFRα: ii. NADPH sequences are targeted by SSc autoantibodies.

Materials and Methods: The interaction between PDGFRα and NADPH oxidase subunits was evaluated by immunoprecipitation of fibroblast total lysate with commercial anti-human PDGFRα, anti-human NADPH, SSc-IgG and human monoclonal anti-PDGFRα antibodies (Moroncini et al., A&R 2015). To predict the amino acid sequences of PDGFRα and NADPH oxidase involved in the formation of a molecular complex, *in silico* molecular docking between homology-modeled monomeric human PDGFRα (Moroncini et al., A&R 2015) and NADPH was performed. A conformational peptide library comprising PDGFRα and NADPH motifs, designed upon molecular docking prediction, was synthesized and tested with serum samples of SSc patients and healthy donors (HD). HLA I-peptide complexes were immunoaffinity purified from peripheral blood mononuclear cells (PBMC) of SSc patients and HD and the bound peptides were obtained by chromatography and analysed by mass spectrometry. Specific peptide sequences and scrambled controls were used as markers.

Results: PDGFRα forms a complex with NADPH as assayed by anti-PDGFRα, NADPH oxidase subunits specific antibodies and by SSc-IgG. Using as guide an *in silico* model that describe the interacting domains of PDGFRα and NADPH oxidase, we screened a conformational peptide library with SSc serum samples. We identified SSc-specific immunodominant epitopes belonging to both proteins. By MS spec analysis we demonstrated the presence in SSc lymphocytes of spliced peptides of both proteins. We were not able to identify the same peptides in cells derived from HD.

Conclusions: This is the first demonstration of the existence of spliced peptides in an autoimmune disease. Moreover, these data show the participation of T cells in SSc immunorecognition.

37. Generation of human PDGFRα-transgenic mouse: a novel experimental model of skin fibrosis

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Background: Platelet Derived Growth Factor (PDGF) Receptor α (PDGFRα) is a target of the autoimmune response in scleroderma (SSc). Both total serum IgG (SSc-IgG) and anti-PDGFRα antibodies cloned from memory B cells of SSc patients (SSc-Mabs) [Moroncini G. et al., Arthritis & Rheumatology 2015] demonstrated the ability to increase collagen gene transcription in healthy donor skin fibroblasts and to induce fibrosis *ex vivo,* in skin grafts in SCID mice [Luchetti M. et al., Arthritis & Rheumatology 2016]. In order to replicate these findings *in vivo*, we generated human PDGFRα-transgenic mice.

Materials and methods: Full length human PDGFRα cDNA was knocked-in into the ubiquitously expressed Rosa26 locus on mouse chromosome 6. Correctly targeted C57BL/6 ES cell clones were selected for blastocyst microinjection, followed by chimera production. F2 heterozygous C57BL/6-hPDGFRα transgenic mice were used to establish the colony. Twelve weeks-old male mice were injected into the back skin at days 0, 3, 6 and 9, either with 0.02 mg/ml of SSc-Mabs (VH_{PAM}-V_κ16F4 or VH_{PAM}-V_κ13B8), or with 2 mg/ml of SSc-IgG or IgG purified from serum of healthy donors (HD-IgG). Vehicle only injection control was included. Age- and sex- matched C57BL/6 wild type mice were used as controls. Animals were sacrificed at day 14. Human PDGFRα transgene expression and collagen amount were assessed in explanted skin tissue.

Results: Transgenic mice were phenotypically normal, fertile, and did not display any apparent pathological features. Human PDGFRα mRNA and protein were detectable in the skin of all examined transgenic mice. Intradermal injection of stimulatory human SSc-Mab VH_{PAM}-V_κ16F4 or SSc-IgG resulted in dermal thickening and increased collagen deposition, whereas non-stimulatory human SSc-Mab VH_{PAM}-V_κ13B8 or HD-IgG did not induce any significant skin tissue alterations compared to vehicle control. C57BL/6 wild type mice did not show any significant skin tissue changes with any antibodies.

Conclusions: We generated a novel humanized mouse model of skin fibrosis based on the concomitant expression of human PDGFRα and injection of stimulatory anti-PDGFRα antibodies. This model may be useful to identify new therapeutic strategies for SSc and for their preclinical validation.

38. Analysis of profibrotic serum markers across the scleroderma spectrum shows subset and stage specific profiles of fibrogenesis

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Background: There is striking heterogeneity in skin fibrosis in scleroderma that is likely to reflect the balance between pro- and anti-fibrotic pathways underlying spontaneous regression of skin fibrosis in late stage diffuse SSc. We have studied potential serum markers of profibrotic activity in SSc, with a view to understanding their relationship with progression of fibrosis as measured by the MRSS to better define cases likely to respond to fibrosis-targeted therapies.

Methods: We prospectively recruited a cohort of well characterised patients (the BIOPSY cohort) from across the scleroderma spectrum. In total 67 subjects were included (21 early dcSSc (<5 years disease duration), 14 established dcSSc, 16 lcSSc, 16 healthy controls (HC)). MRSS was recorded at the time of sample collection. Standard and novel measures of serum or plasma markers were undertaken by immunoassay (20 in total) reflecting ECM turnover or cytokine drivers of fibrosis.

Results: Our results confirmed that 13 analytes showed significant differences in concentration by subgroup using one-way ANOVA. Markers of collagen synthesis were significantly different ($p<0.05$) between the subgroups (Pro-C6, Pro-C3, PIIINP), while markers of collagen degradation were not significantly altered (C3M, C6M, C4M2, C7M). This difference was most significant between the early dcSSc subgroup compared with the other subgroups (students t-test with bonferroni correction). There was significant upregulation of IL-6, MCP-1, and oncostatin M in SSc compared to HC.
There were significant correlations between several candidate profibrotic serum markers and MRSS: Pro-C3, Pro C6, PIIINP, and IL6 (all $p<0.01$).

Conclusion: Our results show the utility of extended patient cohorts to delineate fundamental biology in SSc. We identified key pro-fibrotic molecular markers as being upregulated in SSc and found these to correlate with the extent of skin fibrosis. Markers of collagen III and collagen VI synthesis are particularly raised, especially in the early stages of the disease. Unlike previous work, we did not find any significant difference between SSc subgroups and healthy controls for markers of collagen degradation. These promising cross-sectional data suggest therapies targeting drivers of fibrosis are most likely to show benefit for skin in early dcSSc. This will be further explored longitudinally in the BIOPSY cohort.

39. Parallel analysis of systemic sclerosis and keloidal morphea skin biopsies delineates the hallmark profibrotic gene expression profile for scleroderma in vivo

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Background: We examined whole skin biopsy gene expression by RNAseq in a rare subgroup of scleroderma with both systemic sclerosis (SSc) and concurrent keloidal morphea (KM). We hypothesised that this subtype of localised scleroderma would provide exceptional insight into fibroblast activation in vivo relevant to skin fibrosis in systemic sclerosis and suggest new potential molecular markers for classification.

Methods: 4mm skin biopsies were taken from forearm skin of SSc cases; both lcSSc (n=5) and dcSSc (n=7) and healthy individuals (n=4). In 4 SSc cases there was concurrent KM and 4mm skin were also taken from these lesions. Whole genome expression analysis was performed by RNAseq. Gene expression analysis was undertaken and differentially expressed genes were compared across the clinical subgroups using ANOVA with Benjamini-Hochberg post-hoc correction. The 500 most differentially expressed genes were identified, and unsupervised clustering was performed using CIMminer (Bethesda, Maryland, USA). Integration of the candidate genes from ANOVA, and principal component analysis (PCA) was carried out to identify instructive genes for a molecular classifier.

Results: RNAseq identified over 13000 expressed genes. PCA discriminated 4 unique clusters, with KM and HCs being the most distinct. PC1 accounted for 33% and PC2 for 22% of variation. Initial analysis identified over 3000 significantly different genes expressed by paired analysis. The 500 most significantly differentially expressed genes (all with $p<0.001$) were selected. Unsupervised hierarchical clustering of gene expression based on these results, showed clear clustering of the KM group, and the HCs. The majority of lcSSc and dcSSc patients clustered to their patient subgroups.

Correlation between ANOVA and PCA results highlighted 100 key genes shared across both analysis results. These included genes upregulated in SSc:SFRP4, THY1, COMP, ADAM12, THBS4, ADAMTS12 and others with lower expression than in HC:WIF1, KLF5. These include several implicated in pathogenesis or included in recent candidate biomarkers for skin disease in SSc.

Conclusion: We show the high value of RNAseq and the unique strength using skin biopsies from SSc with concurrent keloidal morphea, histologically characterised by dense fibro-proliferation, to define profibrotic genes relevant to SSc. This provides powerful insight into pathogenesis and candidate molecular markers for classification across the scleroderma spectrum.

40. A Semi-quantitative Scoring System for Picrosirius Red Stain Distinguishes Skin and Antinuclear Antibody Defined Subsets of Systemic Sclerosis

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Background/Objectives: Picrosirius red (PSR) staining can be used for histological demonstration of fibrillar collagen in tissues by bright field or polarised light imaging. There is significant interobserver variability when recording MRSS, which can limit its use as a measure of skin severity and in clinical trials. Since skin biopsy is increasingly performed for molecular subset definition of SSc in clinical trials, we developed a novel semi-quantitative scoring system for PSR staining to establish the extent of collagen deposition within the SSc spectrum.

Methods: 48 prospective patients (20 early dcSSc, 13 established dcSSc, 15 lcSSc) and 16 healthy controls were recruited into the BIOPSY cohort. 4mm punch biopsies from forearm skin were stained with PSR. 2 independent observers assessed samples on brightfield viewing. Scores were recorded as 1-5 (1=Normal like, >50% gaps in the tissue, fine collagen bundles, and faint hypodermal and subepidermal staining; 5=<5% gaps, densely thickened fibrotic collagen bundles, and deep staining of the hypodermis). Polarised light evaluation on the same samples was analysed using ImageJ.

Results: Analysis confirmed significantly different scoring by patient subgroup on ANOVA ($p<0.0001$), with highest scores in early dcSSc. There was significant correlation between the PSR score and MRSS ($r=0.37$, $p=0.002$). There was also significant difference between PSR scores and autoantibody subgroups ($p=0.0004$), with ATA and ARA having higher scores, compared to ACA and HC groups.

Polarised light analysis compared proportion of colour distribution. The proportion of red, orange and green collagen fibres were significantly different between patient subgroups ($p=0.01$, 0.04 and 0.04 respectively, yellow=NS). Only the proportion of red fibres negatively correlated with MRSS ($p=0.022$). There were no significant correlations between PSR scores and the polarised light colour proportions.

Conclusion: Simple brightfield grading for PSR stained tissue reflects overall severity of skin disease assessed by MRSS and differentiates skin and ANA based disease subsets. The results suggest that the PSR scoring gives a robust and generalisable quantification of the extent of collagen, compared to the more complex analysis required through in polarised light. This has the potential to be applied to skin biopsies for purposes of staging and assessing treatment response in the trial or clinical setting.

41. Periostin as a novel marker of heart involvement in scleroderma

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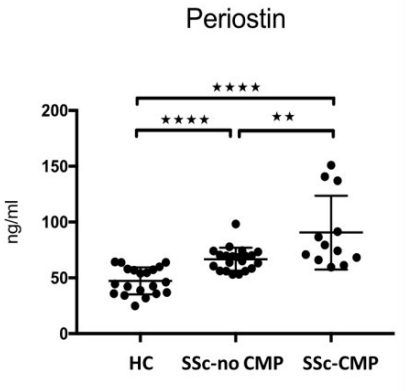
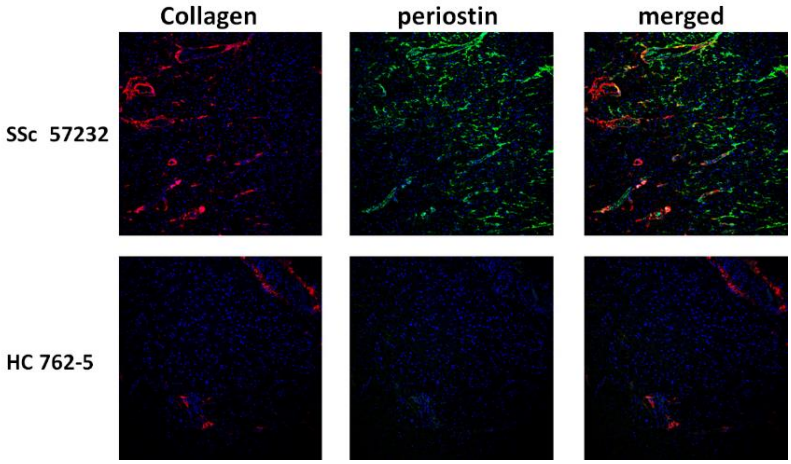
Background: Widespread myocardial fibrosis with diastolic dysfunction, a common SSc complication, is a strong predictor of death. Often subclinical, diastolic dysfunction is difficult to diagnose by conventional means, due to normal ejection fractions and minor elevations in BNP. To identify a new, more accurate marker to diagnose this SSc complication, we performed multiplex analysis of several markers known for their association with SSc or heart failure in the serum of patients with and without SSc cardiomyopathy (CMP).

Materials and methods: Serum samples from patients with and without SSc-CMP (pCWP >13, TPG<12, PVR <240, by RHC and LVEF >50%) and healthy, matched controls (HC), were analyzed for the levels of CXCL13, endothelin-1, Fas, FGF basic, IL-1 alpha, IL-18, IL-6, MMP-3, osteoprotegerin, pentraxin 3, periostin, renin, ST2, TNF-alpha, galectin-3, MMP-2, MMP-9 and MPO, using multiplex magnetic bead immunoassays. Quantitation was performed on a Luminex Magpix instrument with xPONENT 4.2 software and results were compared using one-way ANOVA. Periostin levels were confirmed by ELISA and checked in two additional patient groups: SSc-ILD and SSc-PAH, to assess for specificity. Paraffin embedded human heart tissue (National Disease Research Interchange, NDRI) from SSc patients and controls was stained for periostin (n=4 each) and co-stained for periostin and collagen type I (n=2 each) using immunofluorescence.

Results: Periostin, a matricellular protein recently shown to be a specific marker for cardiac myofibroblasts, was the only significantly increased circulating marker in SSc patients with isolated CMP compared with SSc patients without CMP. Additionally, no significant difference was found in the SSc-ILD and SSc-PAH groups when compared to SSc without internal organ involvement. Immunofluorescence staining of SSc hearts showed patchy periostin expression in all SSc patients, but not in controls. Furthermore, there was extensive periostin expression even in areas without collagen deposition, while all established fibrotic areas showed colocalization of collagen and periostin.

Conclusions: Periostin is elevated in SSc heart in vivo and circulating levels of periostin are increased in patients with SSc-CMP compared to SSc patients without heart involvement. These results suggest that periostin could be a potential marker of SSc-CMP.





42. Profibrotic Macrophage Activation in Systemic Sclerosis is Dependent on the Mechanosensing MRTF-A Pathway

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Background: An M2-like alternative activation state of macrophages has been linked to pathogenesis of systemic sclerosis (SSc). MRTF-A is an essential mechanosensing factor involved in cellular responses to stiff fibrotic tissue, but its role in macrophage polarisation is unknown. Genome profiling identifies the promoter region of IL13Rα1 (critical to M2 macrophage activation) as a target of MRTF-A. Thus, IL13Rα1 is predicted MRTF-A responsive and IL4R predicted non-responsive.

Materials and methods: MRTF-A knockout (KO) and wild type (WT) control mice (n=3) were subject to excisional wound healing and *ex vivo* polarisation of bone marrow derived macrophages with interleukin 4 and 13, to model M2-dependent responses. Peripheral blood mononuclear cells from SSc patients (n=9) were cultured in M-CSF (4ng/ml) for 7 days to derive M2-like macrophages. 50kPa Softwell gels were used to reproduce stiff tissue microenvironments. Morphology (elongated M2-like, round M1-like), and gene expression by qPCR, were used to profile the macrophage activation state (IFNγ for M1-like, Arg1 and CD206 M2-like). TGFβ secretion was assayed by ELISA. CCG-257081 (CCG)(10μM) was used to inhibit MRTF-A.

Results: In mice, MRTF-A WT (3.54±0.337, mean±SEM) and MRTF-A Het macrophages (4.10±0.720) exhibited significantly higher M2/M1 cell morphology ratio compared to MRTF-A KO macrophages (0.445±0.0903) (p=0.0026 and p=0.0007 respectively). Excisional wound repair was also delayed (day 11, WT 3.3 ±1.1, KO 27.4 ± 4.1 p<0.011, % basal wound area). SSc macrophages showed an M2-like activation signature under basal conditions, which was partially reversed by CCG, reducing CD206 (basal 9.16 ± 4.26, CCG treated 1.79±0.98, p=0.0039), suppressing the elongated M2-like morphology (SSc basal 4.04, ±0.74, cells per field, CCG treated 0.46±0.18, P=0.0017), and inhibiting the secretion of TGFβ (SSc basal 7.0± 4.9 pg/ml, CCG treated 0±1.7 P=0.039). IFNγ (M1 marker) was increased by CCG treatment consistent with repolarisation (SSc basal 2.02±0.54, CCG treated 10.4±3.6, p=0.0039). As a direct target of MRTF-A, IL13Rα, was decreased by CCG (21.5±3.39) compared to control (83.2±19.9, p=0.0252), and in mice KO macrophages showed decreased IL13Rα1:IL4R ratio, indicating a pathway selective effect.

Conclusions: Loss of MRTF-A results in reduced IL-13Rα1 and attenuated cytoskeletal changes associated with M2-like polarisation. Experiments with the CCG-257081 inhibitor indicate possible therapeutic potential in inhibiting the MRTF-A mechanosensing pathway.

43. Prevalence and characteristics of Raynaud's phenomenon in patients with peripheral neuropathy

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Background: Raynaud's phenomenon (RP) is a common condition affecting approximately 5% of the general population; and can be either primary or secondary in nature. The cause of RP is not known, but it is thought that there is a neurological component to pathophysiology. Anecdotally, many patients with peripheral neuropathy report cold sensitivity, yet the relationship between peripheral neuropathy and RP has been little studied. Our main aim was to explore the prevalence of RP in patients with peripheral neuropathy: specifically, to investigate the hypothesis that prevalence is increased.

Methods: In this cross-sectional study, 188 patients with a diagnosis of peripheral neuropathy (diagnosed by a consultant neurologist) were sent a short questionnaire asking questions about their condition and any RP symptoms they may be experiencing. Basic demographic data and clinical data were collected. Patients were asked if they experienced any unusual colour changes in their fingers in an attempt to identify the typical biphasic or triphasic pattern (white, red, blue) classically seen in patients with RP. Descriptive statistics were used to describe the study population and presence of RP.

Results: Seventy-nine patients with peripheral neuropathy (42%) returned the questionnaire with a mean age of 58 years (SD 13.2) and median peripheral neuropathy duration of 8 years (interquartile range 4-10). The majority of patients (70%) stated their fingers were sensitive to the cold. Fifty patients (63%) reported colour changes in their fingers, with 35 (44%) indicating such colour changes were in response to the cold. Of the fifty patients reporting a colour change, eleven patients (22%) reported typical biphasic or triphasic RP with eight (16%) also reporting symptoms in the thumb. Forty-four patients out of 79 (56%) also stated they experienced similar issues with sensitivity to the cold in their feet or toes.

Conclusion: This study highlights a high prevalence of RP and of cold sensitivity in patients with peripheral neuropathy. Further investigation into the association between RP and peripheral neuropathy could potentially provide further insights into the pathogenesis of both conditions.

44. Effects of mechanosensing inhibitors on the expression of Cadherin-11 and Collagen 1 in fibroblasts and macrophages in scleroderma patients

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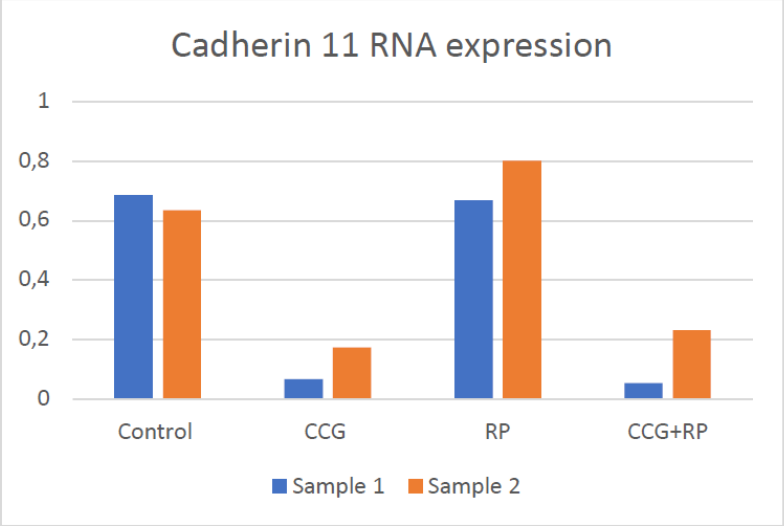
BACKGROUND: Scleroderma is complex diseases characterized by the overproduction of collagen and increased expression of adhesive molecules such as cadherin, which belong to the transmembrane population of adhesive molecules dependent on calcium. Their expression is regulated in large part by the MRT-F mechanosensing pathway. Cadherin 11 (CDH11) is one of those adhesive molecules and is highly expressed in ECM of scleroderma patients, but not in normal healthy individuals (except bone matrix). It has been also reported that CDH11 is one of the markers for epithelial-mesenchymal transition, and a possible measuring factor of the invasiveness of the cells. We investigated the effects of two drug inhibitors (CCG-targets mechanosensing, and RP832c-targets Nfκb) on the expression of CDH11 and Collagen I in cell culture of fibroblasts and macrophages on 50 kPa plates

Materials and methods: Blood samples were taken for both healthy and scleroderma patients and isolation of macrophages was performed after which the cells were co-cultured for four days with fibroblasts and were treated with RP832c and CCG inhibitors, both individually as well as combined. At this point, the RNA extraction was performed to perform qPCR analysis for collagen I and CDH 11 (5′- AACAAACCGGTTTGGGCCCCTCGAGGGATA-3′ sense and 5′- ACAAGCTAGCGGTCCGGAATTCCCGGGATA-3′ antisense).

Results: Collagen and CDH11 production were higher in co-cultures that were not treated withmechanosensing inhibitors. The RP832c reduced collagen I (basal…, RP832 treated..). The effect of CCG was lesser on the expression on collagen I but more effective on inhibiting the expression of CDH11. RP did not show a significant effect on lowering the expression of CDH11, but the results wereslightly lower than of the untreated co-culture. Expression of RNA in wells that were treated with both inhibitors showed almost 5-fold decrees in the overall expression of CDH11



Conclusions: It would seem that although the RP832c inhibitor does lower the expression of collagen I, its effect on CDH11 is not in the same range. While the CCG inhibitor is more effective in bringing down the CDH11 expression. This study has also demonstrated that CDH11 expression is mostly regulated by MRTF mechanosensing pathway

45. Impact and associates of digital pitting in patients with systemic sclerosis – a pilot study

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Background: Despite being a cardinal clinical sign of systemic sclerosis (SSc), digital pitting has been little studied. Our objective was to test the hypothesis (in a pilot study) that pitting is painful, and associated with digital vascular disease severity.

Methods: Fifty patients with SSc were recruited: 25 with and 25 without digital pitting. Fingertip pain was assessed, using a questionnaire, on a 0-10 scale. Thermography of both hands assessed surface temperature, allowing calculation of the distal dorsal difference (DDD, temperature gradient) for each finger. Nailfold capillaroscopy was performed on each finger using a dermatoscope, and graded on a 0-3 scale (0 normal, 3 grossly abnormal). Thermography and nailfold capillary data were analysed at both 'patient level' and 'finger level'.

Results: In the 25 patients with digital pitting, a total of 65 fingers were affected (mainly the index and middle fingers). Pain scores were higher in 'pitting' patients (median 4 [interquartile range 3-8] versus 0 [0-2], $p<0.001$), with pain often described as sharp and shooting. Pitting patients reported that pitting impacted on activities of everyday living, with 20 (80%) of patients reporting that their “pitted” fingers were “always” or “often” more painful than fingers without pitting. Temperature gradients along the fingers did not differ significantly between patients with and without pitting ($p=0.248$). Pitting patients were more likely to have grossly abnormal capillaries than those without pitting, and less likely to have ‘no/mild’ nailfold capillary changes. Patients with pitting had a longer SSc disease duration than those without (median 13.1 versus 6.5 years, $p=0.028$) and were more likely to have a history of digital ulcers than those without (14 versus 3 patients, $p=0.002$).

Conclusions: Digital pitting is painful and impacts on hand function and quality of life. Capillaroscopy findings provide further support for an association between pitting and severity of digital vascular change. The association of digital pitting with longer disease duration and history of digital ulceration further suggests chronic ischemia as the likely underlying pathophysiology. Larger, more comprehensive studies are now required to examine pathophysiology of pitting and to pave the way to therapeutic intervention, ideally including preventative strategies.

46. CCR2^{+} circulating monocytes contribute to the survival of ADSC in bleomycin-induced skin fibrosis

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Background: Monocytes and monocyte-derived cells play a crucial role both during homeostasis and development of various inflammatory diseases including skin fibrosis. Ly6C^{hi} inflammatory monocytes formed in the bone marrow enter circulation as C-C Chemokine Receptor 2 (CCR2^{+}) cells and exert their function by their ability to differentiate into macrophages and other myeloid cell types in the peripheral tissues. Our lab previously showed upregulation of monocytes and monocyte-derived macrophages followed by loss of adipose-derived stromal cells (ADSC) in bleomycin-induced skin fibrosis. CCR2 is implicated to have a pathogenic role in multiple fibrosis models and hence we hypothesized that CCR2^{+} monocytes that infiltrate the skin following bleomycin treatment play an important role in modulating ADSC maintenance and associated bleomycin-induced skin fibrosis changes.

Materials and Methods: C57BL/6J wild type (WT) and CCR2 knockout (CCR2KO) mice were injected with either PBS (control) or 100ug of bleomycin subcutaneously on lower back skin for indicated time periods. Skin was assessed for cellular changes by flow cytometry and for fibrotic changes by collagen measurements.

Results: Consistent with the idea that monocyte accumulation in skin is dependent on CCR2 and that macrophages are monocyte-derived, we observed a robust increase in monocytes and monocyte-derived macrophages in WT but not CCR2KO mice at day 21 after bleomycin treatment. Contrary to expectations, however, while bleomycin-treated WT mice showed ~55% reduction in ADSC numbers compared to PBS control, CCR2KO mice under the same conditions showed further severity in ADSC loss (~80% reduction). CCR2 KO mice also showed reduced collagen levels, suggesting less fibrosis. These results suggest a role for CCR2^{+} cells, potentially monocytes and monocyte-derived macrophages, in maintaining ADSC numbers while contributing to bleomycin-induced skin fibrosis.

Conclusion: Overall, our results suggest a mixed role for CCR2, potentially by mediating the accumulation of monocytes and macrophages and maintaining ADSC numbers and yet promote bleomycin-induced skin fibrosis. Further studies will focus on understanding the significance of CCR2-mediated ADSC maintenance to skin health.

47. Role of B cells and anti-angiotensin receptor type-1 antibodies in lung and skin infiltration

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Background: Systemic sclerosis (SSc) is an autoimmune connective tissue disease featured by autoimmunity, fibrosis and vasculopathy. Although many autoantibodies have been detected in the sera of patients with SSc, it is not clear whether they play a role in the pathogenesis of disease. It has been reported that autoantibodies against the angiotensin-II receptor type 1 (AT1R) are present in the sera of SSc patients and are associated with several clinical symptoms of the disease, suggesting that these autoantibodies may act as pathogenic drivers. Recently, our group has developed a novel mouse model for SSc by immunizing mice with human AT1R (hAT1R). From this model we were able to generate functional monoclonal antibodies agonizing AT1R.
Objectives: In the current study, we aim to clarify, whether B cells and antibodies directed against AT1R are involved in the pathogenesis of experimental SSc in vivo.

Methods: To investigate the role of B cells in the hAT1R-induced mouse model of SSc, we immunized B-cell deficient mice with hAT1R. Nine weeks after the first immunization, mice were sacrificed and sera and tissues were collected for further evaluation. To investigate the pathogenicity of anti-AT1R antibodies in the disease, monoclonal autoantibodies against hAT1R were applied to the ear of C57Bl/6 mice by single or repetitive injection. Mice were sacrificed 24 hours or 14 days after the first injection for single and repeated application, respectively, and ear and lung tissues were collected for further evaluation.

Results: Compared to the wild type C57Bl/6 mice, hAT1R-immunized B-cell deficient mice were resistant against experimental SSc with regard to autoantibody production, inflammation in the lung and skin, and skin fibrosis. Furthermore, both single and repetitive injection of monoclonal antibodies against hAT1R induced inflammation in ears of mice. Despite this local effect, repetitive injection of anti-AT1R monoclonal antibodies provoked also inflammation in the lung of mice.
Conclusions: Our data demonstrate that i) B cells are indispensable for the pathogenesis of the hAT1R-induced mouse model for SSc and ii) monoclonal antibodies against hAT1R can induce inflammation in mice. Therefore, our results support a role of autoantibodies against AT1R in the pathogenesis of SSc.

49. NKX2-5 contributes to EndoMT and endothelial dysfunction in SSc-PAH

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Background: Inflammation within blood vessels can result in endothelial-to-mesenchymal transition (EndoMT), which is observed in patients with scleroderma-associated pulmonary hypertension (SSc-PAH). The homeobox transcriptional factor NKX2-5 is fundamental for cardiovascular development. However, NKX2-5 expression has not been reported yet in endothelial cells (ECs) of adult pulmonary blood vessels.

Materials and methods: Human pulmonary artery endothelial cells (HPAECs) were treated with a cocktail of TGF-β (5 ng/mL), TNF-α (5 ng/mL), and IL-1β (0.1 ng/mL) for 5 days. Immunofluorescence was used to detect NKX2-5 and other markers in ECs. Western blotting and qPCR evaluated, respectively, protein and gene expression. Lentiviral transduction forced NKX2-5 expression in the cells. Transendothelial electrical resistance (TEER) measurements evaluated endothelial barrier function. Pharmacological inhibition was performed to determine the pathways that lead to NKX2-5 activation. Casein kinase 2 (CK2)-inhibition (CX4945) of a chronic hypoxia mouse model of PAH was used to assess right ventricular systolic pressure (RVSP).

Results: Immunofluorescence showed a strong expression of NKX2-5 in the endothelium of SSc-PAH human lungs ($p<0.0001$). Western blot analysis demonstrated a 5.3-fold downregulation of CD31 ($p<0.001$), and an increased production of NKX2-5 (5.6-fold, $p<0.0001$) and of Procollagen I (12-fold, $p=0.0009$) after 5 days of cytokine stimulation on HPAECs. Relative mRNA expression has shown a 3-fold gene downregulation of CD31 ($p=0.0002$) and a reduction of VE-Cadherin (2.3-fold, $p=0.0008$) and of vWF (10.4-fold, $p=0.003$) in EndoMT, whereas gene expression of COL1α2 (8.5-fold, $p<0.0001$) and of NKX2-5 (1.5-fold, $p=0.003$) were upregulated. Immunofluorescence of cells has revealed a decreased VE-Cadherin expression concomitant with upregulation of NKX2-5 in EndoMT cells. Forced expression of NKX2-5 downregulated endothelial markers and endothelial barrier function was impaired whereas proliferation rate of cells was increased. Inhibition of PI3K, ERK5, ALK5 and CK2 reduced NKX2-5 protein expression within cells. CK2-inhibited mice under hypoxia conditions resembled the normoxia mice group by normalising RVSP.

Conclusions: HPAECs undergoing EndoMT express NKX2-5 in vitro and in vivo, via mediation of CK2, TGF-β, ERK5 and PI3K signalling. NKX2-5 downregulates key adherence junctional proteins, disrupting endothelial barrier function. This study highlights the involvement of NKX2-5 in EndoMT and in endothelial dysfunction, leading to vascular disease progression in SSc-PAH.

50. Identification of ZNF416 as a Novel Mechanoregulator of Lung Fibroblast Activation

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Introduction: Myofibroblasts are critical for tissue homeostasis and remodeling. However, if their activation is sustained, these cells can lead to fibro-contractile diseases such as pulmonary fibrosis. Increased tissue rigidity has been shown to accompany and promote fibrosis in multiple organs, including lungs, by amplifying pro-fibrotic signaling while also diminishing anti-fibrotic signaling. Recent evidence from our lab indicates matrix stiffness directs epigenetic changes in fibroblasts, but whether matrix stiffness regulates fibroblast activation by altering chromatin accessibility is currently unknown.

Materials and Methods: To address whether matrix rigidity alters chromatin accessibility of lung fibroblasts, we employed fibroblasts freshly isolated from Col1a1-GFP+ mice. Using fluorescence assisted cell sorting (FACS), we isolated lung mesenchymal cell populations that were CD45-, CD31-, CD326-, and Col1a1-GFP+. Cells were seeded on collagen I coated tissue-culture plastic or 0.2 kPa PDMS substrates for 8 days, after which ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) was performed. To evaluate ZNF416's role in human lung fibroblasts, ZNF416 was knocked down using siRNA for 72 hours after which RNA and protein was collected for analysis.

Results and Discussion: We found that culture on tissue culture plastic relative to soft PDMS increased chromatin accessibility at 2,114 sites in the genome and decreased accessibility at zero sites. Analysis of the genomic loci exhibiting increased accessibility demonstrated statistical enrichment for key fibrotic pathways. Transcription factor motif analysis of the 2,114 genomic loci of increased chromatin accessibility identified ZNF416 as a putative novel transcriptional effector of mechanosignaling in lung fibroblasts. Knockdown of ZNF416 in human lung fibroblasts reduced transcript levels for pro-fibrotic genes (e.g. *YAP1*, *ACTA2*) and increased transcript levels for anti-fibrotic genes (e.g. *PGC1α*) suggesting it plays a key role in lung fibroblast homeostasis.

Conclusions: We conclude that matrix rigidity globally alters chromatin accessibility of key pro-fibrotic pathways in the mouse genome. Motif enrichment of these chromatin sites revealed ZNF416 as a novel key regulator of lung fibroblasts. Knockdown of ZNF416 in human lung fibroblasts demonstrated that it plays a role in the transcription of pro- and anti-fibrotic genes, consistent with ZNF416 contributing to lung fibroblast function and responses to matrix stiffness.

51. Prolyl 3-hydroxylase 2 IS a candidate gene in scleroderma involved in collagen synthesis and fibrosis

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Background: The prolyl 3-hydroxylase 2 enzyme encoded by *P3H2* is believed to play a role in modifying proline residues in the helical region of procollagen chains, facilitating triple helical chain alignment prior to secretion. We aimed (i) to investigate any possible association between *P3H2* genetic polymorphisms and SSc susceptibility and (ii) to assess the role of *P3H2* in experimental fibrosis.

Methods: In order to explore genetic associations with *P3H2*, four SNPs; rs7612998, rs1447936, rs1018343 and rs696065 were analysed in DNA samples from SSc patients (n=3564) and healthy controls (n=6606). Fibrosis was modelled *in vitro* using dermal fibroblasts from SSc patients treated with TGFβ or estradiol or *in vivo* using the bleomycin skin fibrosis mouse model that were either wild type (WT), single knockout (SKO) or double knockout (DKO) for *P3H2*. Dermal fibroblasts from WT, SKO and DKO mice were cultured from explants and the secreted collagen analysed by atomic force microscopy nano-indentation and by Western blotting.

Results: An association between SSc susceptibility and the minor allele at rs696065, was identified (p=0.0038, OR 0.89, 95% CI 0.82-0.96). SNP analysis demonstrated that a haplotype, identified as CTAA across the 4 SNPs, was associated with increased risk of SSc development (OR 3.45, p<0.0023). In cultured dermal fibroblasts, P3H2 protein levels were raised in SSc samples and induced to SSc levels in control fibroblasts by treatment with the pro-fibrotic growth factor TGF-β, or by the addition of estradiol. Preliminary experiments demonstrated reduced triple helical collagen I synthesis in *P3H2* deleted cells. In genetically modified *P3H2* knockout mice, resistance to bleomycin skin fibrosis was seen, and cultured explants of dermal fibroblasts exhibited selectively reduced triple helical collagen I and reduced secreted ECM stiffness (WT had a median Young's modulus of 781.66 ± 16.09 MPa, SKO 750.69 ± 15.71 MPa, and DKO 142.61 ± 2.45, p<0.05 vs WT MPa.

Conclusions: Taken together, these results indicate a possible role for *P3H2* in SSc pathogenesis and support the notion that this enzyme is involved in triple helical collagen I synthesis in skin fibroblasts. Therapies which target the enzyme or inhibit *P3H2* expression might help reduce pathologic fibrosis in SSc.

52. EphB2 receptor tyrosine kinase promotes dermal fibrosis in systemic sclerosis

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Background: Systemic sclerosis (SSc, scleroderma) is an autoimmune disease characterized by fibrosis of the skin and internal organs. We previously reported that the receptor tyrosine kinase EphB2 is involved in liver fibrosis. However little is known about the implication of EphB2 in skin fibrosis during SSc. The goal of this study is to test the hypothesis that EphB2 activation could promote skin fibrosis in SSc.

Methods: The study received approval from the institutional review board of the University of Utah. Immunofluorescence confocal microscopy was used to detect EphB2, phospho-EphB1/B2 and αSMA in skin biopsy specimens of healthy volunteers (n=3) and patients with SSc (n=8). The regulation of EphB2 expression on primary human dermal fibroblasts following TGFβ-1 stimulation in the presence or absence of TGFβ/SMAD inhibitors (SB525334 and SIS) and MAPK inhibitor U0126 was evaluated by western blot, quantitative PCR, and immunofluorescence. CRISPR/Cas9 system was used to knockout EphB2 in human dermal fibroblasts and expression of pro-fibrotic genes were evaluated by qPCR and western blot following TGFβ-1 exposure. ELISA was used to detect serum EphrinB2, a ligand for EphB receptors in healthy (n=40) and SSc patients (n=132). Finally, histology, hydroxyproline levels, qPCR and western blots were used to assess the development of skin fibrosis in *EphB2-/-* and *EphB2+/+* mice following subcutaneous injection of bleomycin to induce skin fibrosis.

Results: Using immunofluorescence confocal microscopy, we showed that EphB2 and phospho-EphB1/B2 are highly expressed in skin biopsies of patients with SSc compared to healthy control skin biopsies and partially co-localized with the fibroblast marker αSMA. *EphB2* was also elevated in some gene expression data sets of SSc patients. Normal human dermal fibroblasts upregulate EphB2 upon TGF-β exposure and this is potentially mediated via the canonical TGF-β/SMAD signaling. Plasma EphrinB2, a ligand for EphB2 is elevated in patients with SSc. Finally, we showed that EphB2 is a critical promoter of skin fibrosis in mice because *EphB2-/-* mice exhibit a drastic reduction of bleomycin-induced dermal fibrosis compared to their +/+ wild-type littermate controls.

Conclusions: Our findings unveil novel information regarding the potential implication of EphB2 receptor signaling during SSc fibrosis in both human and mice.

53. Increased Expression of CD62L in Systemic Sclerosis Monocytes May Indicate Homing to Tissues

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Background: An altered monocyte transcriptome profile was reported in patients with systemic sclerosis (SSc). Circulating monocytes/macrophages expressing M2 and M1/M2 surface markers could distinguish SSc patients from healthy controls (HC). A recent report identified classical CD14+ monocyte percentage as a promising biomarker for outcome in fibrotic diseases, among others, also in SSc. The purpose of the present study was to investigate the expression of selected surface molecules in different monocyte subsets from SSc patients and correlate them with the presence of SSc-related anticentromere (ACA) and anti-topoisomerase antibodies (ATA).

Materials and methods: Monocytes from 10 SSc patients (5 ACA and 5 ATA positive) and 10 age- and sex-matched HC were analysed using flow cytometry. Monocyte subsets were subsequently identified and tested based on their expression of CD14 and CD16 as classical (CD14++, CD16-), intermediate (CD14++, CD16+) and non-classical (CD14+, CD16++) markers. Surface expression of CD11b, CD62L, CD163 and CD169 was determined in each subset. Presence of ACA and ATA was detected in patients' sera using anti-nuclear antibodies HEp-2 test (Immuno concepts, USA) and *in house* counter immunoelectrophoresis, respectively.

Results: We observed higher surface expression of CD62L in monocytes from SSc patients compared to HC (MFI ± SD 30.74±8.70 vs. 13.51±6.55, $p<0.0001$), with the largest significant change observed in classical (36.11±11.10 vs. 15.92±7.41, $p<0.0001$), followed by non-classical, (1.32±0.55 vs. 0.90±0.25, $p<0.05$), but no change in the intermediate subset. No difference was observed in the surface expression of CD11b, CD163 and CD169. None of the surface markers correlated with ACA or ATA in any of the subsets. The frequency of total monocytes and monocyte subsets was not altered in SSc vs. HC, or in ACA vs. ATA positive patients.

Conclusions: The expression of CD62L was highly increased in monocytes from SSc patients, most significantly in the classical monocyte subset, independently of ACA or ATA. These findings indicate that CD62L, which has a role in extravasation of monocytes during inflammation, might lead to enhanced recruitment of these cells to tissues. Targeting this process might enlighten novel treatment strategies in SSc.

54. Defining the transcriptional profile of the skin in pediatric localized scleroderma (LS)

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Background: Scleroderma is an autoimmune disorder involving inflammatory driven fibrosis which encompasses systemic sclerosis (SSc) and localized scleroderma (LS). LS and SSc share histological characteristics, inflammatory, and fibrotic processes, but given divergent clinical phenotypes likely have unique pathways involving interaction between inflammation and fibrosis. Recent research indicates that T cells, macrophages, and related cytokines interact with fibroblasts to initiate an inflammatory phase followed by fibrosis. Identifying inflammatory cells expressing IFNγ-associated genes of interest and unique fibroblast populations will allow mechanistic studies of inflammatory-driven LS fibrosis, leading to more effective therapies.

Materials and Methods: Single cell RNA sequencing (scRNAseq) was performed on fresh and freshly frozen CryoStor® preserved skin (n=11 LS; 5 peds & 6 adult, n=10 healthy). Samples were collected under IRB #PRO11060222. Library preparation was done using a 10X Genomics® Chromium instrument and sequencing performed on Illumina NextSeq or HighSeq instruments. ScRNA-seq reads were examined for quality then transcripts were mapped to reference human genome GRCh38 and assigned to cells of origin using the Cell Ranger pipeline (10X Genomics®). R-language analyses using Seurat identified and visualized distinct cell populations by clustering methodologies.

Results: LS and healthy enzymatically digested skin cells clustered into 28 unique cell population groups. Inflammatory genes in LS populations were highly expressed in 3 main clusters: endothelial, lymphocyte/NK, and macrophage/DC. Sub-clustering showed high IFNγ signaling with CXCR3 and related chemokines (CXCL9 and CXCL10) being expressed from T cell and M1/M2 macrophages. Since chemokine expression from these inflammatory cells might stimulate fibroblasts and affect the degree of LS fibrosis, fibroblast populations were also studied. LS cells formed unique fibroblast clusters defined by COL1A1, SFRP2, and CXCL12 that expressed both inflammatory genes, like CXCL9 and 10, and fibrotic genes, like IGFBP5.

Conclusions: IFNγ associated gene transcripts including CXCR3 ligands (CXCL9 and CXCL10) are prevalent in macrophage, lymphocyte, and fibroblast populations in LS skin. The unique fibroblast subsets expressing these CXCR3 ligands, only found in LS skin, co-express reticular dermis fibroblast markers. Progression of inflammatory expression of these populations will be further investigated using advanced analysis techniques to determine the cellular trajectory and interaction of LS cells.

55. Antibodies Versus Skin Fibrosis Extension In Systemic Sclerosis: A Case-Control Study

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Background: Little is known of anti-centromere antibodies (ACA) diffuse cutaneous systemic sclerosis (dSSc) and of anti-Scl70 antibodies (Scl70) limited cutaneous systemic sclerosis (lSSc).

Materials and Methods: A retrospective monocentric case control study of patients with SSc fulfilling ACR/EULAR classification criteria was performed. Patients with dSSc and ACA or patients with lSSc and Scl70 were included in the study and compared to patients with ACA lSSc and Scl70 dSSc.

Results: Of 1040 patients followed in Cochin University Hospital Internal Medicine department, 12 (1.1%) patients had ACA dSSc and 93 (8.9%) patients had Scl70 lSSc. Patients with ACA dSSc had a more severe disease than patients with ACA lSSc including a larger skin extension ($p<0.01$) and more frequent organ involvement ($p<0.05$) than patients with ACA lSSc. Oppositely, these patients had less frequently interstitial lung disease (ILD) ($p<0.01$) than patients with Scl70 dSSc. After a median follow-up of 5 years, patients with ACA dSSc remained with a more important skin extension ($p<0.01$) than patients with ACA lSSc and with less frequent ($p=0.05$) ILD than patients with Scl70 dSSc. Patients with Scl70 lSSc had a more severe disease than patients with ACA lSSc including a more important skin extension ($p< 0,0001$) and more frequent ILD ($p< 0,0001$) than patients with ACA lSSc. Oppositely, these patients had a more limited skin extension ($p< 0,0001$) and less organ involvement ($p<0.01$) than patients with Scl70 dSSc. After a median follow-up of 5 years, patients with Scl70 lSSc remained with more frequent ($p< 0,0001$) and more severe ($p<0.0001$) ILD than patients with ACA lSSc but less severe ($p<0.01$) than Scl70 dSSc patients. Patients' survival rate was different between the four groups, Scl70 dSSc patients having the worst prognosis ($p< 0.01$). Interestingly, ACA SSc patients' survival did not differ from Scl70 SSc patients' survival ($p=0.122$) whereas dSSc patients' survival differed from lSSc patients' survival ($p< 0.001$).

Conclusion: Scl70 lSSc and ACA dSSc are rare subgroups of SSc and show intermediate patterns of SSc. Scl70 antibodies are associated to ILD regardless of skin extension which determines the severity and the prognosis of the disease.

56. Histologic Features Correlate with the Modified Rodnan Skin Score, Serum Inflammatory Markers, and Patient Reported Outcomes in Patients with Early, Diffuse Cutaneous Systemic Sclerosis

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Background: The association between systemic sclerosis (SSc) skin histology and clinical findings is not fully characterized. In two trials, we developed a scoring system to evaluate histologic change. The purpose of this study is to determine (1) reliability of histology scores and (2) if histologic features correlate with clinical findings.

Materials and methods: Skin biopsies were analyzed from Nilotinib and Belimumab trials. Our scoring approach assesses thickness, follicle count, infiltrate, collagen density, alpha-smooth muscle actin (aSMA) and CD34 staining, and global histologic severity. Blinded pathologists (CM, YZ) scored biopsies. Intraclass correlation coefficients (ICC) were calculated for reliability. Spearman correlations were used to correlate histology scores and clinical variables (local and total modified Rodnan skin score (MRSS), erythrocyte sedimentation rate (ESR), c-reactive protein (CRP), 36-item Short Form Health Survey (SF-36), and physician global assessment (PGA)).

Results: 56 biopsies were analyzed from 26 SSc patients (median (IQR) disease duration 0.8 (0.54) years). Median (IQR) baseline MRSS was 25 (9). Reliability was excellent for follicle count and intra-rater CD34; good for thickness, inter-rater CD34, intra-rater collagen, and intra-rater global score; and moderate for infiltrate, intra-rater collagen, aSMA, and interrater global.

Histologic scores correlated moderately with MRSS, ESR, CRP, and SF-36 (Table 1). Post-treatment local MRSS correlated with thickness (r=0.674, p< 0.001), collagen density (r=0.497, p=0.04), global histologic severity (r=0.467, p=0.02), follicle count (r=-0.550, p=0.01), and CD34 staining (r=-0.653, p=0.001). Local MRSS did not correlate with infiltrate or PGA and weakly correlated with aSMA staining (r=0.337, p=0.01). In stratified analysis, collagen density correlated with local MRSS in patients with low but not high infiltrate score (r=0.578, p<0.001 vs. r=0.253, p=0.256). Total MRSS correlated with collagen density (r=0.425, p=0.001) and CD34 staining (r=-0.460, p< 0.001). Total MRSS and thickness correlated positively in patients with low infiltrate (r=0.326, p=0.05) but negatively in patients with high infiltrate (r=-0.456, p=0.03).

Conclusions: Our scores demonstrate moderate – excellent reliability. Histologic features correlate with MRSS, inflammatory markers, and patient reported outcomes. However, infiltrate does not correlate with MRSS and confounds the correlation of MRSS with collagen density and thickness. This supports further study of skin histology as an SSc outcome measure.

Table 1. Correlation between clinical variables and histologic features in diffuse systemic sclerosis

Clinical Feature	Significant* Spearman's Correlation Coefficients (r_s) for Histologic Features	p-value
Local modified Rodnan skin score	Collagen density (0.470)	<0.001
	CD34 staining intensity (-0.518)	<0.001
	Post-treatment thickness (0.674)	<0.001
	Post-treatment collagen density (0.497)	0.04
	Post-treatment global histologic severity (0.467)	0.02
	Post-treatment follicle count (-0.550)	0.01
	Post-treatment CD34 staining intensity (-0.653)	0.001
Total modified Rodnan skin score	Collagen density (0.425)	0.001
	CD34 staining intensity (-0.460)	<0.001
	Baseline infiltrate score (0.435)	0.03
	Post-treatment thickness (0.486)	0.02
	Post-treatment collagen density (0.425)	0.04
	Post-treatment CD34 staining intensity (-0.487)	0.02
C-reactive protein	Thickness (0.409)	0.01
	Post-treatment thickness (0.655)	0.002
	Post-treatment global histologic severity (0.475)	0.04
	Post-treatment CD34 staining intensity (-0.539)	0.02
Erythrocyte sedimentation rate	Follicle count (-0.430)	0.001
	Baseline follicle count (-0.410)	0.04
	Post-treatment thickness (0.526)	0.01
	Post-treatment CD34 staining intensity (-0.501)	0.01
36-Item Short Form Health Survey (SF-36)	Post-treatment global histologic severity (-0.567)	0.004

Legend: If no time point specified, reported data are from combined baseline and post-treatment samples.
*$p \leq 0.05$ and $r_s \geq 0.4$.

57. Eosinophilic fasciitis, a monocentric retrosepctive study of 30 patients

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Background: To describe clinical, histological features, treatments and outcome of 30 patients with eosinophilic fasciitis (EF).

Material and Methods: We reviewed the files from 30 patients with EF followed in the Internal Medicine and/or Dermatology departments of Cochin hospital. Clinical and histological data, treatment regimens and outcome were collected.

Results: Thirty patients were included of age at diagnosis of 52 [37; 61] years old, median [IQR] and follow-up of 63[36; 107] months. At the time of diagnosis, 28 patients (93 %) had skin sclerosis, 20 (67 %) had edema and 19 (63 %) had weight loss and 6 (20%) had morphea associated. Hypereosinophilia was present in 22 (73 %) of patients. Fascia biopsy was performed in 26 (87 %) patients with inflammatory infiltrates in all of patients and interstitial myositis in 7/17 (41 %). Muscle MRI was performed in 19 (63 %) patients, showing fascia hypersignal in 14/19 (74 %). All patients except one received corticosteroids (CS) and 14 (47%) developed CS dependence. Twenty-two patients (73 %) received methotrexate (MTX), 7 patients (32 %) as first line treatment in combination with CS, 14 (64 %) patients after CS initiation and 6 (27 %) after failure of another treatment. Under MTX, 14 (64 %) patients had weaning of CS therapy, 3 (14 %) were in remission and 14 (64 %) improved.

Conclusion: Our retrospective study highlights importance of early initiation of CS sparing treatment in patients with EF. MTX seems to be the most effective immunosuppressant in this indication.

58. A 3D human triculture system models immune activation of cutaneous fibrogenesis in systemic sclerosis

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Background: The development of precision therapeutics for Systemic sclerosis (SSc) has been hindered by lacking models that accurately mimic human disease and clinical heterogeneity in SSc patients. Previously, we used two complimentary bioengineered models, human skin equivalents (hSE) and self-assembled tromal tissues (SAS), to study profibrogenic mediators by incorporating SSc dermal fibroblasts (SScDFs) into these 3D skin-like systems and compare them to normal dermal fibroblasts (NDFs). To recapitulate complexity of cutaneous fibrogenesis, we developed an innovative 3D self-assembled skin equivalent (saSE) that not only retains both the epithelium and stroma crosstalk in hSE and *de novo* ECM synthesized by fibroblasts in SAS, but also incorporates monocytes to allow polarization of SSclike macrophages in a 3D skin-like microenvironment.

Methods: SScDFs and NDFs were mixed with SSc monocytes (SM, N=6) or control monocytes (CM, N=6) respectively, followed by the addition of SM/CM donor-matched plasma for one week. Keratinocytes were then seeded to establish saSE with fully stratified epithelium. IHC was used to assess tissue morphology, ECM deposition and macrophage differentiation. Atomic force microscopy was used to measure the stromal rigidity of saSE. saSE were dissociated using enzymatic digestion to isolate cells for single cell RNA-sequencing (scRNA-seq) and flow cytometry.

Results: saSE with SScDFs generated a thicker and stiffer dermis compared to NDFs, regardless of monocyte addition. Increased dermal thickness was induced by SM compared to CM within saSE containing either SScDFs or NDFs, but no significant change in stromal stiffness was detected with incorporation of SM. Three distinct cell populations were identified by scRNA-seq: CD90+ fibroblasts, CD163+/HLA-DRA+ macrophages, and KRT+ keratinocytes. SScDFs clustered based on differential expressions of collagen remodeling genes, such as DCN/CTSK, COL6A2, COL1A1/MMP1, and fibronectin. Interestingly, one subtype of SScDFs was enriched for expression of CLEC2B, which is consistent with prior observations demonstrating upregulation of inflammatory and innate immune response-related genes in SScDFs compared with NDFs. Additionally, an increase number of CD163+ cells was identified in SScDFs-saSE compared with NDFs-saSE.

Conclusion: saSE may serve as a new platform for preclinical therapeutic testing and molecular characterization of individual SSc patients by recapitulating interactions observed *in vivo* between macrophages and fibroblasts.

59. Link between metabolic and endothelial factors with antinuclear antibodies in systemic sclerosis

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Background: Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by vascular impairment, prolonged inflammation and progressive fibrosis. One of the major features of immune dysregulation are autoantibodies which are associated with distinct clinical subsets of the disease. In recent years several proteins including adipokines (mediators of importance in metabolism synthetized mostly by adipose tissue) and signaling molecules secreted by endothelial cells were reported to have a possible association with SSc pathogenesis. The aim of this study was to investigate serum level of metabolic adipose tissue factors: adiponectin, resistin, leptin and endothelial proteins: endothelin-1, fractalkine and galectin-3 in correlation with particular antinuclear antibodies and to evaluate the role of these proteins in the pathogenesis of SSc subtypes.

Materials and methods: Concentrations of adiponectin, resistin, leptin, endothelin-1, fractalkine and galectin-3 were determined in the sera of SSc-patients (n=100) and healthy controls (n=20) using ELISA (R&D Systems, Minneapolis, MN, USA). Antinuclear antibodies were detected on indirect immunofluorescence and confirmed by immunoblotting. The results were assessed by the Mann-Whitney *U*-test and Spearman's correlation test.

Results: The incidence of the most prevalent antinuclear antibodies detected by immunoblotting presented as following: anti-centromere (ACA, 44%), anti-topoisomerase I (anti-TOPO I, 40%), anti-RNA polymerase III (anti-RNAP III, 20%), anti-Ro52 (15%), anti-PM/Scl (9%). The strongest correlations between increased concentrations of particular factors in SSc-patients versus healthy controls were determined as following: endothelin-1 with ACA (r=0.47, p=0.000275, median 2.21 vs 1.31 pg/ml), fractalkine with anti-TOPO I (r=0.64, p=0.00001, median 3.68 vs 1.68 ng/ml), galectin-3 with anti-TOPO I (r=0.45, p=0.001, median 6.39 vs 3.26 ng/ml), leptin with anti-PM/Scl (r=0.44, p=0.028, median 31677 vs 14210 pg/ml), resistin with anti-RNAP III (r=0.66, p=0.00003, median 15.13 vs 8.54 ng/ml). The most decreased concentration of adiponectin was found in patients with anti-RNAP III antibodies (r=-0.75, p=0.00002, median 2894 vs 8847 ng/ml).

Conclusion: In systemic sclerosis with anti-TOPO I and anti-RNAP III, both metabolic and endothelial dysfunction contribute to the disease pathogenesis, whereas in ACA-positive subset the most prevalent is vascular impairment. In patients with anti-PM/Scl the major cause may be connected with inflammation process as correlation with leptin has shown.

60. Identification of Transcriptional Regulatory Networks in Systemic Sclerosis

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Background: Gene expression associated with inflammation and innate immune system activation exists in multiple affected organ systems in systemic sclerosis (SSc). The networks of activating transcription factors (TFs) and microRNAs that underlie SSc molecular subsets or are associate with specific clinical co-variates have not been characterized in detail. Here we analyzed transcriptional regulatory networks underlying the intrinsic subsets of SSc and their associated clinical covariates.

Materials and methods: Gene expression profiles were downloaded from Milano *et al* (75 samples), Pendergrass *et al* (89 samples), Hinchcliff *et al* (165 samples), and Assassi *et al* (102 samples). The C3 target gene set (836 regulators) was downloaded from the MSigDB. Gene expression and target gene profiles were integrated using the BASE algorithm to calculate regulator activity scores. TF activity scores were positively correlated and miRNAs activity scores were negatively correlated with gene expression. Correlations were calculated between activity scores and samples' mRSS in each dataset. Clinically relevant regulators were identified with the top 50 highest mean correlations.

Results: Clinically relevant regulators were identified for inflammatory and fibroproliferative patients in all datasets. Examples include SMAD4 and NFAT for inflammatory, and MYC and FOX TFs fibroproliferative samples. A clinically relevant regulator interaction network was created to provide novel insights for SSc and visualize the connectivity between different components of the regulator network. A more severe subgroup of patients within the inflammatory intrinsic subset was identified by analyzing regulator pairs. Inflammatory patients with higher SMAD4 and NFAT activities, demonstrating strong fibrotic and immune activation, had significant more severe skin and lung disease than samples with low expression of both pathways ($P<0.01$).

Conclusions: Using the activities of specific transcriptional regulators, we identified a subset of patients within the intrinsic subsets that show increased expression of innate immune and fibrotic pathways. Patients with high activities of these two pathways had increased disease severity. This further highlights the role of innate immune and fibrotic transcriptional programs in the pathogenesis of SSc. The association between the activation of regulators and multiple clinical variables of SSc could be applied to personal diagnostic and treatment paradigms for patients with SSc.

61. Metabolic Intermediate Alpha-Ketoglutarate Attenuates TGFβ-driven Profibrotic Responses of Dermal Fibroblasts

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Background: Metabolic perturbations are emerging as drivers of fibroblast activation in fibrosis. Our aim was to identify metabolic alterations in fibroblasts that could associate with fibrotic tissue process. Additionally, we studied how key metabolic intermediates, like dimethyl alpha-ketoglutarate (αKG) influence the profibrotic responses in dermal fibroblasts (DF).

Methods: We analyzed publicly accessible transcriptomic data (GSE40839**)** from lung fibroblasts of healthy controls (HC) and patients with systemic sclerosis (SSc), using metabolic KEGG gene sets and STRING protein networks for gene set enrichment analysis (GSEA). Human DF from HC (n=3-7) and SSc patients (n=4-8) were treated with TGFβ and/or αKG (6 mM). Gene expression was analyzed by qPCR. Protein amounts were measured with Western blot. Apoptosis was assayed with flow cytometry (Annexin V assay). Contractile properties of DF were assessed by gel contraction assay. Significance ($p<0.05$) was determined by one sample t test or ANOVA with Tukey's correction.
Results: Bioinformatic analysis of GSE40839 dataset showed enrichment of glycolysis pathway ($p = 0.0007$) and altered expression of genes from tricarboxylic acid (TCA) cycle (IDH2, ACLY) and oxidative phosphorylation (ATP6V0B, ATP5G1) in lung fibroblasts of SSc patients. In line with this, TGFβ-treated DF significantly upregulated mRNA expression of the core components of glycolysis (*GLUT1, PGK1, PGAM1, ENO, LDHA*) and the TCA cycle (*SUCLA, MDH)*. Additionally, the mRNA expression of *HIF1α*, an inducer of metabolic reprogramming, was enhanced (Fig. 1, $p=0.0001$) whereas the mRNA expression of *PGC1α*, the regulator of mitochondrial biogenesis and cellular energy metabolism, was decreased in TGFβ-stimulated DF (Fig. 1, $p<0.0001$). αKG reversed the TGFβ-driven upregulation of *HIF1α* (Fig. 1, $p=0.06$) and significantly repressed the TGFβ-driven profibrotic responses of DF, including the secretion of fibronectin ($p=0.047$) and the production of *αSMA* mRNA ($p=0.07$) and protein ($p=0.02$). αKG reduced the contractile capacity of TGFβ-stimulated DF ($p=0.003$), while had no effect on apoptosis.

Conclusion: TGFβ perturbed the gene expression of key metabolic regulators in DF. Meanwhile, modulating cell metabolism with αKG, the key TCA cycle intermediate, attenuates the TGFβ-driven profibrotic responses of DF. This suggests an intimate crosstalk between metabolic and fibrotic pathways in skin. Targeting perturbed metabolism could offer novel anti-fibrotic strategies in SSc.

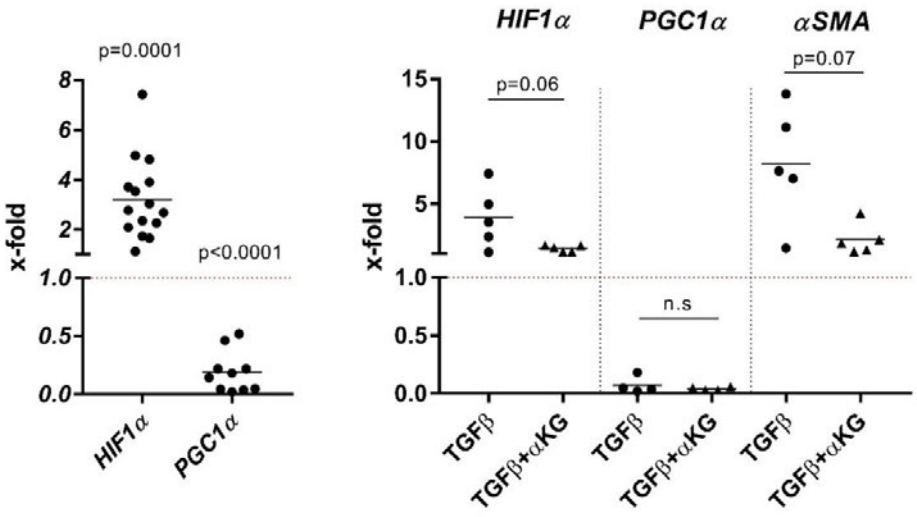

Figure 1. Gene expression analysis of *HIF1α*, *PGC1α* and *αSMA* in dermal fibroblasts stimulated with TGFβ in the presence or absence of alpha-ketoglutarate (αKG). The results are shown as x-fold change of normalized gene expression vs. untreated cells (set to 1, as denoted with red line).

62. The Diversity and Community Metrics of the Esophageal Microbiome of SSc Patients

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Background: Systemic Sclerosis (SSc) is an autoimmune disease where organ systems including the skin, gastrointestinal tract, vasculature, and lungs are affected. Gene expression analyses have identified four distinct molecular subtypes (inflammatory, fibroproliferative, normal-like, and limited). Microbial dysbiosis has been implicated previously in SSc skin and the lower GI tract, but esophageal microbial characterization remains understudied. This work assesses the esophageal microbial diversity and community patterns in SSc patient samples.

Materials and Methods: Metagenomic reads were extracted from 20 SSc patient and 3 healthy control upper and lower esophageal tissue biopsies using Integrated Metagenomic Sequence Analysis (IMSA). After non-spurious hits were removed, samples were rarefied to the lowest read count. Metrics such as the alpha diversity, a measure of species richness and the Firmicutes/Bacteroidetes ratio (associated with gut inflammatory diseases), were taken. Partition Around Medoids (PAM) clustering based on k-clusters was executed to assign patient microbiomes into groups based on metagenomic features. Analyses were executed in R v3.4.1.

Results: The alpha diversity of SSc samples was lower than that of control samples across the upper and lower esophagus (Wilcoxon Signed-Rank Test, n.s). When stratified by esophageal site and molecular subtype, the alpha diversity was higher in upper esophageal inflammatory samples (Kruskal-Wallis Test, $p<0.05$) and slightly elevated in lower esophageal normal-like and fibroproliferative samples. The Firmicutes/Bacteroidetes ratio, typically higher when inflammation is present, was elevated in SSc patient samples compared to healthy controls. When stratified by SSc molecular subtype, Firmicutes/Bacteroidetes ratios were higher in SSc upper esophageal normal-like and fibroproliferative samples, and higher in SSc lower esophageal inflammatory and normal-like samples (Kruskal-Wallis test $p<0.05$ and n.s., respectively). Samples clustered to two groups regardless of site, and there is a significant association between molecular subtype and cluster membership (Fisher's Exact Test, $p<0.05$).

Conclusion: There are microbial profile differences that distinguish SSc and healthy control esophagus, and SSc molecular subtypes. Several significant associations affirm a potential connection between the microbiome and SSc. The microbiome potentially motivates disease etiology and progression, indicating points of potential intervention for individuals with SSc such as restoring microbial diversity or membership.

63. Receptor expression of angiotensin type-1 and 2 are decreased in patients with systemic sclerosis and pulmonary arterial hypertension (PAH) and correlated with serological levels of NT-proBNP

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Background: Previous studies identified functional autoantibodies against the angiotensin receptor type-1 (AT1R) and the endothelin receptor type A (ETAR) in about 85% of the patients with systemic sclerosis (SSc, 1). The antibodies are cross-reactive, agonistic and functionally active by increasing the effects of the natural ligands as well as by specific activation of the receptors (2, 3). The levels of the antibodies are associated with clinical findings such as pulmonary arterial hypertension (PAH). Patients with highest antibody levels show worst prognosis and do not respond well to receptor blocker therapy (2-4). Several in vitro effects of the antibodies depend on the antibody levels and on the cell type bearing the receptors. Receptor expression of ETAR and AT1R was highest in early disease (3).

Materials and methods: The current study analyzed the serological levels of anti-AT1R and anti-ETAR antibodies and the extracellular and intracellular expression of AT1R, AT2R, ETAR and ETBR on circulating CD4pos T cells, CD8pos T cells, CD14pos Macrophages, CD15pos Granulocytes and CD19pos B cells in SSc (n=41) using sandwich ELISA and flow cytometry. Clinical data (PAH, history of digital ulcers, digital-ulcers score, mRSS, pulmonary fibrosis, therapy) and serological markers (ESR, CRP, NT-proBNP) were gathering at the time of serum sampling and every three-month up to 27month after baseline.

Results: Patients with PAH demonstrated a lower AT1R MFI and AT1R/AT2R MFI ratio on all PBMC. Levels of NT-proBNP correlated negatively with the AT1R MFI and AT1R/AT2R MFI ratio on all PBMC. The levels of anti-AT1R ab correlated with the NT-proBNP in SSc patients with levels of NT-proBNP <300pg/ml. AT1R MFI on T cells, Granulocytes and Macrophages distinguished between pathological and physiological levels of NT-proBNP. Using Log-rank test and Mantel-Cox proportional hazards model, decreased expression of AT1R MFI on T cells, Granulocytes and Macrophages identified trends of deterioration of NT-proBNP over 50%.

Conclusion: Expression of AT1R and AT2R on PBMC could be of diagnostic value identifying clinical progress and/or subgroups in SSc. Their role in the pathophysiology, e.g. their impact of endothelial damage, has to be further investigated in SSc.

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64. Pro-fibrotic Activation of Human Macrophages in Systemic Sclerosis

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Background: Genome-wide gene expression studies implicate macrophages as mediators of fibrosis in systemic sclerosis (SSc), but little is known about how these cells contribute to fibrotic activation in SSc. Here, we characterized the activation profile of SSc patient monocyte-derived macrophages and assessed their interaction with SSc patient fibroblasts.

Methods: Plasma and PBMCs were obtained from whole blood of SSc patients and healthy age and gender-matched control subjects. Monocytes were cultured with either autologous or allogeneic plasma for 7 days to differentiate the cells into macrophages. For reciprocal activation studies, macrophages were co-cultured with fibroblasts using Transwells.

Results: Using a bioinformatics approach, we showed that the gene expression profile of blood-derived human SSc macrophages is significantly enriched in SSc patient skin. SSc macrophages expressed surface markers associated with activation and released CCL2, IL-6, and TGF-β under basal conditions. Differentiation of healthy donor monocytes in SSc patient-derived plasma conferred the immunophenotype of SSc patient macrophages, Transwell experiments demonstrated that co-culture of SSc macrophages with SSc fibroblasts induced fibroblast activation.

Conclusion: These data demonstrate the activation profile of SSc macrophages is pro-fibrotic. SSc macrophages are activated under basal conditions, and these cells release mediators and express surface markers associated with both alternative and inflammatory macrophage activation. Moreover, these results suggest that activation of SSc MØs arises from soluble factors in local microenvironments. These studies implicate macrophages as likely drivers of fibrosis in SSc and suggest therapeutic targeting of these cells may be beneficial in ameliorating disease in SSc patients.

65. Change in calcinosis over 1 year using the SCTC Radiologic Scoring System for Calcinosis of the hands in patients with Systemic Sclerosis

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[11]Stanford University School of Medicine and Palo Alto VA Health Care System, Department of Immunology and Rheumatology and Dermatology (by courtesy)

Background: Calcinosis cutis is a debilitating complication of systemic sclerosis (SSc). We previously developed a radiographic scoring system to assess severity of calcinosis affecting the hands in patients with SSc. We sought to further validate our radiographic scoring system to assess for change over 1 year and to identify factors associated with improvement or progression.

Methods: Baseline and 1-year antero-posterior hand radiographs were obtained in 39 SSc patients with calcinosis prospectively enrolled at 6 centers within the US, Canada, Mexico and Australia. Two blinded readers scored all radiographs using the calcinosis scoring system. We defined progressive calcinosis as >10% increase in score from baseline at 1 year, stable calcinosis as change in score between -10% to 10%, and improvement of calcinosis as decrease in score by >10%.

Results: Inter-rater reliability was high with intra-class correlation coefficient of 0.93 (0.89-0.95). The median percentage of change from baseline to 1 year was 12.8% (range -89.3-290.2%). Twenty-one patients (54%) experienced progression of calcinosis over 1 year; 10 (26%) remained stable; and 8 (20%) had improvement (Figure 1). Patients with progressive calcinosis had lower mean modified Rodnan skin score (mRSS) (3.81 vs. 6.5, *p*=0.0446) and lower prevalence of pulmonary artery hypertension (PAH) by right heart catheterization than patients who did not progress (0 % vs 23%, *p*= 0.022)(Table 1). They also exhibited a trend toward having more digital pitting scars (76% vs. 50%, *p*= 0.0892), and arthritis (52% vs. 34%, *p*= 0.0694). Patients whose calcinosis improved had higher mean mRSS (7.13 vs. 4.52, *p*=0.061), less arthritis (0 vs. 50%, *p*=0.0154), greater prevalence of antibodies against PM-Scl (43 vs. 3%, *p*=0.018) and a trend toward having less gastrointestinal disease (50 vs. 84%, *p*=0.0651) than patients whose calcinosis did not improve. In multivariable analysis, a trend for anti-PM-Scl antibodies to be a predictor of calcinosis improvement persisted (OR 13.5, *p*=0.051)(Table 2).

Conclusions: We confirmed the excellent inter-rater reliability of our radiographic calcinosis scoring system. More than half of patients experienced >10% progression of calcinosis over one year; however, 20% of patients improved and these patients were more likely to be positive for the PM-Scl antibody.

Figure 1. Distribution of change in calcinosis score over a year

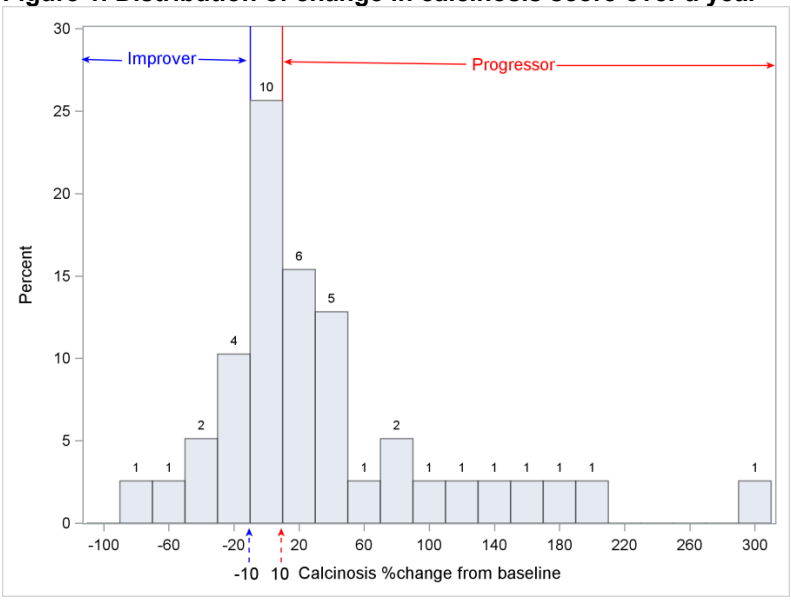

Table 1. Characteristics of patients whose calcinosis progressed and did not progress and whose calcinosis improved and did not improve

	Not progressed n (%)	Progressed n (%)	*p*-value	Not improved n (%)	Improved n (%)	*p*-value
Total	18 (46.15)	21 (53.85)		31 (79.49)	8 (20.51)	
Age (mean years ± SD)	60.1 ± 9.8	58.3 ± 8.7	0.5353	59 ± 9	60 ± 10	0.8685
Female gender	17 (94.4)	16 (76.2)	0.1897	26 (83.9)	7 (87.5)	1
Race			0.8514			
Caucasian	12 (66.7)	15 (71.4)		21 (67.7)	6 (75)	0.1938
Asian	1 (5.6)	0 (0)		0 (0)	1 (12.5)	
Hispanic	5 (27.8)	6 (28.6)		10 (32.3)	1 (12.5)	
SSc Subtype			0.8514			
Diffuse	5 (27.8)	6 (28.6)		8 (25.8)	3 (37.5)	0.7354
Limited	13 (72.2)	15 (71.4)		23 (74.2)	5 (62.5)	
mRSS (mean ± SD)	**6.5 (5.2)**	**3.81 (3.8)**	**0.0446**	**4.52 (4.5)**	**7.13 (5.1)**	**0.0611**
Disease duration from first non-Raynaud Phenomenon (mean years ± SD)	16 (11.5)	17.7 (11.6)	0.6829	16.9 (11.25)	17 (13.00)	0.8483
Raynaud's phenomenon	18 (100)	20 (95.2)	1	30 (96.8)	8 (100)	1
Digital ulcers	5 (27.8)	10 (47.6)	0.323	14 (45.2)	1 (12.5)	0.1214
Digital pitting scars	**9 (50)**	**16 (76.2)**	**0.0892**	21 (67.7)	4 (50)	0.4237
Loss of digital pulp	5 (27.8)	9 (42.8)	0.3278	11 (35.5)	3 (37.5)	1
Abnormal nailfold capillary exam	14 (77.78)	17 (85)	0.821	24 (80)	7 (87.5)	1
Puffy fingers	10 (55.6)	14 (66.7)	0.4771	17 (54.8)	7 (87.5)	0.1214
Sclerodactyly	12 (66.7)	14 (66.7)	1	20 (64.5)	6 (75)	0.6942
Telangiectasias	15 (83.3)	20 (95.3)	0.3183	29 (93.6)	6 (75)	0.1803
Osteopenia or Osteoporosis	3 (25)	9 (45)	0.1175	9 (32.2)	3 (75)	0.42
PAH echocardiogram	3 (16.7)	2 (9.5)	0.8186	4 (12.9)	1 (12.5)	1
PAH RHC	**3 (23.1)**	**0 (0)**	**0.026**	2 (6.9)	1 (25)	0.5139
Pulmonary fibrosis	7 (38.9)	6 (28.6)	0.3969	10 (32.3)	3 (37.5)	1
Any GI involvement	12 (66.7)	18 (85.7)	0.2552	**26 (83.9)**	**4 (50)**	**0.0651**
Myositis	2 (11.11)	0 (0)	0.3911	2 (6.45)	0 (0)	1
Arthritis	**4 (23.5)**	**11 (52.4)**	**0.0694**	**15 (50)**	**0 (0)**	**0.0154**
Positive Scl-70	3 (18.8)	2 (9.5)	0.6419	3 (10)	2 (28.6)	0.3647
Positive Anti-centromere	9 (56.3)	11 (52.4)	1	15 (50)	5 (71.4)	0.7859
Positive PM-Scl	3 (20)	1 (4.8)	0.169	**1 (3.5)**	**3 (42.9)**	**0.0183**
Positive Anti-RNA polymerase III	1 (6.3)	3 (14.3)	0.8025	3 (10)	1 (14.3)	0.319
Positive U1 RNP	2 (13.3)	1 (4.8)	0.3966	2 (6.9)	1 (14.3)	0.3632
Positive ANA	14 (93.3)	20 (95.2)	0.6667	27 (93.1)	7 (100)	1

SD=Standard deviation, mRSS=modified Rodnan Skin Score, PAH=Pulmonary artery hypertension, RHC=Right heart catheterization

Table 2. Predictors for progression and improvement of calcinosis of the hands in multivariable analysis

	OR (95% CI)	*p*-value
Predictors for improvement		
Modified Rodnan Skin Score	1.1 (0.85 - 1.32)	0.6232
Positive PM-Scl	13.5 (0.99 - 183.14)	0.0511
Predictors for progression		
Modified Rodnan Skin Score	0.8 (0.72 - 1.03)	0.1103
Arthritis	3.6 (0.81 - 15.8)	0.0919

66. IL-17A dissociates inflammation from fibrogenesis in scleroderma

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BACKGROUND: Systemic sclerosis (SSc) is an auto-immune, inflammatory, connective tissue disease in which skin fibrosis is a major hallmark. While levels of interleukin-17A (IL-17A) are increased in SSc skin and other organs, the role of this cytokine in SSc pathogenesis is highly debated [1]. Since epithelial cells are preferential targets of IL-17A, we aimed at investigating the crosstalk of IL-17A and TGF-β in the interactions between epidermis and dermis, taking an advantage of keratinocyte-fibroblast interaction model and organotypic skin cultures.

Material and Methods: Primary human keratinocytes were primed with IL-17A and/or TGF-β and conditioned-media were used to stimulate healthy donors (HD) and SSc fibroblasts. Alternatively, organotypic cultures of HD full human skin were challenged with these cytokines. Responses were assessed by quantifying inflammatory mediators, fibronectin and type I collagen (col-I) levels. The factors produced by keratinocytes were identified by a proteomic approach and their contribution was evaluated by neutralization assays. Changes in gene expression in full human skin induced by IL-17A and TGF-β were analysed by high-throughput RNA sequencing (RNAseq).

Results: Keratinocyte-conditioned media (KCM) tilted the balance of col-I to matrix metalloproteinase-1 (MMP-1) production by fibroblasts in favor of MMP-1, significantly more so in HD than in scleroderma resulting in enhanced ECM turnover, further increased by IL-17A. In organotypic skin, TGF-β induced an extensive pro-fibrotic gene signature including the enhanced expression of several collagen genes associated with Wnt signaling. IL-17A strongly promoted the expression of pro-inflammatory genes, with no direct effects on collagen genes, while attenuating Wnt signaling induced by TGF- β. In this model at the protein level, IL-17A significantly decreased col-I production.

Conculsions: Our data strongly support a pro-inflammatory and anti-fibrogenic activity of IL-17A in the context of keratinocyte-to-fibroblast interaction and in full skin. These data help in directing and interpreting targeted therapeutic approaches in scleroderma.

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67. Frequency and predictors of meaningful decline in forced vital capacity during follow up of a large cohort of systemic sclerosis associated pulmonary fibrosis patients

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Background: Pulmonary fibrosis (PF) is common in systemic sclerosis (SSc). Serial pulmonary function tests (PFTs) are used for routine PF monitoring and forced vital capacity (FVC) decline reflects progression in PF. We explore the changes in FVC over time in a cohort of patients with SSc-related PF receiving standard management.

Materials and methods: Only SSc patients with CT-confirmed PF were included. FVC changes over the first 10 years from disease onset were assessed using linear mixed effect models. We analysed time to development of threshold FVC levels on a time scale starting at first available FVC, if this was within 5 years from onset, using Kaplan-Meier estimates and Cox regression.

Results: We identified 505 patients with PF, 21.6% male, 49.3% with diffuse cutaneous SSc (dcSSc). The most common autoantibody was anti-topoisomerase I (ATA) in 40.4% subjects, followed by anti-RNA polymerase in 11.7%, anti-PmScl in 5.2%, anti-centromere in 7.1% and anti-U3RNP in 3.0%; 16.4% had positive ANA, but no ENAs were identified (ANA+ENA-). Mean period between PFTs was 13 months.

Average FVC at 12 months from onset was 80.1%. FVC fluctuated over time, although there was a small but statistically significant absolute decline of approximately 0.32% per year (p=0.007) at a group level. There was no significant correlation between baseline FVC and subsequent change. Multivariable analysis demonstrated significant associations between FVC and age at onset, sex, cutaneous subset and antibodies (Table 1).

The proportion of PF subjects to develop FVC<70%, if they had FVC≥70% at first available test, was 7.7%, 13.6%, 17.2%, 18.7% and 19.8% at 1,2,3,4 and 5 years from first FVC assessment. For FVC<50% this was 2.1%, 4.4%, 5.8%, 7.6%, and 10.5%. In a multivariable model, factors that associated with increased risk for FVC drop below 70% were male sex, ATA positivity and low baseline FVC, while the only predictor of drop in FVC to 50% or lower was low baseline FVC (Table 2).

Conclusion: This study develops a model that may help predict those most at risk of significant decline. We show that ATA positivity, male gender and diffuse subset are associated with greater long-term decline in FVC.

Table 1. Multivariable mixed effect model for FVC

Fixed effects parameters	β	95% CI		p-value
Time, years (centred at 1 year)	-0.50	-1.12	0.13	0.121
Age at onset, years (centred at 45 years)	0.32	0.19	0.45	<0.001
Male	-3.28	-7.69	1.13	0.145
Male*Time (centred at 1 year)	-0.62	-1.19	-0.05	0.034
Diffuse cutaneous subset	-5.57	-9.24	-1.90	0.003
Antibodies				
Anti-RNA polymerase	ref.			
Anti-centromere antibody	-12.48	-21.96	-3.00	0.010
Anti-topoisomerase antibody	-14.60	-20.41	-8.78	<0.001
Anti-U3RNP antibody	-9.09	-20.63	2.46	0.123
Anti-PMScl antibody	-13.40	-22.94	-3.87	0.006
ANA+ ENA-	-15.12	-21.92	-8.32	<0.001
Other antibodies	-15.59	-22.49	-8.70	<0.001
Antibodies*Time (centred at 1 year)				
Anti-RNA polymerase	ref.			
Anti-centromere antibody	1.19	0.03	2.35	0.045
Anti-topoisomerase antibody	0.09	-0.63	0.80	0.811
Anti-U3RNP antibody	0.78	-0.70	2.26	0.301
Anti-PMScl antibody	0.97	-0.30	2.23	0.133
ANA+ ENA-	-0.07	-0.94	0.80	0.873
Other antibodies	1.01	0.18	1.85	0.017
Constant	95.50	89.49	101.52	<0.001
Random-effects parameters				
SD Time	1.79	1.58	2.03	
SD Constant	18.18	16.86	19.60	
Correlation (Time, Constant)	-0.16	-0.30	-0.02	
Residual SD	6.73	6.50	6.96	

Table 2. Multivariable Cox regression analysis for predictors of time to development of FVC<70% and FVC<50% in systemic sclerosis patients with pulmonary fibrosis confirmed on CT.

	HR	95% CI		p-value
FVC<70% prediction				
Baseline FVC	0.84	0.79	0.89	<0.001
Male	1.92	1.16	3.16	0.011
Anti-topoisomerase antibody	1.68	1.05	2.69	0.030
Baseline FVC*Time (years)	1.02	1.01	1.03	<0.001
FVC<50% prediction				
Baseline FVC	0.91	0.89	0.94	<0.001

68. Modelled patient level skin score trajectory predicts risk of death or major organ-based complications in diffuse cutaneous systemic sclerosis

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Background: Skin thickening contributes significantly to diffuse cutaneous systemic sclerosis (dcSSc) morbidity. We explore the association between skin change over time and outcomes in a cohort of early dcSSc patients.

Materials and methods: Subjects with at least one mRss assessment within the first 5 years from onset were included. Random effect models were fitted to evaluate continuous changes in mRss over time. Model-predicted individual patient intercept and slope were used to assess association between absolute mRss at baseline (12 months from onset), mRss change and outcome.

Results: Of the 467 patients, 22.7% were male and mean age of disease onset was 45.5 (SD 13.2) years. Most frequent autoantibodies were anti-topoisomerase I antibody (ATA) in 30.2% and anti-RNA polymerase antibodies (ARA) in 30.0% of the subjects. Other antibodies included anti-U3RNP in 6.9%, anti-PmScl in 4.5%) and 15.6% of the subjects were ANA positive, but ENA negative (ANA+ENA-).
Average mRss at 12 months from onset was estimated to be 25 and there was consistent decline over subsequent years, which slowed down with longer disease duration (3.4, 2.7, 1.9 and 1.2 units at years 2, 3, 4 and 5). There was a weak negative correlation between mRss at 12 months and subsequent change (correlation coefficient -0.3), suggesting higher initial mRss associates with greater subsequent decline **(Table 1).**
Both higher intercept and higher slope predicted increased risk of death with 8% increase in hazard for every unit higher baseline mRss and 4% increase for every unit higher change per year (Table 2). Pulmonary fibrosis and pulmonary hypertension development associated with higher change in mRss, but not with baseline absolute mRss values (3.5% and 7% increase in the hazard respectively for one unit higher mRss change over 12 months). It appeared that higher slope associated with lower hazard of scleroderma renal crisis, while we found no associations between skin and cardiac SSc.

Conclusions: Skin changes over the initial 5 years of disease vary between patients. At a group level there is an improvement, however, for individual patients, slower improvement or deterioration in skin predict increased risk of pulmonary complications and higher mortality rates**.**

69. Nintedanib reduced decline in forced vital capacity across subgroups of patients with systemic sclerosis-associated interstitial lung disease: data from the SENSCIS® trial

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Background: In the SENSCIS® trial, nintedanib reduced the progression of interstitial lung disease associated with systemic sclerosis (SSc-ILD) compared with placebo, as demonstrated by a significantly lower rate of decline in forced vital capacity (FVC) over 52 weeks (primary endpoint). A total of 576 patients were treated (288 in each group). Most (75.2%) of patients were female, 51.9% had diffuse cutaneous SSc, and 48.4% were taking mycophenolate at baseline. Mean ± SD age was 54.0 ± 12.2 years, and 21.4% of patients were aged ≥65 years. Here we assess the effect of nintedanib on the rate of decline in FVC in the SENSCIS® trial across prespecified subgroups defined by baseline characteristics.

Materials and methods: Patients with SSc-ILD with onset of first non-Raynaud symptom <7 years before screning and ≥10% fibrosis of the lungs on a high-resolution computed tomography scan were randomised to receive nintedanib 150 mg twice daily or placebo double-blind. The annual rate of decline in FVC (mL/year) assessed over 52 weeks (primary endpoint) was analysed in the overall population using a random coefficient regression model (with random slopes and intercepts) including anti-topoisomerase I antibody status, age, height, gender and baseline FVC as covariates and treatment-by-time and baseline-by-time interactions. Analyses in subgroups by baseline characteristics included terms for treatment-by-subgroup and treatment-by-subgroup-by-time interaction.

Results: Generally, nintedanib had a consistent effect on reducing the rate of FVC decline across prespecified subgroups defined by baseline characteristics (P>0.05 for all treatment-by-time-by-subgroup interactions) (Figure). The treatment effects estimates were comparable to the estimates of the primary analysis, and the confidence intervals were overlapping. The analysis did not indicate a difference in the treatment effect of nintedanib across all subgroups assessed.

Conclusion: Nintedanib is effective at reducing ILD progression in a broad range of patients with SSc-ILD.

Figure:

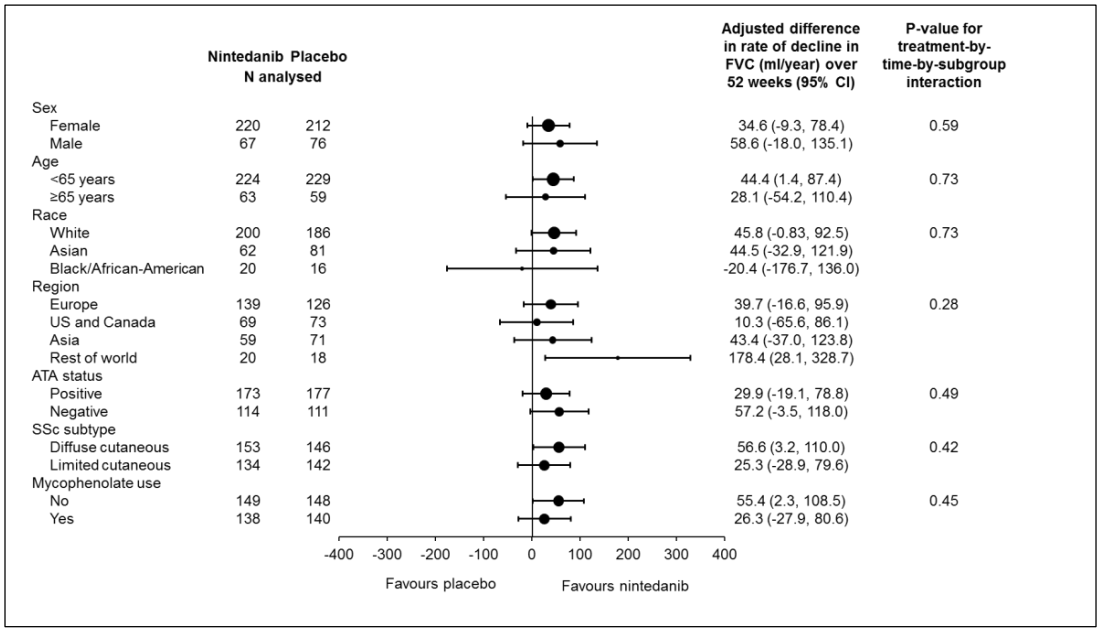

Table 1. Multivariable mixed effect model for change in mRss over time

	β	95% CI		p-value
Time (years, centred at 12 months)	-3.75	-4.80	-2.69	<0.001
Time (years, centred at 12 months)^2	0.36	0.10	0.62	0.006
Constant	25.01	23.90	26.13	<0.001
Random-effect parameters				
SD Time (years, centred at 12 months)	6.66	5.64	7.85	
SD Time (years, centred at 12 months)^2	1.42	1.16	1.75	
SD Constant	9.04	8.15	10.04	
Correlation (Time, Time^2)	-0.92	-0.95	-0.88	
Correlation (Time, Constant)	-0.30	-0.45	-0.13	
Correlation (Time^2, Constant)	0.05	-0.16	0.27	
Residual SD	4.82	4.57	5.08	

Table 2. Associations between time to death or organ complication development and mRss

mRss trajectory	**HR**	**95% CIs**		**p-value**
Death				
Intercept	1.080	1.038	1.123	<0.001
Slope	1.040	1.007	1.075	0.017
Intercept*time (years)	0.9954	0.9912	0.9995	0.029
Clinically-significant pulmonary fibrosis				
Slope	1.035	1.001	1.070	0.045
Pulmonary hypertension				
Slope	1.069	1.002	1.140	0.042
Scleroderma renal crisis				
Slope	0.751	0.599	0.942	0.013
Slope^2	0.300	0.101	0.885	0.029

70. 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 characterized 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 RNAibased 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 RNAibased 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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71. No primary genetic mosaicism detected in whole genome sequencing of linear morphoea epidermis

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Background: Linear morphoea (LM; also referred to as linear scleroderma) is a subtype of morphoea which causes fibrosis of the skin in a linear distribution. Cumulative evidence strongly suggests linear morphoea follows Blaschko's lines of epidermal development, hence suggesting it may represent primary somatic genetic mosaicism.
The aim of this study was to investigate the genetic aetiologic basis of LM and its potential epidermal mosaic origins.

Materials and Methods: Paired biopsies from lesional and site-matched contralateral non-lesional skin, from patients with adult onset LM were obtained. Epidermis was chemically (whole skin soaked in 3.8% ammonium thiocyanate for 20 to 30 minutes) and physically separated from underlying dermis. DNA was isolated from 5 epidermal tissue-pairs (10 isolates) and underwent whole genome sequencing (Illumina HiSeq X Ten System) to a mean coverage of 60-70×. Sequencing reads were aligned with Burrows-Wheeler Alignerv0.7.17 to human genome build 37(hg19) and read duplicates were marked with Sambamba. Somatic variant calling was performed using MuTect2 as a part of Genome Analysis Toolkit v.3.8 and v.4 Beta (somatic variant calling). Variants were annotated with ANNOVAR.

Results: Four tissue-pairs were successfully sequenced. No single common single nucleotide variant (SNV) or commonly affected gene was identified across all epidermal-pairs. A total of 861 SNVs were identified in LM affected epidermis, but absent in paired unaffected epidermis. 119 were protein-coding, 72 were nonsynonymous. All protein-coding SNVs had MAF of <1% and a number had CADD-scores >20 along with pathogenicity rated as deleterious by PolyPhen-2, PROVEAN and Sift-algorithms. These included; ADAMTS16, ADAMTSL1, CBX2, CNTNAP3, DEF8, HES6, NDST2, PRDM9, SDR39U1, SPTBN1 and USP22. When graded according to disease relevance, ADAMTS16 (p.C1206V) and ADAMTSL1 (p. A322T), occurring in two separate patients, were deemed to be of the highest disease relevance. Other members of the ADAMTS superfamily are implicated in embryological morphogenesis, skin development, wound healing, fibrotic pathways, pulmonary fibrosis, liver fibrosis, systemic sclerosis and importantly, LM. ADAMTS8 has previously demonstrated overexpression in LM explanted fibroblasts, with validation on whole-skin immunofluorescence. Finally, a number of non-protein coding variants had high disease relevance, including SMAD4, SMAD6, CCL5, COL6A3, FGF9 and HBEGF.

Conclusion: The absence of a common SNV or affected gene across all epidermal-pairs investigated in this study provides significant evidence against LM representing primary genomic epidermal somatic mosaicism. WGS has instead provided support for a more complex and likely multicomponent aetiopathogenesis. However, in the context of our robust intra-patient paired sample methodology, the presence of a number of deleterious nonsynonymous protein-coding and highly disease relevant non-protein coding epidermal SNVs, does add weight to the potential role of the epidermis and could suggest more complex polygenic cutaneous mosaicism. If present, this could be in keeping with the clinical heterogeneity observed in morphoea. This study has highlighted the possible role of ADAMTS proteases and ADAMTSL proteins in LM.

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72. Energy metabolism and mitochondrial morphology in skin fibroblast cultures from scleroderma patients

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Introduction: One of the most characteristic pathological manifestations of scleroderma is the connective tissue fibrosis. This is especially marked in the diffuse cutaneous form of the disease, where overproduction of collagen and other extracellular matrix proteins (ECM) by connective tissue fibroblasts results in excessive ECM deposition. Stimulated by the inflammatory milieu, the fibroblasts become hyperproliferative and differentiate into myofibroblasts. Hyperactive fibroblasts involved in other fibrotic diseases such as idiopathic pulmonary fibrosis and cirrhosis appear to increase their glycolytic flux – a Warburg-like effect – and show changes in mitochondrial morphology. We hypothesise that skin fibroblasts derived from scleroderma lesions show similar metabolic energy re-programming to sustain their hyperactive status.

Objective: To characterise the energy metabolism and mitochondrial morphology of skin fibroblasts derived from scleroderma patients.

Methodology: Seahorse respirometry, confocal microscopy and western blot analysis were performed on fibroblasts cultures from both lesional skin and uninvolved skin of five scleroderma patients and five age-matched healthy controls.

Results: Fibroblasts from scleroderma patients showed increased mitochondrial respiration coupled to ATP production and decreased spare respiratory capacity in comparison to healthy controls; however, no changes in glycolytic flux and glycolytic enzyme levels were observed. Furthermore, the patient cells had hyperfused mitochondria and lower levels of the mitochondrial fission promoting protein DRP1 than control cells but other proteins involved in maintenance of mitochondrial morphology were unaffected.

Discussion: Our findings suggest that fibroblasts from scleroderma patients modulate mitochondrial respiration to produce more ATP for the support of their hyperactive phenotype. The observed hyperfusion of mitochondria may allow the cells to increase their mitochondrial ATP production and/or be a response to stress.

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