Figure:

Inhibition of type I collagen by Nintedanib and Tofacitinib in dermal fibroblast stimulated with PDGF and/or TGFβ 8 and 7 days after Nintedanib and Tofacitinib initiation.

Disclosure of Interests: Anne Sofie Siebuhr Employee of: Nordic Bioscience, Morten Karsdal Shareholder of: Nordic Bioscience A/S., Employee of: Full time employee at Nordic Bioscience A/S., Pernille Juhl Employee of: Nordic Bioscience, Anne-Christine Bay-Jensen Shareholder of: Nordic Bioscience A/S, Employee of: Full time employee at Nordic Bioscience A/S.

DOI: 10.1136/annrheumdis-2020-eular.4078

AB0168

NINTEDANIB (TYROSINE-KINASE INHIBITOR) INHIBITS THE TRANSITION OF CIRCULATING FIBROCYTES ISOLATED FROM SYSTEMIC SCLEROSIS PATIENTS INTO MYOFIBROBLASTS: AN IN VITRO STUDY

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Background: Systemic sclerosis (SSc) is a chronic connective disease characterized by microvascular alterations, dysregulated immune response and fibrosis [1,2]. Myofibroblasts are alpha-smooth muscle actin (alphaSMA)⁺ cells and play a crucial role in fibrosis, through the excessive synthesis and deposition of extracellular matrix (ECM) proteins, in particular fibronectin (FN) and type I collagen (COL1) [3]. Despite myofibroblasts primarily derive from resident fibroblasts transition and differentiation, another important source is represented by circulating fibrocytes [4]. Nintedanib is a tyrosine kinase inhibitor approved for the treatment of idiopathic pulmonary fibrosis that interferes with the signalling pathways involved in the pathogenesis of fibrosis [5].

Objectives: To investigate the possible effects of nintedanib in contrasting the ability of cultured mature fibrocytes from SSc patients to differentiate into profibrotic myofibroblasts.

Methods: Circulating fibrocytes were obtained from peripheral blood mononuclear cells isolated from 5 limited cutaneous SSc patients (mean age 68 +/- 10 years) and then plated on FN-coated tissue culture dishes in growth medium

(DMEM at 20% of fetal bovine serum, 1% of penicillin-streptomycin and 1% L-glutamine), to allow the adhesion of fibrocyte precursors. Adherent cells were maintained in growth medium for 8 days in order to allow their differentiation into fibrocytes. Differentiated fibrocytes were treated with nintedanib at the concentrations of 100nM and 1000nM for 3 and 24 hours (hrs) or maintained in growth medium without any treatment. The differentiation of fibrocytes into myofibroblasts was determined evaluating the gene expression of alphaSMA, fibroblast specific protein-1 (S100A4) COL1, FN and CXCR4 by quantitative real-time polymerase chain reaction, and the protein synthesis of alphaSMA, COL1 and FN by western blotting.

Results: Nintedanib inhibited alphaSMA and S100A4 gene expression already at the concentration of 100nM in cultured fibrocytes and after 3 hrs of treatment, when compared with untreated cells. Furthermore, both concentrations of nintedanib (100nM and 1000nM) reduced the gene expression of COL1 and FN, whereas only 100nM downregulated the CXCR4 gene expression. At protein level, nintedanib 100nM and 1000nM reduced the synthesis of alphaSMA and COL1 after 24 hrs of treatment, whereas FN synthesis was reduced only by the nintedanib concentration of 1000nM.

Conclusion: The preliminary results show that nintedanib may inhibit the *in vitro* transition of SSc fibrocytes into myofibroblasts and their profibrotic activity, through the reduction of specific myofibroblast phenotype markers and ECM protein production. The results seem to suggest fibrocytes as further possible target of the antifibrotic action of nintedanib in SSc.

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Disclosure of Interests: Stefano Soldano: None declared, Giulia Martinelli: None declared, Samuele Tardito: None declared, Sabrina Paolino: None declared, Massimo Patané: None declared, Emanuele Gotelli: None declared, Claudio Corallo: None declared, Carmen Pizzorni: None declared, Alberto Sulli Grant/research support from: Laboratori Baldacci, Carlotta Schenone: None declared, Vanessa Smith Grant/research support from: The affiliated company received grants from Research Foundation - Flanders (FWO), Belgian Fund for Scientific Research in Rheumatic diseases (FWRO), Boehringer Ingelheim Pharma GmbH & Co and Janssen-Cilag NV, Consultant of: Boehringer-Ingelheim Pharma GmbH & Co, Speakers bureau: Actelion Pharmaceuticals Ltd, Boehringer-Ingelheim Pharma GmbH & Co and UCB Biopharma Sprl, Maurizio Cutolo Grant/research support from: Bristol-Myers Squibb, Actelion, Celgene, Consultant of: Bristol-Myers Squibb, Speakers bureau: Sigma-Alfa

DOI: 10.1136/annrheumdis-2020-eular.4613

AB0169

ENHANCED PLATELET ACTIVATION AMONG PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: The function of platelets (PLT), which can serve as reservoirs of transforming growth beta, leads to the question whether they are activated in systemic sclerosis (SSc) to such an extent as to associate with clinical severity.

Objectives: To compare the level of PLT activation in SSc and to correlate with physical findings

Methods: Blood was sampled from 12 patients participating in the randomized clinical trial LIGHT (ClinicalTrials.gov NCT040045743) evaluating the safety and efficacy of bermekimab in SSc. Patients had to meet the 2013 ACR/EULAR classification criteria and have a modified Rodnan skin score (mRSS) between 15 and 40. Sampling was done before allocation to treatment. Nil patient was on DMARDs. Ten healthy donors were used for comparison. Citrated whole blood was exposed to 0.1 mM adenosine diphosphate (ADP) to stimulate PLT. Total platelet populations were defined as CD42b-positive population; P-selectin (CD62p) served as an activation marker.

Results: Expression of 42b(+)/62p(+) PLT was greater among patients (Figure 1). Mean fluorescence intensity (MFI) of 42b(+)/62p(+) PLTs was 0.16 +/- 0.08 in comparators and 0.51 +/- 0.07 in patients (p= 0.015). Negative correlation was found between the expression of 42b(+)/62p(+) on PLT and mRSS (r= -0.73, p= 0.028). Patients with grade 2 findings at capillaroscopy had greater expression of 42b(+)/62p(+) than patients with grade 3 findings (45.9 +/- 7.2 % vs 27.2 +/- 6.7 %; p: 0.041).