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.

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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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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).

Conclusion: PLTs are greatly activated in SSc and this is associated with disease progression. Findings suggest that this activation is greater at less severe patients.

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AB0170

PHENOTYPIC CHARACTERIZATION OF ENDOTHELIAL PROGENITORS CELLS OF SYSTEMIC SCLEROSIS (SSc) PATIENTS: ROLE IN ENDOTHELIAL-TO-MESENCHYMAL TRANSITION PROCESS

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Background: Endothelial-to-mesenchymal transition (EndoMT), a newly recognized type of cellular transdifferentiation, seems to be involved in Systemic Sclerosis (SSc) pathogenesis. In this process endothelial cells lose their specific markers, and acquire a mesenchymal phenotype, thus expressing cell products such as alpha smooth muscle actin (α -SMA) (1,2). Circulating endothelial progenitors cells (EPCs) derive from bone marrow stem cells and contribute to *de novo* vessels formation. Several studies, although with conflicting results, have shown that EPCs in the peripheral blood of patients with SSc are impaired in their number and function (3).

Objectives: to assess phenotypic characteristics of EPCs from SSc patients and from patients with Very Early Diagnosis of SSc (VEDOSS) compared with healthy controls (HC). In particular we want to evaluate the expression of α -SMA, as marker of a pro-mesenchymal switch (EndoMT) in:

1. Circulating Early (CD34+KDR+CD 133+) and Late EPCs(CD34+KDR+) in the peripheral blood using flow cytometry
2. Cultured EPCs using Western blot analysis

Methods: we enrolled 11 patients (6 SSc and 5 VEDOSS), classified according to the classification criteria for SSc (4) and for VEDOSS not fulfilling SSc criteria (5), and 5 HC. Phenotypic characterization was performed as previously described by Vasa et al. using a FACS Calibur (BD Immunocytometry Systems). EPCs number was expressed as a percentage of cells within the lymphocyte gate. 5×10^6 PBMCs were plated on human fibronectin-precoated (10 μ g/ml Sigma-Aldrich) 6-well plates and cultured for 7-12 days to obtain EPCs. PBMCs from one HC were also cultured with 20% SSc patient serum. Collected EPCs were lysed and a Western blot analysis for α -SMA detection was performed.

Results: we found a significant higher percentage of α -SMA positive Early EPCs in all patients respect to HC ($0,06\% \pm 0,03$ vs $0,03\% \pm 0,01$; $p=0,0149$) particularly in VEDOSS patients ($0,07\% \pm 0,01$ vs $0,03\% \pm 0,01$ $p=0,008$). Similarly we found a significant higher expression of α -SMA protein in all patients and VEDOSS

patients respect to HC ($0,1895 \pm 0,16$ vs $0,07 \pm 0,06$ $p=0,0342$; $0,3075 \pm 0,14$ vs $0,07 \pm 0,06$ $p=0,0159$). After the incubation of HC PBMCs with SSc serum, the α -SMA protein expression seems to be increased respect to its expression in the PBMCs of the same HC cultured without SSc serum ($0,33$ vs $0,1$).

Conclusion: we found higher percentage of Early α -SMA positive EPCs and a higher expression of α -SMA protein in cultured EPCs in patients group (SSc and VEDOSS) than in HC. So we hypothesized a predominant pro-mesenchymal phenotype of this kind of EPCs. This could be considered the expression of the involvement of EPCs in the EndoMT process and it better explain the controversial role of EPCs in SSc pathogenesis. Moreover the modified expression of α -SMA in HC EPCs co-cultured with 20% SSc serum could suggest the presence of a factor inducing the EndoMT process in the disease.

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AB0171

THE IMMUNOMODULATORY AND ANTI-INFLAMMATORY EFFECTS OF BOSENTAN IN SYSTEMIC SCLEROSIS

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Background: Plasma endothelin-1 (ET-1) levels are increased in patients with systemic sclerosis (SSc), playing a central role in the development of fibrosis, vasoconstriction and inflammation¹. While the beneficial effect of Bosentan, the endothelin receptor antagonists, have been demonstrated on vasoconstriction and fibrosis, its potential anti-inflammatory and immunomodulatory activity needs to be further investigated.

Objectives: To assess whether Bosentan can modulate the gene expression profile of immune cells in sample of patients with limited and diffuse SSc and active digital ulcers.

Methods: We enrolled 34 patients affected by SSc. Twenty-four patients were affected by limited SSc and 12 by diffuse SSc. Blood samples were collected from patients before and after 24 weeks of treatment with Bosentan, in the absence of immunosuppressive therapies. All patients received Bosentan 125mg twice a day for 24 weeks. Gene expression profiles were assessed by GeneChip® Human Transcriptome Array 2.0 microarray technology. Significantly (p -value<0.05) and differentially (FC>1.5) expressed genes pre/post treatment were obtained by paired t-statistics, as implemented in Partek Genomics Suite ver. 6.6. These genes were subjected to functional enrichment analysis by Ingenuity Pathway Analysis. The effect of Bosentan on patients was studied on the "diffuse" and "limited" sub-cohorts, individually, as well as on the whole cohort.

Results: Contrary to the limited cohort where differentially expressed genes resulted to be all non-coding genes which are almost all over-expressed before treatment, the diffuse cohort was characterized by 19 differentially expressed genes that enrich biological functions and pathways related to the immune system and its organic response (in particular T-cells). Comparing the limited to the diffuse cohort, pre- and post- treatment, a distinct genetic fingerprint emerges, that characterizes the response to Bosentan by the latter cohort as increased apoptosis of lymphocytes (z -score=3.28) and a decreased quantity of antigen presenting cells (from z -score=1.06 (pre) to -0.75 (post)).

Conclusion: The presence of an inflammatory microenvironment, as occur in SSc, influence the relative expression of ET-1 receptors on immune cells, which in turn further contribute to the amplification of cellular responses to inflammation. The observed difference response to therapy between the two cohorts of patients was attributed to influence of ET-1 levels on the relative expression of ET-1 receptors on immune cells surface. Interestingly Bosentan, beside the already-known effect on promoting antigen presenting cells apoptosis, seem to exert its immunomodulatory activity also by deregulating functions that mainly involves the T cells and by promoting their apoptosis, which in turn reflect also its anti-inflammatory properties.