Conclusion: The newly described antibodies against GPCR, GF and GFR are highly correlated. Associations with morbidity- and mortality-determining organ involvement indicate their possible functional relevance and novel pathophysiological mechanisms. As new biomarkers, some of the ab have prognostic value for SSc; for other manifestations, their value should be evaluated in further studies.

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OP0245

ANTI-S100A4 MONOCLONAL ANTIBODY TREATMENT AMELIORATES SKIN FIBROSIS IN INFLAMMATORY AND NON-INFLAMMATORY PRE-CLINICAL MODELS OF SYSTEMIC SCLEROSIS

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Background: AX-202 is a monoclonal antibody that inhibits the bioactivity of S100A4. S100A4 is an alarm signal that is released from cells in response to stress or injury and functions as an amplifying mechanism of inflammation and fibrosis in the diseased tissue microenvironment. Previous in vitro studies have found that S100A4 induces fibroblast activation, sensitizes fibroblasts to the effects of TGF β , drives epithelial-mesenchymal transition, and stimulates monocyte cytokine release (1-3). Moreover, S100A4^{-/-} mice are protected from fibrosis in several animal models (1). In patients with systemic sclerosis (SSc), S100A4 is elevated both in lesional tissue and systemically and correlates with skin involvement, disease activity, and pulmonary function.

Objectives: The aim of this study was to assess the antifibrotic effects of murine AX-202 in two pre-clinical models of SScs reflecting both inflammation-mediated and inflammation non-mediated fibrosis and confirm the in vivo activity of humanized AX-202.

Methods: We first evaluated the effects of murine AX-202 in the bleomycin-induced skin fibrosis model and the tight-skin 1 (Tsk-1) model. In the bleomycin (BLM) model, fibrosis was induced by 3 weeks of BLM s.c. injections followed by 3 weeks of AX-202 treatment in parallel with continued BLM s.c. injections. The control groups included NaCl s.c. injections for 6 weeks, BLM s.c. injections for 6 weeks, or BLM s.c. injections for 3 weeks, followed by NaCl s.c. injections for 3 weeks. Three dosing regimens of AX-202 were tested: 3.75, 7.5, or 12.5 mg/kg i.p. every 3rd day. In the Tsk-1 model, treatment with 7.5 mg/kg i.p. every 3rd day was administered from week 5 until week 10. The control groups included pa mice, Tsk-1 mice, and Tsk-1 mice treated i.p. with isotype IgG. We subsequently evaluated the effects of humanized AX-202 in the model of BLM-induced skin fibrosis in a similar design as used for the murine AX-202 study. Three dosing regimens were tested: 8 mg/kg and 16 mg/kg i.p. every 3rd day and 24 mg/kg i.v. once weekly.

Results: In the BLM model, murine AX-202 (7.5 mg/kg) was effective both in the prevention of progression of pre-established skin fibrosis and in the induction of regression of fibrosis as assessed by the dermal thickness (-55%, p<0.0001 vs BLM for 6 weeks, and -23%, p<0.0001 vs BLM for 3 weeks), myofibroblast count and hydroxyproline content. Murine AX-202 also ameliorated fibrosis in the Tsk-1 model as assessed by the hypodermal thickness (-24%, p=0.01 vs Tsk-1 isotype control), myofibroblast count, and hydroxyproline content. In both models, the antifibrotic effects were associated with a reduction in pSMAD3 expression. Humanized AX-202 was effective in the prevention of progression of pre-established skin fibrosis in all doses tested across all endpoints (dermal thickness, myofibroblast counts, hydroxyproline content). In the two groups treated with

16 mg/kg i.p. and 24 mg/kg i.v., humanized AX-202 also induced regression of fibrosis (-83%, p<0.001, and -61%, p<0.001 vs BLM for 3 weeks, respectively). Both murine and humanized AX-202 were well tolerated in all study groups in both models.

Conclusion: We demonstrate that AX-202 confers potent antifibrotic effects in complementary models of SSc. These results confirm and expand previous data showing that inhibition of S100A4 by AX-202 is a promising potential therapeutic candidate for disease modification in SSc or other fibrotic conditions.

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OP0246

PREDICTIVE VALUE OF LABORATORY AND INSTRUMENTAL FINDINGS IN THE VERY EARLY DIAGNOSIS OF SYSTEMIC SCLEROSIS. ROLE FOR CXCL4 CHEMOKINE

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Background: Systemic sclerosis (SSc) is a rare and progressive autoimmune disease, whose diagnosis is difficult in the early stages because of the lack of specific signs and symptoms. Criteria for a Very Early Diagnosis of SSc (VEDOSS) have been proposed to identify those patients affected by undifferentiated connective tissue disease (UCTD) at risk to develop SSc [1]. For the diagnosis of SSc a strict clinical and laboratory follow up is mandatory [2]. CXCL4 chemokine recently proved to be higher in early SSc [3][4][5].

Objectives: Aim of our study was to evaluate at baseline the main clinical-demographic and laboratory parameters in a group of VEDOSS patients, comparing these features during the follow-up, to detect any difference between progressors (P) and non-progressors (NP) into SSc. Furthermore, we dosed plasma levels of CXCL4.

Methods: We included 27 VEDOSS patients, defined by EUSTAR 2011 Criteria and not fulfilling the 2013 ACR/EULAR classification criteria of SSc, attending the Rheumatology Unit of Policlinico Umberto I in Rome from 2009 to 2020. Demographic, laboratory and instrumental features were analyzed, and, after a mean follow-up of 5.7 $\pm$ 1.7 years, we compared the P to NP patients. Having obtained written informed consent, blood samples were taken at baseline to measure plasma levels of CXCL4 chemokine using an ELISA assay.

Results: At baseline the 27 VEDOSS patients (mean age 53.2 $\pm$ 13.5 years, all females) had ANA positivity in 25 (93%) cases and Raynaud's phenomenon in 25 (93%) cases. In a mean follow-up time of 43.5 $\pm$ 23.1 weeks from the first clinical examination, 15 (55%) patients were classified as P into SSc. These P patients showed a significant association with SSc specific antibodies such as anti-Centromere, anti-Scl70 and anti-RNAPIII (p=0.014) as well as with a specific "Scleroderma pattern" at the nailfold capillaroscopy (p=0.022) respect to those NP into SSc. A group of 6 P patients evolved in less than 24 months (mean 19,6 $\pm$ 6,8 weeks) and were defined "Fast Progressor". They were also significantly associated with those autoantibodies considered as having a worse prognosis such as anti-Scl70 and anti-RNAPIII (p=0.005), and had a shorter duration of RP (88 vs 189.3 months) and a younger mean age (49.5 vs 55.4 years) respect to "Slow Progressor" (SSc evolving in >24 months). At baseline we detected significantly higher median plasma levels of CXCL4 in the 27 VEDOSS patients compared to 10 healthy subjects (9024 $\pm$ 10559 pg/ml versus 348,5 $\pm$ 684,3 pg/ml; p=0.0047). We also noticed a trend for lower CXCL4 levels in the "Fast Progressor" than in

the "Slow Progressor" (3303 ± 6065 pg/ml vs 13300 ± 10308 pg/ml; $p=0.052$) without reaching a significant value, due probably to the low number of cases.

Conclusion: Our study confirms that the presence of specific autoantibodies and capillaroscopic abnormalities correlate to an increased risk of developing SSc in patients with UCTD [6]. Beside we found significantly higher levels of CXCL4 in our 27 VEDOSS patients respect to controls, in agreement with other authors showing the association of this chemokine with early stages and specific organ involvement [4][5][7]. The finding of CXCL4 lower levels in "fast progressor" cases is consistent with our recent report of anti-CXCL4 antibodies in patients with early SSc, determining lower levels of this antigen [7]. We need deeper investigations to better evaluate the role of CXCL4 in the different stages of SSc.

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OP0247

IN MYOSITIS MUSCLE FIBRE PLAYS A DIRECT AND CRITICAL ROLE IN THERAPEUTIC RESPONSE TO GLUCOCORTICIDS

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Background: Myositis are rare autoimmune diseases, affecting more women than men, characterized by chronic inflammation of skeletal muscle causing muscle weakness, decreased quality of life and increased mortality.

Glucocorticoids (GC) are potent anti-inflammatory drugs, and are the first line treatment of myositis. They improve muscle strength of myositis patients (therapeutic effect), yet muscle recovery is generally only partial. Moreover, GC have an iatrogenic effect on skeletal muscle fibre leading to steroid myopathy. Thus myositis care has to be improved. Despite the autoimmune terrain of myositis, our team has recently shown that muscle fibres themselves develop immuno-metabolic modifications that participate to muscle weakness and perpetuation of the disease¹. GC effects are mediated by the glucocorticoid receptor (GR), which is expressed in various cell types including immune cells and myofibres, but the cells mediating therapeutic responses remain to be determined.

Objectives: Unravel the mechanisms underlying the therapeutic effect of GC in myositis, particularly elucidate the role of skeletal muscle fibres.

Methods: Experimental myositis was induced in eight to ten week-old C57BL/6J female mice by a single intradermal injection of part of skeletal muscle fast-type C protein along with Freund's adjuvant and an intraperitoneal (IP) injection of pertussis toxin, as previously described². Prednisone (PDN) was administered 14 days (D) after the immunization at 1 mg/kg/day for 7 days by gavage. Mice were euthanized 21 days after myositis induction. Muscle strength was assessed by grip test at D 0, before the 1st PDN administration (D 14) and the day before sacrifice (D 20). To investigate whether the PDN effects are mediated by myofibre, we generated transgenic mice carrying two LoxP sites within the GR gene in muscle, expressing the tamoxifen-inducible Cre-ERT2 recombinase selectively in skeletal muscle fibre (HSA-CreERT2/GR L2/L2). Tamoxifen (1 mg/day for 5 days by IP injection) was administered 9 days after immunization to induce GR ablation selectively in skeletal muscle fibres (GR(i)skm^{-/-} mice). Similar treatments were applied to GR L2/L2 that do not express Cre-ERT2, and served as controls.

We compared 4 groups of myositis mice, GR L2/L2 treated by PDN (n=9) or vehicle (n=9) and GR(i)skm^{-/-} treated by PDN (n=10) or vehicle (n=10), by grip test and at the histological level (hematoxylin-eosin (HE) and Gomori trichrome (GT) staining). Moreover, LC3 expression was studied by RTqPCR and western blot.

Results: Muscle strength was decreased in both GR L2/L2 and GR(i)skm^{-/-} myositis mice from D 14 to D 20. GR L2/L2 myositis mice recovered muscle strength after PDN treatment; no significant difference compared to D 0 was detected. In contrast, PDN did not improve muscle strength in GR(i)skm^{-/-} myositis mice (Figure 1).

HE and GT staining did not reveal quantitative differences in inflammatory infiltrate. Necrotic and degenerative fibres were detected in the 4 groups. At RTqPCR, LC3, an autophagy marker, was upregulated in PDN-treated GR L2/L2 myositis mice compared to untreated GR L2/L2 myositis mice; moreover it was 2-fold more expressed in PDN-treated GR L2/L2 myositis mice compared to PDN-treated GR(i)skm^{-/-} mice.

Conclusion: GR in skeletal muscle fibre is crucial to mediate the therapeutic response to GC in a murine model of myositis. Autophagy is one of the candidate pathways controlled by myofibre GR underlying this effect.

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Figure 1. Grip test analysis in GR L2/L2 and GR(i)skm^{-/-} myositis mice. ns, not significant; * $p<0.01$; ** $p<0.001$; *** $p<0.0001$

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OP0248

DEVELOPMENT OF A 3D SKIN MICROTISSUE MODEL FOR FIBROTIC DISEASES

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Background: Traditional preclinical approaches, such as two-dimensional cell culture and animal models, are often inadequate to mimic the pathophysiological features of complex diseases such as systemic sclerosis (SSc). Human specific targets, such as the recently described pro-fibrotic long non coding RNA (lncRNA) H19X¹, are becoming increasingly relevant in preclinical research, creating the need of new strategies and tools in translational medicine. The employment of novel three-dimensional (3D) culture systems, where multiple cell types are included, is filling an important gap left by the traditional preclinical methods.

Objectives: To develop an easy to produce 3D fibrotic skin microtissues model for translational proof of concept studies.

Methods: Two thousand five hundred dermal fibroblasts isolated from skin of SSc patients were seeded in ultra-low attachment 96-well plates. Fibroblast were let to aggregate into spheres for 48h. Two thousand five hundred primary normal human keratinocytes were added to the culture and let to layer onto the fibroblast spheres for 72h. H19X silencing experiments were used as proof of concept studies. H19X silencing with antisense oligonucleotides or transfections with a scrambled control were performed in fibroblasts prior to the sphere formation for 24h. TGF β (10 ng/ml) was added to microtissue to exacerbate the fibrotic phenotype. Haematoxylin eosin staining as well as immunohistochemistry staining for vimentin and cytokeratin 10 was performed. Skin microtissues were processed for RNA and protein isolation. Pro-collagen I α 1 and fibronectin were quantified in the supernatants with ELISA.