Systemic sclerosis
A bird's eye review of the recent literature

edited by A. Della Rossa

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Title: Autologous non-myeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised phase 2 trial.
Lancet 2011; 378 (9790): 498-506. Epub 2011 Jul 21.
Summary: Systemic sclerosis is an autoimmune disease with cardinal features consisting of diffuse vasculopathy, autoimmune activation and tissue fibrosis. At present no treatment has proven to be effective in randomised controlled trials in controlling disease progression. In this phase-2 open-label study, the authors aimed at assessing safety and efficacy of autologous non-myeloablative haematopoietic stem cell transplantation (HSCT) compared with intravenous cyclophosphamide (CPM) in diffuse cutaneous systemic sclerosis (dc-SSc) (disease duration <4 years) and internal organ involvement. To this end patient were randomised in a one-to-one ratio to receive HSCT or monthly pulse CPM for six months (1g/m^2 per month). As for HSCT, peripheral blood mononuclear cells were mobilised with intravenous CPM (2 g/m^2) and subcutaneous filgrastim 10 μg/kg. The conditioning regimen was CPM 200 mg/kg and intravenous rabbit anti thymocyte globulin 6.5 mg/kg 3 pulses of methylprednisolone were administered before rabbit antithymocyte infusions. The primary outcome for all enrolled patients was improvement at the 12-month follow-up, defined as decreased modified Rodnan skin score (mRSS) by 25% or improvement of forced vital capacity by more than 10%. Patient in the CPM arm with disease progression (worsening of skin score by more than 25% or decrease of FVC by more than 10%) were allowed to enter the HSCT arm. 19 patient were enrolled, 10 allocated to HSCT and 9 to CPM. All ten patients who were allocated to transplant arm improved within 12 months compared to none of the 9 controls (p=0.00001). Treatment failure occurred in 8 out of 9 controls as compared to none of the 10 patients treated with HSCT (p=0.0001). All but two patients in the HSCT arm demonstrated a sustained benefit on mRSS improvement and forced vital capacity after a mean of 2.6 years. One patient, after an initial benefit, developed renal crisis and skin and lung deterioration and one patient exhibited dichotomous response, with ongoing improvement of mRSS but FVC deterioration. Seven out of 8 patients with treatment failure underwent HSCT, and in one patient transplant was contraindicated for constrictive pericarditis. All seven patients with treatment failure who were switched to HSCT had an improvment in mRSS and FCV after 12 months. The present study demonstrated the superiority of HSCT over standard of care in early dc-SSc with internal organ involvement. The authors underlined that a careful pre-transplant cardiac assessment might be useful to reduce transplant-related morbidity and mortality. Despite the beneficial effect of HSCT in patients failing CPM treatment, the authors state that early HSCT is able to elicit sustained improvements in lung and skin involvement. On the other hand, delaying transplantation through treatment with standard of care might favour disease progression, increase transplantation risks or contraindicate HSCT.

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Title: Screening for pulmonary arterial hypertension in patients with systemic sclerosis.
Arthritis Rheum 2011; 63 (11): 3522-30.
Summary: Pulmonary arterial hypertension (PAH) is a severe complication of systemic sclerosis (SSc) associated with high mortality. Early detection and treatment might improve the outcome of this ominous complication. However most of the studies on outcome and response to treatment in PAH are derived from cohorts mostly comprised of idiopathic pulmonary hypertension. In these cohorts, only small subsets of patients have SSc PAH and results are underpowered to evaluate SSc PAH as a separate entity. The aim of the present work was to compare two incident groups of SSc-PAH in the same treatment era (2002/2003), gathering them from two large datasets of SSc-PAH, the French PAH registry and the ItinerAIR programme.
The first cohort (designated the routine practice cohort) comprised consecutive adults patients in whom PAH was suspected on the basis of symptom and or signs during routine clinical assessment and confirmed by right heart catheterism (RHC) at time of recruitment in the French PAH registry, the second cohort (designated the detection cohort) comprised consecutive patients who entered a systematic detection algorithm comprising, regardless of symptoms, echocardiography with tricuspidal Jet (TRjet) velocity sampling by expert cardiologists. Patients with TR jet velocity >3 m/s and unexplained breathlessness underwent RHC to confirm PAH. Clinical characteristics at diagnosis and subsequent 8-year mortality were compared between the 2 cohorts.
Thirty-two cases with incident SSc-PAH were identified, corresponding to 16 patients in the routine practice cohort and 16 in the detection cohort. At the time of PAH diagnosis, patients in the detection cohort had less advanced pulmonary disease compared to routine practice patients as evidenced

by more patients being in the New York Heart Association class I and II, a lower mean pulmonary artery pressure and pulmonary vascular resistance index and a higher cardiac output. Moreover, patients in the detection cohort, despite no difference in PAH specific therapies, were less likely to receive warfarin and diuretics. The 1-, 3-, 5- and 8-year survival rates were 75%, 31%, 25% and 17%, respectively, in the routine practice cohort compared with 100%, 81%, 73% and 64%, respectively in the detection cohort (p=0.0037).
In conclusion, PAH detection programmes are able to identify patients with milder disease subsets and allows earlier management. This in turn could improve long-term survival. However, the authors outline that further studies are warranted to confirm their data and to determine whether factors such as lead time bias might have contributed to the survival advantage in the detection cohort patients.

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Title: Patterns and predictors of change in outcome measures in clinical trials in scleroderma. an individual patient meta-analysis of 629 subjects with diffuse scleroderma.
Arthritis Rheum 2012 Feb 10 [Epub ahead of print].
Summary: Most of clinical trials performed on diffuse cutaneous systemic sclerosis (dc-SSc) over the past 15 years have failed to demonstrate therapeutic efficacy. This failure might be due to a lack of complete understanding of molecular and cellular basis for disease pathogenesis, but it is possible that a bias in trial design, including the choice of patient population and outcome measures, might have occurred.
The population of patients enrolled in clinical trials is different from the whole population of SSc patients for a number of factors, such as the small size of cohorts that limits the spectrum of disease manifestations, duration of trials, usually less than one year, that might blunt changes occurring after longer periods, patterns of disease activity and severity, with enrolment of more active and less severe patients that do not reflect the full spectrum of disease status. The utility and responsiveness of currently used outcome measures in treatment trials of scleroderma remains uncertain. Modified Rodnan skin score (mRSS) is a key outcome measure in most clinical trials, but has inherent bias related to the high inter-observer variability, question as to the measure's sensitivity to change, and the need to take into consideration the natural history of scleroderma skin disease. Similarly, the ability of other validated outcome measures, including the modified Health Assessment Questionnaire (HAQ) and patient and physician global assessments (pGA), to measure substantive change in a trial setting has not been fully demonstrated for scleroderma.
To better understand the range, responsiveness, and other test characteristics of the mRSS, and other key outcomes, in the context of clinical trials, an individual patient data meta analysis was conducted using the pooled data from 7 recently completed clinical trials of scleroderma that utilised identical outcome measures. Data of 629 patients were collected. Information of the outcome measures was available for most patients at baseline and for the majority at the 6-month follow-up. Due to the variable length of the studies, fewer patients had data at the 6-month follow-up. All endpoints and covariates were balanced within studies. Major demographic data were homogeneous across studies. A number of variables were not balanced across studies. The most striking difference was for disease duration, due to the different eligibility criteria in different settings. Outcomes tended to improve during trials for patients with more severe disease at study entry and worsen for patients with less severe disease at entry. Multivariate mixed models did not demonstrate that any baseline variables were strongly predictive of subsequent outcome. These results did not differ when comparing trials of early vs. late disease or trial "completers" *vs*. "non-completers".
Disease duration was mildly negatively predictive of change in mRSS at 6 months (r=-0.27; p<0.0001) and substantial bi-directional variation in change in mRSS and HAQ was seen over the spectrum of disease duration. Overall, mRSS scores improved during observation periods while HAQ and lung function were mostly static, although there were wide variations in individual changes in these measures. None of these variables, including disease duration, reliably identify groups of subjects whose mRSS will predictably increase or decrease in the course of a clinical trial. These findings have important implications for clinical trial design in scleroderma. Although there are limitations, linked to factors such as the lack of comprehensiveness and the involvement of "negative" trials, this study is of pivotal importance, since the sample size of 629 treated patients is the largest group of patients with scleroderma enrolled in therapeutic clinical trials ever studied. Data were collected with standardised protocols using the same set of validated outcome measures and patients were all evaluated at academic centres in several countries, each centre with expertise and experience in the evaluation and management of dc-SSc.
These data should help guide researchers to develop more sophisticated studies of promising new agents for scleroderma. Consideration might be given to additional methods of patient selection beyond baseline mRSS and disease duration. Factors of potential interest include use of scleroderma-specific autoantibodies which have prognostic value. Similarly, there are multiple biomarkers under development that attempt to provide both quantification of disease activity and prognostic information using tests related to the underlying pathophysiology of scleroderma. Work on a composite outcome measure for scleroderma may obviate some of the limitations of organ-specific measures currently in use.

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Title: Pulmonary arterial hypertension therapy may be safe and effective in patients with systemic sclerosis and borderline pulmonary artery pressure.

Arthritis Rheum 2012; 64 (4): 1257-62. Epub 2011 Nov 11.
Summary: Exercise induced mean pulmonary artery pressure (PAP) increase above 30 mmHg at right heart catheterisation (RHC), might be a risk factor for overt pulmonary arterial hypertension in systemic sclerosis (SSc) patients. The aim of this pilot study was to invasively monitor SSc patients with borderline PAH (defined as mean RHC PAP after exercise above 30 mmHg with rest PAP <25 and pulmonary artery wedge pressure <15) and to investigate the effect of Bosentan on haemodynamics and exercise capacity in these patients. To this end 10 SSc subjects underwent RHC at baseline and after 12 months. This was followed by 6 months of full dose treatment with Bosentan and new RHC at the end of treatment. The primary end point of the study was the change in mean PAP at 50 W during the course of the treatment period as compared to the observation period. Mean PAP at rest, at 50 W and during maximal exercise increased significantly during the observation period and tended to decrease during the treatment period. The changes during the observation period versus the therapy period were significantly different, both at 50 W and during maximal exercise. The changes in resting pulmonary vascular resistance were also significantly different during the observation period versus during the therapy period. Changes in resting pulmonary artery wedge pressure were not significantly different between the observation period and the treatment period, despite the significant increase during the observation period. The authors conclude that in SSc patients with borderline PAH, the early use of Bosentan might prevent pulmonary haemodynamics deterioration. These exploratory data, however, need to be confirmed in the context of a randomised placebo controlled study adequately powered for endpoints.

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Title: Stimulating healthy tissue regeneration by targeting the5-HT2B receptor in chronic liver disease.
Nat Med 2011; 17 (12): 1668-73.
Summary: Diminished hepatocyte regeneration is a feature of liver disease and is associated with fibrogenesis that leads to liver cirrhosis and cancer. The mechanisms that dictate the balance between cell regeneration and fibrogenesis are not well understood. Hepatic stellate cells (HSC) are able to transdifferentiate in activated myofibroblasts in diseased liver and secrete numerous soluble factors that might influence hepatocyte proliferation, including hepatocyte growth factor (HGF), TGF-β1 and interleukin-6 (IL-6). Although it is suggested that HSC are pivotal in hepatocyte regeneration, no conclusive *in vivo* evidence has been provided.
The aim of the present work was to estabilish the role of HSC in animal models of liver injury. Selective depletion of HSC resulted in enhanced hepatocyte proliferation in Bile Duct Ligation (BLD) induced liver damage. Then, the paracrine signalling between HSC and hepatocytes was investigated. Functional 5 Hydroxytriptamine 2B (5-HT2B) receptors are expressed on activated HSC in diseased liver. The administration of a highly specific molecular antagonist of 5HT2B (SB-204741) resulted in enhanced hepatocyte proliferation in progressive induced BLD liver damage and in liver injury by acute carbon tetrachloride (CCl4). The administration of ketanserine, a selective antagonist of 5HT2A and 5-HT 2C had no effect on hepatocyte proliferation in CCl4 liver injury. Gene deletion or blockade after liver injury enhanced liver regeneration after partial hepatectomy (PHX). Similar effects were obtained in mice lacking Jun D. HSC seem to be the predominant cell type through which 5HT2B negatively influences hepatocyte regeneration. Activation of HSC by serotonin activates the expression of transforming growth factor β1 (TGF β1) a strong suppressor of hepatocyte proliferation, through signalling by mitogen activated protein kinase1 (ERK) and the transcription factor JunD. Antagonism of 5HT2B attenuated fibrogenesis and improved liver function in CCl4 and BDL progressive liver disease.
5HT 2B is selectively expressed in activated human HSC and the signalling pathway described in animal models is conserved in human HSC. Potent and selective antagonists of 5HT 2B are available and have been reported to be safe for clinical use in humans. This class of medications might therefore have therapeutic potential in chronic liver disease, both as stimulants of hepatocyte regeneration and as antifibrotic agents.

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Title: Involvement of functional autoantibodies against vascular receptors in systemic sclerosis.
Ann Rheum Dis 2011; 70 (3): 530-6. Epub 2010 Nov 15.
Summary: Systemic sclerosis is a chronic incurable disease of unknown origin. Vasculopathy, autoimmunity and fibrosis are the pivotal pathogenetic points of the disease. The vascular involvement is precocious and typical of the disease. According to the vascular theory of the disease, vascular dysfunction leads to skin and organ fibrosis. In the hypothesis that autoantibody mediated vascular receptor activation could contribute to the pathogenesis of the disease and to its clinical expression, the authors measured anti-angiotensin II type 1 receptor (anti AT_1R) antibodies and anti-endothelin A type 1 receptor (anti ET_AR) antibodies in three independent cohorts of SSc patients, 298 from Berlin, 137 from Pecs and 43 from Florence. Control serum samples were obtained from 372 healthy subjects and 311 diseased patients, including 208 rheumatoid arthritis, 33 morphea, 32 Raynaud's phenomenon and 38 primary Sjögren's syndrome patients.
Levels of both antibodies were higher in SSc patients than in sex age matched control group. Comparison of autoantibodies values from the Berlin cohort, showed similar values to the cohort of Pecs and of Florence. A strong correlation between AT_1R antibodies and anti ET_AR antibodies was present

in SSc patients. In immunoprecipitation experiments, IgG from SSc patients tested positive for AT_1R and ET_AR antibodies precipitated both AT_1R and ET_AR, while, in contrast, IgG from matched controls tested negative failed to precipitate AT_1R and ET_AR. Autoantibodies, moreover, initiated canonical ERK1/2 *in vitro* signal by means of microvascular endothelial cells.

Quantitative differences in the levels of autoantibodies were linked to different disease manifestations and to the risk of development of specific manifestations in the Berlin cohort. Lower positive levels were linked to anti scl-70 antibodies and renal crisis, higher levels were associated with a high risk of diffuse SSc and for late complications such as pulmonary hypertension, lung fibrosis and digital ulcers. In a smaller cohort of patients prospectively followed up, the levels of autoantibodies predicted disease-related mortality 48 months after testing. In conclusion, the authors demonstrated a high prevalence of antibodies against AT_1R and ET_AR in SSc patients. These antibodies were able to activate microvascular endothelial cell through an increase of profibrotic TGF-β expression and to precipitate their receptor. Their biological effect was attenuated by their respective antagonist. High antibodies levels were linked to more severe disease subsets and mortality. Therefore these molecules might represent the link between autoimmunity, vascular involvement and fibrosis. Their assessment may warrant a better characterisation of SSc patients, moreover the pharmacological blockade of these molecules may represent a promising future therapeutic tool for this disease.

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Title: Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis.

Nat Commun 2012; 3: 735.

Summary: The activation of fibroblasts and their differentiation in myofibroblasts is a key point in most idiopathic fibrotic diseases, whether fibrosis is restricted to a single organ (such as in idiopathic pulmonary fibrosis IPF) or it is systemic (such as in systemic sclerosis SSc). TGF-β is a central regulatory cytokine of fibroblasts activation. However, inhibition of downstream pathways (such as smad proteins, MAP-kinases, focal adhesion kinases, c-ABL and early growth response-1) does not completely abrogate the effect of TGF-β on fibroblasts, indicating that other mechanisms are at play. Accumulating evidence indicates that increased activation of canonical Wnt signalling might have an important role in fibrogenesis. Wnt proteins transmit their signal across membrane by linking the frizzled receptors and low density lipoprotein-receptor-related protein co-receptor (LPR5/6). Upon binding to their receptor, Wnt induce a cascade of intracellular signalling events that culminates in the stabilisation of β-catenin. B-catenin translocates into the nucleus where induces the transcription of Wnt genes. To avoid uncontrolled activation, Wnt signal is tightly regulated. Among negative regulators, Dikkopf proteins (Dkk1-4) play a central role, the best studied of which is Dkk1.

The aim of the present study was to explore the role of canonic Wnt signalling in fibrotic disease, both evaluating human disease and animal models. Skin biopsies from 12 subjects with SSc and 10 healthy controls, lung biopsies from 8 subjects with IPF and 6 matched controls, liver biopsies from 5 subjects with alcoholic liver cirrhosis and 5 matched controls were assessed. Transgenic (tg) Mice overexpressing Wnt 10 and Dkk1 were the animal models. The role of Wnt cascade was investigated in 3 different models:

1. In the bleomicin induced fibrosis
2. In the tight skin-1 model
3. In a model of TGF-β mediated fibrosis

Nuclear β-catenin, an expression of canonical Wnt pathway, was overexpressed in skin fibroblasts from SSc patients as compared to healthy subject. This increase of expression was also demonstrated in IPF and liver cirrhosis as compared to non-fibrotic tissues. On the other hand, the expression of Dkk1 was virtually absent in fibrotic tissues as compared to matched non-fibrotic samples. The canonical Wnt signalling was also activated in different experimental models of fibrosis, with increased nuclear accumulation of β-catenin in bleomicin induced dermal fibrosis and tight skin-1 mice. Wnt stimulates a dose dependent increase of collagen production by fibroblasts. Cycloheximide blocked collagen m-RNA increase, indicating that the stimulatory effect is indirect. Activation of canonical Wnt pathway by R1-Spondin enhanced bleomicin induced dermal fibrosis in wild type mice. On the other hand, overexpression of Dkk1 in Dkk1 tg mice, protected from bleomicin induced fibrosis. Transgenic expression of Wnt10 induced dermal fibrosis. Interbreeding of Wnt10 and Dkk1 resulted in double mutant mice in which dermal thickening and hydroxyproline and myofibroblast content at 12 week were comparable to wild type control mice. Treatment of Dkk1 tg mice with neutralising antibodies against Dkk1 resulted in significant exacerbation of fibrosis. TGF-β reduced the levels of Dkk1 and indirectly enhanced Wnt mediated fibrosis. On the other hand, when fibroblasts were stimulated by TGF-β in the presence of Dkk1, Wnt signalling was blunted.

In this work the author demonstrated a key role for canonical Wnt signalling in fibroblast activation and collagen release in fibrosis and a cross-talk between TGF-β and the canonical Wnt pathway.

These observations might have important implications in the future therapeutic approach to fibrotic diseases. Indeed, the inhibition of the canonical Wnt pathway might prove an effective approach to target TGF-β signalling.