University College London (UCL)

MD (Res) Degree

Treatment of Diffuse Cutaneous Systemic Sclerosis with Hyperimmune Caprine Serum

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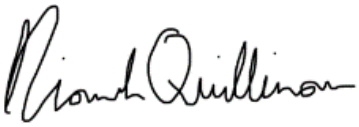
Declaration

I, Niamh Patricia Quillinan, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed:  Date: 17/03/2016

Abstract

Systemic sclerosis (SSc) is a multisystem autoimmune rheumatic disorder with high morbidity and the highest case specific mortality of the rheumatic diseases. There is no currently approved unequivocally effective treatment for SSc and therefore there is a huge unmet medical need for novel and effective therapies. Hyperimmune caprine serum (HCS) is a goat serum extract derivative produced from goats vaccinated with a detergent-inactivated HIV viral lysate. It contains caprine immunoglobulins and small molecular weight proteins as well as a CRH, α-2 macroglobulin (α-2M) and lipoprotein-related peptide-1 complex.

In this thesis we explore the hypothesis that hyperimmune caprine serum improves skin and other measures of disease severity in established dcSSc by modulating immunological function that determines persistence of clinical disease. This hypothesis is explored through 1) a prospective clinical trial, 2) long-term clinical use and 3) detailed assessment of serum growth factors and cytokines, as well as established and exploratory markers of disease.

The primary objective of the clinical trial was to explore safety and tolerability of HCS in established diffuse cutaneous systemic sclerosis (dcSSc). Secondary objectives included assessment of potential efficacy and biological activity and exploration of candidate biomarkers.

There were no safety concerns and frequency of adverse events was not different between HCS and placebo group. MRSS improved in the HCS group and worsened in the placebo group, with more responders in the HCS group at 26 weeks. Neuropathic pain improved in the HCS group compared to placebo. There was a trend to benefit for lung function indices. Cluster analysis revealed changes in a number of cytokines in the HCS group compared to placebo, in parallel with the skin changes. In particular, α-MSH and ACTH were significantly increased in the HCS group leading use to hypothesise that improvement in MRSS may have been mediated through the melanocortin system.

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Glossary

1p2a3sg	1-peptidyl-2- arachidonoyl-3-stearoyl glyceride
α-2M	α-2 macroglobulin
α-MSH	α-Melanocyte stimulating hormone
αSMA	α smooth muscle actin
ACA	Anti-centromere antibody
ACTH	Adrenocorticotropin releasing hormone
AD	Alzheimer's disease
AE	Adverse event
AIM2	Absent in melanoma 2
ALS	Amyotrophic lateral sclerosis
ALSAQ	ALS Assessment Questionnaire
ALSFRS-R	ALS Functional Rating Scale (revised)
ALS-QOL	ALS Quality of Life
ANG2	Angiopoietin 2
APP	Amyloid precursor protein
ASC	Apoptosis associated speck-like protein containing a CARD
ASSIST	**A**utologous **S**tem cell **S**ystemic sclerosis **I**mmune **S**uppression **T**rial
AST	Aspartate aminotransferase

ASTIS	**A**utologous **S**tem cell **T**ransplantation **I**nternational **S**cleroderma
ATG	Anti-thymocyte globulin
BAL	Broncho-alveolar lavage
BLM	Bleomycin
BMI	Basal Metabolic Index
c-Abl	c-Abelson
cAMP	Cyclic adenosine monophosphate
CARD	Caspase recruitment domain
CCL-2	Chemokine C-C motif ligand-2
CCR6	C-C chemokine receptor type 6
CD	Cluster of differentiation
CENP-B	Centromere protein-B
CSF-I	Caprine Serum Fraction-Immunomodulator
CI	Type I Collagen
CIDP	Chronic Inflammatory Demyelinating Polyradiculoneuropathy
CIMT	Carotid intima media thickness
COMP	Cartilage oligomeric matrix protein
CRF	Case report form
CRP	C-reactive protein
CRH	Corticotrophin releasing hormone
CRHBP	Corticotrophin releasing hormone binding protein
CT	Computerised tomography
CTGF	Connective tissue growth factor

CTLA4Ig	Cytotoxic T lymphocyte antigen 4 immunoglobulin
DC	Dendritic cell
DcSSc	Diffuse cutaneous systemic sclerosis
DDX	Doctors and Dentists Exemption
DLCO	Diffusing capacity of the lungs for carbon monoxide
EBMT	European Blood and Bone Marrow Transplantation registry
ECG	Electrocardiogram
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EDSS	Expanded Disability Status Scale
EGF	Epidermal growth factor
Egr-1	Early growth response-1
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EPCs	Endothelial progenitor cells
EQ5D	EuroQol-5 dimension questionnaire
ERA	Endothelin Receptor Antagonist
ERK	Extracellular signalling related kinase
ESR	Erythrocyte sedimentation rate
ET-1	Endothelin-1
EULAR	European League Against Rheumatism
EUSTAR	EULAR Scleroderma Trials and Research group
FAST	**F**ibrosing **A**lveolitis in **S**cleroderma **T**rial

FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FGF	Fibroblast growth factor
Fra-2	FOS related antigen-2
FVC	Forced Vital Capacity
FZD	Frizzled
GCP	Good clinical practise
GFAP	Glial fibrillary acidic protein
GI	Gastrointestinal
gp130	Glycoprotein 130
GROα	Growth related oncogene α
GWAS	Genome wide association studies
HAQ	Health Assessment Questionnaire
HAQ-DI	Health Assessment Questionnaire- Disability Index
HCS	Hyperimmune caprine serum
HDF	Human dermal fibroblasts
HGF	Hepatocyte growth factor
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-pituitary-adrenal
HSP47	Heat shock protein 47
HMGB-1	High mobility group box-1
HRCT	High resolution computerised tomography
HRV	Heart rate variability

HRV-TI	Heart rate variability Triangular index
HSCT	Haematopoietic stem cell transplantation
IAD	Inflammatory airways disease
IB	Investigator's Brochure
IFN	Interferon
IFN-α	Interferon-α
IFN-β	Interferon-β
IFN-γ	Interferon-γ
IgG	Immunoglobulin G
IIRF	Innate immune regulatory factor
IL	Interleukin
IP-10	Interferon γ inducible protein 10
I-QOL	Incontinence on Quality of Life
IRF-5	Interferon regulatory factor-5
ITT	Intention to treat
IV	Intravenous
IVIG	Intravenous immunoglobulin
JAK	Janus kinase
LC	Langerhans cells
LcSSc	Limited cutaneous systemic sclerosis
LFB	Luxol fast blue
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide

LRD	Lower respiratory disease
LRP	Low density lipoprotein receptor-related protein
MAPK	Mitogen-activated protein kinase
MC1-5R	Melanocortin 1-5 receptor
MCA	Middle cerebral artery
MCP	Monocyte chemoattractant protein
mRNA	messenger ribonucleic acid
MIG	Monokine induced by Interferon γ
MIP	Macrocyte inflammatory protein
miRNA	micro ribonucleic acid
MMF	Mycophenolate mofetil
MMP	Matrix metalloproteinase
MMRM	Mixed models repeated measures
MND	Motor Neuron Disease
MRC	Medical Research Council
MRSS	Modified Rodnan skin score
MS	Multiple Sclerosis
MSFC	Multiple Sclerosis Functional Composite
MSIS	Multiple Sclerosis Impact Scale
MSWS	Multiple Sclerosis Walking Scale
MTX	Methotrexate
MyD-88	Myeloid differentiation primary response gene 88
n	Number

NALP	NACHT, LRR and PYD domains-containing protein
NF-κB	Nuclear factor-κB
NHS	National Health Service
NK	Natural killer
NLR	NOD-like receptor
NLRP	Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing
NOD	Nucleotide-binding and oligomerization domain
NRES	National Research Ethics Service
NT-proBNP	N-terminal pro-B type natriuretic peptide
PAH	Pulmonary arterial hypertension
PARC	Pulmonary and activation-regulated chemokine
PAS	Periodic acid Schiff
PASAT	Paced Auditory Serial Addition Test
PASP	Mean estimated pulmonary artery systolic pressure
PBMC	Peripheral blood mononuclear cell
pDC	plasmacytoid dendritic cell
PDE5	Phosphodiesterase 5
PDGF	Platelet derived growth factor
PFTs	Pulmonary function tests
PIIINP	Procollagen type III N-terminal propeptide
POMC	Proopiomelanocortin
PPAR-γ	Peroxisome proliferator-activated receptor gamma

PPI	Proton pump inhibitor
Pred	Predicted
Prob or p	Probability
PRR	Pattern recognition receptor
QD	Daily
QoL	Quality of life
RANTES	Regulated on Activation, Normal T Expressed and Secreted
RMANOVA	Repeated measures analysis of variance
RMSSD	Square root of the mean squared differences in the R-R intervals
RNA	Ribonucleic acid
SAE	Serious adverse event
SAM®	Significance analysis of microarray
SCOT	**S**ystemic sclerosis: **C**yclophosphamide **O**r **T**ransplantation
SD	Standard deviation
SDRR	Standard deviation of the R-R intervals
SEC	Size exclusion chromatography
SF-36	Short form-36 questionnaire
SGOT	Serum glutamic-oxaloacetic transaminase
sICAM	Soluble intercellular adhesion molecule
sIL-2R	Soluble interleukin 2 receptor
siRNA	Silencing ribonucleic acid
SLE	Systemic lupus erythematosus
SLS	Scleroderma Lung Study

SNIP	Sniff nasal inspiratory pressure
SNPs	Single nucleotide polymorphisms
SOD	Superoxide dismutase
SPMS	Secondary Progressive Multiple Sclerosis
SRC	Scleroderma renal crisis
SSc	Systemic sclerosis
SSc-FS	Scleroderma Functional Score
SSc-ILD	Systemic sclerosis related interstitial lung disease
ssDNA	Single stranded deoxyribonucleic acid
STAT	Signal transducers and activators of transcription
sVCAM	Soluble vascular adhesion molecule
t1/2	Half life
TACE	TNFα converting enzyme
TARC	Thymus and activation-regulated chemokine
TG	Transgenic
TGA	Therapeutic Goods Administration
TGF- β	Transforming growth factor-β
Th1	T-helper type 1
Th2	T-helper type 2
Th17	T-helper type 17
Th22	T-helper type 22
TIMP	Tissue inhibitor of metalloproteinases
TLC	Total lung capacity

TLR	Toll-like receptor
TNFα	Tumour necrosis factor-α
TPI	Tripeptidic immunostimulant
Treg	Regulatory T cell
TRPC	Transient receptor potential cation channel
TSH	Thyroid stimulating hormone
VAS	Visual analogue scale
VEGF	Vascular endothelial growth factor
VEP	Visual Evoked Potential
VR	Valsalva ratio
vWF	von Willebrand Factor
WBC	White blood cell
WT	Wild type

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1 Introduction

1.1 Systemic sclerosis

1.1.1 Epidemiology and classification

Systemic sclerosis (SSc) is a multisystem disease that is associated with inflammation, fibrosis and vasculopathy. It is clinically heterogeneous although certain clinical and investigational features are common to the majority of cases. It is uncommon, affecting approximately 1 in 10,000 in the UK but has a very high morbidity and the highest case-specific mortality of any rheumatic disorder with 50% of patients dying or developing major internal organ complications within 3 years of diagnosis (1).

Only a few studies on SSc prevalence and incidence have been reported due to the low frequency of disease, large variability in clinical manifestations and severity, large variability in study design and lack of uniform diagnostic criteria. A recent systematic literature review looked at all reported studies in a 55 year period (2). Huge differences in prevalence and incidence were found in different geographical locations. The USA and Australia had the highest prevalence rates compared with Japan and Europe. Europe also displayed a north-south gradient with the lowest prevalence in Northern Europe. The prevalence in the USA and Australia is in the region of 250 cases per million, while in Japan it is 38 per million for definite SSc. Studies in Greece and France showed a prevalence of 154 and 158 cases per million respectively, while in Northern Europe, studies in the UK and Iceland showed a prevalence of 88 and 71 per million respectively.

Incidence rates in the USA appeared to have substantially increased from 0.6 cases per million per year in 1947 to 19 cases per million per year in 2001, and has remained stable since then. In Australia, the incidence increased from 12

cases per million per year in 1982 to 22 cases per million per year in 1999. In Europe the incidence rates have been stable at about 3.7 cases per million per year in the Northern countries, however a study in Greece revealed a much higher incidence rate at 11 cases per million per year and in Spain 23 cases per million per year (3) (2).

Several studies have also found ethnic variability with a lower age of onset and worse disease in black and Hispanic patients compared to Caucasians. SSc is rare in childhood and increases with age to a peak incidence in the 5th decade. It is more frequent in women than men and has an earlier age of onset in women (2). A twin study has found low concordance of SSc in twins (4.7%, monozygotic and dizygotic twins were similar) but a high concordance of anti-nuclear antibodies in monozygotic twins (4).

Environmental factors, particularly organic solvents and silica exposure, appear to increase the risk of SSc, especially in men. There is no evidence of an increased risk with breast implants. There also appears to be an increased risk of malignancy in SSc patients, particularly in lung, skin, hepatocellular, oropharangeal and oesophageal cancers as well as hematopoietic malignancies (3). Recent studies have also shown a link with malignancy in SSc and the RNA polymerase III antibody with a close temporal relationship between the onset of SSc and the onset of cancer (5) (6) (7) (8).

The first standardised classification criteria for SSc were published in 1980 (9). The classification criteria were developed to ensure that patients enrolled into research studies had definite disease. However classification criteria are not the same as diagnostic criteria, though they list many of the same features. Diagnostic criteria are often more inclusive as they are based on physician diagnosis. The 1980 classification criteria were developed using a population of long-standing SSc patients. Therefore, they do not perform well in patients with early SSc or in patients with the limited cutaneous form of the disease. Since

the publication of the 1980 criteria, recent advances in laboratory testing for autoantibodies and nailfold capillaroscopy have improved the ability to diagnose SSc early. Therefore a new set of classification criteria were published in the last year which have a higher sensitivity and specificity than the 1980 criteria (10).

The 2013 classification criteria are presented as an 11 item list with weighting of items. If a patient has skin thickening of the fingers of both hands that extends proximal to the metacarpophalangeal joints, the classification system assigns 9 points for this one item alone, which is sufficient to classify the patient as having SSc with no further application of the point system needed. Otherwise a points system applies. The maximum score is 19 and patients with a score of ≥9 are classified as having SSc. All cases that were classified as SSc by the 1980 criteria were also classified as SSc by the new criteria as well as a few cases that were not classified as SSc by the 1980 criteria. The system also performs well in early disease (10).

1.1.2 SSc Clinical Features

Systemic sclerosis is clinically heterogeneous. All cases manifest Raynaud's phenomenon and most have features of gastro-oesophageal reflux. There are two major subsets based on extent of skin fibrosis, limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc) (11). Approximately one fifth of cases of SSc also manifest features of another autoimmune rheumatic disease. These are designated as SSc overlap syndromes. The commonest overlap feature is myositis but other cases manifest Sjogrens, vasculitis or inflammatory arthritis. Clinical or serological features of systemic lupus erythematosus (SLE) may also be present. This project involves patients with dcSSc only, without overlap features.

In patients with lcSSc, Raynaud's phenomenon usually precedes the onset of skin fibrosis by many years, whereas in contrast, dcSSc patients usually

develop Raynaud's contemporaneously with their skin symptoms. Persistent vasospasm can lead to ischaemia, digital ulceration and infarction or gangrene.

Gastrointestinal involvement is the commonest visceral involvement. Almost all patients have gastro-oesophageal reflux, ranging from mild to severe. Some have difficulty maintaining adequate nutrition and require naso-gastric or naso-jejunal feeding. Gastric antral vascular ectasia may occur, requiring laser therapy and multiple blood transfusions. Other symptoms include bacterial overgrowth in the midgut causing malabsorption and diarrhoea, severe gut dysmotility causing constipation and anal incontinence.

Scleroderma renal crisis (SRC) is one of the most important major organ complications. SRC most often develops in patients with early dcSSc in association with rapidly worsening skin disease. It is associated with anaemia, new cardiac events, anti-polymerase I and III antibodies and high dose preceding medications such as steroids and non-steroidal anti-inflammatory drugs. Apart from SRC, many patients have some degree of renal impairment.

Interstitial lung disease (SSc-ILD) and pulmonary arterial hypertension (PAH) are now the two major causes of morbidity and mortality. For this reason, annual screening tests such as echocardiogram, ECG and lung function tests are performed on all patients as early treatment improves survival and morbidity. Early SSc-ILD is often asymptomatic but in later stages can present with dyspnoea, chest tightness, cough and fatigue. SSc-ILD is more commonly found in dcSSc patients. PAH can also be asymptomatic until late in its course and has similar symptoms to SSc-ILD, though cough is more suggestive of SSc-ILD (12).

1.1.3 Diffuse cutaneous systemic sclerosis (dcSSc)

DcSSc represents approximately one third of SSc cases. Common features of dcSSc include proximal skin thickening (i.e. skin thickening that extends to

upper arms, thighs, trunk and/or back), a maximum modified Rodnan skin score (MRSS) of >18 and a short history of Raynaud's syndrome. It is usually associated with anti-topoisomerase or anti-RNA polymerase III antibodies. The various hallmark autoantibodies occurring in SSc are mutually exclusive and several studies have demonstrated that individuals carrying each of these autoantibodies are associated with different frequencies of internal organ complications (13) (14). This also allows patients who are at increased risk of pulmonary, cardiac or renal complications to be identified. Pulmonary fibrosis and renal crisis are more commonly associated with the dcSSc disease subset.

DcSSc typically is most active in the first 3 years from onset (early stage disease) and this is the time during which major organ complications develop. The skin disease usually plateaus or improves over 1 to 2 years. Skin disease is the hallmark feature of SSc and can be associated with major disability. The severity and extent of skin involvement correlates to internal organ involvement (15). Skin inflammation causes oedema, pruritis, hypo-/hyper-pigmentation, and shiny inflamed skin resulting in skin thickening and fibrosis. Skin sclerosis can lead to joint contractures and loss of function. Although there is understandable focus on the high burden of severe skin and internal organ involvement in early stage diffuse SSc, with less than 3 years disease duration, there is also substantial burden at later stages and this has been highlighted in recent cohort studies (16).

1.1.4 Pathogenesis and Pathobiology

The pathogenesis of SSc is still poorly understood although it seems likely that there is interplay between inflammation, vascular and fibroblast dysfunction, lymphocyte activation, autoantibody production and connective tissue fibrosis. This leads ultimately to accumulation of the constituents of extracellular matrix, which replaces the normal tissue architecture in skin and internal organs, leading to organ failure (17) **Figure 1.1**.

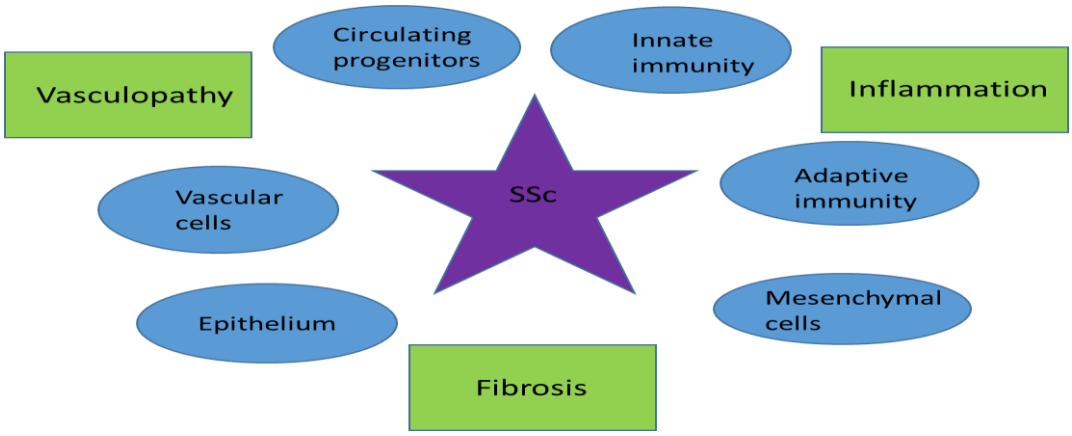


Figure 1.1: The pathogenesis of SSc is a complex interplay between vasculopathy, inflammation and fibrosis.

Genetic factors are thought to confer susceptibility to disease which is triggered by a combination of environmental factors such as solvents, toxins, infections and oxidative stress. Several genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in genes associated with SSc (18) (19) (20) (21) (22) (23) (24) (25). Other studies have identified changes in cytokine profiles and signalling pathways associated with SSc.

Traditional models of pathogenesis have suggested that early vascular events associated with autoimmunity and inflammation lead to subsequent fibrosis. Although this is plausible and supported by preclinical mechanistic studies it is clear that a broad range of biological processes interact in SSc and that these include involvement of key pro-fibrotic cytokines such as transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) as well as pro-inflammatory cytokines such as IL-6 and TNFα. There is also increasing evidence of an imbalance in Th1/Th2/Th17/Treg system promoting inflammation and fibrosis and activation of B cells promoting production of autoantibodies (26).

DcSSc is often categorised as early-stage or established/late-stage disease and it is possible that the pathogenic factors underlying the distinct phases of the disease are different. In particular, pathogenic drivers of late-stage disease are

less clear, but there is emerging evidence that persistent perturbation of immune cell function is increasingly important (27).

Vasculopathy is one of the hallmarks of the disease and peripheral vasculopathy or Raynaud's phenomenon is one of the first symptoms and is present in almost all patients. As the disease progresses, loss of microvasculature occurs, causing tissue hypoxia and endothelial injury. This normally initiates vasculogenesis and angiogenesis. However, in SSc vasculopathy results from an inappropriate repair process after endothelial injury causing vasoconstriction, adventitial and intimal proliferation, inflammation and thrombosis. It involves all layers of the vessel wall and is characterised by fibrotic intimal hyperplasia. Endothelial dysfunction plays a key role and chronically impaired production of vasoactive mediators, such as nitric oxide and prostacyclin, combined with over-expression of vasoconstrictors such as endothelin-1 (ET-1) affect vascular tone and promote vascular remodelling (28). The expression of ET-1 is induced by TGF-β, and ET-1 is considered to be a downstream mediator of some profibrotic TGF-β responses (29).

Vasculopathy in SSc displays a number of organ-specific features but also shares similarities in pathogenesis. Plexiform lesions develop in pulmonary arterial hypertension, which consist of endothelial cells and myofibroblasts. In renal crisis, the renal arteries display characteristic overgrowth of the epithelium, fibrinoid necrosis, onion-skin lesions and deposition of scar tissue in the blood vessels (17). In digital ulceration, vascular remodelling leads to progressive occlusion of the blood vessels and this, combined with reduced capillary density, results in hypoxia, necrosis and tissue loss (30).

Vasculogenesis appears to be impaired in SSc. Endothelial progenitor cells (EPCs) and monocytic EPCs have a reduced ability to form new blood vessels in SSc. This is combined with impaired angiogenesis in SSc in spite of overexpression of angiogenic factors such as vascular endothelial growth factor

(VEGF), fibroblast growth factor (FGF) and interleukin-8 (IL-8).The dysregulated response to these angiogenic factors in SSc is a complex combination of multiple pathways. For instance, the angiogenic response to VEGF is affected by the downregulation of kallikrein 12 and the overexpression of Fra-2. Similarly, FGF is affected by pentraxin 3, urokinase plasminogen activator receptor and junctional adhesion molecule-A (31).

Fibroblasts maintain the structural integrity of connective tissue, secreting fibrillar procollagens, fibronectin, and regulating the turnover and composition of the extracellular matrix (ECM). Following tissue injury, quiescent fibroblasts are activated during the wound healing and inflammation phase, producing granulation tissue and a provisional matrix, a process that is subsequently reversed to remodel the scar. In SSc, this scar is not properly remodelled and fibroblasts continue to promote a pro-fibrotic microenvironment rich in growth factors and ECM, resulting in excessive scar formation and fibrosis.

In SSc, activated fibroblasts are responsible for the development of fibrosis and the accumulation of ECM molecules (17). Fibroblasts explanted from lesional skin in SSc synthesise increased collagen and fibronectin in vitro. Moreover, they show constitutive production of cytokines and chemokines and spontaneous myofibroblast transdifferentiation (32). Activated fibroblasts in SSc may be derived from a number of different origins. Mesenchymal precursor cells may be recruited from the bone marrow via the circulation or resident tissue-specific percursors can be utilised from the surrounding tissues (33). Quiescent fibroblasts can be activated in a number of different ways including direct cell-to-cell contact, stimulation by soluble mediators or by cell-matrix interaction (17).

Hypoxia, TGF-β and Wnts promote the transition of precursor and non-fibroblastic cell types towards an activated myofibroblast phenotype and PPAR-γ (peroxisome proliferator-activated receptor gamma) promotes cellular

quiescence. TGF-β is the pre-eminent signal for connective tissue synthesis and is considered the core pathway in wound healing and pathological fibrosis. In normal fibroblasts, TGF-β induces a Smad-independant activation of c-Abl (c-Abelson, a non-receptor tyrosine kinase). Endogenous c-Abl is required for profibrotic responses induced by TGF-β in vitro. An important downstream target of c-Abl is Egr-1. Fibroblasts lacking Egr-1 show loss of collagen stimulation in response to TGF-β and lesional skin biopsies from patients with SSc show increased Egr-1 expression and activity, making it a potent fibrogenic mediator in SSc (32).

The Wnts constitute a large family of secreted signalling proteins important in embryonic organogenesis. While active in embryogenesis, the Wnts are normally tightly controlled in adults. Canonical Wnt signalling is initiated by ligand binding to Frizzled (FZD) and low density lipoprotein receptor-related protein (LRP) surface receptors, stabilising cytosolic β-catenin, blocking its degradation, which stimulates fibroblast activation. Abberant Wnt signalling is important in SSc (32).

PPAR-γ modulates TGF-β signalling and mesenchymal cell plasticity. Studies show that PPAR-γ is a cell-intrinsic anti-fibrotic pathway and activation of PPAR-γ ligands resulted in abrogation of TGF-β induced collagen production and Smad-3 dependant transcriptional responses (34). PPAR-γ also blocks the activation function of Egr-1 (35). PPAR-γ plays a fundamental role in regulating mesenchymal cell lineage fate determination and can shift progenitor cell differentiation along fibrogenic or nonfibrogenic pathways. Animal studies have shown that reduction in PPAR-γ causes increased fibrosis and PPAR-γ expression and activity are impaired in lesional skin in SSc. PPAR-γ expression is also inversely correlated with TGF-β signalling (35). A schematic representation of the major players in the pathogenesis of SSc is presented in **Figure 1.2.**

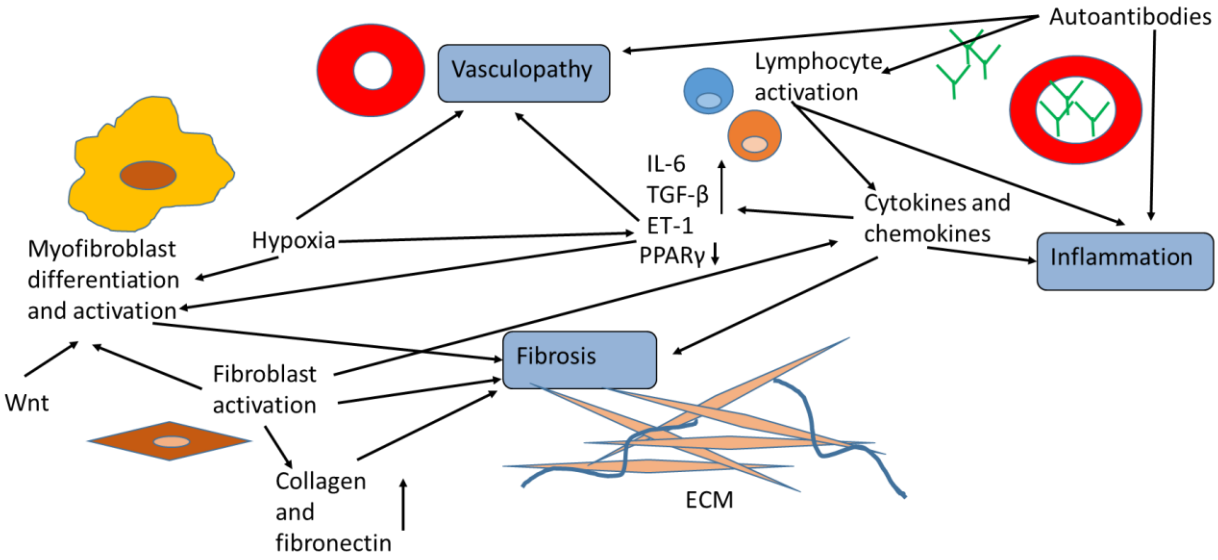


Figure 1.2: Schematic representation of pathogenesis of SSc..

1.1.5 Immune abnormalities

The immune system is also involved in the pathology of SSc. Cytokines play a major role in regulating the production of ECM by fibroblasts. Elevated levels of growth factors (TGF-β, connective tissue growth factor (CTGF), VEGF, FGF), interleukins (IL-2,4,6,8,10 and 13), chemokines (CCL-2, also called monocyte chemoattractant protein-1 (MCP-1)) and cytokines (tumour necrosis factor-alpha (TNF-α), fractalkine and others) have been found in SSc patients (17). Interferon gamma (IFN-γ) inhibits, while IL-4 and IL-13 enhance collagen synthesis.

Many recent studies have shown preferential accumulation of T cells producing high levels of IL-4 and IL-13 belonging to a Th2-like subset in SSc. Th2 cytokines favour collagen production, are pro-fibrotic and pro-angiogenic, whereas Th1 cells are anti-fibrotic and anti-angiogenic but it is unknown what drives Th2 differentiation in SSc. MCP-1 is produced in large amounts by SSc skin fibroblasts and has a direct role on collagen and matrix metalloproteinase-1 (MMP-1) production. It is produced by both Th1 and Th2 cells and is present at

sites of ongoing fibrosis (36). Other studies suggest a Th1 response especially in early inflammatory SSc, which seems to decrease in later disease whereas Th2 responses appear to actively promote fibrosis throughout (37).

Th17, as well as IL-17, appears to be increased in SSc patients. TGF-β, IL-1 and IL-6 have a role in Th17 priming and are all increased in SSc patients. Th17 appears to be anti-fibrotic and pro-inflammatory but several studies suggest that SSc fibroblasts may be resistant to the effects of IL-17. Regulatory T cells (Tregs) are reciprocally linked to Th17 cells and data are conflicting in SSc. Tregs are reported to be reduced in number and defective in function in SSc.Th22 cells (producing IL-22) also appear to be increased in SSc, though little is known about these cells in SSc. There was a positive correlation between Th22 cells and CCR6 and SSc-ILD was strongly associated with an increased number of Th22 cells (37).

B cell activation is enhanced by a Th2 environment and Th2 cytokines enhance immunoglobulin production by B cells. B cells, in turn, promote Th2 cell production with their antigen presenting capacity. B cells can also affect dendritic cells as IL-10 produced by activated B cells inhibits IL-12 production by dendritic cells, promoting Th2 differentiation. Activated B cells also produce IL-6, and several studies have found increased IL-6 in skin and serum of SSc patients (38) (39). In addition to autoantibody production, hypergammaglobulinema and polyclonal B cell hyperactivity, other B cell abnormalities are detected in SSc. Total B cells are increased, with naive B cells increased and memory B cells and plasmablasts reduced. There is also over-expression of CD19 (40).

Autoantibodies are detectable in the vast majority of SSc patients. Currently there are 5 major autoantibodies associated with SSc, being mutually exclusive and associated with different patterns of internal organ involvement. These are anti-topoisomerase antibody (anti-Scl70), anti-centromere antibody, anti-RNA

polymerase III antibody, anti-U1 and U3 (anti-fibrillarin) RNP antibodies. There is also anti-PMScl antibody, which is associated with overlap SSc and polymyositis, and a number of other antibodies under investigation such as antibodies against MMP1 and 3, heat shock protein 47 (HSP47), anti-fibrillin 1, anti-fibroblast antibody (36), and anti-endothelial cell antibodies (which may also have a pathogenic role) (41). Ahmed et al showed that SSc sera from both dcSSc patients (with anti-topoisomerase antibodies) and lcSSc patients (with anti-centromere antibodies) contain anti-endothelial cell antibodies, which can trigger apoptosis and are associated with increased caspase-3 activity as well as the re-expression of endothelial cell fibrillin 1.

Another recent study (42) shows higher levels of anti-angiotensin II type 1 receptor antibodies and anti-endothelin-1 type A receptor antibodies in SSc sera compared to healthy controls and other autoimmune diseases. A strong correlation between the 2 autoantibodies was found. Furthermore, the authors show that the autoantibodies induce ERK 1/2 and induced gene expression of TGF-β, blocked by their respective blocking agents. Patients with high levels of the two autoantibodies had a higher risk of developing dcSSc, late onset PAH, ILD and digital ulcers and a higher risk of dying from SSc-related causes. Lastly, the 2 autoantibodies have similarities to anti-endothelial cell antibodies since endothelial cells express both receptors. In another study by the same group (43), the authors found that these 2 autoantibodies induce activation of fibroblasts, increased expression of IL-8 and increased neutrophil migration into target tissues. Animal studies with passive transfer of the antibodies showed marked structural alteration of the lungs with increased interstitial cellular density and wound migration studies showed reduced wound repair correlating to the two autoantibodies. The authors also show increased type 1 collagen expression in response to the autoantibodies, attenuated somewhat by their respective blockers.

Different antibodies are also associated with different organ complications. Anti-topoisomerase antibodies are associated with interstitial lung disease, while

anti-RNA polymerase III is associated with scleroderma renal crisis and a predisposition to malignancy. Anti-U3 RNP is most often seen in males and African Americans and is associated with cardiac and skeletal muscle disease, **Table 1.1** (44).

Table 1.1: Auto-antibodies in SSc and their clinical associations

Autoantibody	Clinical associations	Frequency	Specificity for SSc
Anti-topoisomerase	dcSSc>lcSSc, SSc-ILD, severe digital vasculopathy	9-39%	97-100%
Anti-centromere	lcSSc, isolated PAH, Primary biliary cirrhosis, protective for SSc-ILD and SRC	16-39%	99.8-100%
Anti-RNA polymerase III	dcSSc, SRC, malignancy	4-25%	98-100%
Ant-U3 RNP	dcSSc>lcSSc, severe disease, muscle involvement, PAH	1-6%	-
Anti-PMScl	Polymyositis/dermatomyositis overlap, arthritis overlap, ILD	0-6%	45%
Anti-Th/To	lcSSc, PAH, SSc-ILD	1-7%	-
Anti-U1 RNP	Overlap syndromes	5-35%	-
Anti-Ku	Myositis, arthritis	1-3%	-
Anti-U11/U12 RNP	ILD	1.6-5%	-

Abbreviations: dcSSc; diffuse cutaneous systemic sclerosis, lcSSc; limited cutaneous systemic sclerosis, SSc-ILD; systemic sclerosis associated interstitial lung disease, PAH; pulmonary arterial hypertension, SRC; scleroderma renal crisis

Over the past few years, through research we have a better understanding of the role of the innate immune system in autoimmune disorders such as SSc, particularly the link between inflammation and fibrosis. The innate immune system provides immediate defence against a variety of pathogens and endogenous danger signals based on recognition of a variety of microbial patterns and acts to trigger inflammation and promote development of specific adaptive immune responses to pathogens. The innate immune system responds rapidly to the presence of certain patterns that microbes possess more commonly than mammalian hosts and these patterns trigger pattern recognition receptors (PRRs) which are widely expressed on cells of the immune system, epithelial and mesenchymal cells (such as fibroblasts).

Toll-like receptors (TLRs) are important PRRs. All TLRs except TLR-3 signal through the adaptor molecule MyD-88. TLRs are divided into 2 groups; one group based on the cell surface and recognise bacterial, fungal, mycobacterial and parasitic patterns (these include TLR-1, -2, -4, -5, -6 and -10) and the second group is found intracellularly in endosomes and recognise bacterial and viral patterns (these include TLR-3, -7, -8, and -9). Of the first group, TLR-2 and -4 are most relevant to SSc and bleomycin has been recently found to be a TLR-2 ligand. The second group have also been implicated in SSc as well as SLE. In SLE, anti-DNA antibodies bind to the Fc receptor on plasmacytoid dendritic cells (pDCs) and this leads to internalisation and delivery of the nucleic-acid containing immune complex to an endosomal compartment where TLR-9 is activated by a pattern on the ssDNA. Similar activation with immune complexes containing RNA has been identified and these have been shown to activate TLRs -7 and -8, which also reside in the endosome. There have been several recent advances in SSc research that show a similar mechanism may be occurring in SSc (45).

After activation of the endosomal TLRs, the pDC produces a burst of type I interferon (IFN). An “interferon gene signature” has been found in both SLE and SSc and in SLE correlates with disease activity, though a correlation has not been found in SSc. Type I IFN includes IFNα and β, and their production is

initiated early in the innate immune response. IFNα and β also increase type II IFN (IFNγ) by T cells and dendritic cells (DC). Polymorphisms associated with SSc (both anti-topoisomerase and anti-centromere positive) have been found in the genes for IRF-5 (IFN regulatory factor-5), which regulates IFN genes. IRF-5 polymorphisms have also been associated with SSc-ILD (45).

TLR-3 stimulation increased fibrosis and endothelin-1 production in fibroblasts and endothelial cells. Type I IFN also increases TLR-3 expression on healthy and SSc fibroblasts, potentially extending the inflammatory and fibrotic response. Human microvascular endothelial cells also express TLR-3 on the cell surface as well as in endosomes. The association between endothelial cell apoptosis and autoimmunity has been strengthened by the finding that apoptotic endothelial cells localize the centromere protein, CENP-B, to apoptotic blebs. CENP-B is the protein target of anti-centromere antibodies commonly found in patients with limited SSc (45). A recent study also demonstrated that IFN genes are up-regulated in skin from SSc patients (as opposed to PBMCs) and incubation with the synthetic TLR-3 agonist poly (I:C) induces both the IFN signature and TGF-β responsive genes (46).

A role for TLR-4 has been demonstrated in SSc patients in which monocytes derived from SSc patients with interstitial lung disease have an enhanced pro-fibrotic phenotype and can differentiate into fibrocytes (CD45+ CD34+) and secrete higher collagen after exposure to the TLR-4 agonist LPS, implying that TLR-4 is inducing a pro-fibrotic situation in the monocytes. In a recent study it was demonstrated that TLR-4 is elevated in SSc biopsies from both skin and lung and in vitro stimulation with dermal fibrosis with LPS produced global gene changes related to wound healing. It was also shown that in vitro stimulation of dermal fibroblasts with LPS and TGFβ leads to synergy in the production of collagen and that this is abrogated with knockdown of TLR-4, the LPS receptor. It is unlikely that in SSc LPS is the agonist responsible for TLR-4-mediated fibrosis but more likely that endogenous signals derived from damaged or redox ‘stressed’ cells are responsible for the fibrosis. One ‘danger signal’ released

from damaged cells that binds TLR-4 is HMGB-1 and this is elevated in tissue and serum from SSc patients and correlates with the skin score (46).

The inflammasome is an intracellular association of proteins, which act as a receptor for multiple 'danger signals' and results in triggering of the caspase system and release of IL-1β. The NLR (NOD (nucleotide-binding and oligomerization domain)-like receptor) family are part of the PRR system that are localized to the cytoplasm. Here these receptors recognize intracellular motifs. Upon ligation they lead to the activation and initiation of NF-κB (nuclear factor κB) and MAPK (mitogen-activated protein kinase) ultimately leading to the expression of pro-inflammatory cytokines. NALPs, when activated, create a complex that recruits pro-caspase-1, which is activated, resulting in recruitment of the adaptor protein ASC (apoptosis associated speck-like protein containing a CARD (caspase recruitment domain)) and activation of the 'inflammasome', a molecular platform, resulting in the activation and secretion of IL-1β and IL-18 via caspase-1. Environmental/occupational exposure to silica has been associated with SSc as mentioned above. The mechanism by which silica causes inflammation was recently described to involve activation of the NALP3 inflammasome. The NALP3 inflammasome is also activated in other inflammatory disorders such as gout and pseudogout and autoinflammatory disorders such as Muckle-Wells (46).

Polymorphisms in NLRP1 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 1) are associated with SSc-related pulmonary fibrosis and anti-topoisomerase-positive SSc patients. Furthermore, in SSc dermal fibroblasts, elevated levels of NOD2, NLRP3 and AIM2 (absent in melanoma 2) were observed. AIM2 is an intracellular bacterial and a viral DNA sensor. A recent study demonstrated that the inflammasome is necessary to mediate fibrosis induced by bleomycin. Using the bleomycin model of fibrosis they showed that NALP3-knockout mice had significantly reduced fibrosis compared with wild-type mice receiving bleomycin. Furthermore, they demonstrated that uric acid is the trigger for the activation of the inflammasome

as treatment of the mice with allopurinol reduced fibrosis in the wild-type mice given bleomycin. It is known that IL-1 is essential for pulmonary fibrosis in the bleomycin model of lung fibrosis (46). Furthermore, Artlett et al (47) inhibited caspase 1 using small molecule inhibitors or siRNA which showed that IL-1β, IL-18 and collagen secretion were attenuated in the SSc fibroblasts and also the hydroxyproline levels were lower. Also, their knockout mice were resistant to bleomycin induced fibrosis. Interestingly, in the formation of α-smooth muscle actin, the myofibroblast marker was also attenuated.

Most cells can perform functions of the innate immune system, however mast cells, macrophages, dendritic cells and natural killer (NK) cells are noteworthy. In addition to histamine, mast cells secrete several cytokines and other mediators associated with fibrosis such as TGF-β, IL-4, IL-13, platelet-activating factor, PDGF, MCP-1, IFN-α and endothelin-1. Mast cells have recently been identified as an important source of TGF-β in the skin of SSc patients. In SSc, mast cells are found in increased numbers in clinically uninvolved skin from early SSc patients, and these mast cells already express markers of activation and are degranulated. Later in the disease course, when the skin is atrophic, the density of mast cells appears to be decreased. Monocytes and macrophages have long been recognised as one of the predominant inflammatory cells present in the dermis of SSc patients. The macrophages are activated and produce CCL-2, TGF-β and PDGF. Natural killer cells are cytotoxic lymphocytes that can be rapidly activated and proliferate upon stimulation with type I IFN. Like mast cells and macrophages, NK cells can secrete several factors implicated in the pathogenesis of fibrosis such as IL-13, IL-10 and TGF-β. Circulating NK cells are increased in SSc and have an activated phenotype, though their function is controversial (45).

Dendritic cells are the ‘professional antigen-presenting cells’ of the immune system. The two main types of DCs in humans are the pDC and the conventional or myeloid DC that express TLR-2 and TLR-4 and secrete IL-12 on activation. pDCs express CD123 and were recently found to be increased in

SSc dermis by Fleming et al (48) as part of a translational analysis from the SSc Cyclophosphamide or Transplantation (SCOT) clinical trial. Following autologous stem cell transplantation treatment, the patients had dramatic clinical improvement and repeat skin biopsy showed decreased pDC density and less IFN-α mRNA. Langerhans cells (LCs) are a type of DC that circulate between the epidermis, dermis and lymphoid tissue. Several studies have shown a relative paucity of LC in SSc compared with healthy controls, although the differences were more pronounced in lesional skin. LC were found to promote regulatory T cells and IL-10 production (45).

IL-6 is a classic proinflammatory cytokine that is often dysregulated in autoimmune diseases. IL-6 is also associated with the wound healing pathway and deserves particular mention. It can be synthesised by a wide variety of cells and can have effects on many different cell types including B cells. The IL-6 receptor (IL-6R) can bind IL-6 in low affinity binding; however signal transduction requires a signalling molecule gp130, which is expressed on virtually all cells. IL-6 signalling is complex; classic signalling involves dimerisation of IL-6R with gp130, leading to activation of Janus kinases (JAKs), signal transducers and activators of transcription (STATs) and ERK and is restricted to hepatocytes and lymphocytes expressing membrane bound IL-6R. However there is a second signalling pathway called trans signalling which can occur in all cells expressing gp130 via soluble IL-6R. Soluble gp130 is the natural inhibitor of trans signalling but does not affect the classic pathway. IL-6 can upregulate αSMA and in vitro blockade of IL-6 reduced fibroblast production and secretion of collagen. Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of matrix metalloproteinases (MMPs) which break down ECM. TIMP-1 is elevated in SSc and IL-6 enhances TIMP-1 production in a STAT-3 dependant manner (49). Recently it has been shown that IL-6 trans signalling is mediated through a TGF-β signalling and downstream Smad3 (50).

Khan et al report that high levels of IL-6 correlate with thrombocytosis in dcSSc patients and serum IL-6 levels positively correlate with CRP and platelet count.

When IL-6 and IL-6R are added to cultured fibroblasts, there was an increase in collagen expression and also in αSMA and CTGF. This group confirmed a correlation between skin score and IL-6 levels but there was also a moderate correlation between peak skin score and IL-6 at time of presentation. In a subgroup of dcSSc patients, serum IL-6 at presentation correlated with MRSS at 36 month follow-up and furthermore, high IL-6 levels at presentation predicted higher mortality with 15 year survival 30% in these patients compared to 93% in the group with low IL-6 at presentation (39). Serum IL-6 has also been shown to be an independent predictor of DLCO decline in SSc-ILD with a threshold level of 7.67pg/ml. In a larger cohort IL-6 levels >7.67pg/ml was predictive of FVC and DLCO decline within the first year and predictive of death within the first 30 months. When stratified according to ILD severity (FVC<70), serum IL-6 was predictive of functional decline or death in the first year in patients with mild disease only (38).

1.1.6 Immunomodulatory treatment

The cornerstone of management of early stage diffuse SSc is broad spectrum immunosuppression. Currently, no treatment is proven to be effective in preventing progression of disease, reversing fibrosis or improving long-term outcome. Several studies have reported effectiveness of immune modulating drugs in the treatment of this disease, although these have mostly been in open, uncontrolled trials. These drugs include azathioprine, cyclosporin, methotrexate, mycophenolate mofetil and cyclophosphamide (51). However, an observational study looking at outcomes of 5 different treatment protocols in early dcSSc found no significant difference between the treatment protocols (52).

Emerging data support the benefit of immunosuppression for skin and lung fibrosis in SSc, especially when given at the early stages of disease The EULAR Scleroderma Trials and Research group (EUSTAR) recently published a set of core recommendations for treatment of SSc. Cyclophosphamide (2-3 mg/kg), in combination with low-dose prednisone is recommended for skin disease in dcSSc and for lung fibrosis. Methotrexate can be used in skin

disease or in patients with features of overlap inflammatory arthritis. Mycophenolate mofetil is increasingly being used in skin and lung disease with azathioprine as an alternative option (53). The main currently used treatment approaches are discussed below.

1.1.6.1 Cyclophosphamide

Two open label trials of cyclophosphamide have shown possible benefit in scleroderma lung disease (54) (55). A further open label trial in 18 patients showed stabilisation of lung function for up to 3 years after 1 year treatment (56). Two retrospective case series also show stabilisation in lung function with cyclophosphamide (57) (58). Two landmark randomised, double-blind, placebo controlled trials for cyclophosphamide in SSc-ILD, the Scleroderma Lung Study (oral) (59) and the FAST trial (intravenous) (60), showed beneficial effects on lung function. The 2 year data from the Scleroderma Lung Study showed a sustained benefit in dyspnoea index and difference in FVC of 6.8% predicted in the cyclophosphamide treated group at 18 months but this benefit was no longer significant at 2 years, suggesting that longer term immunosuppression may be warranted to maintain benefit (61). There were also beneficial effects on skin and this has also been seen other open-label uncontrolled trials (62) (63).

Berezne et al report stabilisation or improvement in 70% and 51.8% of their patients after 6 months and 2 years, respectively with intravenous cyclophosphamide followed by oral maintenance immunosuppression in a multicentre retrospective cohort (64). However, a meta-analysis by Nannini et al concluded that while previous trials with cyclophosphamide show a statistically significant improvement in lung function, they do not show a clinically significant improvement (>10% change in lung function) (65). A further recent long term observational study found that 5 of 13 patients relapsed after 1 year and concluded that long term immunosuppression maintenance will be necessary to maintain remission (66) and an open label study found improvement or stabilisation in 31 of 36 patients (67).

1.1.6.2 Methotrexate

Methotrexate has been reported to be effective in a case-report (68), three open studies (69) (70) (71) and two placebo-controlled double-blind trials (72) (73). In these studies improvement was observed in approximately 70% of patients, either in skin involvement or arrest of progression of internal organ involvement.

The largest of these trials was considered to be underpowered (72). Although there was statistically significant improvement, it was not considered clinically significant. In this study, the dose of methotrexate was modest at 15mg and was not increased in cases of inefficacy. There is a lack of data about progression or stabilisation of lung fibrosis with methotrexate, although this study shows minor non-significant improvements. Using the data from the Pope study for reanalysis using Bayesian methods, a recent article by Johnson et al suggest that, in fact, methotrexate has a high probability of beneficial effects in SSc for skin disease and global assessment (74).

Currently, methotrexate is the treatment of choice in patients with SSc/myositis or SSc/inflammatory arthritis overlap syndromes. A rare but severe side effect of methotrexate is pneumonitis. Methotrexate should therefore be used cautiously in scleroderma patients with advanced pulmonary fibrosis who have diminished respiratory reserve.

1.1.6.3 Mycophenolate Mofetil

Mycophenolate mofetil (MMF) has been used in various autoimmune diseases particularly systemic lupus erythematosus with good results. In a small case series, benefit in skin scores was reported with MMF when given as maintenance treatment after anti-thymocyte globulin (ATG) induction treatment (75). A further report from a large retrospective study showed similar efficacy in skin improvement and reduced progression to severe lung disease in SSc when compared to other immunosuppressive drugs, with a good safety profile (76).

A small retrospective analysis found an improvement in SSc-ILD with MMF, in keeping with similar findings in other small case series (77). One retrospective case control study comparing cyclophosphamide and mycophenolate found no change in PFTs in either group but a radiological decline in the MMF group (78). A further recent retrospective observational study on 98 patients also showed an improvement in MRSS compared to historical controls taken from other studies (79).

Two recent prospective observational trials in 25 (80) and 15 (81) early dcSSc patients reported significant improvement in skin score and though not powered for lung, showed stable/slight improvement in lung function tests. Two prospective open label trials in connective tissue disease-associated lung fibrosis including 9 patients (82) and 14 patients (83) with SSc-ILD suggest benefit or stabilisation with MMF. The Scleroderma Lung Study 2 (SLS2) is currently underway with a study protocol assessing efficacy of cyclophosphamide versus mycophenolate mofetil.

1.1.6.4 Azathioprine

Most of the published data regarding azathioprine in SSc refers to maintenance immunosuppression using azathioprine after cyclophosphamide induction therapy (60) (64) (84). One study reported stabilisation of skin score and lung parameters with azathioprine maintenance treatment, but there was no control group in this study (84). One unblinded study reported better efficacy of cyclophosphamide compared to azathioprine (85) and two retrospective studies reported stabilisation of lung function with azathioprine treatment (86) (87).

1.1.6.5 Tolerance to human Type I Collagen

Several studies have identified a variety of autoantigens in SSc patients. One of these autoantigens is type I collagen (CI). A previous open-label study using bovine CI to induce immune tolerance to human CI showed very promising results, with a reduction in skin score of 23% (88). Patients with diffuse cutaneous SSc did better than the limited subset. Recently, a larger placebo controlled trial in diffuse cutaneous SSc showed no significant differences in

skin score in the total number of patients treated. However, a subgroup of patients with late stage diffuse cutaneous SSc had a significant reduction in skin scores. The authors concluded that it may be beneficial in selected patients and that further study of this interesting treatment is warranted (27). The results of this study were important for generating a hypothesis and the planning and setup of this trial.

1.1.6.6 Intravenous Immunoglobulin

Human pooled immunoglobulin contains polyclonal IgG antibodies against pathogens, foreign antigens and autoantigens. It is used in low doses as replacement therapy in immunodeficiency syndromes and in high doses as immunomodulatory therapy for autoimmune conditions such as dermatomyositis and idiopathic thrombocytopenia purpura. A number of open-label trials have shown an improvement in skin score with intravenous immunoglobulin (IVIG) (89) (90) (91) (92). A recent retrospective single centre observational study of 30 patients with refractive dcSSc (with and without immunosuppressants) showed significant reduction in MRSS at 24 months, indicating that it may be an effective adjunctive treatment (93). To date, only one randomised double blind trial has been completed. In this trial, a single 5-day course of IVIG did not show significant improvement but a retreatment with a second course showed an improvement in skin score. The authors suggest further trials with repeated courses of IVIG should be considered (94).

1.1.6.7 Haematopoietic Stem Cell Transplant (HSCT)

Cases have been reported of patients with autoimmune diseases and coexisting haematological conditions, treated with HSCT and also experienced improvement in their autoimmune diseases. The first reports from the European Blood and Marrow Transplantation/European League against Rheumatism (EBMT/EULAR) Registry of HSCT in SSc showed a significant improvement in skin score, however, transplant related mortality was 17% (95). Transplant-related mortality dropped to 8.7% on analysis of a second cohort, 3 years later and skin score improvements continued to be significant (96). A long-term follow-up study of 27 patients in the US, using a regime which included total body irradiation showed a skin score reduction of 39% at 12 months and a

continuing trend after this. Estimated progression-free survival was 64% at 5 years (97).

The long-term follow-up of two trials from the Netherlands and France with a median follow-up was 5 years showed 81% patients demonstrated a clinically beneficial response. 73% patients had a >25% reduction in skin score in the first year and 5 and 7 year survival was 96.2% and 84.8%, respectively (98). Another single centre study of 26 patients in Germany showed treatment related mortality 11% and progression free survival at 74% (99).

The ASSIST trial (open-label) reported a much better outcome for skin and lung function in the HSCT arm compared to the cyclophosphamide arm. 19 patients were enrolled, 10 in the HSCT arm and 9 on cyclophosphamide alone. In the cyclophosphamide arm, 8 of 9 patients progressed and 7 of these were treated with HSCT after 1 year. Mean MRSS was higher in HSCT group at baseline and improved after 1 year, but worsened in the cyclophosphamide group after 1 year. The MRSS in the patients who crossed over to HSCT also improved. There were no deaths during the study period, possibly due to relatively mild disease at entry and a small sample size (100). Burt et al also report a retrospective analysis of 90 patients who receive HSCT as part of a study or on compassionate basis in the US. Five of 90 patients died from treatment related causes (6%), 4 of these from cardiac causes. HSCT improved MRSS and FVC in treated patients. 5 year relapse free survival was 70% (101).

The results of two further trials (ASTIS and SCOT) have been reported at international conferences. The Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial was the first phase 3 trial enrolling 156 patients over 8 years (2001-2009) in 29 centres. Patients were randomised to HSCT or 12 monthly pulses of IV cyclophosphamide. Patients randomised to HSCT experienced more events and higher mortality in the first year compared to controls but had a significantly better long-term event free and overall

survival. Treatment related mortality was 10% in the HSCT group. There were no treatment related deaths in the control group, but most deaths occurred due to disease progression. 8 patients in the cyclophosphamide group received rescue HSCT and 2 in the HSCT group received rescue IV pulsed cyclophosphamide (102) (103). Mean change in baseline to 2 years follow-up for MRSS showed significant benefit for the treatment group compared to the control group (MRSS improvement by 19.9 in treatment group vs 8.8 in control group, $p<0.001$). There was also significant improvement in FVC, TLC, HAQ-DI, physical component of SF-36 and the EQ5D, whereas creatinine clearance was significantly worse in the treatment group. Sensitivity analysis showed loss of significance for FVC, TLC, HAQ-DI and SF-36 due to missing data and smaller patient numbers when data was missing because of death. 7 of the 8 patients who died due to treatment in the HSCT group were current or former smokers (103).

The Scleroderma: Cyclophosphamide or Transplantation (SCOT) trial is a randomised controlled phase 3 trial still ongoing in North America enrolling patients from 2005-2011, with similar endpoints and control treatment to ASTIS but a different protocol for HSCT which includes total body irradiation and equine anti-thymocyte globulin (instead of the rabbit form in ASTIS) as part of conditioning. Renal toxicity was observed in 12% HSCT patients and so bilateral lung and kidney shielding was employed as a modified protocol. The results of this trial are still awaited (102).

1.1.6.8 Biological Agents

A retrospective study of etanercept in SSc-associated inflammatory joint disease showed a good response of joint disease with a trend towards improvement in skin score (104). A more recent prospective open-label pilot study of infliximab showed a stabilisation of skin score and a fall in two laboratory markers of collagen synthesis, but no clear benefit was seen (105). A further observational study on 10 patients with SSc and inflammatory arthritis concluded that etanercept was effective in inflammatory arthritis associated with SSc, but there was no change in MRSS in this study (106).

Rituximab, a monoclonal antibody against CD20 present on mature B cells, is a B cell depleting agent. Four small open label trials have been reported in SSc with conflicting results. Three report benefit in skin (107) (108) and lung function (109) while another found no improvement is skin disease in SSc (110). However one of these trials, the patients remained on other treatments and the Rituximab and conventional treatment groups were not matched (109).

Two further open label trials, each with 8 patients and for 2 years, again report significant improvement in skin and lung disease (111) (112). A recent nested case-control study from the EUSTAR group has reported improvement in skin and stabilisation in lung disease, providing further encouraging results (113). Further double blind randomised trials are needed to confirm efficacy.

The first case report on 2 patients treated with Tocilizumab, an IL-6 receptor antibody, showed improvement of MRSS in these patients (114). The same author reported another case of dcSSc that improved with both skin score and joint range of motion with Tocilizumab (115). This is particularly interesting in view of the data above on IL-6 associated with the pathogenesis of SSc and IL-6 being a predictor of worse disease and mortality in SSc patients. Abatacept is cytotoxic T lymphocyte antigen 4 immunoglobulin (CTLA4Ig). EUSTAR have reported finding from an observational study showing significant improvement in joint counts in patients treated with tocilizumab and abatacept, but no change in skin or lung parameters and no improvement in SSc-related myositis (116).

A randomised double-blind clinical trial of Tocilizumab in SSc is currently underway and full results are eagerly awaited. Interim 24 week data were presented at the American College of Rheumatology annual meeting 2014. 87 patients were enrolled, 43 tocilizumab and 44 placebo. There was a trend to improvement in MRSS ($p=0.09$) and numerically more patients in the

tocilizumab group achieved a clinically important improvement in MRSS (>4.7 units improvement), though the primary endpoint was not met. More patients in the placebo group had worsening of lung function compared to the tocilizumab group. The frequency of AEs and SAEs were similar, though there were more infectious complications in the tocilizumab group (117).

1.1.6.9 Other Treatments

Two open-label trials for imatinib have been reported, the first showing some efficacy in SSc-related lung disease, but with a note of caution regarding the large number of adverse events from the medication (118). The second open-label trial also showed improvement in skin and lung function (119) and an extension phase recently published showed continued improvement in skin score with most adverse events that were attributed to the medication being graded mild to moderate (120). Two randomised double-blind trials have also been reported, showing efficacy in skin disease in one, though the cohort enrolment criteria were not uniform with regard to skin disease and classification of SSc (121) and in the second study, imatinib was very poorly tolerated so efficacy could not be assessed properly, though skin score did not change between those who completed 6 months of treatment and those who did not (122).

Two further studies were reported at 2010 and 2011 American College of Rheumatology conferences. The first trial in 7 patients showed improvement in skin score (123). The second trial showed no efficacy at 24 weeks and trend to improvement of skin score at 48 weeks, but when compared to a historic cohort, the trend to skin improvement was not significant (124) (125) (126). 2 other recent reports, the first a case series of 6 patients (127) and the second a trial in 30 patients with SSc-ILD, suggest that low dose imatinib (127) may be tolerated better and may improve SSc symptoms.

A recent randomised single blind pilot trial comparing rapamicin with methotrexate in SSc reported improvement in skin score in both groups, however further follow-up studies will need to confirm efficacy (128).

Other novel approaches include rilonacept (anti-IL-1), pomalidomide, ambrisentan, tadalafil, LPA receptor antagonists, PPARγ agonists, and anti-IL-13 monoclonal antibody which are all potential treatments affecting different targets and are currently in trials or trials are being planned (129).

1.1.7 SSc general management approaches

As SSc is a clinically heterogeneous disease, management is tailored to the individual patient, depending on stage, severity and organ complications. A general approach to management is outlined in **Figure 1.3.** Once a patient has a potential diagnosis of SSc, full clinical history and examination including MRSS, serological profile, chest radiograph, pulmonary function tests, echocardiogram and ECG should be performed to investigate for organ based complications and overlap features (12). After diagnosis, the benefit of regular screening tests should not be underestimated. A report by Nihtyanova et al demonstrated that regular screening has led to better ascertainment of organ-based complications, with earlier diagnosis and treatment of these complications, leading to better survival in these patients. Therefore, it is recommended to perform non-invasive screening tests on an annual basis in stable patients, and more frequently if the patient becomes progressively symptomatic. These tests should include an ECG, routine blood tests, echocardiogram and pulmonary function tests. If these indicate possible organ complications, then further tests such as right heart catheter, high resolution CT chest scan or cardiac MRI may be ordered (16).

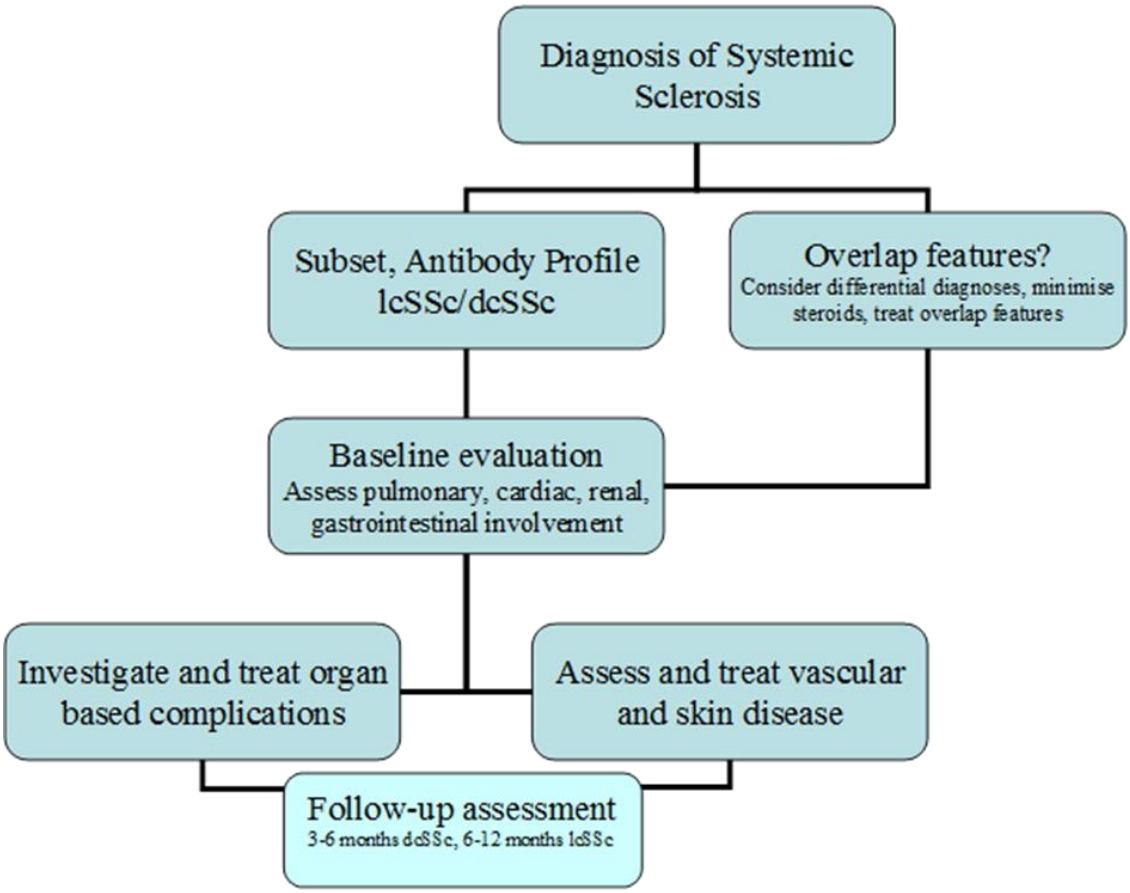


Figure 1.3: General management of SSc

Immunosuppressants remain the cornerstone of management of skin disease and organ complications in SSc. Treatment of skin disease is usually indicated in the first few years of disease in those who have active dcSSc and can be discontinued in most patients after a few years, once the disease becomes less active. The choice of immunosuppressant, as discussed above, depends on other organ complications and whether there are any overlap features. If there are no organ complications or overlap features, MMF or MTX are the most appropriate choices. In very severe cases, cyclophosphamide or HSCT may be considered. If there is evidence of SSc-ILD, where FVC >70 on lung function tests, MMF is normally used, while in more extensive lung disease IV cyclophosphamide is more appropriate. In patients unresponsive to treatment, Rituximab is considered. Patients with myocarditis are treated with IV cyclophosphamide or MMF and if immunosuppression is required in renal crisis, low dose MMF is considered. In patients with overlap, immunosuppression

appropriate to the overlap syndrome is considered. For example, if there are features of joint synovitis or myositis, MTX is more appropriate (130).

Apart from immunosuppression, it is important to consider symptomatic treatment of skin inflammation and other symptoms. Pruritis is treated with topical measures, antihistamines, and if severe, low dose glucocorticoids and/or leukotriene receptor antagonists. Telangiectases can be treated with laser. Minocycline can be considered for calcinosis but, unless severe, surgery is not usually indicated as calcinosis tends to recur. Calcinotic deposits may become infected and ulcerated so may require courses of antibiotics.

Raynaud's syndrome is often very debilitating and treatment includes lifestyle changes such as stopping smoking, avoiding cold and wearing layers of warm clothing and gloves. Calcium channel blockers, angiotensin II receptor blockers and selective serotonin reuptake inhibitors may be helpful. Iloprost IV, phosphodiesterase 5 (PDE5) inhibitors and endothelin receptor antagonists (ERAs) are considered for severe Raynaud's especially if associated with digital ulceration or critical digital ischaemia. Sympathectomy may also be considered in severe cases.

Gastrointestinal symptoms are extremely common and most patients have some element of gastroesophageal reflux which is treated with proton pump inhibitors. Many patients have severe symptoms requiring higher doses to control symptoms or the addition of histamine 2 receptor antagonists. Prokinetics may be prescribed for dysphagia and rotating courses of antibiotics for small bowel overgrowth causing diarrhoea. Constipation may be treated with regular laxatives and anal incontinence may require a sacral nerve stimulator.

In addition to immunosuppression discussed above, N-acetylcysteine may be beneficial in SSc-ILD. Low dose glucocorticoids and alternative

immunosuppression with azathioprine may be considered. In severe cases, lung transplantation may be appropriate. Treatment of PAH includes, diuretics, anticoagulation, oxygen and digoxin. Monotherapy with advanced therapies such as prostanoids, PDE5 inhibitors and ERAs is considered first and combination therapy is appropriate if monotherapy fails. Riociguat, a guanylate cyclise agonist, is a new class of drug, which has recently reported benefit in PAH. Renal crisis is treated by removing any known trigger, angiotensin converting enzyme inhibitors and supportive care including dialysis. Renal function can improve up to 18 months after SRC, therefore renal transplantation should not be considered for at least 2 years (130) (12).

1.2 Hyperimmune Caprine Serum

1.2.1 Hyperimmune caprine serum manufacture and composition

Hyperimmune caprine serum (HCS) is a goat serum extract derivative supplied frozen and thawed to a liquid for immediate injection. It is produced in goats raised and housed at a licensed facility in Tasmania, Australia. The animals are vaccinated using detergent- inactivated HIV viral lysate. The caprine model is an ideal vehicle for this as it cannot propagate the HIV virus in vivo. Serum is shipped frozen to the manufacturing facility in Victoria, Australia where the sera are pooled, fractionated and diafiltered to preserve various macromolecules, immunoglobulin species and low molecular weight components prior to further processing nanofiltration and vialing.

The final product contains principally caprine immunoglobulins but also various small molecular weight species including cytokines. ELISA characterisation of the serum has revealed the presence of a range of components including the cytokines IL-4 and IL-10, proopiomelanocortin (POMC), arginine vasopressin, β-endorphin and corticotrophin-releasing hormone (CRH). Previous studies have shown that when peripheral blood mononuclear cells (PBMCs) are isolated and incubated with serial dilutions of HCS, raw hyperimmune serum and heat-inactivated sera induced the release of IL-10 in vitro {Investigator Brochure,

Daval International, personal communication}. The following sections describe mechanism of action and previous animal and human studies with HCS. Most of the data are unpublished, apart from abstracts presented at international meetings, as indicated below.

1.2.2 Anti-Inflammatory action

The therapeutic efficacy of HCS is based on the presence of a novel stable multi-protein complex, whose key components include; CRH, α-2 macroglobulin (α-2M) and lipoprotein-related peptide-1 (LRP-1). α-2M, a well-known protease inhibitor, protects CRH from normal proteolytic degradation. The stabilisation of the CRH peptide allows it to have a longer half-life (t1⁄2) in the body (14-16 hours vs. 2.5 to 4.5mins), leading to its sustained-release within homeostatic boundaries.

HCS's reparative qualities are thought to be as a result of the stabilised complex's targeting of the proximal hypothalamic-pituitary-adrenal (HPA) axis and extra-hypothalamic sites where the CRH-receptor-1 is expressed centrally. HCS's subcutaneous delivery results in sustained release of CRH which is the principal neuroregulator of the basal and stress-induced production of adrenocorticotropin releasing hormone (ACTH), β-endorphin and several other POMC related peptides. HCS's ability to re-establish the HPA axis's normal responsiveness to biologic and environmental stressors is the hallmark of a drug {Investigator Brochure, Daval International, personal communication}.

The stabilised protein-peptide complex that incorporates CRH and LRP-1 is made possible by the structure created by the α-2 macroglobulin. The degree of penetration of activities associated with different domains on α-2 macroglobulin, may be regulated asynchronously by conformational change in α-2 macroglobulin and by other regulatory proteins in the cellular microenvironment, allowing a novel complex to be formed in a caprine vehicle exposed to a specific attenuated inoculate as seen with the medicinal product. The ability of α-2 macroglobulin to "trap" caprine CRH affords the neuropeptide "protection" from rapid proteolytic degradation (131).

Analysis and characterisation of the composition of the medicinal product has been carried out using Size Exclusion Chromatography (SEC), which shows that approximately 95% is in the caprine IgG size while the remainder is composed of higher molecular weight components. The molecular and proteomic analysis of the medicinal product has been carried out in collaboration with the Public Health England (PHE) using SELDI-TOF mass spectrometry and 1-D and 2-D gel electrophoresis with proteomic analysis. Peptide-capture from the medicinal product using immobilised monoclonal or polyclonal antibodies (where appropriate) was used to detect peaks that matched the predicted molecular mass for specific molecular species. Specificity was demonstrated and confirmed with the use of a number of irrelevant control antibodies.

1.2.3 Sodium channel effect

Previous exploration of the potential applications of HCS has focused on its neurological properties. Some 400 patients with multiple sclerosis (MS) have taken the medication for periods of up to five years. In excess of 50,000 doses have been given over this period, without significant side-effects (Daval International Ltd., personal communication). Uncontrolled (open-label) observations suggest that many such MS patients experience an improvement in motor function, fatigue and bladder control, and there have been reported instances of marked improvement in colour vision and balance. Findings on threshold tracking / electrotonus testing on a patient with Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) before and within two hours of her first treatment with HCS, showed a reduction in triggering voltages of sodium channels in peripheral nerves and a prolongation of their opening, thus favouring conduction in damaged or demyelinated axons (132) (Abstract 549, XVIIIth World Congress of Neurology 2005) (133).

A trial in optic neuritis showed a non-significant trend for improvement of automated visual field measurements in MS patients with chronic visual symptomatology, under double-blind conditions (134) (Abstract 71, Proceedings of the Association of British Neurologists 2005). In another study, reversal of

conduction block within optic nerve fibres was demonstrated in one of six patients, using visual evoked potential studies, with a significant and sustained improvement in colour vision within the group as a whole (135) (Abstract 77, Joint Sino-British Neurology meeting, Beijing 2004). These rapid improvements in neurological function seem also to occur in patients with muscular dystrophy (136) (Abstract 272, XVIIIth World Congress of Neurology 2005) and myasthenia gravis (137) (Abstract 306, XVIIIth World Congress of Neurology 2005) consistent with a unifying sodium channel effect.

1.2.4 Animal studies

1.2.4.1 Murine and Equine lipopolysaccharide (LPS) model

In a murine and equine lipopolysaccharide (LPS) model of inflammation/endotoxaemia, HCS significantly decreased production of several pro-inflammatory cytokines including TNF-α and led to improved clinical outcome when compared to controls. HCS increased survival in the murine LPS model. In addition, Dr. Simon Bailey, a Veterinary Pharmacologist at the University of Melbourne, Australia, administered either HCS, placebo, or a registered intravenous anti-inflammatory comparator (Flunixin®) to standard bred horses at the University of Melbourne, following injection of according to a double- blind “low dose” protocol. He found that HCS and Flunixin significantly reduced peak body temperature compared with placebo and that HCS, in particular, lead to an earlier recovery of blood leukocyte counts post-acute margination, although there was also an effect seen in HCS treated animals only, where peak TNF-α levels were significantly reduced (abstract in 3rd AVA/NZVA Pan Pacific Veterinary Conference, Equine stream, June 2010).

1.2.4.2 Bleomycin Lung Model

For this study, there were 5 groups; a NAIVE SERUM group that received no bleomycin (BLM) challenge and no treatment, and a second control that received no BLM challenge and no treatment with NAIVE SERUM (i.e. saline only). The remaining study animals were treated with either saline (negative control), NAIVE SERUM (negative control for compound activity), or HCS along with BLM. All mice used in this project were C57BL/6 females, 8 to 10 weeks old, obtained from Charles River Laboratories, Hollister, CA. After 3 days of

acclimation, animals were treated with of saline, NAIVE SERUM (100 µg) or HCS (100 µg) via subcutaneous injection delivered under the skin on the back of the mice beginning on day 1, administered QD for 28 days (28 injections total per mouse). Mice also received either BLM (0.045 units/50 µl) or saline control (50 µl) via intratracheal instillation on day 1 following saline, NAIVE SERUM or HCS delivery. On Day 28, animals were anaesthetized and bled via retro-orbital sinus and followed by tissue and broncheal-alveolar lavage (BAL) fluid collection.

No statistical changes were observed in any experimental group when compared to any control group when analysing lung homogenates for α-MSH, CRH, CRH2, CRHBP, MC1R, MC4R, IL17F, LRP1, hydroxyproline or PIIIINP. Serum samples were assayed for circulating cytokine levels by ELISA; no statistical changes were observed in any experimental group when compared to any control group when analysing for CRHBP. An increase in α-MSH was observed though not significant in the BLM/HCS group. Serum MMP-1 and MMP-13 showed a trend to decrease in the BLM/HCS group but this was not found to be significant.

Serum MMP-9, BAL fluid IL-12p70, MCP-1 and TNFα analysis revealed that there were significant differences between groups; a statistically significant decrease was noted in the BLM/HCS group compared to the BLM/NAIVE SERUM and BLM/Saline groups. The difference was more significant comparing BLM/HCS and BLM/saline. Under the conditions of this study, injury to the lung and the consequent fibrosis were focal to multifocal and of generally minimal or mild severity. The two control groups (saline and NAIVE SERUM) had no increase in pulmonary fibrosis. Among the groups with increased pulmonary fibrosis, the lowest mean group severity scores for fibrosis was present in the BLM/HCS group. The lowest mean group severity scores for all findings were present were also present in the BLM/HCS treatment group (unpublished data, Daval international, personal communication).

1.2.4.3 Bleomycin Lung Function Model (Buxco)

Lung function, including respiratory volume changes and rate of volume change, was assessed in conscious unrestrained mice by use of the BUXCO plethysmograph system. Baseline functional data was collected while mice were breathing air, while stressed in a 5% CO2 atmosphere and then during recovery in air. This study was separate from the above assays, and used mice treated with BLM/HCS (n=9), BLM/NAIVE SERUM (n=9), and untreated control mice (n=3), with functional analysis at 10 and 28 days after study initiation. No significant changes were observed between the BLM/NAIVE SERUM and BLM/HCS groups at any time point or during CO2 stress or recovery. The BLM/HCS group exhibited a significantly lower tidal volume and minute volume following a 5% CO2 stress challenge at 28 days versus the BLM/NAIVE SERUM treatment group. The profile of change in the BLM/HCS group was in the same direction as the normal controls, that is, with an associated decrease in residual and minute volumes post-CO2 exposure. Interestingly, the changes were paradoxical within the BLM/NAIVE SERUM treatment group at 28 days. The animals recovered to near the control group when returned to air (unpublished data, Daval international, personal communication).

1.2.4.4 Bleomycin Skin Model

After 3 days of acclimation, animals were treated with of saline, NAIVE SERUM (100 µg) or HCS (100 µg) in 100 µl volume via subcutaneous injection beginning on day 1, administered QD (daily) for 52 days (52 injections total per mouse). Mice also received either BLM (0.09 units/50 µl) or saline control (50 µl) via subcutaneous injection QD for 45 days, beginning on day 1 until day 45 of the study. On Day 52, animals were anaesthetised with inhaled isoflurane, and bled via retro-orbital sinus and tissues collected. Blood was processed into serum and kept frozen at -80ºC until analysed. Skin, lung, brain, adrenal, heart, and retroperitoneal adipose tissues were collected from a subset of the animals in each study group, and serum samples were collected from all mice.

CRH levels in skin homogenates were significantly lower in the BLM/HCS group versus the BLM/NAIVE SERUM group and BLM/Saline group (p=0.014). Tissue

CRH2 analysis showed that no statistically significant difference between the BLM/NAIVE SERUM and BLM/HCS treatment groups (p=0.432). However, a strong trend was observed with an increase in CRH2 in the BLM/Saline group (p=0.067). CRH-binding protein as measured in the skin homogenate exhibited no significant difference in any of the treatment groups. These data likely point to a net rise in the “free” active fraction of CRH1/2 in situ.

A potential downstream mechanism of action of HCS was observed, as evidenced by the maintenance of protein levels of α-MSH, likely due to increased proteolytic cleavage of POMC by regional cells. Skin exposed to BLM has been shown to reduce intrinsic α-MSH expression, which may have a negative impact on cell survival.

α-MSH levels were significantly increased and/or maintained in the BLM /HCS (171±17pg/mL) treatment group versus BLM/Saline (87±10 pg/mL) or BLM/NAIVE SERUM (129±9 pg/mL) treatment groups (p=0.013 and p=0.003) respectively. MC4R expression was significantly lower in the BLM/HCS group (4392.7±1645 pg/mL) when compared to the BLM/saline and BLM/NAIVE SERUM groups (p=0.017). In contrast, there were no significant differences in the levels of MC1R expression between any of the BLM treatment groups. The changes in MC4R may have been due to a compensatory response that reflected the greater fall in α-MSH levels in both the BLM/NAIVE SERUM and BLM/Saline treatment groups.

Of the three markers of skin fibrosis that were assayed in the skin homogenates, interesting observations were made with both intrinsic (local) LRP-1 expression and total hydroxyproline content. Both showed significant changes that favoured a reduced local fibrotic response. In contrast, analysis of skin tissue homogenate levels of PIIINP expression showed no significant differences between the various BLM-treatment arms. The hydroxyproline content was significantly lower in the BLM/HCS group (2351.7±348.2 µg per mg

of protein) compared to the positive control (BLM/Saline, 5958.7±1226.5 µg per mg of protein, p=0.012) or the BLM/NAIVE SERUM (4526.4±669.3 µg per mg of protein, p=0.0133) groups respectively.

Cytokine levels in serum showed analysis of CRH-1 BP revealed that there were no significant differences between the treatment groups. However when a Mann-Whitney test was performed significant differences were seen between the BLM/saline and the saline group (p=0.0080), between the BLM/NAIVE SERUM and the NAIVE SERUM groups (p=0.0095) and between the BLM/HCS and the NAIVE SERUM groups (p=0.0077). No differences were seen between the BLM/HCS, BLM/Saline and the BLM/NAIVE SERUM groups (p=0.2987).

Cytokine analysis in serum showed that there was a strong trend but not significant increase in α-MSH in the BLM/HCS group versus BLM/Saline positive control (p=0.054). Analysis of TGF-β showed no statistically significant differences between the treatment groups. Factors regulating the extracellular matrix remodelling such as MMP-1, MMP-9, MMP-13 and TIMP-1 were all assayed in serum with several mediators providing strong evidence of an anti-fibrotic signal in response to HCS post-BLM in skin. TIMP-1 analysis revealed a strong trend in reduction in expression in the BLM/HCS group compared to the BLM/NAIVE SERUM group (p=0.058) and BLM/Saline (p=0.054), however as can be seen this did not achieve statistical significance. The ulcerating lesion is more florid and larger in the BLM/Saline and BLM/NAIVE SERUM groups as compared with the BLM/HCS treatment arm at day 50 of the experimentation.

In rodents, bleomycin administration induces an inflammatory response characterised by leukocyte infiltration, fibroblast proliferation, and increase in collagen content that can culminate in the development of pulmonary lesions similar to those observed in human idiopathic pulmonary fibrosis. Previous studies have demonstrated a genetic susceptibility to bleomycin-induced pulmonary toxicity based on the close association between mouse strain and

the fibrotic outcome. C57Bl/6 mice are considered to be fibrosis prone. HCS attenuates both the histological and biochemical features associated with the Bleomycin-induced cutaneous fibrotic model (unpublished data, Daval International, personal communication).

1.2.4.5 Superoxide Dismutase (SOD1) G93A Transgenic (TG) Mouse Model of ALS

Amyotrophic lateral sclerosis is a progressive neurodegenerative disorder primarily involving motor neurons. A subset of individuals with familial autosomal dominant forms of the disease have mutations of the copper/zinc superoxide dismutase (Cu/Zn SOD, SOD-1) gene, which encodes a ubiquitously expressed enzyme that plays a key role in oxygen free radical scavenging. The G93A SOD-1 mouse introduces a mutation into the mouse SOD-1 gene that corresponds to one of the changes found in the human gene in familial amyotrophic lateral sclerosis. The original article describing the generation of these mice reported early onset of the disease (~100 days) and rapid decline with the affected mice reaching the end stage on average within 40 days after disease onset (typical survival 130-160 days) (138).

The objective of the study was to determine whether targeting of the HPA axis at a specific site using HCS could elicit measurable efficacy in the G93A SOD1 murine model (Abstract 48.08, Society for Neuroscience 2013, Abstract P221 ALS/MND International Symposium 2013). Age-matched G93A male/female mice n=20 per treatment group (NAIVE SERUM/WT, NAIVE SERUM/TG and HCS/TG animals were injected once daily (100µg subcutaneously) using a double-blind experimental protocol from day 60 days to 150 days and analysed using open-field testing, survival rate, clinical standard methods of assessment as well as utilising a 1H-MRS brainstem study. Significant maintenance was observed in rotarod latency, grip strength and concomitant changes were observed in several key cellular metabolites, using 1H-MRS at 90 and 110 days. Delayed onset of disease and prolonged survival were also observed though not significant.

Onset of disease for each mouse were recorded when they reached a disease stage 4. The mice were carefully examined using clinical scoring three times a week. The clinical scoring system was on a scale of 1 to 5; with 1 as the endpoint for euthanasia, and 5 as healthy with little or no signs of onset of disease. The disease onset was delayed by 6 days in the HCS versus NAIVE SERUM treatment in the TG groups though this was not statistically significant. The survival (%) was not statistically different between the intervention groups with a mean survival time of 105 days.

The data show that at 90 days there was a significant difference noted between the TG/HCS and TG/NAIVE SERUM treated groups where grip strength did not deteriorate in TG/HCS group to such an extent as the TG/NAIVE SERUM treated group and the rotarod latency superseded both that seen in WT and TG/NAIVE SERUM treated groups.

1.2.4.6 Alzheimer's Mouse Model

The objective of the study was to determine whether using novel stabilized HCS could elicit measurable efficacy and if so, by what mechanism in the Tg2576 transgenic mouse model of Alzheimer's disease (AD), which overexpresses a mutant form of amyloid precursor protein (APP), APPK670/671L, linked to early onset familial AD (Abstract 753.18, Society for Neuroscience meeting 2013). Age-matched Tg2576 (Tg) male and female mice, n=15 per treatment group (wild type/NAIVE SERUM, Treatment group/NAIVE SERUM and Tg/HCS), were injected twice weekly with 100 µg HCS s.c. using a double-blind experimental protocol starting from 3-months of age and continued to 5-months of age. Mice were analysed for contextual fear conditioning testing and disease progression at 5-months of age. Levels of hippocampal metabolites were analysed pre- and post-dose using non-invasive in vivo 1H-MRS at 3 and 5 months.

The major protein component of amyloid deposits associated with Alzheimer's disease is a 39-42-amino acid, self-assembling peptide, known as the amyloid

Aβ peptide. Biochemical analysis of the amyloid peptides isolated from Alzheimer's disease brain indicates that Aβ1-42 is the principal species associated with senile plaque amyloids. Significant reduction in soluble amyloid Aβ1-41 and Aβ1-42 was noted in the hippocampus, ventral cortex, CSF and serum of the Tg/HCS treated group vs. Tg/NAIVE SERUM as measured by ELISA. Insulin degradation enzyme activity in the hippocampus by immunocapture activity assay was unchanged. Significant maintenance was observed with open-field testing and with preservation of contextual fear conditioning outcome.

There was significant increase in neurogenesis as evidenced by increased BrdU+ and CD34+ in the hippocampus determined by immunohistochemistry following introduction of HCS. Significant changes in the cellular hippocampal metabolite choline using 1H-MRS at 5-months, indicated increased cellular mitosis.

1.2.4.7 Inflammatory Airways Disease in Horses

The purpose of this study was to determine whether HCS is able to reduce or abolish clinical signs and inflammatory changes associated with inflammatory airway disease (IAD) in horses. The horses recruited for this trial showed the characteristic clinical signs of inflammatory airway disease, and were typical of the large numbers of young horses in early training all over the world which show signs of IAD. These signs included coughing and nasal discharge, either at exercise or at rest, and excessive mucus in their airways and markedly increased numbers of neutrophils in their bronchial fluid. These signs caused interruption to their training program.

HCS treatment produced significant beneficial effects compared with saline between days 0 and 16, assessed by coughing and mucus viscosity, with the amount of mucus accumulation also tending to decrease. Furthermore, the objective data provided by the differential cell counts in the tracheal wash fluid showed that HCS significantly reduced the numbers of neutrophils.

Horses receiving HCS from days 16 to 32, after not responding very well to conventional antibiotic therapy only (from the original saline control group) did not respond as favourably as those horses treated initially with HCS. The injections were generally well tolerated and in the majority of cases, the 10 ml of HCS deposited subcutaneously disappeared within a few hours, as observed in previous studies involving standard bred horses. Unfortunately however, fluid swellings were observed in two of the sixteen fine-skinned thoroughbred. Although these gradually disappeared over 24-72 hours, and no systemic adverse effects were noted, these reactions were unsightly (in the skin) and in one case painful (pectoral muscle). The reason for these reactions is unclear. In the two cases where they did occur, they tended to be more pronounced following repeated administrations, suggesting that they may be immune-mediated, after prior sensitisation. However, the skin swellings were fluid in nature and there was no external evidence of any tissue thickening or cellular tissue reaction either within or beneath the skin (unpublished data, Dr. Bryan Youl, personal communication).

1.2.4.8 Twitcher Mouse Model (Krabbe's Leucodystrophy)

Krabbe's Leucodystrophy disease is a fatal genetic neurodegenerative disorder, predominantly affecting infants, caused by a mutation in the gene encoding for the lysosomal enzyme galactocerebrosidase. An immune and inflammatory involvement has been associated in the pathogenesis of this disease. Treatment options available for this disorder to date are limited. Anti-inflammatory therapies have been shown to reduce excessive activation of inflammatory molecules, which contribute to disease progression in various neurological disorders including potentially in Krabbe's

A study was conducted by the University of Sydney using HCS, to test the safety and efficacy, as well as the clinical and pathological effects of HCS using a Twitcher mice murine model of Krabbe's disease. The Twitcher mouse (C57BL/6J-GALCtwi; twi/twi), an enzymatically authentic model of human Krabbe disease (Kobayashi et al., 1980), was used in the studies. The model involved sacrificing c. 30 mice at 30-35 days (Twitchers twi/twi) and 45 days (normal) that

had been injected with HCS or placebo every second day for 3 days a week, commencing at 10 days to 35 days . Following sacrifice, blood was extracted and analysed, as well as brain, spinal cord and sciatic nerve samples. Luxol Fast Blue (LFB), with Periodic Acid Schiff (PAS) as counterstain, was performed under standard staining procedures to assess the level of myelination of the samples. In addition, glial fibrillary acidic protein (GFAP) immunohistochemical staining was performed to define levels of astrocytosis.

Drug safety was shown, as no adverse effects were recorded in the normal mice that were administered with HCS. Furthermore, no significant difference in measured parameters was noted between HCS and placebo treated normal mice. LFB staining of the medial corpus callosum was more severe for both placebo-treated, and HCS-treated Twitchers, compared to normal mice ($p<0.001$). Importantly LFB staining was more significantly intense in the HCS-treated Twitchers compared to placebo-treated Twitchers ($p=0.043$). Normal mice had fewer, shorter, lightly stained GFAP-positive processes, while an increase in staining was evident in both placebo- and HCS-treated Twitcher mice.

Upon visual examination, astrocytes appeared to be more densely packed, and intensely stained with thicker cellular processes in Twitcher mice that received placebo treatment, than those of HCS-treated Twitcher mice. This observation was supported by statistical evidence to indicate a significant difference in GFAP stained area between placebo-treated and HCS-treated Twitcher mice ($p=0.029$). Less astrocytosis was observed in the HCS versus placebo-treated Twitcher mice. Exaggerated astrocytosis has been shown to be causative in pathological demyelination.

Box testing was also performed in the form of an open field during which exploratory activity is assessed through a series of qualitative and quantitative measures. Twitcher mice in both groups were considerably less mobile than normal mice and were observed to generally tend to stay on the corner of the enclosure and remain there for the duration of the test compared to the normal inquisitive behaviour of mice. Dragging of limbs was observed in both HCS and

placebo treated Twitcher mice thus exhibiting less exploratory activity levels than normal mice.

Although on gross observations, the two Twitcher groups cannot be differentiated, the values recorded for each of the clinical parameters consistently showed HCS-treated Twitcher mice to have the milder clinical condition. Box testing and body weight in the HCS versus placebo treated groups showed either significant, or near significant differences, respectively (Box test: p=0.039, one-tailed t-test, Body weight: p=0.068, one-tailed t-test) (unpublished data, Daval International, personal communication) .

1.2.5 Human studies

1.2.5.1 Case reports

A number of case studies have been reported in patients with MS, ALS (139) (140), CIDP (141) (Abstract, British Society for Clinical Neurophysiology, London 2004), optic neuritis (135) (Abstract 77, Joint Sino-British Neurology meeting, Beijing 2004) and Krabbe's leukodystrophy (142) (Abstract 38, XVIIIth World Congress of Neurology 2005). HCS is being prescribed in patients with non-inflammatory central nervous diseases such as Krabbe's leukodystrophy (for which an Orphan Status designation has been awarded by the Therapeutic Goods Administration (TGA) in Australia) and demyelinating peripheral nervous system disorders such as Charcot-Marie-Tooth disease, Type I and CIDP. HCS also has FDA Orphan drug approval for ALS in MND based on the case report and study below (**Section 1.2.5.5**).

1.2.5.2 UK DDX pre-2004 Multiple Sclerosis Trials

In total, two previous clinical studies received MHRA and ethics approval under the UK DDX system, pre-2004. The first, the Oxford optic neuritis study with 11 patients (134), was completed. The patients had chronic stable visual loss due to multiple sclerosis, and a total of three, weekly doses of either HCS or a human albumin placebo were followed by a wash out period of approximately 6 weeks and then the crossover regimen. A trend towards an improvement in

HCS treated patients in automated visual field scores did not reach statistical significance. No adverse effects of medication or placebo were recorded.

The second, a double blind placebo controlled study in secondary progressive MS, due to run for 2 years, was halted after 6 months due to hospital pharmacy failure to apply correct standards of preservation in handling the study material (HCS must remain deeply frozen until immediately prior to injection). Six month safety data were collected from all 47 patients and there were no adverse events attributable to HCS or the placebo.

1.2.5.3 Phase II Double Blind Multiple Sclerosis Trial

This trial was conducted at the same time as the trial reported in the thesis, but was not completed until later and the data has not yet been published. The data presented here are from Daval International and are being prepared for publication. The primary objective of this Phase II trial was to explore safety and tolerability and measure the effect of regular HCS injections on the symptoms of overactive bladder and several pre-defined non-bladder secondary and tertiary outcome measures in Secondary Progressive Multiple Sclerosis (SPMS). Secondary objectives were to seek signals indicative of efficacy and biological activity.

20 SPMS patients were randomised to receive either HCS (n=10) or placebo (n=10) by subcutaneous injection. One mL, containing 4.5 mg protein per mL, was injected twice weekly for four weeks, followed by a six week wash-out period, and then a crossover to the other treatment for four weeks. Patients were entered into the trial based on a confirmed diagnosis of SPMS together with having no relapses in the preceding year six months prior to enrolment. Patients were ambulatory, at least 18 years old, passed urine at least 8 times per 24 hours and had urinary urgency. Patients had no more than one relapse in the previous 12 months and no relapse in the last six months with respect to their MS. Following completion of the cross over period all patients were then offered open-label use of HCS for an additional 38 weeks.

Safety was assessed by summarising incidence and type of adverse events through to Week 14 and in the case of continued treatment through to Week 56. All patients were included in the safety assessment. Efficacy endpoints included bladder function (a selected primary endpoint), measured by change in average voided volume of urine and percentage improvement was measured using the MSFC (MS functional composite), which produces scores for each of the three individual measures (25 foot walk, 9 Hole Peg Test and Paced Auditory Serial Addition Test – PASAT3) as well as a composite score. Visual acuity and colour vision were also tested. A 12 item multiple sclerosis walking scale (MSWS-12, version 1), Incontinence on Quality of Life (I-QOL) Patient Questionnaire score, a Multiple Sclerosis Impact Scale (MSIS) questionnaire on physical ability and the Expanded Disability Status Scale (EDSS) (which measures for visual, pyramidal, sensory, brain stem, cerebellar, bowel and bladder, and cerebral function) were also performed. Specific biomarker analyses on cytokines, growth factors and miRNA was conducted as part of one of the secondary outcome measures.

HCS was defined as a safe and well-tolerated treatment up to 12 months of analysis. No treatment related severe adverse events were recorded. The mean urinary frequency (voids / day) by visit for the open-label phase showed that HCS significantly decreased the mean frequency from baseline (8.41) to 52 weeks (7.18) (adjusted mean change -1.6; $p<0.0001$). In addition, improved bladder function was observed by the fall in mean urinary incontinence (episodes / day) by visit during the open-label phase, evidenced by HCS significantly decreasing the mean frequency from baseline (2.12±2.94) to Week 26 (1.14±1.51) (adjusted mean change -0.84; $p=0.0009$). HCS did not show a significant difference in bladder function between four weeks of treatment with HCS and four weeks of treatment with placebo in the double blind phase.

An analysis of the mean total MSFC score with treatment difference for the double blind phase showed that HCS significantly increased the total MSFC score from

-0.259±1.771 to 0.177±1.361 (adjusted mean change 0.694; p=0.0215). This compares to the placebo group where a significant increase was not observed in the DBPC phase (from - 0.773±2.695 to 0.116±1.735; p=0.297). The mean total MSFC score by visit for the open-label phase also suggested that a longer period of treatment with HCS significantly increased the total MSFC score from -0.45±1.85 at baseline to -0.127±1.95 at week 52 (adjusted mean change 0.661; p=0.021). The individual components that make up the MSFC were analysed and all 3 components showed significant improvements with HCS in the double blind phase and further improvements in the open label phase suggesting that longer treatment led to improved efficacy.

The visual acuity (LogMar for both eyes combined) showed significant improvement with HCS versus placebo during the blinded phase (p=0.026). The Visual Evoked Potential (VEP) studies, using HCS on P100 latency, provided additional confirmatory evidence on demyelination and inflammation of the optic nerve. The visual acuity (LogMar for individual eyes) showed that HCS significantly improved right eye visual acuity LogMar versus placebo during the open label phase (p=0.005). The mean colour vision score, by treatment period and overall, showed that HCS nearly significantly decreased the mean colour vision score (that is tending to normalisation) during the double blind phase from baseline 138.9±125.2 to end of period 106.1±106.4 (adjusted mean change -18.4; p=0.06).

HCS treatment showed trends to improvement in mean MSWS during the open-label phase and statistically significant improvement in the overall I-QOL during the open-label phase. Improved quality of life was observed when using HCS as evidenced by the patient's Question 2 responses from the beginning of the treatment period for the open-label phase to 26 weeks as shown by a 53.3% improvement in the cohort group; 26.7% showed no change and only 20.0% of patients worsened (reflecting attenuation of the natural course of SPMS where up to 79% of patients worsen by 6 months). A further analysis showed that HCS at 52 weeks saw 42.9% of patients stating an observed improvement, and 42.9% no change and only 14.3% feeling that they were worse. Further functional

improvement, with respect to the mean MSIS, for the double blind and open- label phases was also seen.

This trial was a proof of concept and safety trial, as part of the development of HCS. The medication was found to be well tolerated with minor injection site reactions being the main side effect recorded. Although the trial did not show a difference in bladder function between four weeks of treatment with HCS and four weeks of treatment with placebo, implying that short-term administration of HCS may not be effective for the SPMS indication, during the open- label phase of the study results of bladder and non-bladder assessments showed a number of functional areas of significant improvement, suggesting the need for longer term administration of HCS during future clinical studies {Investigator Brochure, Daval International ,personal communication}.

1.2.5.4 Open Label Multiple Sclerosis Trial

The study was conducted in multiple centres across the United Kingdom with registered clinicians (consisting of neurologists and general practitioners), commencing 2004 (Abstract SC23, CMSC-ACTRIMS meeting May 2013). The objective of the open label study was to retrospectively analyse the safety, tolerability and efficacy of HCS in progressive MS. The open label format of the study allowed the safety and efficacy of HCS to be assessed in MS patients over an extended time period. 154 patients were treated with 1 mL of 4.5 mg/mL. HCS 1-3 times weekly, via subcutaneous injection, on an open-label basis for 2 weeks to 3 years (3 to 150 doses).

Of the 154 patients treated on a named patient basis 140 had adequately documented follow-up and assessment available for review. The patients were ambulant, aged at least 18 years, with progressive MS (the majority with SPMS) for duration of 3 months to 40 years. Adverse events were limited to skin irritation at the injection site. 19 patients reported mild self-limiting reactions: some needing topical antihistamines; 8 reported more persistent reactions needing oral antihistamines; 1 patient withdrew from treatment due to persistent skin irritation. No treatment-related severe adverse events were noted. Of all the EDSS scores

recorded, 117 patients (over 80%) reported clinical benefit in 1 or more areas while 16 patients reported no overall benefit and 9 patients showed overall worsening by 1 point in 1 area. 96 patients had improvement in 2 or more areas and as such were likely to have a change of at least -0.5 in their EDSS score, 35 of these had changes in 4 or more areas indicating a probable EDSS change of -1.0. Clinical benefit was reported largely in the following areas of the EDSS: motor (97 patients), energy (56 patients), bladder (37 patients) and sensory / pain (33 patients). Bladder efficacy was demonstrated in the open label study on longer-term use of HCS. The implications of this are important as they corroborate with the data readout from the open label phase of the SPMS Phase II clinical trial (above).

A second open label study was performed with a total of 14 patients. The mean age was recorded at 53.1±3.76 years with a male-female ratio of 1:4. The average duration of treatment was 12.3±2.35 months. While patients showed a pre-treatment average EDSS score of 30.5±2.73, HCS significantly improved the EDSS score with an mean of -12.79±2.18 resulting in a post-HCS EDSS score of 17.71 ±1.94, ($p<0.0001$) {Investigator Brochure, Daval International, personal communication}.

1.2.5.5 Open Label Amyotrophic Lateral Sclerosis Trial

This is an open label study in amyotrophic lateral sclerosis (ALS), also known as Motor Neuron Disease (MND). ALS was investigated following the identification of a significant efficacy signal from a single long-term ALS patient case study mentioned above in section 1.2.5.1 (139) (140). The primary objective of the study was to prospectively analyse the longer term safety, tolerability and efficacy of HCS in patients diagnosed with ALS. 1 mL of 4.5 mg/mL HCS was administered daily, via subcutaneous injection. Patients were assessed intermittently against a number of standardised criteria over the course of the open-label study (Abstract P320, ALS/MND International Symposium 2013). Patients in the study had been monitored between 4 to 18 months at time of reporting. Mean patient treatment duration was 181 days at time of IB publication. Patients were both ambulant and non-ambulant, aged at

least 18 years, with independently confirmed ALS (based on El Escorial Criteria) and disease duration of more than 24 months from the time of diagnosis. In total 20 patients have taken part in the study at time of reporting.

Adverse events were recorded and collated as change from baseline (prior to therapy) and post dose during the entire treatment period. The ALSFRS-R (revised ALS Functional Rating Scale), the ALS Assessment Questionnaire (ALSAQ-40), ALS score of Hillel and the ALS score of Jablecki were assessed at visits. Pulmonary function testing incorporating standard spirometry, full blood profiles, electrocardiography (ECG) and clinical examination by several clinicians at each study site were performed.

There have been no serious adverse events recorded during the clinical study to date and non-serious adverse events have been limited to a reversible and mild skin irritation at the injection site which was resolved spontaneously after 24hrs. No biochemical or haematological adverse issues were observed. Twenty patients have been enrolled to date. The mean age of the study group was 48.45-yrs, with a disease duration of >2.5-yrs (from the time of diagnosis) and a M: F ratio of 4:1. The patients had been monitored from between 4 to 18 months depending on the time of enrolment by their treating clinician (mean 0.95±0.36 yrs).

In summary, patients showed a significant improvement in ALSFRS-R ($p<0.0001$), FVC ($p<0.011$) and stabilisation in ALS scores of Jablecki and Hillel during the study period. ALS-QOL was also stable. Muscle power (increase of 6.5% from baseline in the dominant hand) using a hand held digital dynameter testing grip strength (dominant and non-dominant hand assessment). This correlated with no deterioration in mid-thigh circumference - a measure of muscle wasting. The study also showed a distinct stabilisation post treatment in BMI with a 1.57% improvement ($p=0.002$).

This study also showed that patients either improved or stabilized irrespective of their stage of ALS. No deterioration was observed in respiratory function testing. Patients showed early improvement in pseudobulbar/bulbar and spasticity within 4-6 weeks of starting treatment. Patients did not develop any tolerance to the medicinal product. Despite a trend of benefit, larger, blinded, studies of the effect of HCS in ALS patients will need to be performed to truly prove efficacy {Investigator Brochure, Daval International, personal communication}.

1.3 Caprine Serum in other contexts

The caprine serum fraction-immunomodulator (CSF-I) mentioned below is a completely different immunomodulatory agent than HCS. It is produced in a different way and has a different mode of action. HCS is produced by injecting goats with an inactivated HIV virus to produce an immune response and serum is then collected, pooled and nanofiltered. HCS is then frozen and thawed just prior to injection. In contrast, CSF-I is produced by collecting serum from goats and is fractionated by collecting material flowing through a dialysis membrane with a cut-off of 6-8kDa, then lyophilised to a powder. The powder is reconstituted with water before injection. It is interesting to note that both induce changes in the cytokine balance and innate immunity in humans and animals and that both have been investigated for therapeutic use.

Caprine serum fraction-immunomodulator has a limited license for veterinary use since 1993. Field trials have shown efficacy of CSF-I against bacterial, viral, and environmental stress challenges as found in bovine shipping fever and respiratory disease, canine parvovirus and lymphoma, and ovine footrot according to the patent application (143).

Willeford and colleagues (144) reported a study on CSF-I in 2000, which they described as a nonadjuvanted immunostimulant derived from goat serum. Their study in chickens was the first to show that material derived from goats had the potential to retard the progression of bacterial infection (pasteurella multocida) in a non-mammal. This infection results in high rates of mortality in chickens,

but their data shows that CSF-I retards pathogenesis and reduces mortality. The higher dose regimen with 2 doses instead of one had the best prognosis.

Hamm et al (145) reported 2 blinded studies and field studies in horses with lower respiratory disease (LRD) using CSF-I in combination with standard antibiotics which was usual standard of care for LRD. Their data show that in study 1 (dose finding study, placebo, 15mg, 30mg and 60mg) all CSF-I treated horses improved but only the highest dose of 60mg produced a statistically significant improvement both at day 7 and day 14. Eight of 10 non-responsive control horses were treated with CSF-I at day 14 and these subsequently improved so the difference between groups was insignificant by day 28. Study 2 (placebo, 60mg, 120mg) showed a significant improvement in all CSF-I treated horses by day 7, but no difference between 60mg and 120mg groups. Again control horses treated at day 14 improved and there was no statistically significant difference by day 21. The field studies in 4 centres confirmed the previous findings and 75% CSF-I treated horses had improved by day 14. This increased to 83% at day 21 compared to 10% in the control population.

Parker et al (146) studied mice injected a tripeptidic immunostimulant (TPI) isolate of CSF-I prior to infection with salmonella. Their data shows no benefit of injection 4 days prior to infection but a benefit was observed if mice were injected at day -2, -1 or day 0. By day 8, the mortality in the control population reached 80%, while groups that received TPI on day -2, -1 and day 0 had mortality rates of 60, 32 and 54%, respectively. The day -1 treatment group had significantly lower mortality than the day 0 and day -2 TPI treatment groups, ($p=0.0193$ and $p=0.0014$, respectively). The prophylactic benefit occurred in a dose-dependent manner, with a maximal effect seen when approximately 15 mg of TPI (a total of 5.6 mg of protein) was administered. The benefit appeared to derive from TPI's proteinaceous components, in light of the observation that all benefit was lost after proteolytic digestion with bromelain and proteinase K or incubation at 85°C, procedures known to denature protein.

Parker et al subsequently reported another study using the same mouse model as 2002 and depleting NK cells prior to treatment with TPI (now called innate immune regulatory factor, IIRF). While 85% of control mice and NK depleted treatment mice died, only 30% of the treated NK cell intact mice died showing that NK cells were involved in IIRF-induced protection. In a NK sensitive mouse model of melanoma the authors also showed that IIRF suppresses cancer metastases twofold and that IIRF is not directly toxic to cancer cells (147).

In a further study in mice, Parker et al (148) showed induction of IL-6 and IL-10 after intraperitoneal injection of IIRF. IL-6 peaked at 3 hours and was back at baseline by 8 hours. IL-10 peaked at 8 hours and returned to baseline by 36 hours. There were no changes in any of the other cytokines measured. Serum levels of haptoglobin and serum amyloid A were also elevated after IIRF administration. IIRF also stimulated production of IL-6 in human monocytes in a dose dependant manner. In a separate experiment on mouse whole blood, NK cells were found to be at least partly responsible for inducing IL-6 production after IIRF treatment but it is unsure whether it is a direct or indirect effect.

Thacker et al (149) reported the chemical structure of the active component of CSF-I, present only in serum and not in plasma. The compound is described as 1-peptidyl-2- arachidonoyl-3-stearoyl glyceride (**1p2a3sg**)**.** To determine the bioequivalence between the natural product **1p2a3sg** and a synthetic version, normal human fibroblasts were exposed to **1p2a3sg** and synthetic **1p2a3sg**, and IL-6, IL-8, MCP-1, and MIP-1R expression was measured. IL-6 mRNA was elevated 20% in response to synthetic **1p2a3sg** and 40% with natural **1p2a3sg** relative to a β-actin control ($p < 0.05$). It was found that 5 ng/mL of synthetic **1p2a3sg** was approximately equivalent to the natural product diluted 1:100. Compound **1p2a3sg** induced IL-8 expression by about 5-fold compared to untreated fibroblasts, in dose-response studies. Both synthetic **1p2a3sg** (3 ng/mL) and the natural product (1:100 dilution) induced a 3-fold increase in

MCP-1 mRNA relative to the β-actin control and increased MIP-1R mRNA expression relative to β-actin by 15%.

The peptide showed identical sequence homology to amino acids 558-574 in the transient receptor potential channel-related protein 1 (TRPC-1). The data also suggest that the role of the diacylglycerol moiety is to facilitate transportation of the peptide across the cell membrane. The cytokine/chemokine expression that is mediated by **1p2a3sg** arises from the peptide alone and that the intracellular peptide signalling from **1p2a3sg** is mediated through the inflammasome and IL-6 and IL-8 production may arise as a downstream event from IL-1β secretion and feedback signalling through the IL-1β receptor.

The authors conclude that this is a new immunomodulatory compound originally isolated from caprine serum, **1p2a3sg**, the peptide portion from which was found to be 100% homologous to a unique region of TRPC-1. The in vitro data suggest that the peptide in **1p2a3sg** may activate the host innate immune response against pathogen infection or other cellular injury and may induce Th17 cell differentiation in vivo. The data suggest a role for **1p2a3sg** and the peptide moiety of **1p2a3sg** as damage-associated molecular patterns in the host response to pathogen infection or other cell injury.

Thacker et al further reported on **1p2a3sg** mechanism of action (150). They reported a significant reduction in white cell count in cows with mastitis treated with **1p2a3sg** and a significant reduction in mortality in mice infected with salmonella (from 100% in control population to 20% in treated mice). In vitro experiments with human monocytes infected with Chlamydia pneumonia showed a reduction of infection from 90% in untreated cells to 12% in cells treated with **1p2a3sg** at 100ng/ml, 35% at 50ng/ml and 40% at 25ng/ml. Using a caspase-1 inhibitor, the authors show that **1p2a3sg** induced a caspase-1-dependent secretion of inflammatory cytokines (IL-1β, lL-18, and IL-33) from

primary human fibroblasts, suggesting that the effects of **1p2a3sg** are mediated through the inflammasome complex and using knockout mice, they confirmed that this was mediated, at least partially, by the NLRP3 inflammasome. Looking at gene signatures in human fibroblasts, **1p2a3sg** had maximal effect at 48 hours and was down regulated at 72 hours. Using a mouse model infected with cutaneous MRSA, the authors show a significant reduction in skin infection in treated mice, combined with elevation of IL-33, IL-8, IL-6 and CCL-2. Their data also show that the compound enhances recruitment monocytes, which then differentiate into macrophages.

In conclusion, CSF-I or 1p2a3sg, as the active component is now known, has been identified as a lipopeptide and is anti-infective with a mechanism of action mediated through the innate immune system and the NLRP3 inflammasome. It is possible that HCS may also have some effect via the lipopeptide mechanism. However, the majority of HCS action is likely through its effect on the HPA axis via the CRH complex and also possibly due to an IVIg effect, though the dose of IVIg is small. It is interesting to note that both induce changes in the cytokine balance in humans and animals and that both have been investigated for therapeutic use.

1.4 Clinical trial design and endpoints

1.4.1 Clinical trial design

Conducting clinical trials in SSc is challenging due to the heterogeneous nature of the disease, lack of validated or highly sensitive outcome measures and SSc is a rare disorder, making recruitment difficult. Recruitment of an adequate number of subjects to trials remains a challenge often necessitating multicentre involvement or extended periods of recruitment. Most clinical trials in SSc focus on either skin or lung fibrosis as the primary outcome, with other organ systems being evaluated as secondary outcomes. For skin disease, efficacy is measured by improvement of skin fibrosis using MRSS and for lung disease a scoring system on HRCT chest and/or lung function tests are used. Composite indices are being developed and could be more responsive and clinically meaningful than current measures (151).

In the past, proof-of-concept studies have often been open label uncontrolled trials. These are of limited usefulness regarding efficacy, but can be used in safety evaluations of therapeutic agents. The general consensus is that clinical trials should have a comparator group and randomised controlled studies are optimal for assessment of therapeutic efficacy. All clinical trials should involve collection and storage of serum and plasma samples for biomarker analysis. Proof-of-concept studies can, at best, provide possible efficacy signals. However, a fully powered randomised double-blind controlled trial is necessary to confirm efficacy. Rarer manifestations of SSc, such as joint disease, myopathy, gastrointestinal disease, cardiac and renal disease, are often neglected in clinical trials and should be included as secondary endpoints in larger controlled trials (151).

Over the past number of years a number of innovative alternative trial designs have been developed, which may improve efficiency and allow smaller numbers to be recruited for trials. Some of these designs have already been used successfully in SSc and other rare diseases, such as the Add-on design, the Early escape design and randomised withdrawal though all have some limitations (152). Recently a group of international experts in SSc have published guidelines on conducting trials in SSc, including many of the points mentioned above and expanding on further issues such as trial duration, selection criteria for subjects (uniformity versus generalisability), outcome measures, statistical analysis and power calculations (153).

1.4.2 Modified Rodnan Skin Score (MRSS)

In 1960, Farmer et al first published the observation that extensive skin change correlated to internal organ involvement in SSc (154). This was a pivotal observation as skin change could then be used as a prognostic factor and a biomarker for severe disease. Rodnan et al published the first standardised semi-quantitative skin score in 1979 (155) by weighing skin biopsies and measuring collagen content. This was a 26 site assessment, graded 0-4.

Subsequently there were 3 further modifications; a 22 site assessment (156), a 10 site assessment (157) and a 17 site assessment (158), all graded 0-3. The final modification, the 17 site modified Rodnan skin score (MRSS), is currently used in clinical trials and clinical practise. There is good correlation between histological appearance on skin biopsy and grade at assessment on skin scoring. The interobserver variability was found to be 25% and intraobserver variability 12% in one study (158). However, with training, the variability can be reduced significantly (159). The gold standard is to have a single assessor for MRSS assessments in clinical trials to reduce variability, but this is rarely feasible.

Subsequent studies have shown that baseline skin score is predictive of outcome and high baseline skin score correlates with increased mortality (157) (160). Studies also show that changes in skin score are prognostic, with improvement in skin score correlating with improved survival (161). Rapid worsening in skin score in the first 2 years after diagnosis is associated with reduced short term survival and renal crisis (162). Skin thickening usually peaks within the first 2 years of disease (163). In a recent analysis of clinical trials that a negative outcome, overall MRSS tended to improve over the course of a clinical trial, however, those with a worse baseline skin score (more severe disease) at study entry tend to improve while those with less severe disease at entry tend to worsen (164). Another study confirmed decline in skin score overall, but found that patients enrolled with a disease duration <6 months had a small but significant increase in skin score before a subsequent decline and patients with a disease duration >2 years had a greater rate of skin score decline than the other groups (165).

Shand et al showed that patients could be grouped into 3 categories according to change in skin score in the first 3 years after diagnosis; a group with low baseline skin score who improve over time, a group with high baseline skin score who improve and a group with high baseline skin score who don’t improve. The group with high baseline skin score who didn’t improve over time

were found to have a significantly higher mortality than the other 2 groups and there were fewer organ based complications in the low baseline skin score group (15). Maurer et al recently published a report looking at predicting patients who will progress with worsening skin scores. They found that a low baseline skin score (<22/51) and shorter disease duration (<15 months) and joint synovitis at baseline predicted worsening skin fibrosis at 1 year. This was validated in a second cohort and resulted in a 4.5 fold increased prediction rate (166).

1.4.3 Key points

SSc is a multisystem rheumatic disease with high morbidity and the highest case specific mortality of any rheumatic disorder. As no treatment is proven to be effective in preventing progression of disease, reversing fibrosis or improving long-term outcome, there is a huge unmet medical need to explore novel therapies for this disease. Late-stage dcSSc has very high morbidity and few studies are specifically designed to look at this stage of disease.

The pathogenesis of early dcSSc is very complex and involves interaction between inflammation, vasculopathy and fibrosis. Key drivers of SSc pathogenesis are immune system dysfunction (pro-fibrotic and pro-inflammatory cytokine activation, such as TGF-β and IL-6 and innate immune system activation), endothelial dysfunction, fibroblast activation and dysregulated collagen turnover. However, pathogenic drivers of late-stage disease are less clear, but there is emerging evidence that persistent perturbation of immune cell function is increasingly important. Therefore, ongoing research into pathogenesis of disease and potential biomarkers is important. Multiplex serum analysis is increasingly being used to identify potential new biomarkers.

Treatment of skin disease with immunosuppressants is usually indicated in the first few years of disease in those who have active dcSSc and can be discontinued in most patients after a few years, once the disease becomes less active. However, some patients may require ongoing immunosuppression and

the balance between benefit and side-effects must be considered. In previous studies, HCS has shown some efficacy in inflammatory diseases such as optic neuritis and multiple sclerosis. Its novel mechanism of action on the HPA axis makes it a useful medication to consider in inflammatory rheumatic diseases, especially as drug-related side effects are infrequent. However, as HCS has not previously been administered to SSc patients, safety was the primary objective, followed by assessment of potential efficacy. The clinical trial design was important as SSc is a rare disease and recruitment of potential patients is challenging. The inclusion of a placebo arm is vital as it enables assessment of potential efficacy signals, which would not be possible with an open label design. The inclusion of cytokine and potential biomarker assessment is also an important part of modern trial design, to further understanding of pathogenesis and improving future treatment of disease.

1.4.4 Study Objectives

The primary objective of the study was to assess safety of using HCS in late-stage dcSSc. The secondary objectives were assessment of possible treatment effect (using clinical outcomes such as modified Rodnan Skin Score (MRSS), SSc Health Assessment Questionnaire Disability Index (SSc HAQ-DI) and Short Form 36 (SF-36) quality of life questionnaire) and the exploration of candidate biomarkers (including vWF, sIL-2R, PIIINP, as well as multiplex analysis of serum and plasma). The study was approved by the NHS NRES Hampstead Local Ethics Committee.

1.5 Hypothesis

The hypothesis addressed in this project is that hyperimmune caprine serum improves skin and other measures of disease severity in established dcSSc by modulating immunological function that determines persistence of clinical disease. Established dcSSc has a high morbidity, and as such, provides a “safety platform” to assess this novel immunomodulatory agent. This is a unique strength of this study. This hypothesis is explored through 1) a prospective clinical trial, 2) long-term clinical use and 3) detailed assessment of serum growth factors and cytokines, as well as established and exploratory markers of disease.

2 Methodology

2.1 Clinical Trial Methodology

2.1.1 Patients and Methods

This is a single centre, placebo controlled, double blind, parallel group pilot randomised controlled trial (167). Eligible patients were identified and recruited from outpatient clinics and chart reviews. The major eligibility criteria are included in sections 2.1.2 and 2.1.3 below. All patients fulfilled eligibility criteria. Case report forms (CRF) were used to collect data and the trial was conducted to Good Clinical Practise (GCP) standards with standard verbal and written informed consent procedures. The trial was approved by the local ethics committee. We treated 10 subjects with established dcSSc using HCS and compared outcome over 6 months with 10 control subjects receiving placebo. Patients were randomised to receive 1ml study drug or placebo subcutaneously twice weekly for 6 months. The first two doses of medication were administered in the study centre under supervision at week 0, day 0 and week 0, day 3.

2.1.2 Inclusion criteria

Full inclusion criteria are included in **Table 2.1**. In brief, patients recruited to the study were required to be >18 years, have dcSSc by LeRoy criteria (11) of at least 3 years duration and fulfil the 1980 preliminary classification criteria for systemic sclerosis of the American Rheumatism Association (American College for Rheumatology) (9). Screening laboratory and radiology tests did not reveal malignancy or infections and were in the range specified by the protocol. Patients adhered to the visit and protocol requirements including contraceptive advice.

2.1.3 Exclusion criteria

Full exclusion criteria are provided in **Table 2.2**. In brief, patients included in the study were not permitted to take immunosuppressant agents and did not have evidence of pregnancy, severe organ disease, infections or malignancy at screening.

Inclusion Criteria
Men and women ≥18 years of age.
Patients must fulfil the 1980 Preliminary Classification Criteria for systemic sclerosis of the American Rheumatism Association (American College for Rheumatology) (9)
Clinical classification must be diffuse cutaneous SSc, as evidenced by skin sclerosis proximal to the elbows or knees and absence of the anti-centromere autoantibody.
At least three years must have elapsed since the first non-Raynaud's manifestation of scleroderma.
Men and women of childbearing potential must use adequate birth control measures (e.g., abstinence, oral contraceptives, intrauterine device, barrier method with spermicide, or surgical sterilization) for the duration of the study and should continue such precautions for six months after receiving the last injection of HCS.
The screening laboratory test results must meet the following criteria: Haemoglobin ≥8.5 g/dL, WBC ≥3.5 x 109/L, Neutrophils ≥1.5 x 109/L, Platelets ≥100 x 109/L, SGOT (AST) and alkaline phosphatase levels must be within twice the upper limit of normal range for the laboratory conducting the test.
The patient must be able to adhere to the study visit schedule and other protocol requirements.
The patient must be capable of giving informed consent and the consent must be obtained prior to any screening procedures.
There must be no radiological evidence of malignancy, infection or (previous) tuberculosis in a chest radiograph performed within three months prior to the first injection of study drug.

Table 2.1: Inclusion criteria

Exclusion Criteria
Women who were pregnant, nursing, or planning pregnancy within one and a half years after screening (i.e., approximately six months following last injection of study drug)
Patients using any investigational drug within one month prior to screening or within five half-lives of the investigational agent.
Patients taking a putative disease modifying drug (such as D-penicillamine, methotrexate, azathioprine, mycophenolate mofetil etc.) within one month of screening.
Treatment with any therapeutic agent targeted at reducing TNF (e.g., infliximab, pentoxifylline, thalidomide, etanercept, etc.) within three months of screening.
Immunosuppressive therapy within one month of screening.
Previous administration of HCS or history of known allergy to animal proteins.
History of serious infections (such as pneumonia or pyelonephritis) in the previous three months.
Less serious infections (such as acute upper respiratory tract infection [colds] or simple urinary tract infection) were monitored to their conclusion or treated, as appropriate, prior to inclusion.
Active hepatitis B or hepatitis C or active tuberculosis.
Opportunistic infections, including but not limited to evidence of active cytomegalovirus, active Pneumocystis carinii, Aspergillosis, histoplasmosis or atypical mycobacterium infection, etc., within the previous six months.
History of lymphoproliferative disease including lymphoma, or signs and symptoms suggestive of possible lymphoproliferative disease, such as lymphadenopathy of unusual size or location (such as nodes in the posterior triangle of the neck, infra-clavicular, epitrochlear, or periaortic areas), or splenomegaly or patients with malignancy within the past five years.
Known recent substance abuse (drug or alcohol).
Poor tolerability of venepuncture or lack of adequate venous access for required blood sampling during the study period.
Presence of a transplanted organ (with the exception of a corneal transplant > three months prior to screening).
Signs or symptoms of severe, progressive or uncontrolled renal, hepatic, haematologic, gastrointestinal, endocrine, pulmonary, cardiac or neurological disease (including demyelinating diseases such as multiple sclerosis).
Patients who, within the previous three months, had either a myocardial infarction, uncontrolled congestive cardiac failure, unstable angina, uncontrolled systemic hypotension or uncontrolled systemic hypertension.
Screening laboratory values which deviated 20% or more from the upper or lower limits of normal or which were considered to be clinically significant to the investigator.

Table 2.2: Exclusion criteria

2.1.4 Concomitant medications

An attempt was made to keep all medications stable for the duration of the trial. Medications contraindicated during the treatment phase included other investigational drugs, drugs targeting TNF or any immunosuppressive agents. Sodium channel blocking agents such as anti-convulsant medications (e.g. lamotrigine, gabapentin, pregabalin) were also contra-indicated. Medications that were allowed during treatment phase included low dose steroid up to 10mg/day, simple analgesics and other medications as required for treatment of Raynaud's and other disease related conditions including intravenous prostacyclin.

2.1.5 Randomisation

Randomisation was achieved by a computer program assigning random numbers, performed by the statistician, Dr. Sockler of Datapharm. The sequence of numbers was transmitted to the company who packaged the medication (Biotec Services International, Bridgend, Wales). The medication was labelled with patient numbers and a specific code to which the site, investigators and patients were blinded and only the packaging company and the statistician had access to. Code break envelopes were produced and kept in the site file in case of emergency.

2.1.6 Double-blind Phase

2.1.6.1 Study Visit Schedule

Figure 2.1 shows the study visit schedule. The visit flow chart is provided in **Figure 2.2** and these visits are discussed in more detail below.

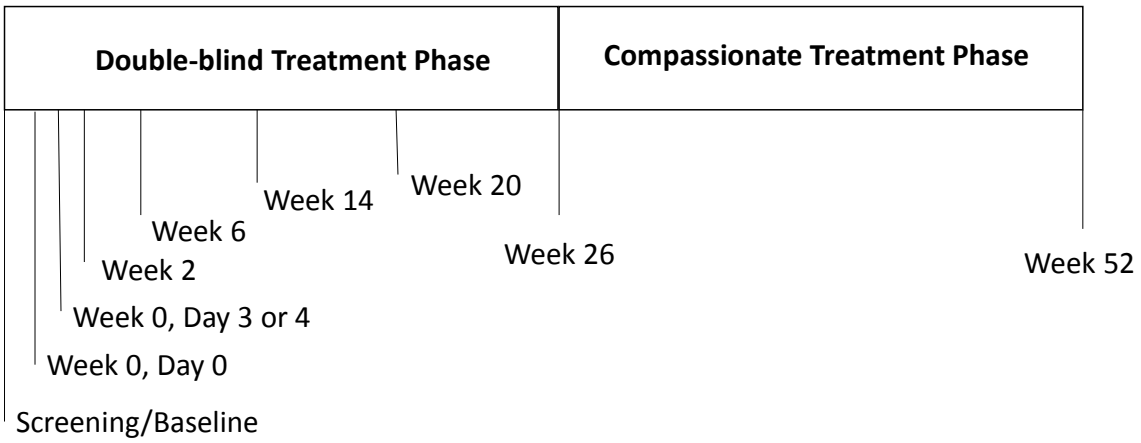


Figure 2.1: Study visit schedule.

	Screening/ Baseline	Week 0 Day 0	Week 0 Day 3 or 4	Week 2	Week 6	Week 14	Week 20	Week 26	Week 52
Consent	X								
Demographics	X								
Disease History	X								
Medical History	X								
Pregnancy Test	X							X	X
Physical Examination and Vital signs	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X
Eligibility Criteria	X								
Chest X-ray	X								
HRCT (if applicable)	X							X	
ECG	X				X			X	X
Echocardiogram	X				X			X	
Pulmonary function tests (PFTs)	X				X			X	
Haematology, Biochemistry, Coagulation	X	X		X	X	X	X	X	X
Cytokine samples	X	X *		X	X	X	X	X	X
Serology	X							X	
Thyroid function tests	X				X			X	X
Study defined biomarkers	X				X			X	X
R-R interval variation		X *						X	
MRC Sum Score		X *			X			X	
MRSS		X			X			X	X
Questionnaires (HAQ, SF-36, UK-FS, Neuropathic pain VAS)		X			X			X	
Physician Global VAS		X			X			X	
Adverse Events reporting		X	X	X	X	X	X	X	X

Figure 2.2: Study visit flow chart. * denotes that the test is done before and 2 hours after the first administration of the study drug.

2.1.6.2 Screening and Baseline

Screening and baseline visits were performed on the same day. Informal consent was obtained prior to the visit and signed informed consent was obtained at the screening visit. Demographic data, medical history, concomitant medications and a physical examination including vital signs and MRSS was obtained and a pregnancy test was performed for women of child bearing potential. The patient had a comprehensive assessment including electrocardiogram (ECG), echocardiogram, pulmonary function tests, routine and study specific bloods, urinalysis and chest radiograph. The patient also completed the study specific questionnaires (see section 2.2.3). Eligibility criteria were confirmed and the patient was randomised to placebo or active medication.

2.1.6.3 Week 0; Day 0 and Day 3 or 4

On Day 0 and Day 3 or 4, a history and physical examination were performed and any changes to medications noted. An injection of placebo or active medication was administered under instruction on both days. Vital signs were performed pre-injection and every 30 minutes thereafter for three hours. On Day 0, serum samples, R-R interval and MRC sum score were performed before and 2 hours after the injection. Follow-up and delivery plans for the medication and home freezer for the storage of medication were arranged.

2.1.6.4 Week 2, 14 and 20

At these visits, a history and physical examination was performed with assessment for adverse events and change in medications. Routine and study specific bloods, vital signs and urinalysis were also obtained.

2.1.6.5 Week 6 and 26

At these visits, a history and physical examination was performed with assessment for adverse events and change in medications. Routine and study specific bloods, vital signs, MRSS and urinalysis were also performed. A comprehensive assessment with echocardiogram, pulmonary function tests, ECG, MRC sum score and study specific questionnaires were completed. A

repeat R-R interval and sample for repeat serology were completed on week 26.

2.1.7 Safety phase and follow-up

At week 52 (26 weeks after trial completion) a safety visit was performed to collect data on new medications and adverse events since trial completion. A full history and clinical examination was performed including routine and exploratory blood tests, ECG and MRSS.

Most of the patients opted to receive a trial of HCS on a compassionate basis after the 6 month double blind phase. Some patients did not receive HCS due to early trial termination or other medical or personal reasons. Between trial termination and the safety visit, patients were seen as per routine practice, depending on severity of disease and symptoms and these visits were documented in the patients hospital notes as per routine practice.

2.1.8 Adverse events

Patients were monitored for the occurrence of adverse events for three hours after the initial and second injections. At each of the study visits, the patient was questioned about the occurrence of new adverse events and changes in concomitant medications since the last visit, or the outcome of any adverse events reported at previous visits. Any pre-existing conditions were recorded in the medical history and pre-existing conditions that worsened in severity or frequency during the study were also recorded as adverse events.

Serious adverse events occurring after the first injection and up to 6 months after the last injection were documented and reported to the sponsor within 24 hours. A serious adverse event was defined as any adverse event occurring at any dose that results in death, life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalisation, persistent or significant disability/incapacity, congenital anomaly/birth defect, or deemed by the principle investigator to be serious.

2.1.9 Compliance

Compliance was assessed by a self-reported patient diary and was calculated as the amount of study medication the patient received according to the diary as a percentage of the amount the patient should have received. If a patient withdrew from the study prematurely, the dose the patient should have received was calculated based on the time the patient was in the study. Exposure to study treatments was calculated as the number of injections received and the total volume of study medication injected.

2.1.10 Statistics

2.1.10.1 General statistical methodology

As this is a parallel group study design, no 'overall' data is presented. Continuous measures are summarised and tabulated using the number of observations (n), mean standard deviation, minimum, median and maximum. Categorical measures are summarised and tabulated using the count and percentage of patients within the group (n (%)). The denominator used to calculate percentages is the number of patients in the group for measures of efficacy. For measures of safety and tolerability, the number of patients in each group at each visit serves as the denominator.

With any patients inadvertently receiving the incorrect treatment, safety population tables are tabulated by the study treatment actually received, whereas ITT population tables are tabulated by the treatment to which the patient was randomised.

Baseline measurements are defined as the last measurement taken prior to the first dose of study medication. Where repeated measurements are taken for the same visit, the latest measure for the visit is summarised and tabulated for pre-treatment measures, while the first measure for the visit is used in post-treatment measures. As the primary outcome of this study is the safety and

tolerability of HCS compared to placebo, no imputation of missing data has been performed.

2.1.10.2 Power calculations

This study represents the first administration of HCS to patients with systemic sclerosis and the primary objective was assessment of safety. Since this was a pilot study to inform potential future larger evaluation, formal power calculation was not considered appropriate. However to provide confidence that we could address our objectives we undertook a limited determination of likely statistical power of the study based upon the number of subjects and known changes in efficacy measurement of MRSS. The main concern was not to miss a potential positive clinical outcome and so, for the purpose of power estimation, a relatively low level of statistical significance was selected of 0.20. Assuming standard deviation of 4.0 units for MRSS and a target effect size of 4.0 units MRSS between treatment arms the sample size was calculated to be n=20, 1:1 randomization, consistent with the executed study design for this trial.

2.1.10.3 Primary efficacy analysis

Key measures of efficacy in this study are the change in SSc Health Assessment Questionnaire (HAQ) overall disability index (DI) from baseline to Week 26, the change in Modified Rodnan Skin Score (MRSS) from baseline to Week 26, the change in the SSc Functional Score (SSc-FS) from baseline to Week 26 and the change in Short Form-36 questionnaire (SF-36) scales from baseline to Week 26. These four measures will be considered as signals for efficacy of HCS in systemic sclerosis. Change in HAQ-DI and MRSS analysed as a continuous variable were the pre-specified efficacy end points. Responder frequency analysis for MRSS was also included post-hoc to capture clinically meaningful change in MRSS.

Inferential testing has been performed to compare groups in the change from baseline to each post-treatment visit. Two-sided p-values <0.05 were considered statistically significant. A mixed models repeated measures (MMRM) analysis was performed on the data in the first instance. In some cases the mixed models algorithm could not converge, so a standard repeated

measures analysis of variance (RMANOVA) was performed. The use of the MMRM also allowed the calculation of probabilities for the adjusted mean change value tested against a standard value of zero, whereas the RMANOVA does not calculate those probabilities, but does provide the 95% confidence interval. Other analysis included a responder frequency analysis to capture individual patient data within the more variable cohort changes in mean MRSS. The unconditional z-pooled test was used to analyse responder frequency analysis, as recommended by Lydersen et al (168).

The baseline values of both the assessment under analysis and the HAQ-DI score and baseline (of assessment) by visit interaction terms have been used as covariates in the analysis. Primary inferences have been made for the change from baseline to Week 26, although the model also provides analysis of all preceding visits as supportive information.

2.1.10.4 Secondary efficacy analysis

Secondary efficacy measures include change from baseline to Week 26 in each of the eight scales of the SSc HAQ, MRC Sum Score, chemokines, cytokines, Scleroderma Physician Global Visual Analogue Scale (VAS) and the Scleroderma Neuropathic Pain Scale VAS. Chemokines and cytokines have been analysed by blinded independent laboratories. The statistical methods used for the multiplex analysis are described in **Chapter 5, Section 5.3**.

2.2 Clinical assessments

2.2.1 Demographics

Demographic data were recorded at the screening visit. These included age, sex, race, smoking habits, height, weight, BMI, time since diagnosis of dcSSc, time since onset of first Raynaud's symptoms, time since onset of first non-Raynaud's symptoms and family history of SSc. SSc functional class was also recorded (169).

2.2.2 Clinical history and examination

Each patient had a comprehensive clinical history taken at the screening visit including current symptoms, SSc diagnosis and symptoms, past medical and

surgical history and family history. This was followed by a full clinical examination. This formed the baseline for comparison at future visits, for the identification of new or worsening symptoms. At each subsequent visit, each patient had a current history taken and a full clinical examination.

Modified Rodnan skin score (MRSS) (158) is a standard method of defining skin involvement in SSc. 17 sites are assessed, and a score of 0 (normal skin), 1 (slight thickening), 2 (moderate thickening) or 3 (hidebound skin sclerosis) is assigned at each of these sites (face, chest and abdomen – a single score each – and bilaterally from upper arm, forearm, hand, fingers, thigh, leg and foot). The score ranges, therefore, from 0 to 51, **Figure 2.3**. MRSS was performed at each study visit by the same investigator throughout the study.

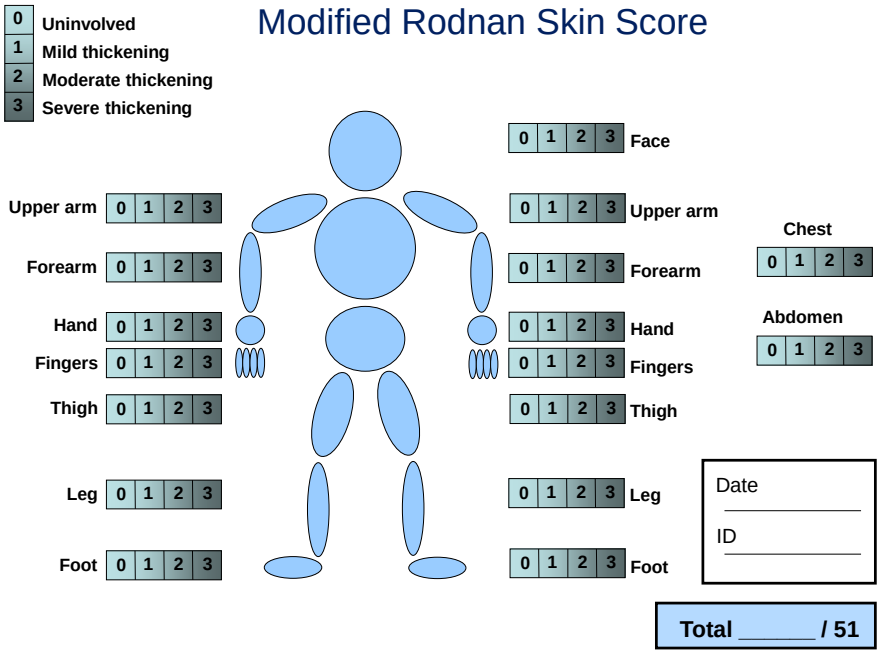


Figure 2.3: An example of the MRSS scoring sheet.

MRC sum score (170) is a global score of muscle strength, measuring 6 muscle groups on each side with a score from 0-5, as described in the boxes below,

Figure 2.4. The global score ranges from 0-60. MRC sum score was performed at 3 intervals during the study.

Muscle Groups (Right and Left)
Abduction of the arm
Flexion of the forearm
Extension of the wrist
Flexion of the leg
Extension of the knee
Dorsiflexion of the foot

0 No visible contraction
1 Visible contraction without limb movement
2 Movement of limb, but not against gravity
3 Movement of limb against gravity over (almost) full range
4 Movement against gravity and resistance
5 Normal

Figure 2.4: MRC sum score

2.2.3 Questionnaires/Quality of Life Assessments

2.2.3.1 Scleroderma Health Assessment Questionnaire (SSc-HAQ)

The Health Assessment Questionnaire (HAQ) is a patient self-administered questionnaire commonly used to assess quality of life in rheumatological conditions, most notably rheumatoid arthritis. It was developed in 1978 by J. Fries, published in 1980 and modified subsequently (171) (172) (173). It is composed of 20 items in eight domains, scored 0-3. The HAQ disability index (HAQ-DI) is calculated by calculating the highest score in each domain and adding these together and dividing by the number of domains; this gives a score between 0 and 3, a lower score is better quality of life. The patient has to answer at least 6 of the 8 domains for the HAQ-DI calculation.

The HAQ has been modified to include a number of visual analogue scales to measure symptoms specific to scleroderma (SSc-HAQ, SHAQ) and this has been validated in a number of studies (174) (175). SSc-HAQ is now routinely used in scleroderma studies as an outcome measure for quality of life (QoL)

and improvement in disease status (176) (177). An example of the SSc-HAQ is given in the **Appendix, Section 8.1.1.**

2.2.3.2 Short-Form 36 (SF-36) Questionnaire

The short-form 36 (SF-36) questionnaire is a 36 item questionnaire in 8 domains with weighted scoring used to measure quality of life in many different diseases (178) (179) (180). The original SF-36 was designed for the Medical Outcomes Study and subsequently modified to the SF-36v2, now in use in many rheumatological trials including scleroderma (181) (182). The health transition index is a question asking the patient to compare their health now with the same time last year and answer one of 5 options: much better, somewhat better, about the same, somewhat worse or much worse. This index is often used as a summary measure in studies for comparison. An example of the SF-36 is given in the **Appendix, Section 8.1.2.**

2.2.3.3 Systemic Sclerosis Functional Score (SSc-FS)

The SSc-FS is an 11 item 4 grade questionnaire, developed specifically to assess functional capacity in scleroderma patients (183) (184). An example of the SSc-FS is given in the **Appendix, Section 8.1.3.**

2.2.3.4 Patient Global and Physician Global Visual Analogue Scales (VAS)

Patient global VAS score is incorporated into the SSc-HAQ. Physician global VAS is usually a separate VAS 10cm scale asking the physician to rate the patients' global condition from very good to very poor in their medical opinion.

Neuropathic pain VAS was also included as the mechanism of action of HCS included sodium channel opening effect, which could, in theory, improve neuropathic pain. It is a 10cm scale rating neuropathic pain from none to very severe.

2.2.3.5 Outcome measures

SSc-HAQ, SF-36, SSc-FS and global VAS scales are all used in current clinical trials. While each alone can be used, each has a distinct validity and combination can give more useful information (185) and correlate to certain aspects of disease and functionality (186).

2.2.4 Physiological assessments

2.2.4.1 Cardiological assessments

ECG and echocardiogram were performed at 3 time periods as described above during the study to monitor for worsening cardiac disease. ECG was performed on the same machine and interpreted by the same investigator throughout the study. The echocardiogram was performed in the cardiology department by a qualified technician and interpreted by a cardiologist.

2.2.4.2 Pulmonary function tests

Pulmonary function tests (PFTs) were performed and interpreted in the pulmonary function laboratory by a qualified technician dedicated to clinical trials at 3 time periods as described above throughout the study.

2.2.4.3 Chest radiograph and High resolution Computer Tomography (HRCT)

Chest radiograph was performed on the screening visit to rule out chest infection, evidence of tuberculosis, severe lung fibrosis and neoplastic disease. HRCT chest is used to define lung fibrosis more completely. During this study, HRCT was only performed in patients who had worsening PFTs or a clinical suspicion of worsening lung symptoms. If HRCT showed worsening fibrosis, further treatment options were discussed with the patient.

2.2.5 Exploratory physiological studies

2.2.5.1 Sniff nasal inspiratory pressure (SNIP)

Inspiratory muscle weakness is a recognised cause of unexplained dyspnoea. Many patients with SSc have dyspnoea and in some, a cause is not found. It is possible that patients with SSc can have respiratory muscle weakness due to

myositis associated with their disease. As HCS has a potential sodium channel opening effect, it was hypothesised that if a patient had inspiratory muscle weakness, it would improve with HCS treatment. Sniff nasal inspiratory pressure (SNIP) is a pulmonary function test that measures inspiratory muscle function. This assessment has been validated in a number of studies (187) (188).

SNIP is performed in a seated position by inserting a catheter tip into one nostril and occluding the other nostril. The catheter is attached to a computer. The patient is asked to take a short, sharp sniff. The test is repeated 10 times with 30 second rest periods (as there is an element of learning) and the maximal SNIP from the 10 tests is recorded as the test value (189), **Figure 2.5**.

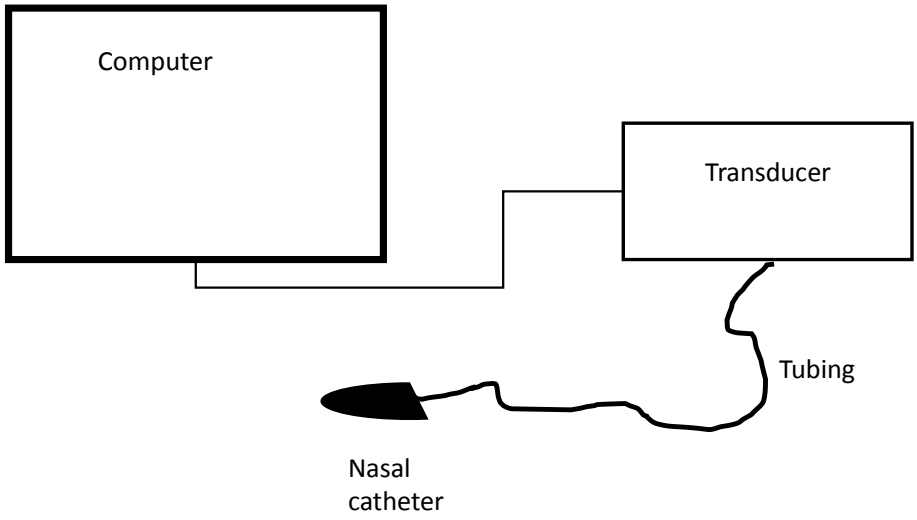


Figure 2.5: Equipment used for SNIP measurement.

2.2.5.2 R-R interval

Heart rate variability (HRV) is governed by vagal tone which is controlled by the autonomic nervous system. Therefore, HRV can be a surrogate marker or biomarker of autonomic dysfunction. Autonomic dysfunction is a well-recognised association with SSc (190) (191) (192). HRV to assess autonomic

dysfunction in SSc was first reported in the mid 1990's (193) (194) (195). HRV is non-invasive and relatively easy to perform and, as an adjunct to other cardiological tests, may provide important diagnostic and prognostic information (196) (197). As HCS has a potential sodium channel opening effect, it was hypothesised that it may improve autonomic dysfunction, and therefore normalise HRV.

HRV is measured in time and frequency domains. Time domain analysis refers to statistics that are derived directly from the measurement of the normal-to normal (N-N) intervals (i.e. intervals between consecutive QRS complexes resulting from sino-atrial discharge) and statistics calculated from the differences between successive N-N intervals. N-N interval is also called R-R interval (R wave to R wave), which is the term used in this study. Premature ectopic beats are ignored in these analyses. R-R interval-based measures are influenced both by short-term factors (e.g. respiratory) and long-term factors (e.g. circadian). The simplest variable to calculate is the standard deviation (SD) of the R-R intervals (SDRR, also called SDNN). SDRR reflects all the cyclic components (i.e., short-term and long-term) that are responsible for variability in the period of recording. The RMSSD, another variable often reported, is the square root of the mean squared differences in successive R-Rs (198) (199). The Valsalva ratio (VR) is calculated as the longest R-R interval within the 30 seconds after the manoeuvre divided by the shortest R-R interval during or within the first 5 seconds after the manoeuvre (max/min). In this study, time domain variables alone were analysed.

The patient was asked to lie on a couch semi-prone at an angle of 45 degrees. 3 electrodes are applied to the skin, one below right clavicle, one in left upper quadrant of abdomen and one on right hand (earth) ensuring the electrodes were picking up good signal and the R waves were upright (electrodes plugged in properly). The electrodes were attached to a computer. The test was performed in 3 separate modes, normal breathing, deep breathing and Valsalva manoeuvre. Each was recorded for one minute. Deep breathing was performed

with the aid of a visual prompt on the computer screen with timed inhalation and exhalation, 6 cycles in a one minute period. Verbal prompts from the investigator were used in tandem with the visual cues. The Valsalva test was performed with the aid of an animation/cartoon. The patient is instructed to breathe normally for the first 10 seconds and then the cartoon will prompt to blow out, whereby the patient is instructed to take a breath in and blow out against the cheeks with the mouth closed. This is demonstrated for the patient, with a trial run until the patient felt comfortable with the instructions. Each set of 3 tests was done a second time to verify results. Variables recorded were SDRR, RMSSD and max-min/mean for normal and deep breathing and VR for the Valsalva manoeuvre.

2.3 Laboratory studies

2.3.1 Routine laboratory assessments

Routine laboratory assessments for safety were performed at every visit apart from Week 0, Day 3 or 4. These included blood samples taken for full blood count, biochemistry, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), thyroid function (on 4 occasions, including week 52 safety visit), coagulation screen (if the patient was on warfarin), pregnancy test (screening/baseline), SSc serology sample (screening/baseline and end of double-blind period) and a urine sample for urinalysis. Blood samples were sent to the main hospital laboratory and urine samples were tested in the clinical trials department with a standard urine dipstick test. Urine samples were sent to the hospital laboratory for further testing if there were any abnormal results with dipstick testing.

2.3.2 Exploratory laboratory assessments

Exploratory blood samples were obtained at baseline, week 0 day 0 (pre- and post-injection of medication), week 26 (end of study) and week 52 (end of safety period). These followed the template of recent expert consensus regarding exploratory biomarker studies in SSc trials (151). A 10ml serum sample, a 10ml plasma sample with EDTA and a 4ml plasma sample with trisodium citrate were obtained. The EDTA and citrate samples were centrifuged immediately at 3000

rpm for 10 minutes. After centrifuging, the plasma was aliquoted into 1ml sample bottles, labelled and stored in a -80°C freezer. The serum sample was allowed to clot for 30 minutes at room temperature and then centrifuged at 3000 rpm for 10 minutes. The serum fraction was then aliquoted into 1ml sample bottles, labelled and stored in a -80°C freezer. All samples were stored until the end of the safety period.

Samples were separated into 2 groups. The first group contained 2ml serum and 1ml EDTA plasma samples and was sent frozen to Quest Diagnostics (Valencia, CA 91355 USA) for analysis of procollagen III N-terminal propeptide (PIIINP), soluble IL-2R (sIL-2R), cartilage oligomeric matrix protein (COMP), TGF-β1 and von Willebrand factor (vWF). The second group contained 1ml serum samples sent frozen to Quansys Biosciences (Utah 84321, USA) for analysis of αMSH, ACTH, ANG2, HGF, PDGF-bb, TIMP-1, TIMP-2, VEGF, FGF basic, Eotaxin, GRO-α, MCP-1, MCP-2, RANTES, I-309, TARC, IP-10, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-23, IFN-γ, TNF-α, TNF-β, IFN-α, IFN-β, Fractalkine and PARC by multiplex analysis. The remaining stored samples were transferred to Daval International for storage.

2.3.2.1 Quest Diagnostics samples

vWF samples were plasma samples and an enzyme linked immunosorbent assay (ELISA) Aushon Biosystems, Inc. (Cat. #84793) and a Searchlight analyser was used. Samples were thawed on ice, centrifuged at 20,000 x g for 5 minutes to remove any residual precipitate and appropriately diluted before placement onto Searchlight plates in duplicate. Samples and standards were incubated at room temperature for 1 hour while shaking. Plates were washed three times using a plate washer, biotinylated secondary antibody added, and incubated for an additional 30 minutes. After three more washes, streptavidin-HRP was added to the plates, incubated for 30 minutes, washed again, and substrate added. Images of the plates were taken within 10 minutes, followed by image analysis using Searchlight array analysis software.

COMP samples were serum samples and an ELISA BioVendor, Inc. (Cat. #RD194080200) and a Tecan Genios Pro analyser were used. Standards, quality controls and diluted samples were incubated in microplate wells pre-coated with monoclonal anti-human COMP antibody. After 60 minutes incubation and washing, biotin-labelled second monoclonal anti-human COMP antibody was added and incubated with captured COMP for 60 minutes. After another washing, streptavidin-HRP conjugate was added. After 30 minutes incubation and the last washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). The reaction was stopped by addition of acidic solution and absorbance of the resulting yellow product was measured. The absorbance is proportional to the concentration of COMP. A standard curve was constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples were determined using this standard curve.

Soluble IL-2R samples were serum samples and an ELISA Thermo Scientific (Cat. #EH2IL2R) and a Tecan Genios Pro analyser were used. An anti-IL-2R monoclonal antibody was pre-coated onto polystyrene microtiter wells. Standards, controls, or patient samples were introduced to the wells followed immediately by the addition of an enzyme conjugated anti-IL-2R monoclonal antibody. The soluble IL-2R present in the standards, controls, or samples was bound to the coated antibody while the conjugated antibody was bound to a second, distinct epitope on the IL-2R molecule completing the sandwich. Unreacted components were removed by washing. A chromogen solution was added to the wells forming a coloured end product that is proportional to the amount of IL-2R present in the sample. The reaction was terminated by the addition of stop solution, and the absorbance at 450nm, or 450 minus 550 nm, was measured. A standard curve was prepared from six IL-2R standards. Unknown values were determined from the standard curve.

TGF-β1 samples were serum samples and an ELISA R&D Systems, Inc. (Cat. #DB100B) and a Tecan Genios Pro analyser were used. This assay employs

the quantitative sandwich enzyme immunoassay technique. TGF-β soluble receptor Type II, which binds TGF-β1, was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any TGF-β1 present was bound by the immobilized receptor. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TGF-β1 was added to the wells to sandwich the TGF-β1 immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of TGF-β1 bound in the initial step. The colour development was stopped and the intensity of the colour was measured.

PIIINP samples were serum samples and a radioimmunoassay Orion Diagnostica (Cat. #06098) was used, with a Cobra-II Auto Gamma Counter to analyse data. The Orion Diagnostica UniQ PIIINP RIA kit is based on the competitive radioimmunoassay technique. A known amount of labelled PIIINP and an unknown amount of unlabelled PIIINP in the sample compete for the limited number of high affinity binding sites of the antibody. After separating the free antigen, the amount of labelled PIIINP in the sample tube is inversely proportional to the amount of PIIINP in the sample. The concentrations in unknown samples are obtained from a calibration curve.

All reagents and samples were brought to room temperature before testing and test tubes labelled appropriately and in duplicate. 200 µL of calibrator, control or patient samples were added to the appropriate tubes and 200µL tracer was added. 200 µL of antiserum was added to all tubes except non-specific binding (NSB) and total and 200 µL of distilled water was added to the NSB tubes. All tubes were mixed on a vortex mixer, then covered with paraffin film and incubated for 2 hours at 37°C. The separation reagent thoroughly by gentle inversion and 500 µL was added to all tubes except totals. The tubes were mixed on a vortex mixer and incubated for 30 minutes at room temperature. All tubes were centrifuged (except totals) for 15 minutes at 2000 g at 4 °C. The supernatants were decanted and the head of each tube, except the totals, was

tapped firmly against absorbent paper. Each tube was counted using a gamma counter for at least 1 minute or until 10,000 counts per tube were accumulated {Quest Diagnostics, personal communication}.

2.3.2.2 Quansys Biosciences samples

Quansys samples were tested on Quansys Biosciences' (Logan, UT, USA) Q-Plex Array™ kits for Human Angiogenesis (#150251HU), Human Chemokine (#120251HU), and Human Cytokine (#110951HU). Both Fractalkine and PARC were custom developed from match pair antibodies available from R&D Systems (Minneapolis, MN, USA). Samples were received, counted, and stored under appropriate storage conditions.

Q-Plex™ technology involves the micro-spotting of individual groups of capture antibody in either a cartesian or polar coordinate system on the bottom of a 96 well plate, each spot being its own micro ELISA. Each well was identically spotted. Standard ELISA incubation steps apply such as initial sample incubation, washing, secondary antibody incubation, washing, incubation with the label and measurement are involved. The label and reporting system used in a Q-Plex Array™ is chemiluminescent.

The Q-Plex™ kits used in the sample testing have undergone extensive validation. Ranges for each assay were determined by dilutions determining upper ranges where high end hook effect and apparent antibody saturation are avoided and lower ranges that are above detection limits (200). Lower limits of detection (LLD) were calculated based off 2x the standard deviation of the background of 20 negative wells. Intra assay precision was measured with acceptance criteria of a coefficient of variation (%CV) of less than 15. Inter assay variability across plates was also determined to be less than 15% CV. Samples from human serum, plasma, or other biological fluid anticipated to have lower concentrations of expressed protein (i.e. cytokines) were tested using a modified, high sensitivity protocol. Antigen standard curves were performed in duplicate diluting the antigen standard 1:3 for 11 points with a

single negative point. The sample and antigen standard incubation was extended from one hour to two hours and the detection or secondary antibody incubation was extended from one hour to two hours. Lower limits of quantification (LLOQ) were determined to be the lowest point of the 10 point positive standard curve where the back-fit regression values are within 20% of the known value.

Samples were thawed on ice and diluted into Quansys Human Sample Dilution Buffer or Mouse Sample Dilution Buffer. The sample buffers were formulated to reduce effects from heterophilic antibodies and other interferants (201). Samples were diluted at ratios of 1 to 2 (sample to buffer) (50%), 1 to 20 (5%) and 1 to 200 (.5%). Each dilution is loaded into three wells and measured in triplicate, a total of 9 wells per sample. The optimal dilution was selected by finding the dilution where the pixel intensity values fall on the most linear portion of the standard curve. Preparatory polypropylene low-binding 96-wellplates were used to prepare the samples and standards prior to loading the Q-Plex™ plate with a multichannel pipettor in order to reduce pipetting error.

A composite or stacked image composed of individual exposures of 30, 60, and 180s with camera noise background subtraction was performed using the Q-View Imager™ and Q-View Software™. Levels of luminescent units or pixel intensity units were measured by the Q-View Software™. The duplicate standard curves are fit by the Q-View Software™ which allows for the selection of multiple non-linear and linear equations to fit the standard curve. Optimal curve fits are determined by visual graph evaluation and comparison of Aikake's information criteria (AIC) values. Measured pixel intensity values are regressed using the selected equation to interpolate concentrations in appropriate units. These concentrations are used in reporting on the sample testing report form {Quansys Biosciences, personal communication}.

3 Clinical Trial – Primary endpoints and key secondary endpoints

3.1 Demographics and patient characteristics

Twenty two subjects were screened and there were two screen failures. Twenty subjects were enrolled into the study, all of whom received at least one dose of study medication. Of these, 17 completed the study and there were 3 withdrawals. None were lost to follow-up, **Figure 3.1**.

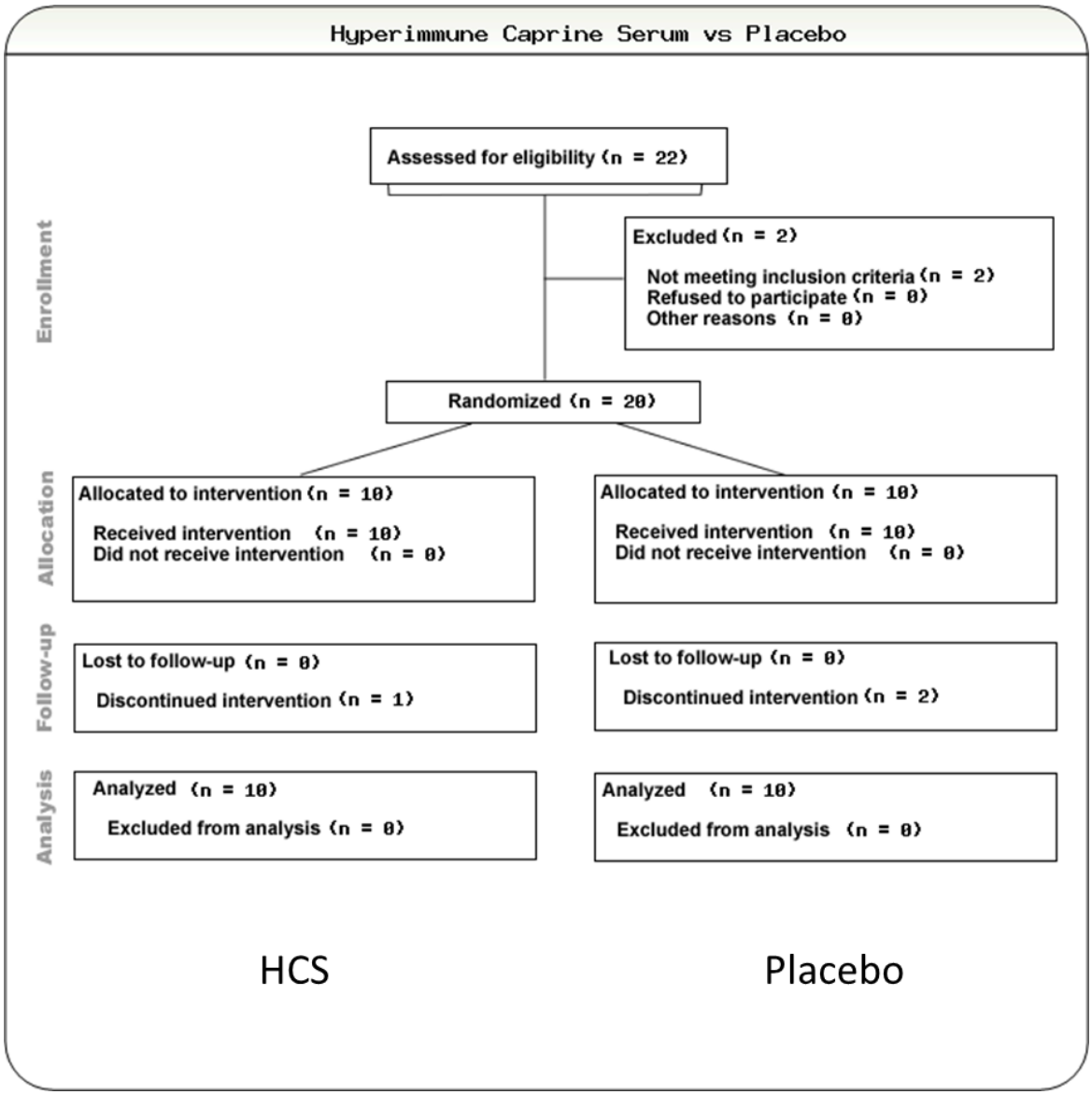


Figure 3.1: Screening, HCS vs Placebo.

Demographic characteristics of the cohort are summarised in **Table 3.1** and disease characteristics at baseline are outlined in **Table 3.2**. These features were as expected for a cohort of subjects with established diffuse SSc.

Table 3.1: Demographics of the study cohort

Characteristic			**HCS**	**Placebo**
Age (years)		n	10	10
		Mean (SD)	53.3 (12.66)	53.6 (13.23)
		Min, Max	35 , 75	29 , 77
		Median	55.7	57.2
Weight (Kg)		n	10	10
		Mean (SD)	75.80 (20.531)	70.00 (14.765)
		Min, Max	51 , 123	52 , 98
		Median	75.50	70.00
Height (m)		n	10	10
		Mean (SD)	1.64 (0.089)	1.63 (0.083)
		Min, Max	1.5 , 1.8	1.5 , 1.8
		Median	1.64	1.62
BMI (kg/m^2)		n	10	10
		Mean (SD)	27.93 (5.484)	26.47 (4.976)
		Min, Max	21.8 , 36.7	20.1 , 32.5
		Median	27.66	26.75
Gender	Male	n (%)	1 (10.0)	1 (10.0)
	Female	n (%)	9 (90.0)	9 (90.0)
Race	Caucasian	n (%)	8 (80.0)	9 (90.0)
	Asian	n (%)	2 (20.0)	0 (0.0)
	Other	n (%)	0 (0.0)	1 (10.0)
Smoking Status	Non-Smoker	n (%)	5 (50.0)	7 (70.0)
	Ex-Smoker	n (%)	4 (40.0)	3 (30.0)
	Current Smoker	n (%)	1 (10.0)	0 (0.0)
Number of Pack Years	Current Smoker	n	1	0 (0.0)
		Mean (SD)	5.3	0 (0.0)
		Min, Max	5 , 5	0 (0.0)
		Median	5.3	0 (0.0)
	Ex-Smoker	n	4	3
		Mean (SD)	14.0 (15.39)	14.5 (20.02)
		Min, Max	1 , 31	1 , 38
		Median	10.0	5.0

Table 3.2: Baseline characteristics of the study cohort

Parameter	Placebo (n=10)	HCS (n=10)
Disease duration, years		
Mean (SD)	10.95 (5.5)	10.21 (8.5)
Median	10.9	7.99
Min, Max	3.7, 20	3, 33
MRSS		
Mean (SD)	13.2 (4.7)	16.9 (9.1)
Median	12.5	12.0
Min, Max	7, 22	6, 31
Autoantibodies, no. (%)		
Anti-Topoisomerase	4 (40)	2 (20)
RNA Polymerase III	3 (30)	5 (50)
Other	3 (30)	3 (30)

MRSS= modified Rodnan Skin Score

9 of 10 patients in each group were female (reflecting the disease predominance in females). The median patient age was similar in each group (55.7 and 57.2 years for the HCS and placebo groups, respectively). In the HCS group, the youngest patient was 35 and the eldest 75 years, while in the placebo group; the youngest was 29 and the eldest 77 years. BMI was also similar in each group with a median of 27.66 and 26.75 in the HCS and placebo groups, respectively. **Table 3.3** summarises disease history of the cohort. The median time since diagnosis of diffuse SSc was higher in the HCS group (10.50 years) than in the placebo group (7.54 years). The median time since diagnosis of first Raynaud's symptoms was again higher in the HCS group, being 12.93 years and 10.14 years in the HCS and placebo groups, respectively. All patients in both groups had visceral involvement, indicating severe morbidity secondary to SSc.

Table 3.3: Disease history

			HCS	Placebo
Time since diagnosis of diffuse cutaneous SSc (years)		n	10	10
		Mean	10.35	9.19
		SD	5.484	7.051
		Min	2.9	2.3
		Median	10.50	7.54
		Max	19	27
Time since diagnosis of first Raynaud's Symptoms (years)		n	10	10
		Mean	12.84	17.31
		SD	6.100	20.856
		Min	3.5	4.4
		Median	12.93	10.14
		Max	22	72
Time since diagnosis of first non-Raynaud's Symptoms (years)		n	10	10
		Mean	10.95	10.21
		SD	5.513	8.527
		Min	3.7	3.0
		Median	10.90	7.99
		Max	20	33
Family History of SSc	Yes	n (%)	0 (0.0)	1 (10.0)
	No	n (%)	10 (100.0)	8 (80.0)
	Unknown	n (%)	0 (0.0)	1 (10.0)

Figure 3.2 shows a schematic of patient progression through the study.

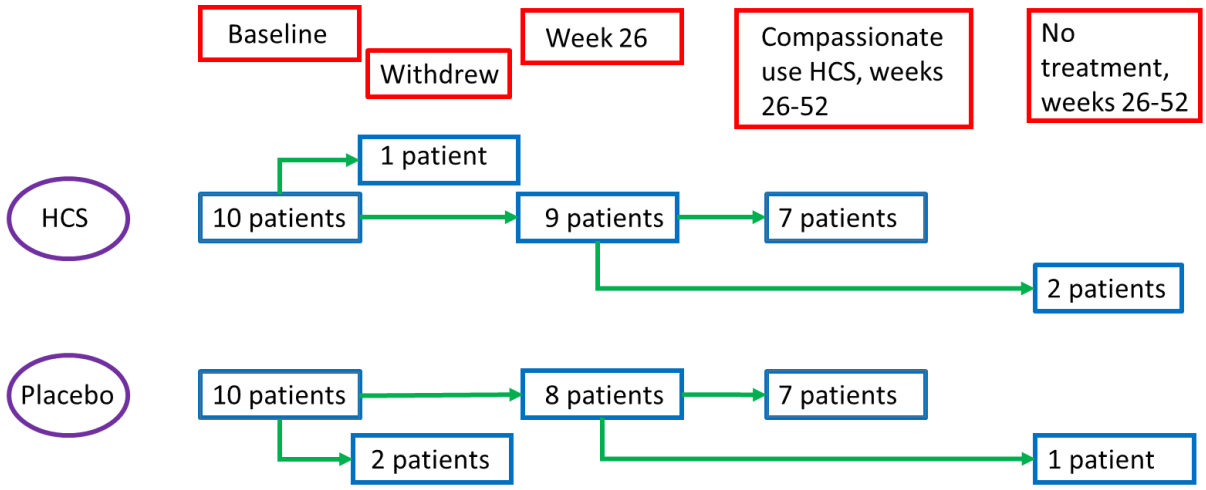


Figure 3.2: Patient progression through the study. There were 3 withdrawals in the double blind phase. At week 26, 3 patients from the whole cohort opted not to receive HCS and the 14 remaining patients received HCS for a further 26 weeks

3.2 Safety assessments

All subjects in both groups had at least one adverse event (AE) and AEs were frequent in both groups in keeping with the high morbidity of the disease. An AE was defined as any untoward medical occurrence in a clinical study participant administered an investigational product, including any clinical or laboratory test value abnormality which occurred during the course of the study, whether considered related to treatment or not. A serious adverse event was defined as any experience which was fatal or life-threatening, was permanently disabling, required hospitalisation or prolongation of hospitalisation, was a congenital anomaly, or was an important medical event that could jeopardise the patient or require intervention to prevent one of those outcomes. There were numerically more AEs in the placebo group compared to the treatment group (though it did not reach statistical significance), 154 in the placebo group and 139 in the treatment group. This supports a conclusion that the study drug was safe and well tolerated. Details of AEs are provided in **Table 3.4**.

Table 3.4: Summary of adverse events

Parameter	Placebo	HCS
Total no. of AEs	154	139
Possibly/Probably related to study medication	18	12
No. of patients reporting Grade 3/4 AEs (severe)	5	4
No. of mild AEs	59	59
No. of moderate AEs	84	76
No. of severe AEs	11	4

The most commonly reported AEs were injection site reactions, cutaneous or musculoskeletal-related issues (such as skin itching, joint pains and ischaemic digital ulcers) and infections. Transient injection site reactions (redness and swelling at the injection site) occurred in both groups, but were more common in the HCS treatment group, occurring in 9 out of 10 patients in this group. Three were graded mild, 5 moderate and 1 severe. An injection site haematoma and chills occurred in one patient, graded moderate, and injection site pain graded mild in one other patient in the placebo group. The frequency of other AEs was similar in both groups. There were no statistically significant differences in the safety laboratory values throughout the study and no differences were noted between the groups in vital signs, physical examination, electrocardiography or echocardiography. One patient had re-emergence of thyroid abnormalities due to known but undertreated hypothyroidism and one patient developed low calcium and magnesium secondary to an increase in proton pump inhibitor given for worsening reflux symptoms.

There were 6 serious adverse events (SAE) in 3 patients in the placebo group and 4 SAEs in 3 patients in the treatment group in the blinded phase. Two patients in placebo group and one in the treatment group withdrew due to AEs or SAEs. None of the SAEs were considered due to HCS treatment. There were no deaths during the course of the study. Details of SAEs are provided in **Table 3.5**.

Table 3.5: Summary of serious adverse events from baseline to week 26.

Parameter	Placebo	HCS
No. of subjects reporting SAEs	3	3
Total no. of SAEs	6	4
Withdrawal due to AEs and SAEs	2	1
SAE by organ system	Intestinal Obstruction x 2	Cerebral infarct
	Panenteric dysmotility	Pulmonary embolus
	Viral meningitis	Atrial Fibrillation
	Pyelonephritis	Respiratory Tract Infection
	Ischaemic digital ulcer	

The only treatment related AEs during extension were reported by patients from the placebo group who commenced HCS treatment after the blinded phase (six (85.7%) of 7 patients) and 5 of these were related to injection site reactions. One patient, placebo to HCS group, discontinued permanently (withdrew) due to an AE (severe injection site reaction). One patient in each of the continuing HCS and placebo follow-up only groups reported a serious adverse event, one was a respiratory tract infection in a patient receiving HCS who continued on HCS and one was an acute episode of digital ischaemia and ulceration in a patient who was on placebo and who chose not to receive HCS and was follow-up only. The respiratory tract infection was not considered to be related to study medication. Both patients were hospitalised and recovered and so these were classified as SAE.

A period of one month off immunosuppression was required for entry into the study. A total of 10 out of the 20 patients enrolled stopped immunosuppression prior to enrolment, 3 in the HCS arm and 7 in the placebo arm. Only 4 patients

started immunosuppression after 26 weeks, all in the placebo arm and 3 of 4 of these had been on immunosuppression prior to the study. Of these 4, 2 patients had worsening lung disease; 1 patient restarted immunosuppression and 1 started immunosuppression after being off it for a number of years. Of the remainder, one had rapidly progressive skin disease and one had worsening GI and cardiac scleroderma.

3.3 Efficacy assessments

3.3.1 Modified Rodnan Skin Score

The difference from baseline score to 26 weeks was analysed first as outcome variable using Student's t-test and corresponding confidence intervals. Using this approach, analysis for the primary data shows mean MRSS fell by 1.4±4.7 units with active treatment but worsened by 2.1±6.4 units on placebo (p=0.181, unpaired t-test) when baseline values were compared to 26 weeks, **Figure 3.3**.

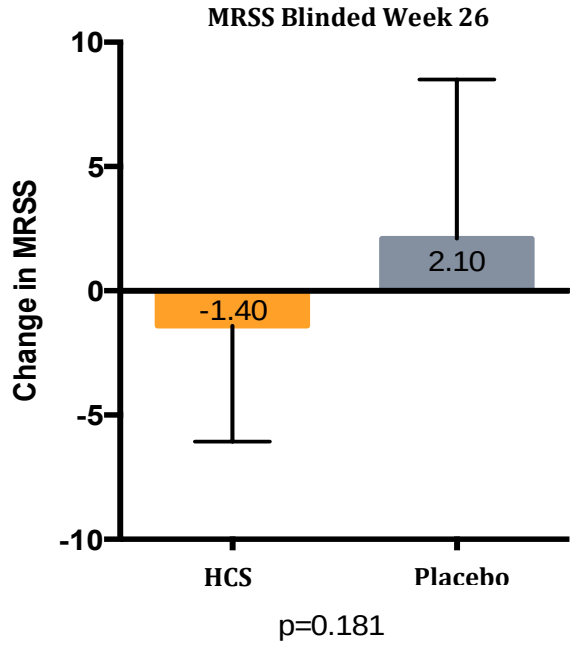


Figure 3.3: Change in MRSS from baseline to 26 weeks, blinded cohort.

Because some patients had demonstrated clinically significant improvement in MRSS, a post hoc analysis of responder frequency in active and placebo treated patients was performed. In the active treatment group one (10%) patient had at least 20% improvement from baseline in MRSS at week 6, and

the number had increased to five (50.0%) at Week 26. In contrast the placebo group had a greater proportion of patients (four patients; 40.0%) with response at week 6, and fewer patients (one patient; 10.0%) at Week 26. The difference between groups at week 26 by the unconditional z-pooled test showed a strong trend towards statistical significance (p=0.067) **Figure 3.4**.

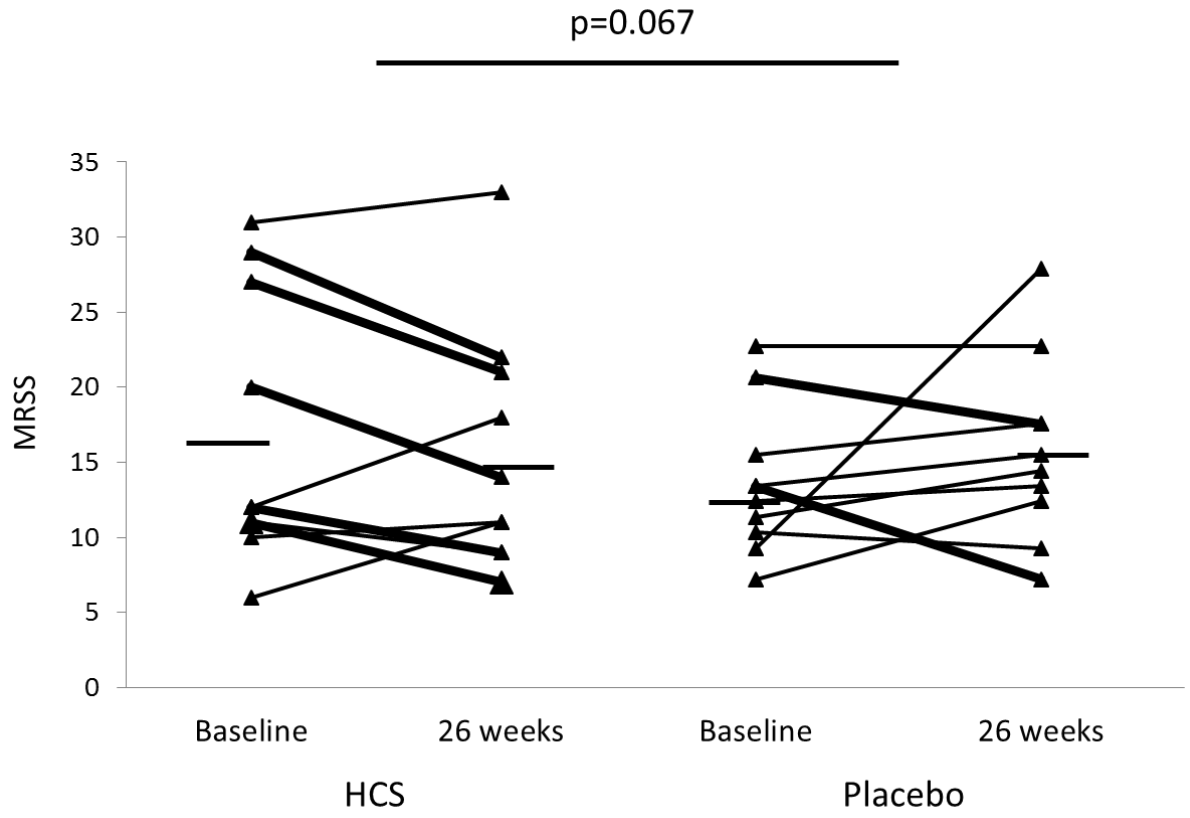


Figure 3.4: Change in modified Rodnan Skin Score (MRSS) from baseline to week 26. Horizontal bars show mean values. There was an increase in mean MRSS in the placebo treated subjects and improvement in those receiving active therapy. This did not reach statistical significance but changes were driven by the larger number of cases on active treatment that showed clinically meaningful improvement in MRSS during the trial (> 4 skin score units and 20% of baseline MRSS). The lines marked in bold show cases with significant improvement on active treatment or placebo. Responder frequency analysis showed a strong trend in favour of active treatment (p=0.067).

As the assessor was blinded to treatment, and remained blinded until after all patients has finished the follow-up phase, there was some degree of blinding in the extension phase. All patients were given a new batch of medication and all patients had the first dose in hospital under supervision. To extend these skin score data and provide greater clarity further analysis from an extended dataset was performed, that is, from patients enrolled in the study but receiving HCS on a compassionate basis for 26 weeks after completion of the double-blind phase of the study. In this extended dataset, the 26 week score for the placebo patients was used as the baseline score and the 52 week score as the 26 week score. All patients had MRSS completed at 52 weeks, whether receiving HCS or not for the safety phase. Whilst these data were not completely blinded they are generally supportive of the trend for improvement seen in the analysis of the 26 week blinded phase. Thus, there is skin score data for 7 additional cases treated for 26 weeks with HCS, and from 3 cases that chose not to take HCS but that were observed for 26 weeks. For this larger patient group the change in MRSS between baseline and 26 weeks was -2.00±1.03 for those treated with HCS (n=17) and +2.39±1.64 in those not receiving active therapy (n=13). Using Student's t-test and corresponding confidence intervals, this difference reached statistical significance (p=0.025), although the limitations of open label data and a post hoc analysis must be considered, **Figure 3.5**.

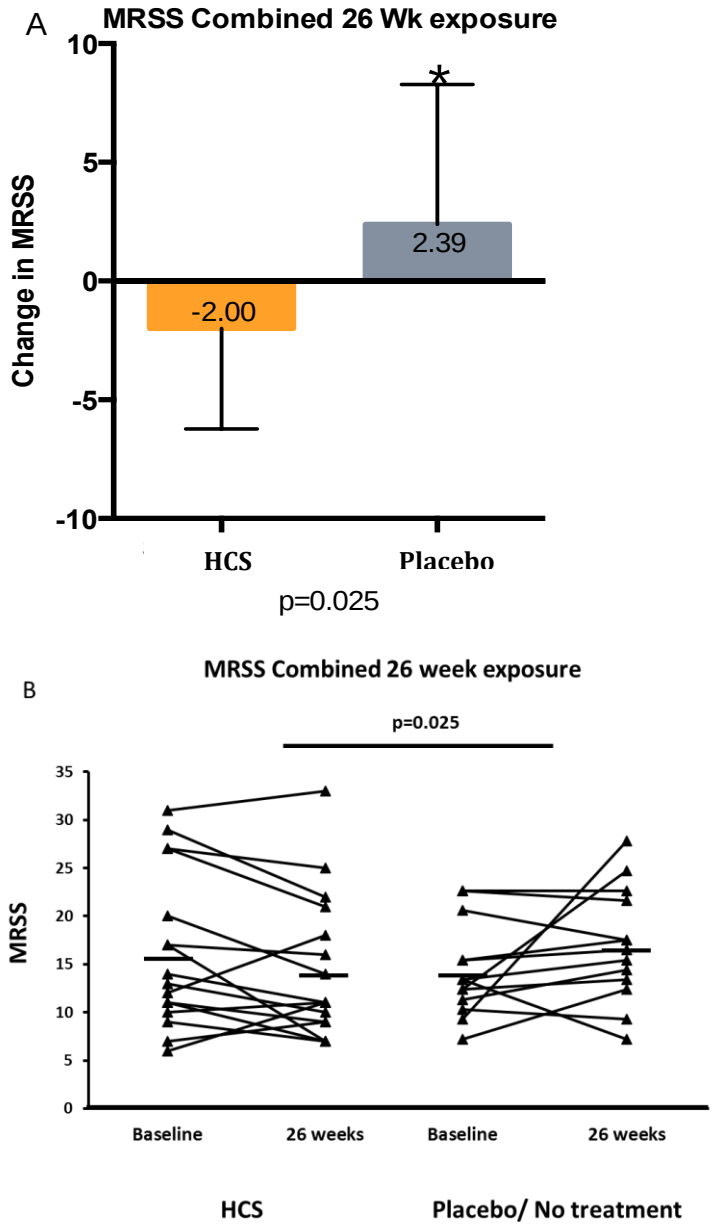


Figure 3.5, A&B: Change in MRSS, baseline compared to 26 weeks of treatment, combined cohort (blinded results combined with compassionate use 26 weeks-52 weeks results). Horizontal bars show mean values.

Furthermore, looking at the 4 groups separately, though the results are not statistically significant, the patients who continued on HCS for a further 6 months and the patients who had HCS in the first 6 months but chose not to continue treatment appear to have stabilisation or slight improvement of MRSS. The patients who had no treatment overall (placebo to no treatment) seemed to have a slight worsening of MRSS, while the patients who were on placebo and

changed to HCS overall seemed to have a slight improvement in MRSS, **Figure 3.6 A-D.**

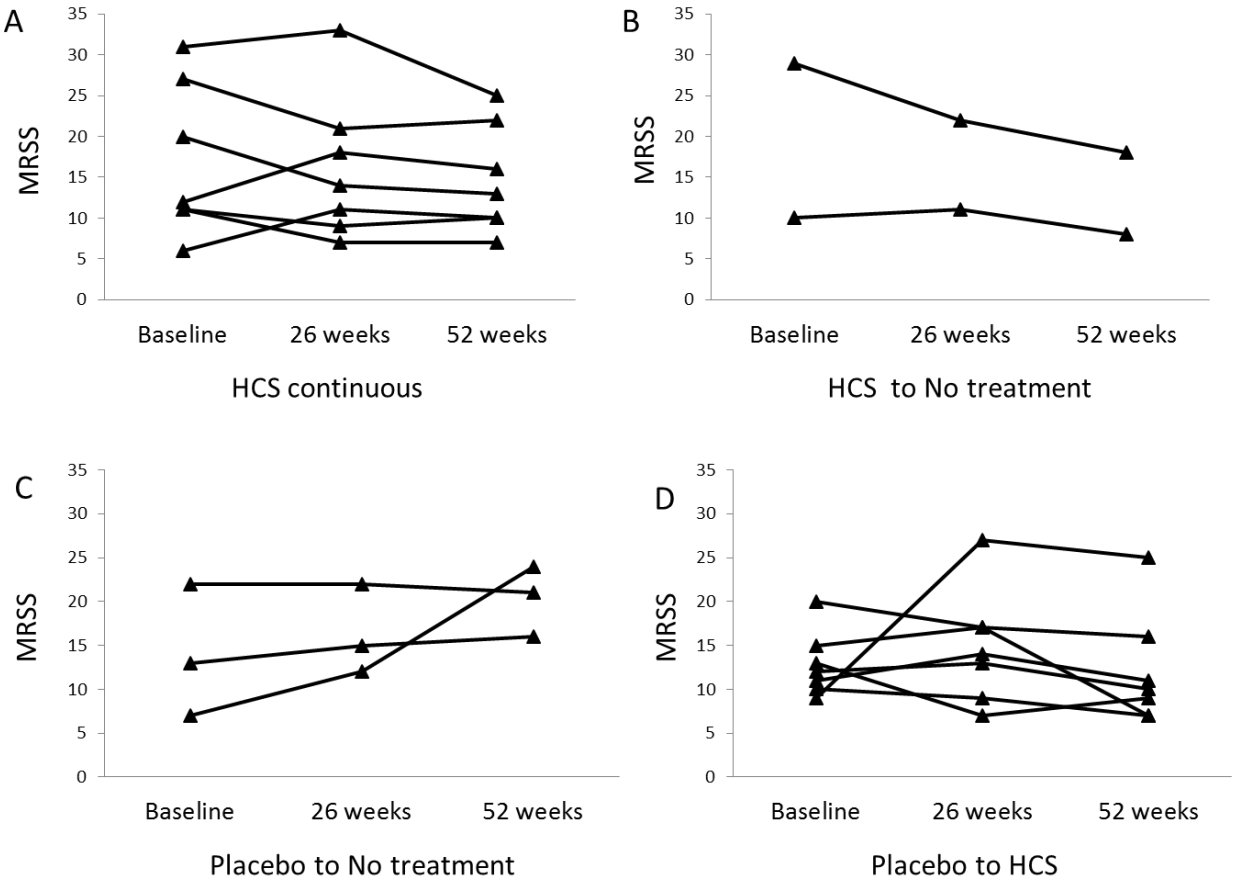


Figure 3.6: Graphic representation of MRSS at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

3.3.2 HAQ and HAQ-DI

The HAQ-DI is calculated from patient responses to the HAQ questionnaire. The questionnaire assesses the performance of 20 daily activities (items) which are grouped into eight categories that represent functional activity. Responses to at least six of the eight categories are required to calculate the HAQ-DI and the highest sub-category score determines the value for each category. The category

scores are averaged into an overall single index of disability (HAQ-DI) ranging from zero to one (representing mild to moderate functional difficulty), one to two (representing moderate to severe functional disability) and two to three (representing severe to very severe functional disability). There was no significant difference between the groups, though the placebo group was slightly worse. Mean ±SD for HAQ-DI at baseline was 1.2±0.07 for the HCS group and 1.6 ± 0.63 for placebo group and at 26 weeks was 1.2±0.98 for HCS and 1.6 ± 0.55 for placebo (p=0.47), **Table 3.6**.

Table 3.6: Summary statistics for HAQ-DI, baseline to week 26. Adjusted mean change for patient number. Higher scores indicate worse disability.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	1.2	0.7	0	1.3	2	
	Week 6	Visit value	9	1.1	0.89	0	1.3	2	
		Change from baseline	9	0	0.28	-1	0	0	
		Adjusted mean change		0	(-0.3, 0.3)				0.9455
	Week 26	Visit value	9	1.2	0.98	0	1.3	3	
		Change from baseline	9	0.1	0.35	0	0.1	1	
		Adjusted mean change		0.1	(-0.1, 0.4)				0.3759
Placebo	Baseline	Visit value	10	1.6	0.63	1	1.5	3	
	Week 6	Visit value	10	1.3	0.51	1	1.3	2	
		Change from baseline	10	-0.3	0.51	-1	-0.1	1	
		Adjusted mean change		-0.2	(-0.5, 0.0)				0.0875
	Week 26	Visit value	9	1.6	0.55	1	1.4	2	
		Change from baseline	9	0	0.31	-1	0	1	
		Adjusted mean change		0	(-0.3, 0.2)				0.8833
Difference between groups	Week 6	Adjusted mean change		0.3	(-0.2, 0.7)				0.2194
	Week 26	Adjusted mean change		0.1	(-0.2, 0.5)				0.473

There are eight functional activity category scores in the HAQ; groom and dress, arise, eating, walking, hygiene, reach, grip and activity. At 26 weeks, no changes were seen in median values for any of these categories for patients in both groups. Statistically there was no overall significant difference between the groups in any of the eight categories: groom and dress, arise, eating, walking, hygiene, reach, grip and activity (p=0.6139, p=0.6560, p=0.3927, p=0.8015, p=0.2506, p=0.5363, p=0.5628, p=0.8133 respectively). See **Appendix Section 8.2** for details.

The SSc-HAQ also has disease-specific VAS items of bodily pain, intestinal symptoms, breathing problems, Raynaud's syndrome and finger ulcers and the results showed no significant differences between HCS and placebo for 4 of 5 scales. The only significant difference found was in the scale for finger ulcers with the HCS group showing an improvement with treatment in percentage change (p=0.0466). However there was no difference in absolute change. There was a large variability in a small sample size. Variability may be explained by the time of year the patients were seen as finger ulcers tend to be worse in colder months, **Table 3.7**.

Table 3.7: Summary statistics of the change in VAS scales of SSc-HAQ, baseline to week 26. Negative mean scores indicate increasing disease burden.

VAS item	Group	n	Mean	SD	Min	Median	Max	95% Confidence interval of mean	Prob (t)
Intestinal problems	HCS	8	8.9	29.73	-12	-4	79		
	Placebo	7	-0.1	35.43	-57	7	46		
	Difference		11.7					(-17.4, 40.7)	0.4062
Breathing problems	HCS	8	4.3	14.92	-8	0.0	41		
	Placebo	5	-9.8	23.71	-66	-3.0	12		
	Difference		14.3					(-3.3, 31.8)	0.1036
Raynaud's symptoms	HCS	8	-0.9	19.81	-33	-2	44		
	Placebo	7	3.8	41.72	-53	0.0	68		
	Difference		-0.9					(-27.6, 25.8)	0.9438
Finger ulcers	HCS	6	-1.3	4.7	-7	0.0	9		
	Placebo	3	-3.9	32.4	-50	0.0	60		
	Difference		4.8					(-18.3, 27.9)	0.6654
Pain	HCS	9	-5.6	4.04	1	-15	6		
	Placebo	9	17.29	6.25	8	20	-19		
	Difference		22.89					(-28.98, 30.9)	0.4844

3.3.3 SF-36

The SF-36 is split into 8 domains, 4 for physical health (Physical functioning, Role Physical, Bodily pain and General Health) and 4 for mental health (Vitality, Social Functioning, Role Emotional and Mental Health). In the 8 domains of SF-36, the only domain to show some change was Role Physical, which showed a worsening in the placebo group and maintenance or stabilisation in the treatment group between baseline and week 26, with trend to significance between the groups (p=0.0685), **Table 3.8**.

For the SF-36 domain scales that mostly contribute to the scoring of the physical health summary outcome, patients in the HCS group reported improvement between baseline and Week 6 in role-physical and general health, however this was not maintained at Week 26 as results indicate that there had been no change from baseline median in role-physical and a worsening in general health. For the two other domain scales, physical functioning and bodily pain in this category there were no changes in median results between baseline and Week 6 or Week 26. Scores for patients in the placebo group at Week 6 mostly declined in all but one (general health) of the domain scales that contribute mostly to the physical health summary outcome. At Week 26 patients in the placebo group either reported continued decline (physical functioning, role-physical) or remained unchanged (bodily pain). However there was a small improvement in median change from baseline for the general health domain. Overall for physical health there was no significant difference at week 6 or week 26 apart from role physical mentioned above. There were no significant changes in the 4 mental health domains between the groups. The Health Transition Index showed improvement in the HCS group at week 6 and week 26, while the placebo group showed initial improvement at week 6, with worsening at week 26, **Table 3.9**. See **Appendix Section 8.3** for further details.

Table 3.8: SF-36 Role Physical with change from baseline by treatment and visit. Adjusted mean change is adjusted for number of patients. Lower scores indicate greater burden of disease.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	38.8	30.16	0	40.6	75	
	Week 6	Visit value	9	54.9	38.37	0	50.0	100	
		Change from baseline	9	16.7	25.77	0	6.3	81	
		Adjusted mean change		14.5	(-5.7, 34.8)				0.1468
	Week 26	Visit value	9	45.8	30.62	0	37.5	94	
		Change from baseline	9	7.6	23.55	-25	0.0	56	
		Adjusted mean change		5.5	(-11.6, 22.6)				0.5044
Placebo	Baseline	Visit value	10	48.8	31.29	6	50.0	100	
	Week 6	Visit value	10	42.5	27.76	0	40.6	88	
		Change from baseline	10	-6.3	34.74	-56	-12.5	63	
		Adjusted mean change		-2.8	(-22.1, 16.5)				0.7609
	Week 26	Visit value	9	27.1	23.18	0	25.0	69	
		Change from baseline	9	-16.0	32.79	-75	-12.5	44	
		Adjusted mean change		-16.7	(-33.5, 0.2)				0.0520
Difference between groups	Week 6	Adjusted mean change		17.4	(-10.7, 45.4)				0.2084
	Week 26	Adjusted mean change		22.2	(-1.9, 46.3)				0.0685

Table 3.9:

A) A summary of the individual SF-36 scales at baseline, week 6 and week 26. Lower scores indicate greater burden of disease.

	Baseline		Week 6		Week 26		Adjusted mean change from Baseline to Week 26		95% Confidence Interval	
	HCS	Placebo	HCS	Placebo	HCS	Placebo	HCS	Placebo	HCS	Placebo
Physical functioning	39.5	33.5	45.6	37	40.6	26.9	0.9	-9.7	-8.9, 10.7	-19.9, 0.4
Role Physical	38.8	48.8	54.9	42.5	45.8	27.1	5.5	-16.7	-11.6, 22.6	-33.5, 0.2
Pain	36.4	52.4	42.2	45.6	38.8	35.6	-2.1	-9.7	-17.5, 13.3	-24.7, 5.5
General Health Perception	36.8	31	40.1	27.3	36.2	24	2	-7.9	-10.4, 14.4	-19.8, 4.1
Vitality	35.5	34	38.9	33.5	38.3	32	2.1	-2.8	-10.1, 14.3	-14.9, 9.2
Social Functioning	53.8	48.8	61.1	51.3	56.9	36.1	6.9	-8.4	-12.4, 26.2	-27.4, 10.6
Role Emotional	45.8	70	72.2	61.7	59.3	57.4	4.8	-4.2	-19.7, 29.3	-28.1, 19.7
Mental Health	58.8	62	61.8	66.4	58.2	57.3	1	-1.7	-11.4, 13.4	-13.9, 10.4

B) Health Transition Index at baseline, week 6 and week 26.

	Baseline		Week 6		Week 26	
	HCS	Placebo	HCS	Placebo	HCS	Placebo
Health Transition Index (n)						
Much better		1		2		1
Somewhat better		2	2	1	3	
About the same	6	4	5	4	4	3
Somewhat worse	4	2	2	2	2	2
Much worse		1		1		3

3.3.4 Scleroderma Functional Score (SSc-FS)

Each item of the Scleroderma Functional Score is scored from 0 (normal) to 4 (impossible to achieve) with an overall sum score of 0 to 33, where a higher score indicates worse function. The median SSc-FS for patients taking HCS remained stable at both Week 6 and Week 26 however, the range of scores was large with the minimum score being 0 and maximum scores 27.0, 24.0 and 26.0 at baseline, Week 6 and Week 26, respectively. In the placebo group the median SSc-FS demonstrated a slight increase at Week 6 (0.50) and again at Week 26 (1.00) indicating worsening of function. Similarly, there was a wide spread in the scores in the placebo group with minimum and maximum scores of 4.0 to 23.0, 5.0 to 22.0, and 6.0 to 19.8 at baseline, Week 6 and Week 26, respectively. No group had an adjusted mean change from baseline at either Week 6 or Week 26 that was significantly different to zero. Analysis of the adjusted mean change in the SSc-FS from baseline to Week 6 or to Week 26 in the ITT population indicates that there was no overall significant difference between the groups or between visit weeks, **Table 3.10.**

Table 3.10: SSc-FS with change from baseline by treatment and visit. Adjusted mean change is adjusted for patient numbers. Higher scores indicate greater disease burden.

Treatment group	Visit	Value	Summary statistics						
			n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	10.73	8.460	0.0	13.00	27.0	
	Week 6	Visit value	9	11.56	9.475	0.0	10.00	24.0	
		Change from baseline	9	1.41	4.711	-4.0	0.00	11.0	
		Adjusted mean change		1.34	(-1.82, 4.51)				0.3822
	Week 26	Visit value	9	11.78	9.985	0.0	14.00	26.0	
		Change from baseline	9	1.63	3.659	-1.0	0.00	10.0	
		Adjusted mean change		1.56	(-0.95, 4.08)				0.2061
Placebo	Baseline	Visit value	10	11.38	6.246	4.0	10.00	23.0	
	Week 6	Visit value	10	10.40	4.904	5.0	10.00	22.0	
		Change from baseline	10	-0.98	4.508	-12.0	0.50	3.0	
		Adjusted mean change		-0.92	(-3.92, 2.08)				0.5253
	Week 26	Visit value	9	11.87	5.697	6.0	13.00	19.8	
		Change from baseline	9	0.34	3.278	-5.0	1.00	5.0	
		Adjusted mean change		0.46	(-2.05, 2.96)				0.7036
Difference between groups	Week 6	Adjusted mean change		2.26	(-2.11, 6.63)				0.2885
	Week 26	Adjusted mean change		1.11	(-2.45, 4.66)				0.5189

3.3.5 Neuropathic pain VAS

Neuropathic pain VAS was indicated by patients on a horizontal line in mm on a scale of 0 (no pain) to 100 (severe pain), with the final score presented in cm. At baseline patients reported a median neuropathic pain of 4.50 cm in the HCS group and 0.60 cm in the placebo group. The median neuropathic pain decreased at Week 6 (-2.0 cm) and Week 26 (-0.90 cm) compared to baseline in the HCS group. There was no change in median pain VAS in the placebo group at Week 6 and an increase (0.05 cm) at Week 26. The analysis of adjusted mean change from baseline in the neuropathic pain VAS indicates that there was significant difference between groups at week 26 with an improvement in the treatment group and no change in the placebo group, p=0.0461, **Figure 3.7, Table 3.11**.

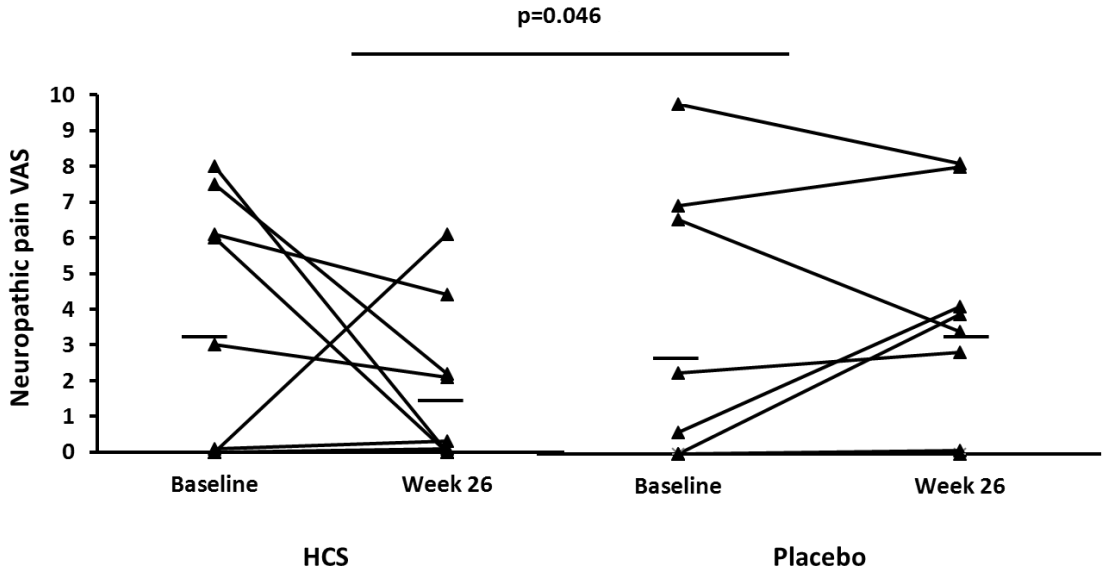


Figure 3.7: Change in Neuropathic pain VAS from baseline to week 26. Horizontal bars show mean values.

Table 3.11: Summary statistics for Neuropathic pain, baseline to week 26. Adjusted mean change is adjusted for patient numbers. Higher scores indicate worse pain.

Treatment Group	Visit	Value	Summary statistics						Prob
			n	Mean	SD	Min	Median	Max	
HCS	Baseline	Visit value	8	3.84	3.478	0	4.5	8	
	Week 6	Visit value	9	1.91	2.9	0	0.1	7.8	
		Change from baseline	7	-1.9	2.217	-6.1	-2	0	
		Adjusted mean change		-1.79	(-3.46, -0.13)				
	Week 26	Visit value	9	1.69	2.243	0	0.3	6.1	
		Change from baseline	7	-1.96	2.615	-6	-0.9	0.2	
		Adjusted mean change		-1.98	(-3.45, -0.51)				
Placebo	Baseline	Visit value	9	2.97	3.901	0	0.6	10	
	Week 6	Visit value	9	1	2.412	0	0	7.3	
		Change from baseline	9	-1.97	3.725	-10	0	0.2	
		Adjusted mean change		-2.05	(-3.52, -0.58)				
	Week 26	Visit value	9	3.47	3.207	0	3.5	8.3	
		Change from baseline	8	0.06	1.987	-3.2	0.05	3.6	
		Adjusted mean change		0.08	(-1.30, 1.46)				
Difference between groups	Week 6	Adjusted mean change		0.25	(-1.97, 2.48)				0.8086
	Week 26	Adjusted mean change		-2.06	(-4.08, -0.04)				0.0461

3.3.6 MRC sum score

No significant changes were noted of any of the six muscle groups (shoulder abduction, forearm flexion, wrist extension, hip flexion, knee extension and foot dorsiflexion) for patients in either group by Week 26. At baseline, pre- and post-treatment administration the muscle strength of patients in both HCS and placebo groups was the same with median scores of 60.0. The MRC score remained unchanged at Week 6 and Week 26. While the scores ranged from 54 to 60 at most visits, there was one patient in the HCS group whose score declined to 33 at Week 26 due to MCA infarction which caused the patient to withdraw from the study at Week 2, **Table 3.12**.

Table 3.12: Summary statistics MRC Sum score comparing Baseline to Week 0/Day 0, week 6, week 26. Lower scores indicate worse function. Adjusted for patient numbers.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	59.4	1.07	57	60	60	
	Week 0/Day 0	Visit value	10	59.6	0.7	58	60	60	
		Change from baseline	10	0.2	0.42	0	0	1	
		Adjusted mean change		0.3	(0.1, 0.5)				
	Week 6	Visit value	9	59.8	0.44	59	60	60	
		Change from baseline	9	0.1	0.6	-1	0	1	
		Adjusted mean change		0.3	(0.1, 0.5)				
	Week 26	Visit value	10	57.2	8.51	33	60	60	
		Change from baseline	10	-2.2	7.7	-24	0	2	
		Adjusted mean change		-2.3	(-5.9, 1.3)				
Placebo	Baseline	Visit value	10	59.2	1.87	54	60	60	
	Week 0/Day 0	Visit value	10	59.6	0.7	58	60	60	
		Change from baseline	10	0.4	1.26	0	0	4	
		Adjusted mean change		0.3	(0.1, 0.5)				
	Week 6	Visit value	10	59.8	0.63	58	60	60	
		Change from baseline	10	0.6	1.26	0	0	4	
		Adjusted mean change		0.5	(0.3, 0.6)				
	Week 26	Visit value	10	59.5	1.27	56	60	60	
		Change from baseline	10	0.3	0.82	-1	0	2	
		Adjusted mean change		0.4	(-3.2, 4.0)				
Difference between groups	Week 0/Day 0	Adjusted mean change		-0.1	(-0.4, 0.2)				0.5537
	Week 6	Adjusted mean change		-0.2	(-0.5, 0.1)				0.2019
	Week 26	Adjusted mean change		-2.7	(-7.8, 2.4)				0.2808

3.3.7 Physician and patient global VAS

There was no significant difference in change from baseline between patients' VAS scores in either HCS or placebo groups in overall disease severity at week 6 or week 26 (p=0.344), **Table 3.13**.

Table 3.13: Summary statistics of the change in Patient global VAS scale of SSc-HAQ, baseline to week 26. Higher scores indicate increased disease burden.

VAS item	Group	n	Mean	SD	Min	Median	Max	95% Confidence interval of mean	Prob (t)
Disease severity	HCS	9	3.0	21.83	-24	3.0	54		
	Placebo	9	10.9	24.85	-20	5.0	61		
	Difference		-10.4					(-32.9, 12.2)	0.3440

In the HCS group the median physician disease severity at baseline decreased slightly at Week 6 (-0.3 cm) and further by Week 26 (-1.1 cm). In the placebo group the baseline median disease severity was higher than in the HCS group, decreased slightly at Week 6 (-0.1 cm) and then increased Week 26 (0.8 cm). The analysis of adjusted mean change from baseline in the global disease severity VAS indicates that there is no significant difference between treatment groups at either Week 6 (p=0.6515) or Week 26 (p=0.3451), **Table 3.14**.

Table 3.14: Summary statistics for physician global score. Higher scores indicate increasing disease burden. Adjusted for patient numbers.

Treatment group	Visit	Value	Summary statistics						Prob
			n	Mean	SD	Min	Median	Max	
HCS	Baseline	Visit value	10	4.7	1.82	2	4.8	8	
	Week 6	Visit value	9	4.7	1.88	1	5.1	7	
		Change from baseline	9	-0.2	1.19	-2	-0.3	2	
		Adjusted mean change		-0.3	(-1.4, 0.8)				
	Week 26	Visit value	10	4.4	2.51	1	3.3	8	
		Change from baseline	10	-0.3	2.77	-3	-1.1	6	
		Adjusted mean change		-0.7	(-2.5, 1.1)				
Placebo	Baseline	Visit value	10	5.7	1.08	4	5.8	7	
	Week 6	Visit value	10	5.7	1.86	3	6	8	
		Change from baseline	10	0	1.79	-3	-0.1	4	
		Adjusted mean change		0.1	(-1.0, 1.1)				
	Week 26	Visit value	10	5.8	2.68	2	6.9	8	
		Change from baseline	10	0.1	2.96	-5	0.8	4	
		Adjusted mean change		0.5	(-1.3, 2.4)				
Difference between groups	Week 6	Adjusted mean change		-0.3	(-1.9, 1.2)				0.6515
	Week 26	Adjusted mean change		-1.2	(-3.9, 1.4)				0.3451

4 Physiological studies

4.1 Routine physiological studies

4.1.1 Vital signs and clinical examination

There were no significant changes between or within HCS and placebo groups in any of the vital signs parameters or clinical examination.

4.1.2 Cardiological assessments

There were no significant changes in ECG or echocardiogram findings between HCS and placebo groups throughout the study. Left ventricular ejection fraction (LVEF) was normal in all patients in both groups. Mean estimated pulmonary artery systolic pressure (PASP) was in the normal range in most patients. Mean PASP was slightly higher at baseline in the placebo group than the HCS group (26.1mmHg versus 19.3mmHg) but did not change significantly. Elevated PASP was reviewed by the assessor in the context of clinical symptoms and worsening lung function tests, particularly DLco. If the patient did not have symptoms and DLco was stable, the patient was monitored as per clinical protocol with a repeat echocardiogram in 6 months. If symptoms developed, a repeat echocardiogram was performed and depending on results the patient either continued to be monitored or was referred for further assessment. If lung function tests and/or clinical symptoms showed deterioration, the patient was referred for cardiological assessment including consideration for right heart catheterisation.

PASP was found to be slightly elevated in 3 patients in the HCS group after the start of treatment, but it was not clinically significant and further tests were stable. PASP was significantly elevated at withdrawal visit in one patient in the placebo group who withdrew due to worsening lung disease. This patient was further assessed per clinical protocol and due to the significant change coupled with her dyspnoea, she went on to have a right heart catheter test which confirmed that her elevated PASP was pulmonary hypertension secondary to her lung disease and not due to PAH.

4.1.3 Chest radiograph and HRCT Chest

Seventy percent of patients in the HCS group and 90% of patients in the placebo group had a normal chest radiograph at baseline. Abnormal findings were due to pre-existing lung fibrosis. 5 patients (2 in the HCS arm and 3 in the placebo arm) had HRCT chest performed due to either worsening of symptoms (dyspnoea, cough, worsening exercise tolerance) and/or worsening of pulmonary function tests (FVC or DLco decrease of 20% compared to last PFT readings), in line with usual clinical practice. HRCTs were reviewed with reference to extent of fibrotic changes i.e. definitely >20% or indeterminate (10-30%) as per staging system suggested by Goh et al (202), which is routine practice. FVC <70% was used as the threshold in patients with indeterminate disease on HRCT. Of the 5 patients who had a HRCT, 1 patient in the placebo group had confirmed worsening of lung fibrosis (when comparing her HRCT to previous scans and using the scoring system mentioned). She subsequently discontinued the study and started immunosuppression.

4.1.4 Pulmonary function tests

Lung function indices showed a trend of benefit for the HCS group compared to the placebo group for those variables that reflect respiratory effort (FVC and FEV_1) for absolute values but not in % predicted values. At Week 26 FEV_1 had increased in the HCS group and decreased in the placebo group resulting in 5.83% difference between groups. A similar pattern was shown in FVC for a 7.37% difference between the groups. This represents an interesting positive trend but did not reach statistical significance due to the small sample. In addition, no significant fold changes were noted in either group. However when background disease was taken into account (pre-existing lung disease worsened in 1 patient in the placebo group), there was no significant difference between the two treatment groups. DLco and TLC did not change during the study, **Table 4.1.**

Table 4.1, A&B: Descriptive statistics for change and percent change from Baseline to Week 26 for pulmonary function parameters.

A

Parameter	Value	Group	n	Mean	95% Confidence limits for Mean	SD	SEM	Prob (t)
FEV_1 (L)	Raw change from Baseline	HCS	9	-0.004	-0.128 - 0.119	0.1603	0.0534	0.9358
		Placebo	10	-0.103	-0.225 - 0.019	0.1702	0.0538	0.0879
		Group diff.		0.099	-0.062 - 0.259	0.1656	0.0761	0.2126
FEV_1 (L)	Percent change from Baseline	HCS	9	0.266	-4.658 - 5.191	6.4066	2.1355	0.9039
		Placebo	9	-5.564	-11.375 - 0.246	7.5592	2.5197	0.0582
		Group diff.		5.830	-1.171 - 12.832	7.0066	3.3029	0.0966
Pred. FEV_1 (%)	Raw change from Baseline	HCS	9	0.34	-4.10 - 4.79	5.780	1.927	0.8626
		Placebo	10	-3.24	-7.78 - 1.30	6.349	2.008	0.1410
		Group diff.		3.58	-2.32 - 9.49	6.088	2.797	0.2172
FEV/FVC ratio (%)	Raw change from Baseline	HCS	9	-1.203	-4.096 - 1.690	3.7638	1.2546	0.3658
		Placebo	10	0.200	-4.502 - 4.902	6.5730	2.0786	0.9255
		Group diff.		-1.403	-6.671 - 3.866	5.4350	2.4972	0.5816

B

Parameter	Value	Group	n	Mean	95% Confidence limits for Mean	SD	SEM	Prob (t)
FVC (L)	Raw change from Baseline	HCS	9	0.027	-0.214	0.139	0.0463	0.5808
		Placebo	10	-0.162	-0.392	0.2745	0.0868	0.0948
		Group diff.		0.189	-0.429	0.2213	0.1017	0.0810
FVC (L)	Percent change from Baseline	HCS	9	1.799	-7.871	5.1202	1.7067	0.3225
		Placebo	10	-5.569	-13.922	9.7311	3.0772	0.1038
		Group diff.		7.368	-15.323	7.9037	3.6315	0.0584
Pred. FVC (%)	Raw change from Baseline	HCS	9	1.64	-7.29	4.74	1.58	0.3284
		Placebo	10	-1.39	-10.18	7.119	2.251	0.5522
		Group diff.		3.03	-11.85	6.116	2.81	0.2953
DLCO (mmol/min/kPa)	Raw change from Baseline	HCS	9	-0.129	-0.452	0.2943	0.0981	0.2253
		Placebo	10	-0.325	-0.796	0.5569	0.1761	0.0981
		Group diff.		0.196	-0.878	0.4527	0.208	0.3590
Pred. DLCO (%)	Raw change from Baseline	HCS	9	-1.48	-6.07	3.951	1.317	0.2944
		Placebo	10	-3.76	-10.68	7.461	2.36	0.1455
		Group diff.		2.28	-11.76	6.068	2.788	0.4244
TLC (L)	Raw change from Baseline	HCS	9	-0.081	-1.011	0.6576	0.2192	0.7210
		Placebo	10	-0.12	-0.524	0.3659	0.1157	0.3268
		Group diff.		0.039	-1.016	0.5238	0.2407	0.8735

4.2 Exploratory physiological studies

4.2.1 Sniff Nasal Inspiratory Pressure

Sniff nasal inspiratory pressure (SNIP) was in the normal range (compared to reference values for normal subjects by age by Uldry et al (189)) for most patients **Figure 4.1** and **Table 4.2, A & B**. SNIP was slightly low throughout compared to normal subjects for 3 patients, patients 2, 3 and 19, all of whom were women in their 70's. There were no significant changes between the groups or within the groups throughout the study. This indicates that respiratory muscle function as measured by SNIP is normal in this group of SSc patients and remained normal throughout.

Figure 4.1: Graphic representation of SNIP values at baseline, week 6 and week 26.

Table 4.2: Descriptive statistics for SNIP, **A:** Baseline, week 6 and week 26 comparisons and **B:** Change from Baseline to Week 26. Normal values lie between 90-110 cmH_2O.

A			**Descriptive Statistics**					
Parameter	**Treatment Group**	**Visit**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**
Sniff nasal inspiratory pressure (cmH_2O)	HCS	Baseline	10	103.2	29.43	67	100.5	171
		Week 6	9	99.0	23.73	67	96.0	148
		Week 26	9	102.4	26.75	72	98.0	157
	Placebo	Baseline	10	103.3	24.10	54	105.5	146
		Week 6	10	102.7	20.16	69	105.0	146
		Week 26	10	105.7	23.06	61	111.0	145

B			**Descriptive statistics**					
Parameter	**Value**	**Group**	**n**	**Mean**	**95% Confidence limits for Mean**	**SD**	**SEM**	**Prob (t)**
SNIP ($cm\ H_2O$)	Raw change from Baseline	HCS	9	-3.8	-17.8 - 10.2	18.23	6.08	0.5514
		Placebo	10	2.4	-2.3 - 7.1	6.62	2.09	0.2812
		Group difference		-6.2	-19.2 - 6.8	13.40	6.16	0.3593

4.2.2 R-R interval

Mean changes in R-R interval variation (also called heart rate variability, HRV) during normal breathing, deep breathing or Valsalva manoeuvre were similar for patients in both HCS and placebo groups throughout the study period. No clinically or statistically significant variations were noted. A number of patients in both groups demonstrated variation in association with ectopic beats or heart block. Patient 13 developed complete heart block at week 26, making his readings ineligible. Software is available to correct for occasional ectopic beats however, any patients who had numerous ectopic beats also had outlying readings, as these were impossible to correct for. The results compare SSc placebo patients to HCS treated patients before and after treatment, therefore patients act as their own controls. In this study there was no normal comparator control group. However, there is some published literature on HRV and autonomic dysfunction in SSc. These studies are mentioned in the relevant sections.

4.2.2.1 Normal breathing

Descriptive statistics for R-R interval are presented in **Tables 4.3 and 4.4**. Due to some outlying values, it is more useful to consider median rather than mean values.

Placebo patients tended to have slightly lower values for SDRR and RMSSD but there was no significant change after treatment in either group. However, though there is no normal control group in this study, values for SDRR and RMSSD appear to be much lower than normal control groups in other studies, suggesting that autonomic neuropathy is present in these patients, in keeping with these and other studies showing evidence of significant autonomic neuropathy in SSc (195) (196) (203) (204).

Table 4.3: Descriptive statistics for normal breathing R-R interval. Normal control values for SDRR are reported to be 0.13-0.167sec and RMSSD are 0.032-0.057sec.

Descriptive Statistics									
Parameter	**Treatment Group**	**Visit**	**Time**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**
Max-min/ mean (%)	HCS	Day 0	pre dose	10	18.6	13.34	7	14.4	54
		Day 0	post dose	10	16.7	11.43	7	13.4	47
		Week 26	N/A	8	15.2	5.92	7	14	23
	Placebo	Day 0	pre dose	10	16.8	9.94	9	11.5	40
		Day 0	post dose	10	18.3	14.24	8	14.1	56
		Week 26	N/A	10	22.9	26.36	9	15.2	97
SDRR (sec)	HCS	Day 0	pre dose	9	0.062	0.0474	0.01	0.053	0.16
		Day 0	post dose	9	0.034	0.0224	0.02	0.028	0.09
		Week 26	N/A	8	0.039	0.0221	0.02	0.033	0.08
	Placebo	Day 0	pre dose	10	0.04	0.0355	0.02	0.024	0.13
		Day 0	post dose	10	0.044	0.0454	0.02	0.023	0.16
		Week 26	N/A	10	0.055	0.0613	0.02	0.025	0.19
RMSSD (sec)	HCS	Day 0	pre dose	9	0.07	0.0794	0	0.031	0.26
		Day 0	post dose	9	0.033	0.0294	0.01	0.021	0.09
		Week 26	N/A	8	0.046	0.0413	0.01	0.035	0.14
	Placebo	Day 0	pre dose	10	0.041	0.0443	0.01	0.018	0.15
		Day 0	post dose	10	0.047	0.0617	0.01	0.019	0.18
		Week 26	N/A	10	0.069	0.1009	0	0.024	0.28

Table 4.4: Change from baseline for normal breathing R-R interval. Normal control values for SDRR are reported to be 0.13-0.167sec and RMSSD are 0.032-0.057sec.

Change from Baseline								
Parameter	**Treatment Group**	**Visit**	**Time**	**Mean**	**SD**	**Min**	**Median**	**Max**
Max-min/ mean (%)	HCS	Day 0	pre dose					
		Day 0	post dose	-1.9	3.91	-8	-2	4
		Week 26	N/A	-4.2	13.78	-31	-4.2	14
	Placebo	Day 0	pre dose					
		Day 0	post dose	1.5	5.44	-5	0.7	15
		Week 26	N/A	6.1	26.53	-21	-0.5	78
SDRR (sec)	HCS	Day 0	pre dose					
		Day 0	post dose	-0.028	0.042	-0.12	-0.018	0.02
		Week 26	N/A	-0.027	0.0358	-0.08	-0.033	0.03
	Placebo	Day 0	pre dose					
		Day 0	post dose	0.004	0.0224	-0.04	0.003	0.05
		Week 26	N/A	0.015	0.0297	-0.01	0.001	0.08
RMSSD (sec)	HCS	Day 0	pre dose					
		Day 0	post dose	-0.037	0.0694	-0.19	-0.01	0.04
		Week 26	N/A	-0.03	0.0588	-0.12	-0.028	0.06
	Placebo	Day 0	pre dose					
		Day 0	post dose	0.006	0.0308	-0.05	0.001	0.07
		Week 26	N/A	0.028	0.0643	-0.03	0.005	0.16

4.2.2.2 Deep breathing

There was no significant difference between or within treatment groups for deep breathing HRV variables. Descriptive statistics are presented in **Tables 4.5** and **4.6**. The published data on HRV deep breathing variables usually compares E/I ratio (the maximum expiration over the maximum inspiration) (205) (206). Unfortunately the software used does not record this variable, therefore conclusions can only be made comparing the values for each time period and comparing between groups. The HRV triangular index (HRV-TI) considers the major peak of the histogram as a triangle with its baseline width corresponding to the amount of RR interval variability, its height corresponds to the most frequently observed duration of RR intervals, and its area corresponds to the total number of all RR intervals used to construct it. The triangular HRV index is an estimate of the overall HRV. One study showed no difference in deep breathing variables (E/I) comparing SSc to controls (207).

Table 4.5: Descriptive statistics for deep breathing R-R interval variables. No normal values are available for comparison on SDRR and HRV-TI. Normal values for RMSSD vary between 0.0724 and 0.1288 depending on age group (208).

				Descriptive Statistics				
Parameter	**Treatment Group**	**Visit**	**Time**	**Mean**	**SD**	**Min**	**Median**	**Max**
SDRR (sec)	HCS	Day 0	pre dose	0.077	0.0541	0.03	0.052	0.18
		Day 0	post dose	0.068	0.0402	0.02	0.061	0.14
		Week 26	N/A	0.064	0.0509	0.02	0.059	0.18
	Placebo	Day 0	pre dose	0.062	0.0528	0.02	0.053	0.2
		Day 0	post dose	0.073	0.0453	0.02	0.065	0.17
		Week 26	N/A	0.088	0.0577	0.03	0.08	0.19
RMSSD (sec)	HCS	Day 0	pre dose	0.08	0.091	0.02	0.039	0.3
		Day 0	post dose	0.059	0.0461	0.01	0.043	0.13
		Week 26	N/A	0.044	0.0481	0	0.039	0.16
	Placebo	Day 0	pre dose	0.062	0.0742	0.02	0.041	0.27
		Day 0	post dose	0.07	0.0669	0.02	0.043	0.23
		Week 26	N/A	0.099	0.1069	0.02	0.055	0.31
HRV-TI	HCS	Day 0	pre dose	0.141	0.046	0.087	0.14	0.23
		Day 0	post dose	0.134	0.073	0.066	0.12	0.32
		Week 26	N/A	0.136	0.079	0.07	0.097	0.33
	Placebo	Day 0	pre dose	0.157	0.073	0.079	0.14	0.3
		Day 0	post dose	0.135	0.046	0.077	0.125	0.23
		Week 26	N/A	0.128	0.054	0.069	0.1	0.27

Table 4.6: Change from baseline for deep breathing R-R interval. No normal values are available for comparison.

				Change from Baseline				
Parameter	**Treatment Group**	**Visit**	**Time**	**Mean**	**SD**	**Min**	**Median**	**Max**
SDRR (sec)	HCS	Day 0	pre dose					
		Day 0	post dose	-0.009	-0.0139	-0.01	0.009	-0.04
		Week 26	N/A	-0.013	-0.0032	-0.01	0.007	0
	Placebo	Day 0	pre dose					
		Day 0	post dose	0.009	-0.0075	0	0.012	-0.03
		Week 26	N/A	0.026	0.0049	0.01	0.033	-0.01
RMSSD (sec)	HCS	Day 0	pre dose					
		Day 0	post dose	-0.021	-0.0449	-0.01	0.004	-0.16
		Week 26	N/A	-0.036	-0.0429	-0.02	0	-0.14
	Placebo	Day 0	pre dose					
		Day 0	post dose	0.008	-0.0073	0	0.002	-0.04
		Week 26	N/A	0.037	0.0327	0	0.014	0.04
HRV-TI	HCS	Day 0	pre dose					
		Day 0	post dose	-0.007	0.027	-0.021	-0.02	0.09
		Week 26	N/A	-0.009	0.033	-0.017	-0.043	0.1
	Placebo	Day 0	pre dose					
		Day 0	post dose	-0.022	-0.027	-0.002	-0.015	-0.07
		Week 26	N/A	-0.029	-0.019	-0.01	-0.04	-0.03

4.2.2.3 Valsalva manoeuvre

The Valsalva ratio (VR) is calculated as the longest R-R interval within the 30 seconds after the manoeuvre divided by the shortest R-R interval during or within the first 5 seconds after the manoeuvre (max/min). There was no significant difference between or within the groups for VR, **Tables 4.7** and **4.8**. Compared to normal comparator control values in two studies, VR was between the normal values in patients in both groups (208) (207).

Table 4.7: Descriptive statistics for Valsalva manoeuvre R-R interval. The mean normal value for VR reported in one study was 1.13-1.19 depending on age group and in another study was 1.6 with no difference between SSc patients and controls.

				Descriptive Statistics					
Parameter	**Treatment Group**	**Visit**	**Time**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**
Valsalva ratio	HCS	Day 0	pre dose	10	1.249	0.1658	1.02	1.228	1.49
		Day 0	post dose	10	1.187	0.1986	1.02	1.11	1.55
		Week 26	N/A	8	1.303	0.3281	1.01	1.22	1.97
	Placebo	Day 0	pre dose	10	1.305	0.2314	1.01	1.415	1.59
		Day 0	post dose	10	1.354	0.2853	1.01	1.388	1.8
		Week 26	N/A	10	1.435	0.3112	1.06	1.465	1.94

Table 4.8: Change from baseline for Valsalva manoeuvre R-R interval. The mean normal value for VR reported in one study was 1.13-1.19 depending on age group and in another study was 1.6 with no difference between SSc patients and controls.

					Change from Baseline				
Parameter	**Treatment Group**	**Visit**	**Time**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**
Valsalva ratio	HCS	Day 0	pre dose	10					
		Day 0	post dose	10	-0.063	0.1893	-0.43	-0.028	0.17
		Week 26	N/A	8	0.099	0.2126	-0.11	0.04	0.51
	Placebo	Day 0	pre dose	10					
		Day 0	post dose	10	0.049	0.0923	-0.1	0.045	0.21
		Week 26	N/A	10	0.13	0.2935	-0.18	0.05	0.89

5 Laboratory studies and candidate biomarker analysis

5.1 Routine laboratory studies

Biochemistry and haematology laboratory studies were largely within the normal range or just slightly abnormal but not clinically significant throughout the study. There were no significant changes between or within the groups for most values. One patient in the HCS group had significantly low calcium and magnesium levels at week 20 and 26, due to an increase in her proton pump inhibitor (PPI) for reflux. These went back to normal after stopping the PPI and recurred with restarting the PPI. One patient had high TSH and low T4 thyroid function tests at baseline due to undertreated concomitant hypothyroidism. These were normal at week 26. There were no other significant changes in thyroid function throughout the study in either group. Urinalysis and eGFR remained largely unchanged throughout the study.

5.2 Trial specified candidate biomarkers

5.2.1 PIIINP

PIIINP is a marker of fibrosis. At week 26, PIIINP was significantly increased from baseline in the HCS group by 8.080 (4.445, 11.715) µg/L [adjusted mean change (95%CI)], (p=0.0002), and relatively unchanged in the placebo group, 1.104 (-2.531, 4.739) µg/mL (p=0.5301). The difference between the groups at Week 26 was significant (p=0.0118) and the treatment groups were significantly different overall (p = 0.0118) **Table 5.1, Figure 5.1**.

When looking at post hoc combined baseline to week 26 (i.e., 7 placebo patients who started on HCS at week 26 and 3 placebo patients who did not take medication, using week 26 as baseline and week 52 as week 26), the results were even more significant with p=0.0085, **Figure 5.2**.

Table 5.1: Descriptive statistics for PIIINP levels (μg/L) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	6.851	3.7638	3.88	5.630	16.31	
	Week 26	Visit value	10	15.442	10.1068	5.89	13.530	34.44	
		Change from baseline	10	8.591	7.8340	0.75	5.955	23.31	
		Adjusted mean change (95% CI)		8.080	(4.445, 11.715)				0.0002
Placebo	Baseline	Visit value	10	5.311	2.4068	2.17	5.330	9.41	
	Week 26	Visit value	10	5.904	2.7205	2.50	5.485	10.72	
		Change from baseline	10	0.593	1.3078	-2.05	1.020	2.32	
		Adjusted mean change (95% CI)		1.104	(-2.531, 4.739)				0.5301
Difference between groups	Week 26	Adjusted mean change (95% CI)		6.975	(1.753, 12.198)				0.0118

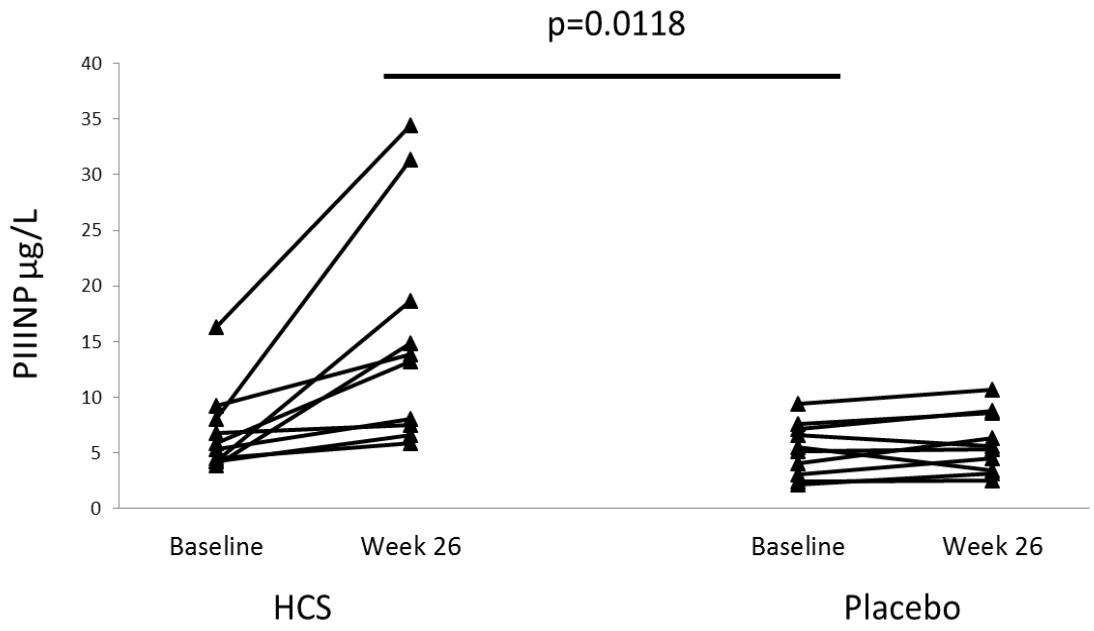


Figure 5.1: Graphic representation of PIIINP levels from baseline to week 26.

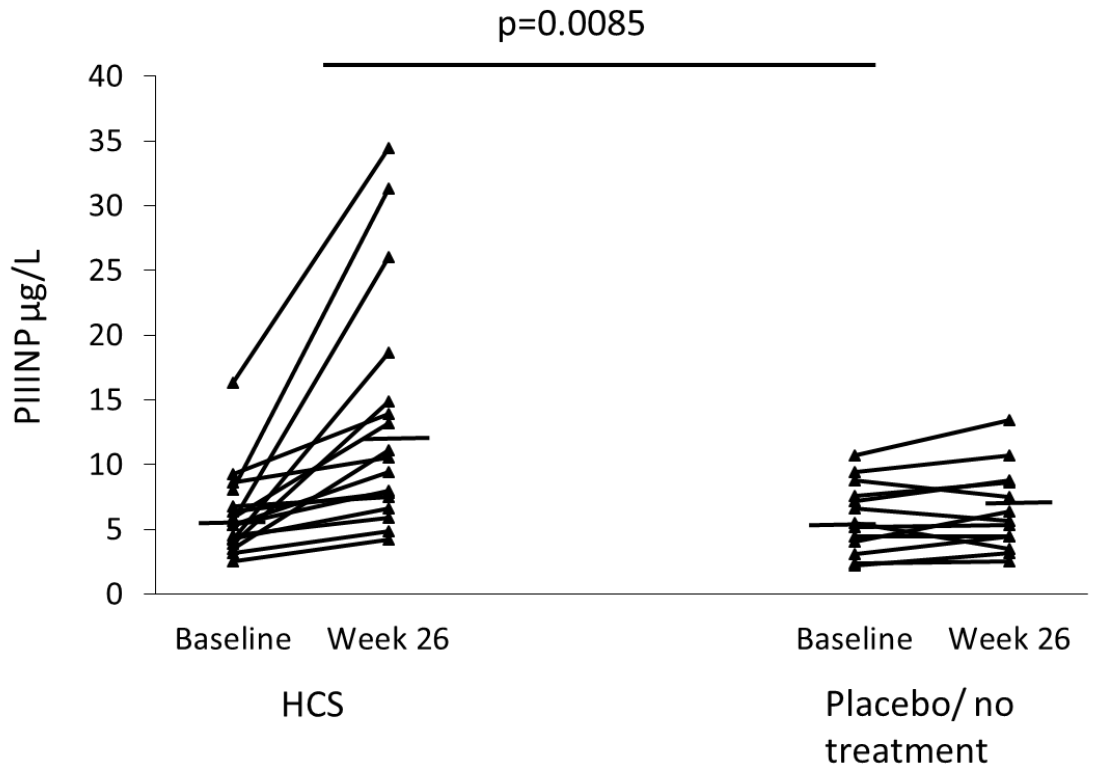


Figure 5.2: Graphic representation of PIIINP levels combined 26 week post hoc data, baseline to 26 weeks of treatment. Horizontal bars show mean values.

Interestingly, the HCS group who continued HCS had a significant reduction in PIIINP from 26 weeks to 52 weeks (p=0.0078) though not back to baseline levels; comparing PIIINP levels at 52 weeks to baseline, there was a strong trend to significant increase in PIIINP (p=0.0527). The HCS patients who choose not to continue on HCS compassionate use also had a significant reduction of PIIINP level from 26 weeks to 52 weeks back to baseline levels, but there were only 2 patients in that group. The placebo patients who chose not to go on HCS at 26 weeks had no significant change in PIIINP levels (3 patients). In the group that switched from placebo to HCS at 26 weeks, significant increases from baseline (and from 26 weeks) were seen in PIIINP at 52 weeks (p=0.0008 compared to baseline). These results are shown graphically in **Figure 5.3 (A-D)**.

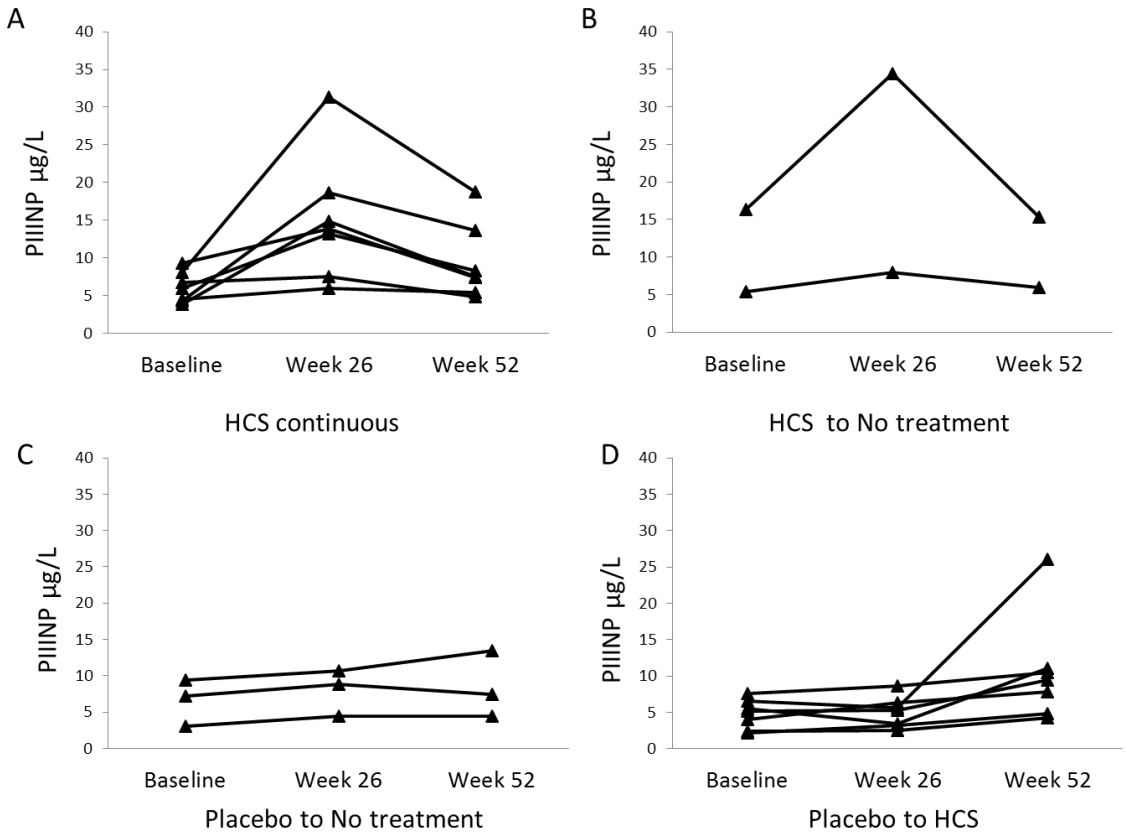


Figure 5.3: Graphic representation of PIIINP levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.2.2 sIL-2R

At week 26, there was no significant difference between the groups for soluble IL-2 receptor (sIL-2R), p=0.7862 **Table 5.2, Figure 5.4**. The combined 26 week data from the post hoc analysis was also not significant, p=0.2218, **Figure 5.5.**

Table 5.2: Descriptive statistics for sIL-2R (U/ml) from baseline to week 26 in HCS and placebo groups.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	1117.05	554.764	495.1	1077.95	2238.1	
	Week 26	Visit value	10	1213.91	946.773	203.1	1177.35	2936.3	
		Change from baseline	10	96.86	644.354	-564.9	-93.2	1738.1	
		Adjusted mean change (95% CI)		92.39	(-243.16, 427.95)				0.5689
Placebo	Baseline	Visit value	10	966.18	496.8	430.3	797.75	2024.5	
	Week 26	Visit value	10	1116.43	445.21	585.9	1011.3	2028.7	
		Change from baseline	10	150.25	243.24	-183.3	115.4	655.9	
		Adjusted mean change (95% CI)		154.72	(-180.84, 490.27)				0.3443
Difference between groups	Week 26	Adjusted mean change (95% CI)		-62.32	(-539.54, 414.89)				0.7862

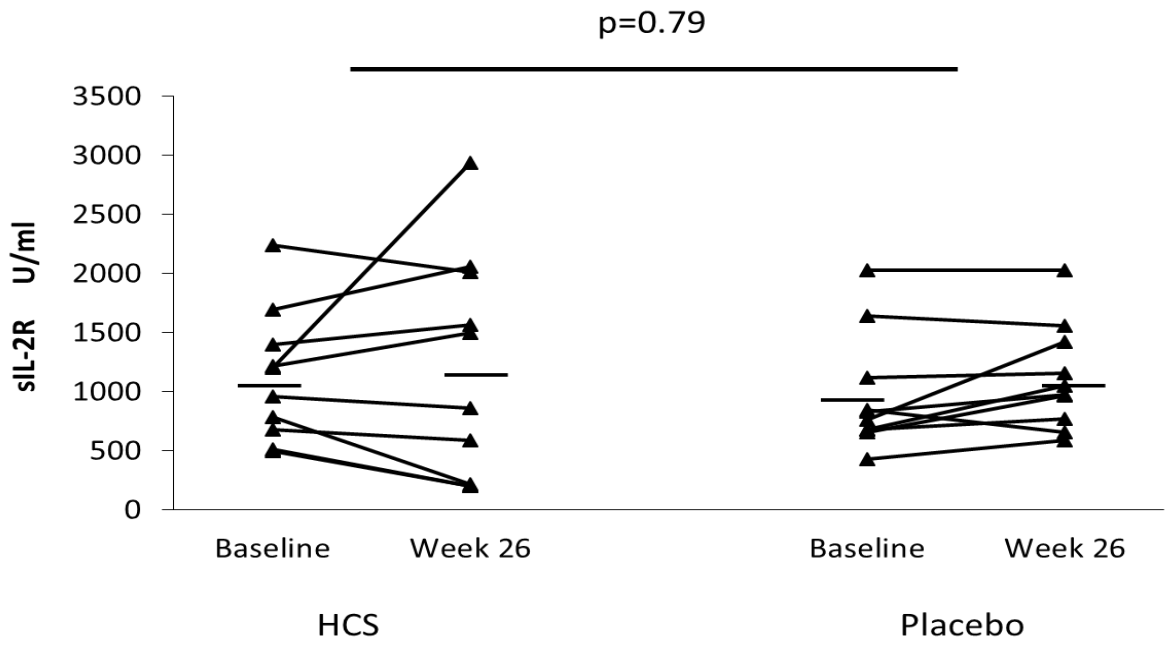


Figure 5.4: Graphic representation of sIL-2R levels from baseline to week 26. Horizontal bars show mean values.

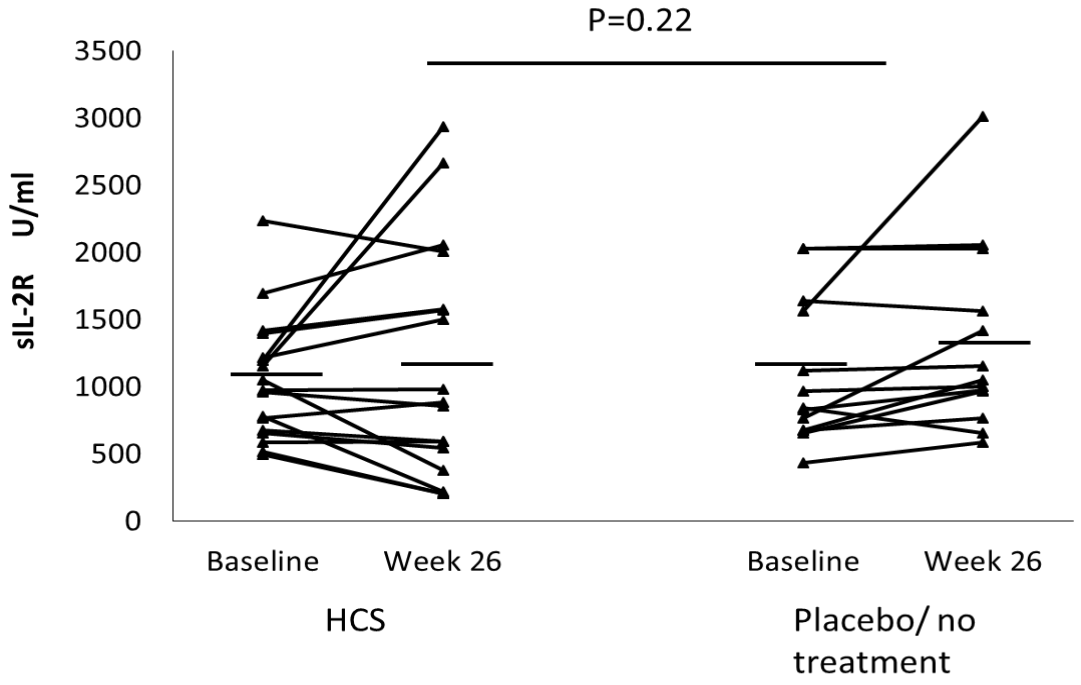


Figure 5.5: Graphic representation of combined 26 week post hoc data, sIL-2R levels from baseline to week 26. Horizontal bars show mean values.

At week 52, there was no significant change in the HCS group who continued a further 26 weeks of treatment or in the HCS group who discontinued treatment.

In the placebo group, there was a trend to significant increase in the patients who did not take HCS (p=0.053). There were only 3 patients in this group and 2 of these did not show a change; the third showed an increase in sIL-2R levels. sIL-2R is a marker for inflammation. The patient who had an increase in sIL-2R was extremely unwell throughout the study, particularly between week 26 and week 52, when he was an inpatient. He died soon after week 52 as a result of progression of his disease. There was a significant increase in sIL-2R in the placebo patients who opted for HCS compassionate treatment for 26 weeks (p=0.0311). This change was not seen in the double-blind HCS patients, mentioned above and the difference may be explained by fewer patients in the group (7 patients versus 10 patients in the original group) with a wider range of results. These results are shown graphically in **Figure 5.6.**

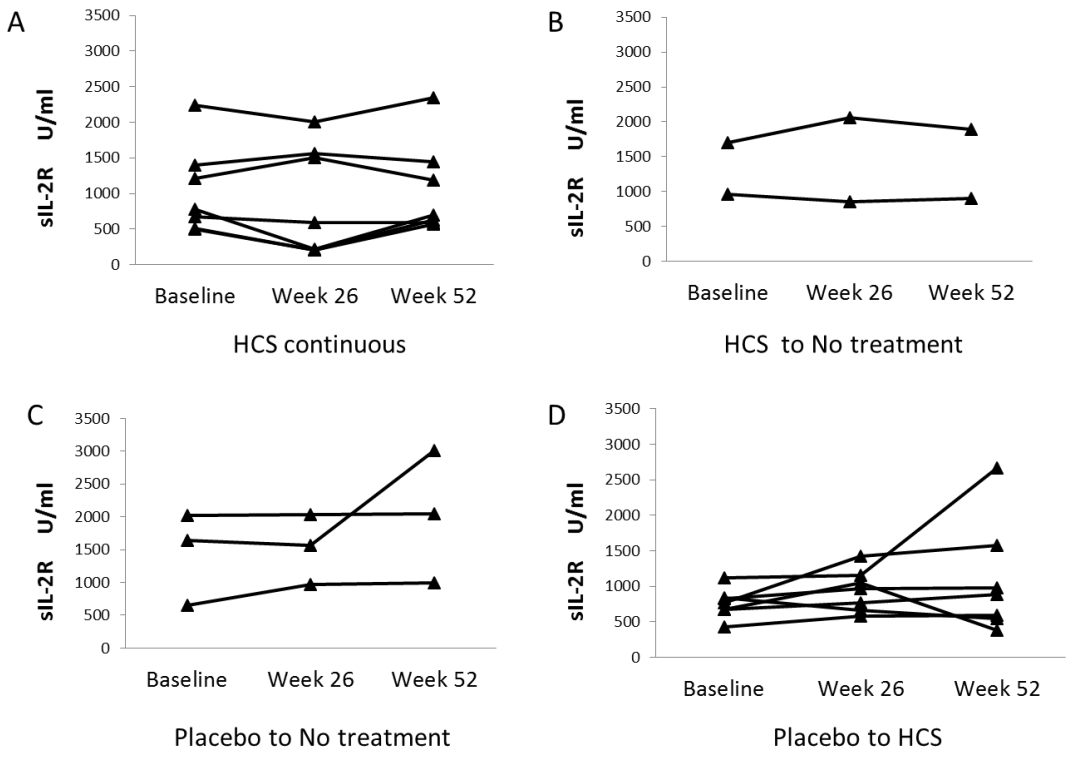


Figure 5.6: Graphic representation of sIL-2R levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.2.3 vWF

At week 26, there was no significant difference between the groups when comparing difference from baseline to week 26 for von Willebrand Factor (vWF), p=0.6875 **Figure 5.7, Table 5.3**.

Table 5.3: Descriptive statistics for vWF (U/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	27.08	24.2704	1.59	18.355	87.65	
	Week 26	Visit value	10	17.64	14.6770	2.65	14.135	52.86	
		Change from baseline	10	-9.43	13.0639	-34.79	-8.575	8.65	
		Adjusted mean change (95% CI)		-16.03	(-33.505, 1.440)				0.0697
Placebo	Baseline	Visit value	10	61.48	75.4690	4.93	13.995	203.89	
	Week 26	Visit value	10	43.76	58.3372	4.60	14.780	183.00	
		Change from baseline	10	-17.72	44.5572	-140.51	-2.410	10.01	
		Adjusted mean change (95% CI)		-11.12	(-28.595, 6.350)				0.1969
Difference between groups	Week 26	Adjusted mean change (95% CI)		-4.91	(-30.226, 20.407)				0.6875

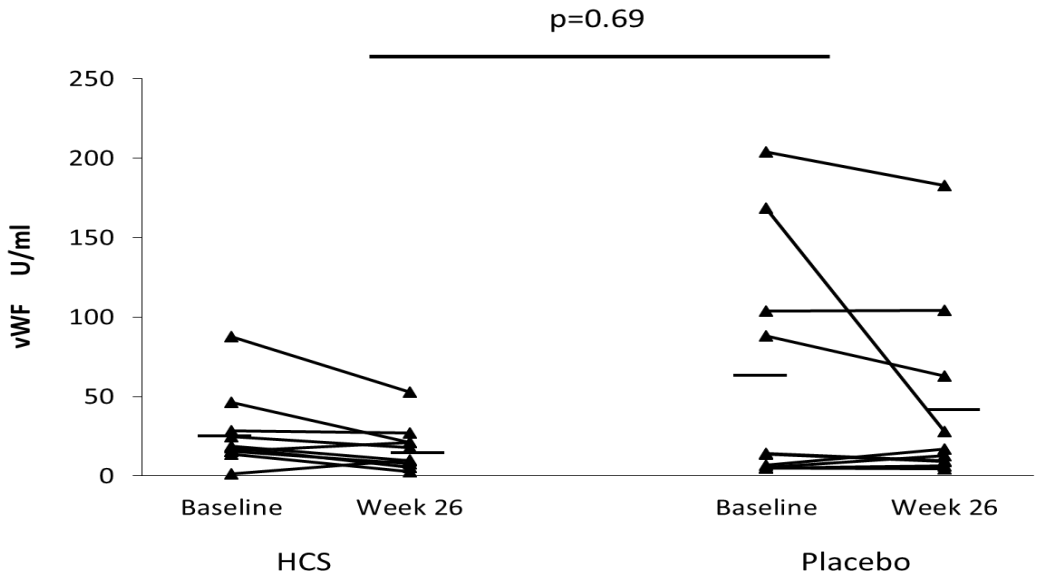


Figure 5.7: Graphic representation of vWF levels from baseline to week 26. Horizontal bars show mean values.

Post hoc combined 26 week data for vWF also revealed no significant difference, p=0.35, **Figure 5.8.**

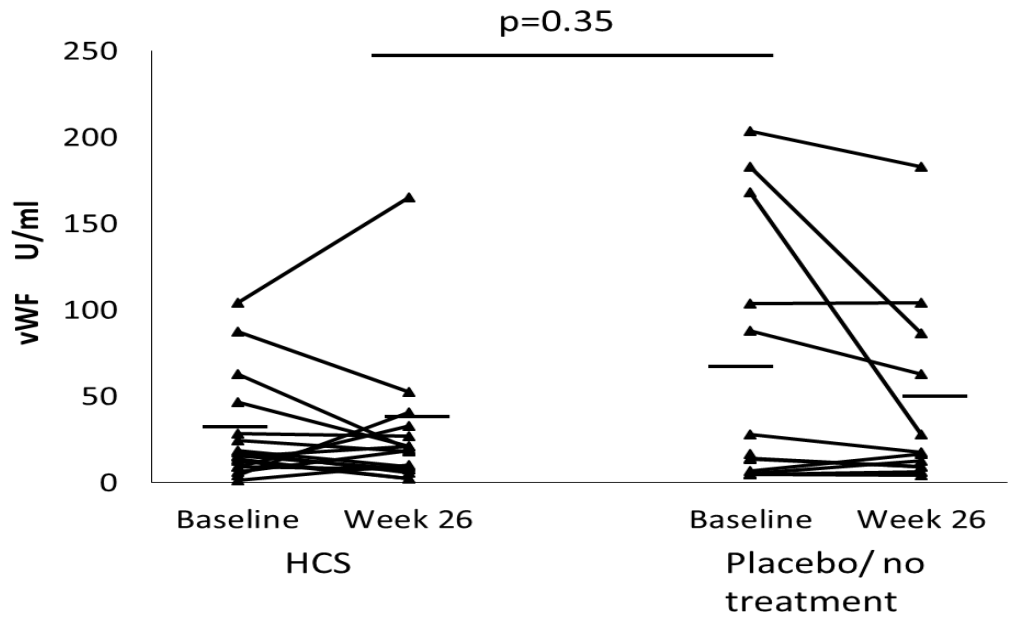


Figure 5.8: Graphic representation of combined 26 week post hoc data, vWF levels from baseline to week 26. Horizontal bars show mean values.

At week 52, there was no significant change in the HCS group who continued a further 26 weeks of treatment or in the HCS group who discontinued treatment. In the placebo group, there was no significant change in the patients who did not take HCS. There was a significant decrease in vWF in the placebo patients who did not take HCS, p=0.003; however, there were only 2 patients in this group. These 2 patients were unwell throughout the study and withdrew at week 14 due to disease progression and/or worsening lung fibrosis. Both went on immunosuppressive agents after week 14, and their disease stabilised. As vWF is a marker for vasculopathy, this could explain the decrease in vWF levels. These data are presented in graphical form in **Figure 5.9.**

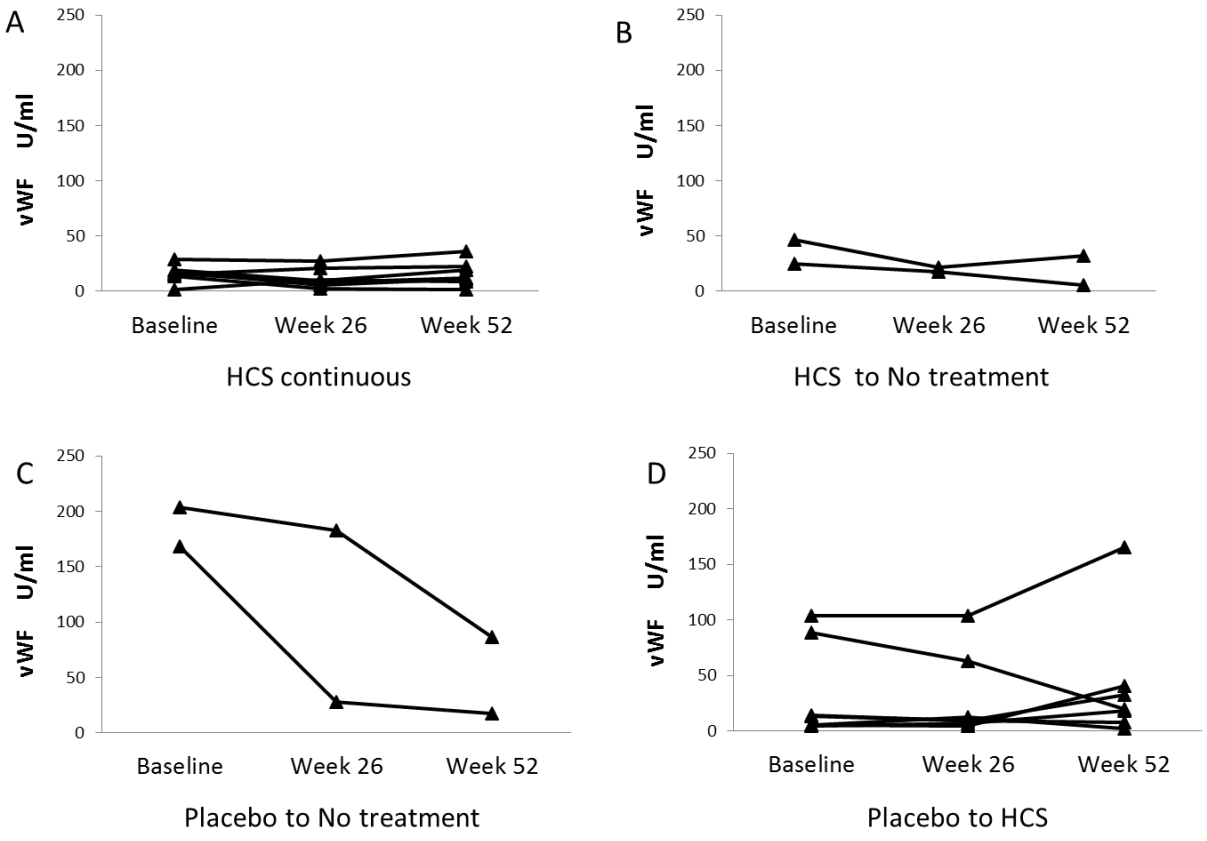


Figure 5.9: Graphic representation of vWF levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3 Multiplex cytokine analysis

The following sections describe individual results, followed by a cluster analysis of the data. The first 3 analytes (MSH, ACTH and FGF) and PIIINP were the top 4 upregulated hormones/growth factors in the HCS group according to cluster analysis, whereas the remainder were downregulated.

5.3.1 α-Melanocyte Stimulating Hormone (αMSH)

At week 26, αMSH was significantly increased from baseline in the HCS group compared to placebo, p=0.0395, **Figure 5.10, Table 5.4**. Post hoc combined 26 week data for MSH revealed a more significant difference, p=0.005, **Figure 5.11.**

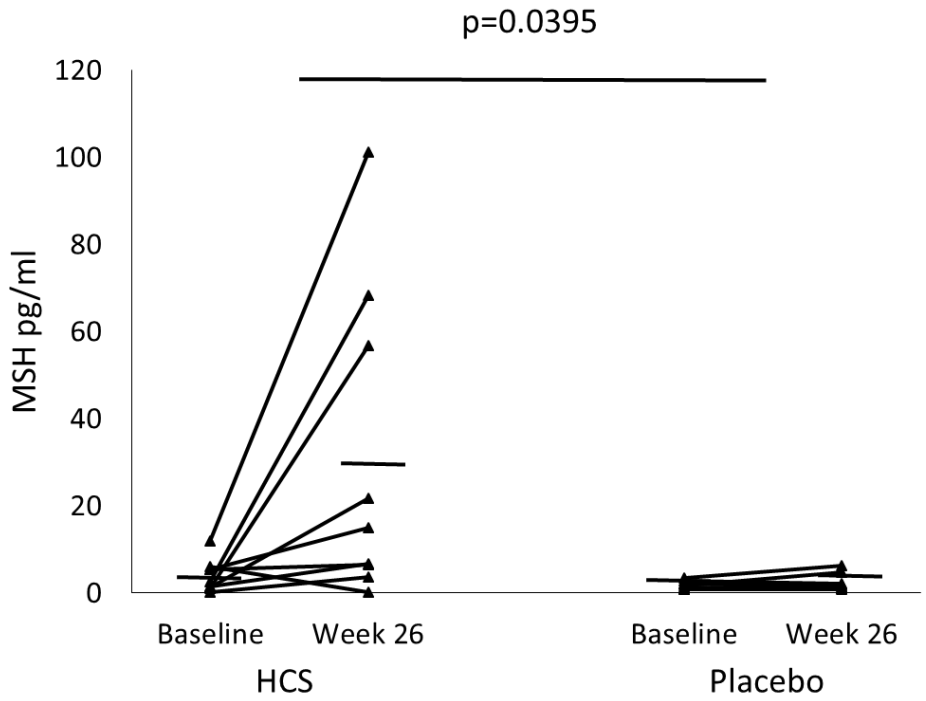


Figure 5.10: Graphic representation of αMSH levels from baseline to week 26. Horizontal bars show mean values.

Table 5.4: Descriptive statistics for αMSH (pg/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	3.71	3.573	0.2	2.03	11.9	
	Week 26	Visit value	9	31.13	35.782	0.2	15.06	101.3	
		Change from baseline	9	27.16	34.206	-6	9.58	89.5	
		Adjusted mean change (95% CI)		24.96	(9.42, 40.49)				0.0035
Placebo	Baseline	Visit value	10	1.78	0.896	0.9	1.55	3.6	
	Week 26	Visit value	10	2.18	1.869	0.8	1.27	6.4	
		Change from baseline	10	0.39	1.365	-0.8	-0.03	3	
		Adjusted mean change (95% CI)		2.27	(-12.50, 17.03)				0.7498
Difference between groups	Week 26	Adjusted mean change (95% CI)		22.69	(1.23, 44.14)				0.0395

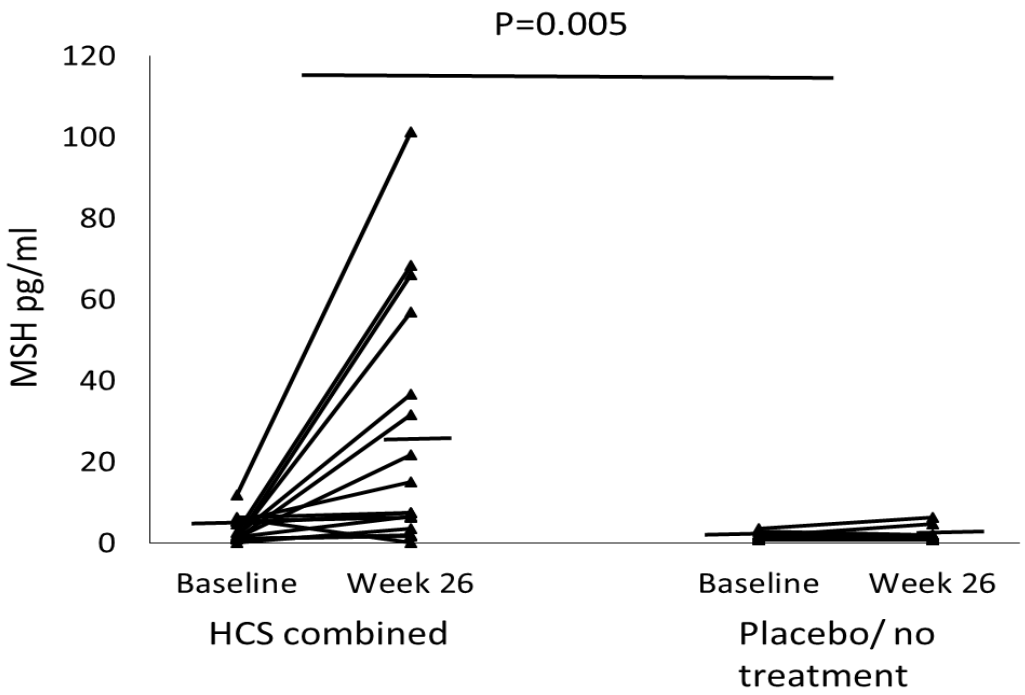


Figure 5.11: Graphic representation of combined 26 week post hoc data, αMSH levels from baseline to week 26. Horizontal bars show mean values.

At week 52, in the HCS group who continued treatment, there was a decrease in αMSH levels compared to week 26, but not back to baseline and the difference was not statistically significant compared to baseline or week 26. The HCS group who did not continue treatment had a significant drop in αMSH back to baseline levels (only 2 patients). There was no change in the placebo group who did not take HCS and in the placebo to HCS group a significant increase from baseline (and week 26) was seen in αMSH, p=0.0014, **Figure 5.12**.

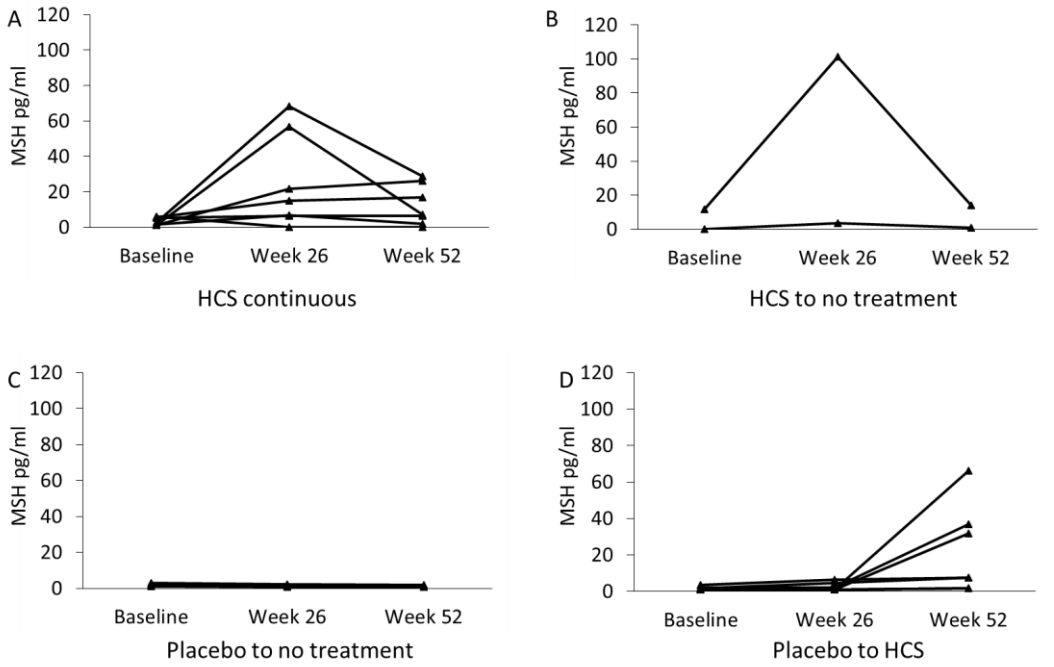


Figure 5.12: Graphic representation of αMSH levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.2 Adrenocorticotrophic Hormone (ACTH)

Mirroring MSH, at 26 weeks ACTH levels showed a strong trend to increase from baseline in the HCS group compared to placebo, p=0.0532, **Figure 5.13, Table 5.5**. Post hoc combined 26 week data for ACTH revealed a more significant difference, p=0.0208, **Figure 5.14.**

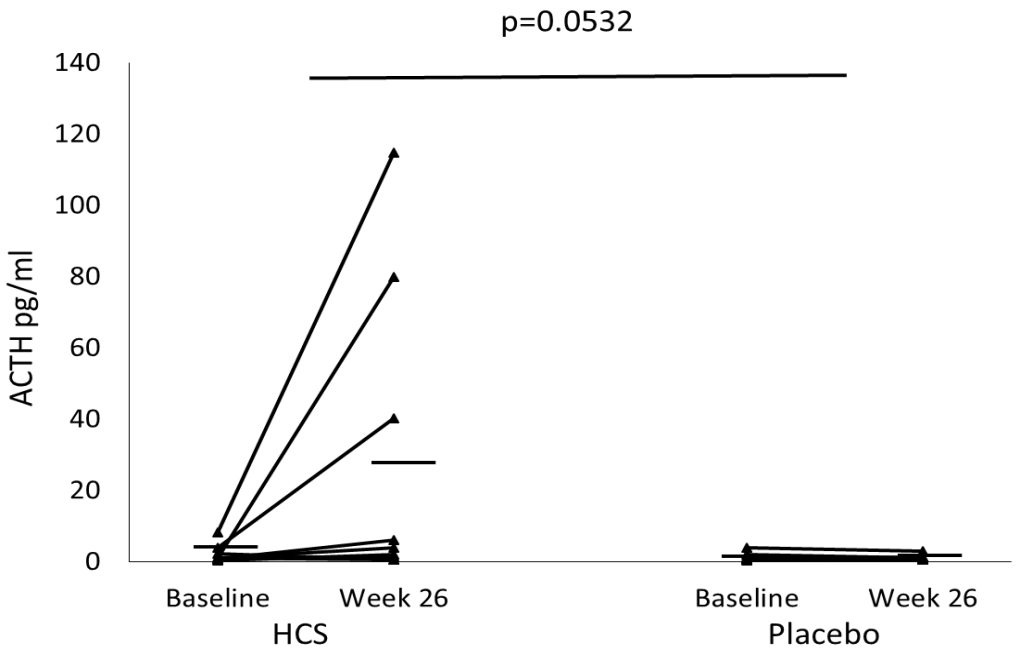


Figure 5.13: Graphic representation of ACTH levels from baseline to week 26. Horizontal bars show mean values.

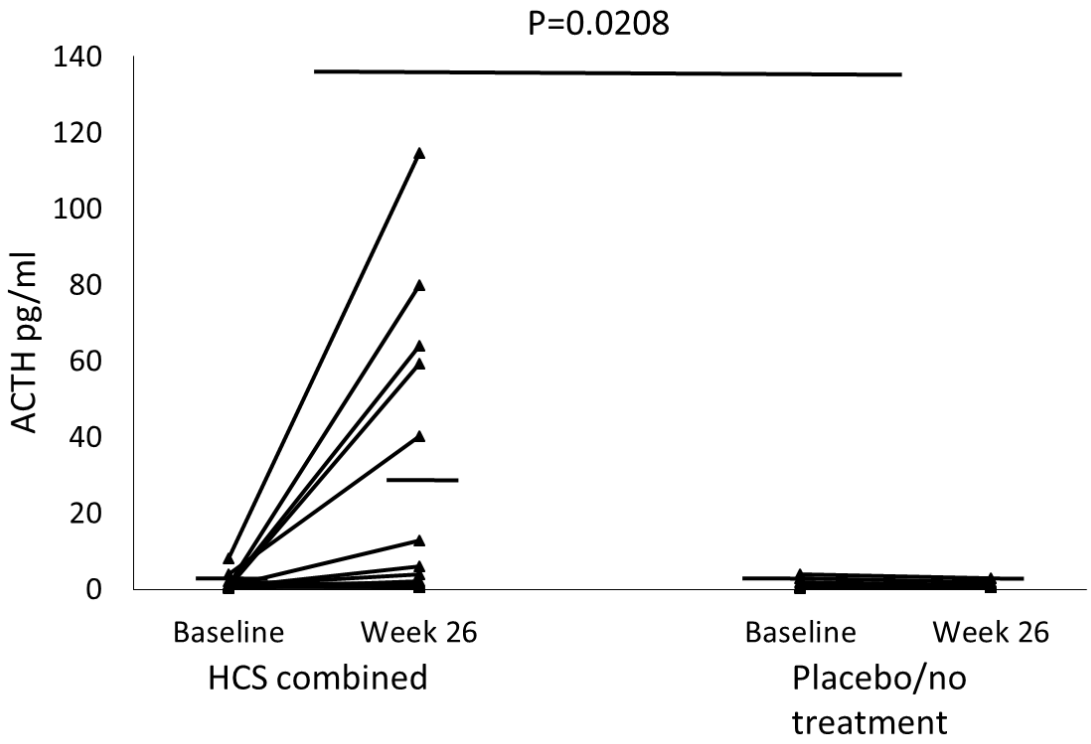


Figure 5.14: Graphic representation of combined 26 week post hoc data, ACTH levels from baseline to week 26. Horizontal bars show mean values.

Table 5.5: Descriptive statistics for ACTH (pg/ml) from baseline to week 26 in HCS and placebo groups

Treatment Group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	1.94	2.434	0.2	0.96	8.2	
	Week 26	Visit value	9	27.63	42.282	0.4	3.94	114.6	
		Change from baseline	9	25.54	40.374	-1.8	2.95	106.4	
		Adjusted mean change (95% CI)		25.41	(6.95, 43.87)				0.0099
Placebo	Baseline	Visit value	10	1.14	1.074	0.4	0.76	3.9	
	Week 26	Visit value	10	0.99	0.679	0.4	0.82	2.8	
		Change from baseline	10	-0.15	0.61	-1.2	-0.24	0.7	
		Adjusted mean change (95% CI)		0.28	(-17.33, 17.89)				0.9736
Difference between groups	Week 26	Adjusted mean change (95% CI)		25.13	(-0.39, 50.65)				0.0532

At week 52, again similar to the αMSH results, in the HCS group who continued treatment, there was a decrease in ACTH levels compared to week 26, but not

back to baseline and the difference was not statistically significant compared to baseline or week 26. The HCS group who did not continue treatment had a significant drop in ACTH back to baseline levels (only 2 patients). There was no change in the placebo group who did not take HCS and in the placebo to HCS group a significant increase from baseline (and week 26) was seen in ACTH, p=0.0022, **Figure 5.15**.

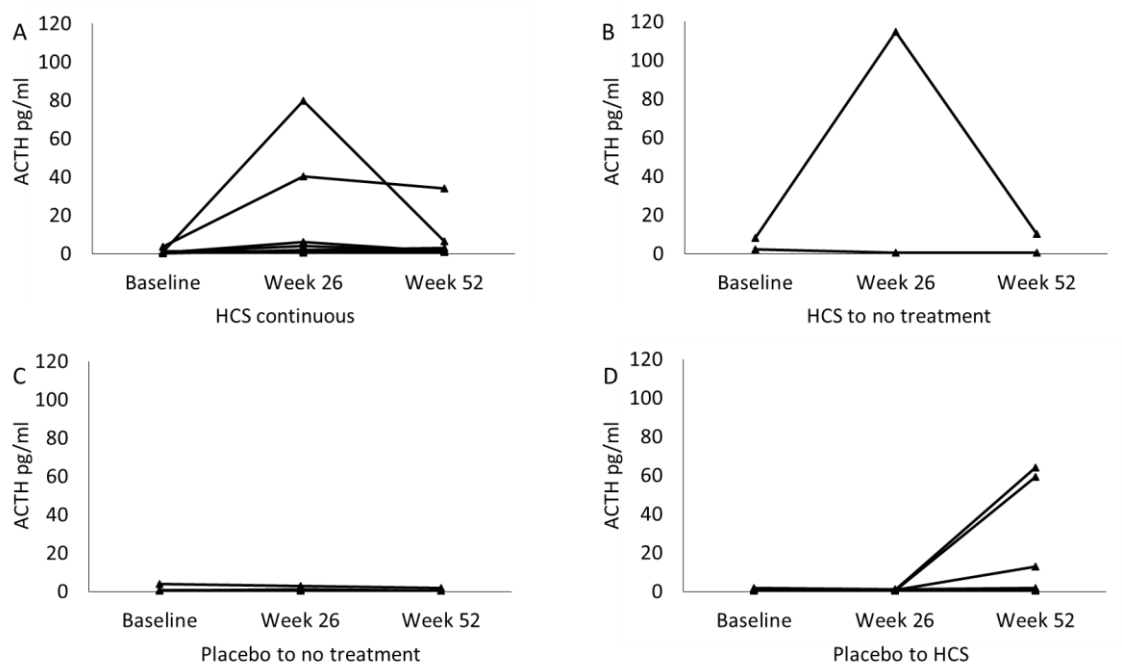


Figure 5.15: Graphic representation of ACTH levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.3 basic Fibroblast Growth Factor (bFGF)

At 26 weeks bFGF (or FGF-basic) levels showed an increase from baseline in the HCS group compared to placebo, but this was not statistically significant, p=0.148, **Figure 5.16, Table 5.6**. Post hoc combined 26 week data for FGF also showed an increase, but again it was not statistically significant, **Figure 5.17.**

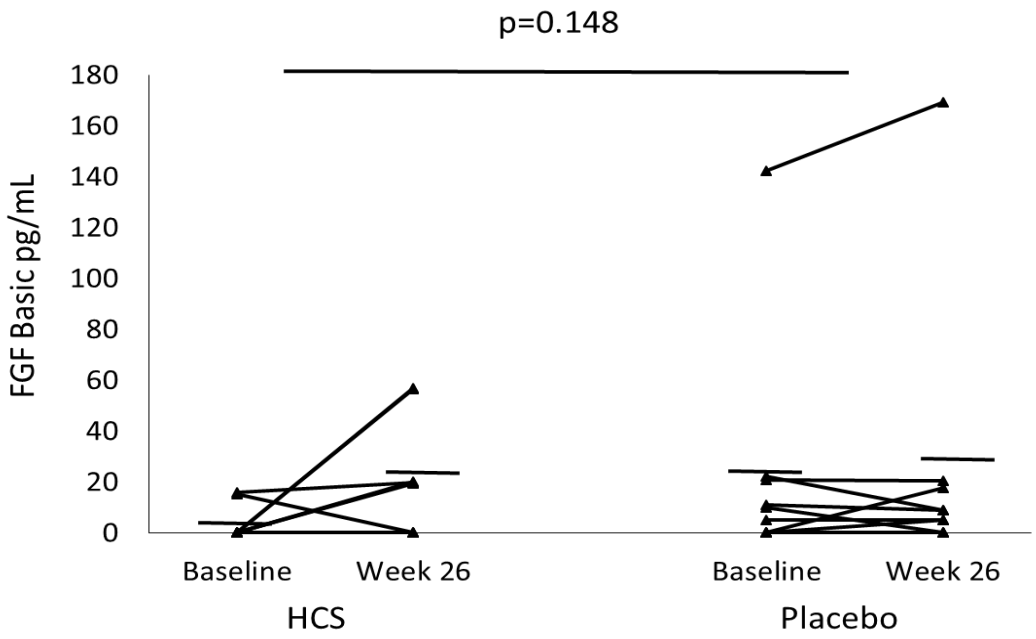


Figure 5.16: Graphic representation of bFGF levels from baseline to week 26. Horizontal bars show mean values.

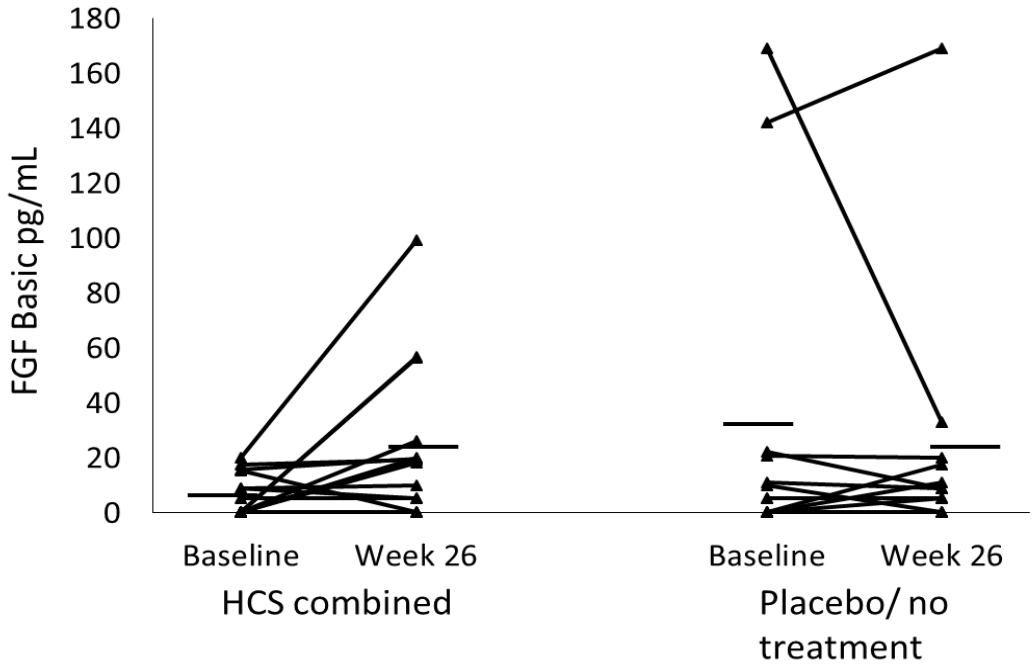


Figure 5.17: Graphic representation of combined 26 week post hoc data, bFGF levels from baseline to week 26. Horizontal bars show mean values.

Table 5.5: Descriptive statistics for bFGF (pg/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	3.37	6.483	0.3	0.3	16	
	Week 26	Visit value	9	21.52	21.921	0.3	19.76	57	
		Change from baseline	9	17.8	24.753	-15.1	19.28	56.7	
		Adjusted mean change (95% CI)		17.03	(3.24, 30.81)				0.0185
Placebo	Baseline	Visit value	10	21.29	43.34	0.3	7.59	142.3	
	Week 26	Visit value	10	23.64	51.652	0.3	7.04	169.3	
		Change from baseline	10	2.35	11.892	-13.5	0	27	
		Adjusted mean change (95% CI)		3.22	(-10.10, 16.54)				0.6165
Difference between groups	Week 26	Adjusted mean change (95% CI)		13.81	(-5.43, 33.05)				0.1484

At week 52, in the HCS group who continued treatment, there was a non-significant increase in bFGF levels compared to week 26. The HCS group who

did not continue treatment had a significant drop in bFGF back to baseline levels (only 2 patients). There was a significant decrease in the placebo group who did not take HCS (only 3 patients) and in the placebo to HCS group a non-significant increase from baseline (and week 26) was seen in bFGF, **Figure 5.18**.

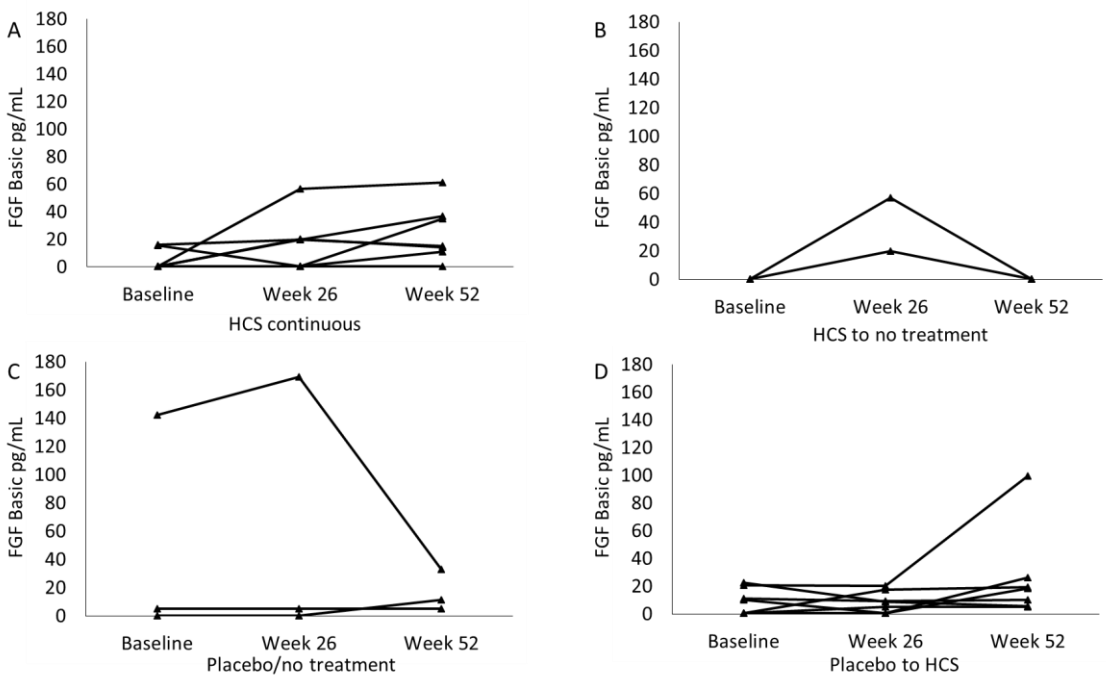


Figure 5.18: Graphic representation of bFGF levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.4 Transforming Growth Factor-β1 (TGF-β1)

At 26 weeks, both groups showed a decrease in TGF-β1 levels while the HCS group showed a slightly bigger decrease. This was not significant with p=0.6009, **Figure 5.19, Table 5.6**. The post hoc combined 26 week data showed an increase in the HCS group but no change in the placebo group and the difference again was not significant, **Figure 5.20**.

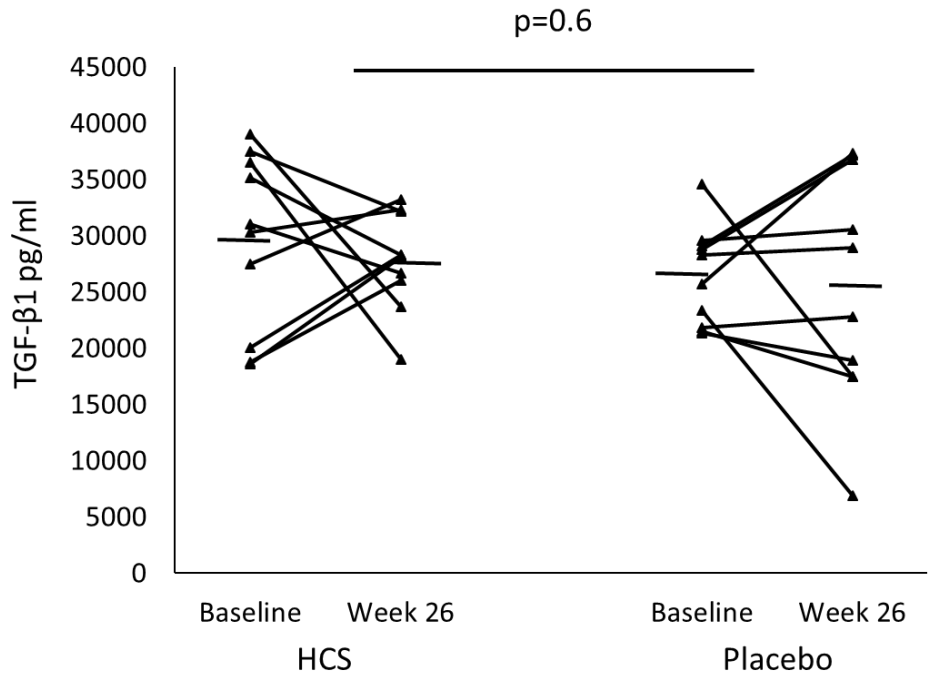


Figure 5.19: Graphic representation of TGF-β1 levels from baseline to week 26. Horizontal bars show mean values.

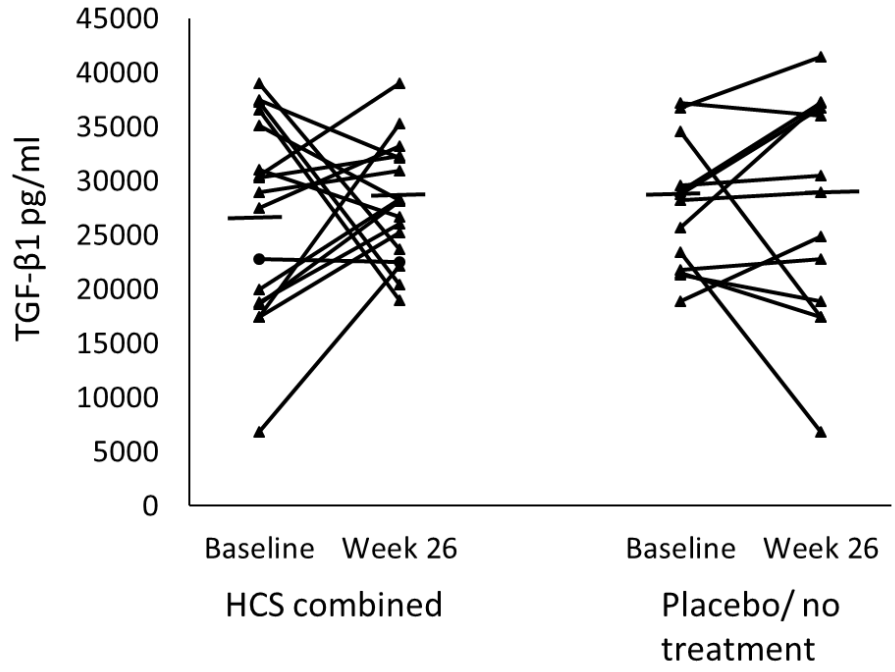


Figure 5.20: Graphic representation of combined 26 week post hoc data, TGF-β1 levels from baseline to week 26. Horizontal bars show mean values.

Table 5.6: Descriptive statistics for TGF-β1 (pg/ml) from baseline to week 26 in HCS and placebo groups.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	29450.8	7931.31	18623	30691.5	39011	
	Week 26	Visit value	10	27777.1	4309.86	19000	28182	33190	
		Change from baseline	10	-1673.7	9751.16	-17545	-1188	9479	
		Adjusted mean change (95% CI)		-329.3	(-5838.7, 5180.2)				0.9011
Placebo	Baseline	Visit value	10	26408	4385.31	21367	26988	34587	
	Week 26	Visit value	10	25424.8	10364.98	6877	25868	37341	
		Change from baseline	10	-983.2	9692.93	-17114	816	11626	
		Adjusted mean change (95% CI)		-2327.6	(-7837.1, 3181.8)				0.3852
Difference between groups	Week 26	Adjusted mean change (95% CI)		1998.4	(-5910.6, 9907.4)				0.6009

At 52 weeks, the group of placebo patients who were followed up only with no other treatment showed an increase in TGF-β1 levels but there were only 3 patients in this group. None of the other 3 groups showed statistically significant changes compared to baseline or week 26, **Figure 5.21.**

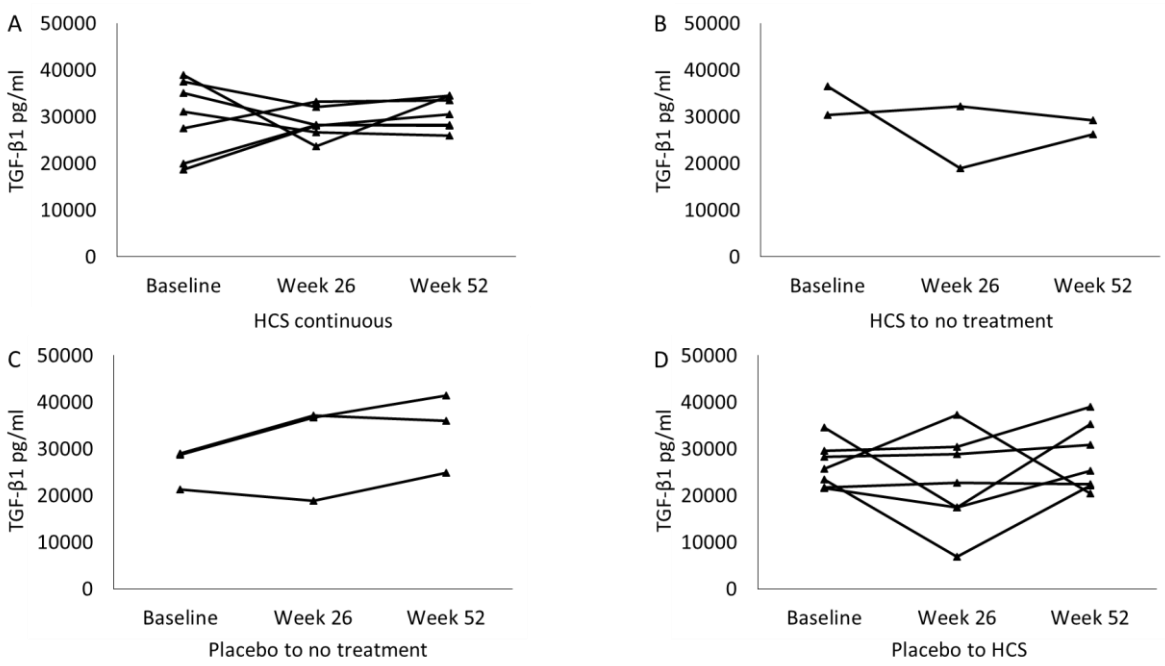


Figure 5.21: Graphic representation of TGF-β1 levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.5 Tissue Inhibitor of Metalloproteinase-2 (TIMP-2)

At 26 weeks there was a slight decrease in TIMP-2 levels compared to baseline in the HCS group and a slight increase in the placebo group. However, the difference was not statistically significant, p=0.1044, **Figure 5.22, Table 5.7.** The combined 26 week post hoc analysis showed a less significant difference, **Figure 5.23.**

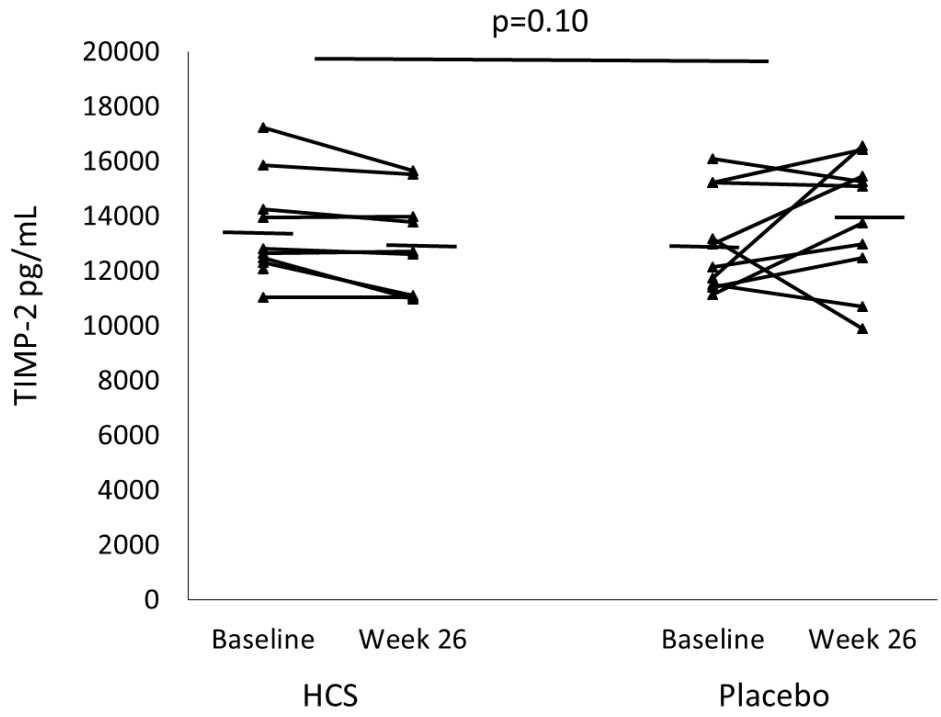


Figure 5.22: Graphic representation of TIMP-2 levels from baseline to week 26. Horizontal bars show mean values.

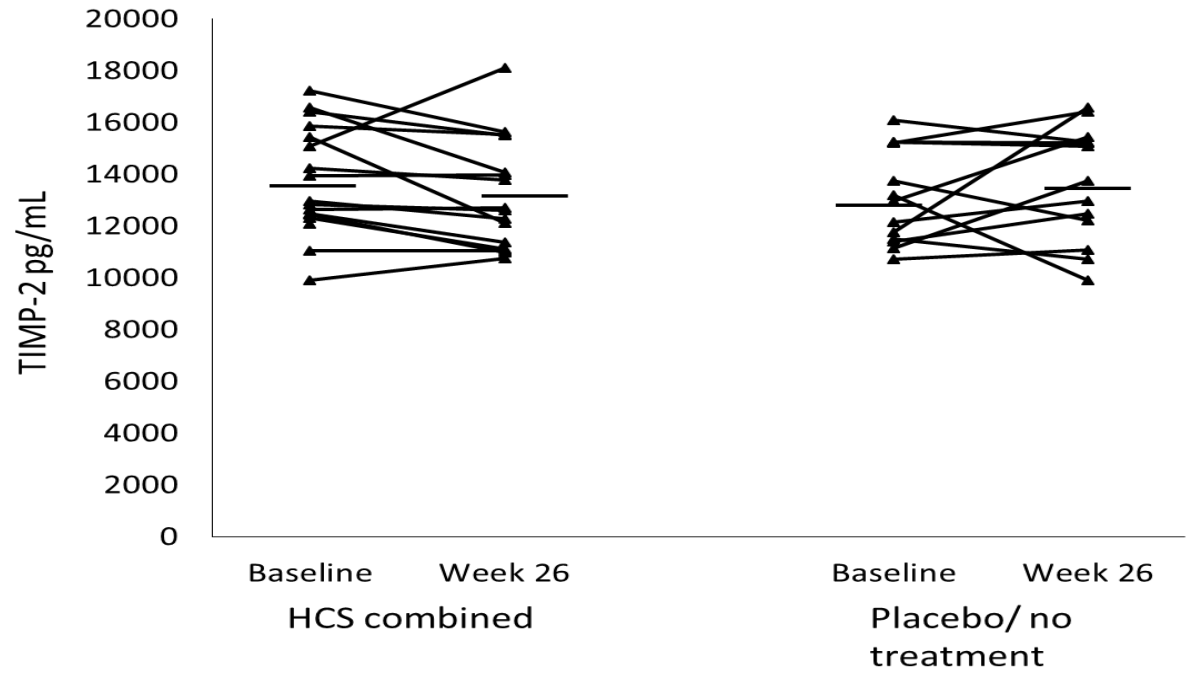


Figure 5.23: Graphic representation of combined 26 week post hoc data, TIMP-2 levels from baseline to week 26. Horizontal bars show mean values.

Table 5.7: Descriptive statistics for TIMP-2 (pg/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	13458.24	1880.143	11046.8	12733.52	17217.1	
	Week 26	Visit value	13038.63	1821.765	10978.2	12713.92	15636.3	
		Change from baseline	-573.59	663.711	-1580.8	-329.67	63.6	
		Adjusted mean change (95% CI)	-630.36	(-1934.2, 673.45)				0.322
Placebo	Baseline	Visit value	13058.01	1825.532	11124.7	12561.83	16082.6	
	Week 26	Visit value	13850.66	2311.337	9901.6	14400.88	16558.6	
		Change from baseline	792.65	2239.923	-3280.8	951.43	4810.8	
		Adjusted mean change (95% CI)	834.62	(-400.72, 2069.96)				0.1721
Difference between groups	Week 26	Adjusted mean change (95% CI)	-1464.98	(-3266.4, 336.40)				0.1044

At 52 weeks, there were no statistically significant changes in any of the 4 groups compared to baseline or week 26, **Figure 5.24.**

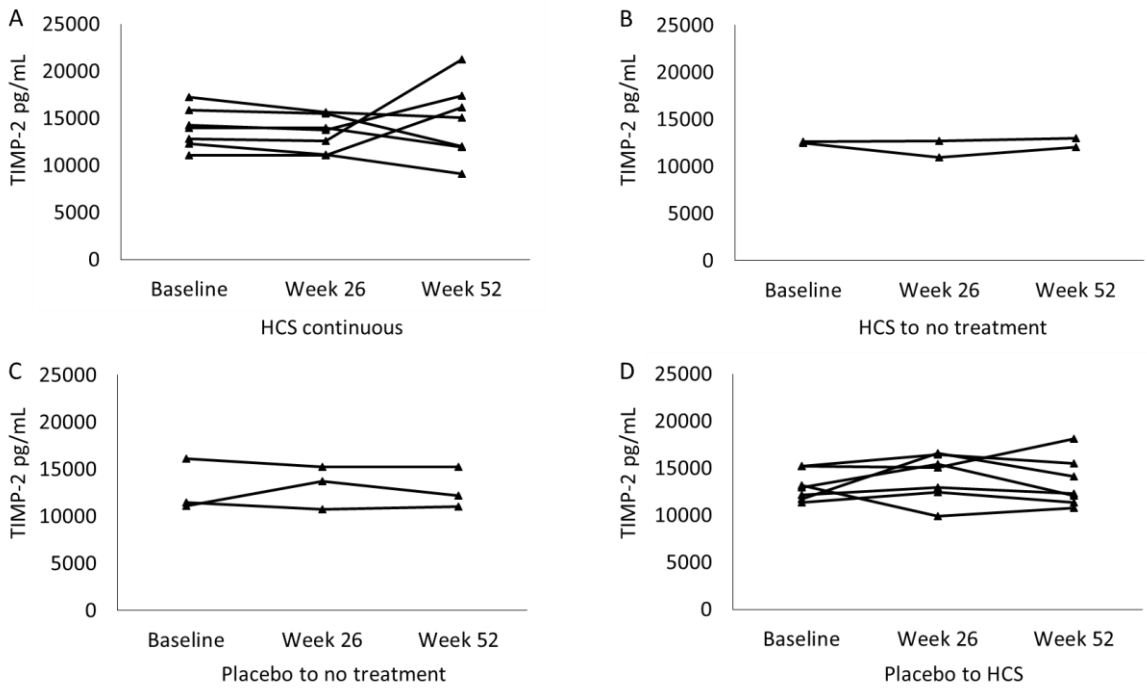


Figure 5.24: Graphic representation of TIMP-2 levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.6 Fractalkine (CX3CL1)

At 26 weeks, there was a decrease in Fractalkine levels in both groups. The HCS group had a bigger decrease, however the difference was not statistically significant with p=0.3179, **Figure 5.25, Table 5.8.** The combined 26 week post hoc analysis showed an even smaller difference, **Figure 5.26.**

At week 52, there were no significant changes in any of the 4 groups compared to baseline or week 26, **Figure 5.27.**

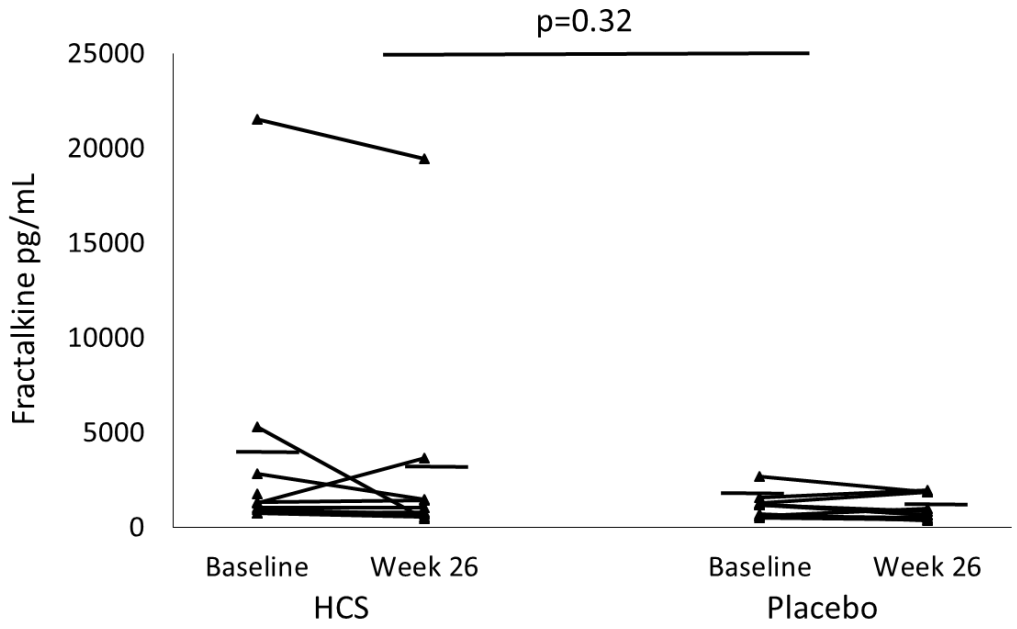


Figure 5.25: Graphic representation of Fractalkine levels from baseline to week 26. Horizontal bars show mean values.

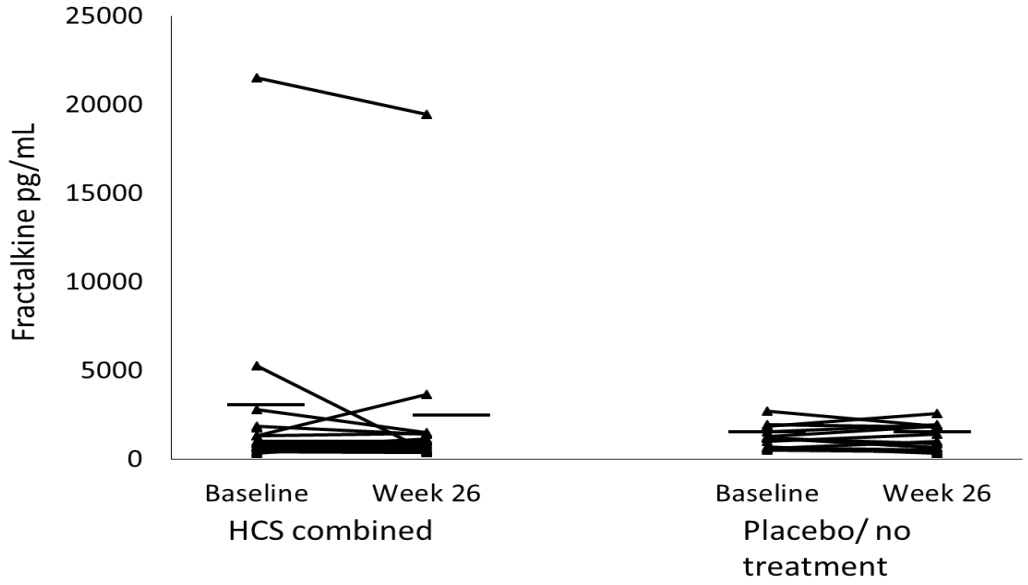


Figure 5.26: Graphic representation of combined 26 week post hoc data, Fractalkine levels from baseline to week 26. Horizontal bars show mean values.

Table 5.8: Descriptive statistics for Fractalkine (pg/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	3736.54	6396.612	734.7	1301.59	21512.1	
	Week 26	Visit value	9	3272.08	6131.862	439.9	1012.26	19418.2	
		Change from baseline	9	-685.9	1965.505	-4833.7	-170.83	2344	
		Adjusted mean change (95% CI)		-793.29	(-1778.1, 191.55)				0.1075
Placebo	Baseline	Visit value	10	1148.46	653.894	485.4	1184.58	2700.9	
	Week 26	Visit value	10	998.76	638.248	333.1	752.29	1952.1	
		Change from baseline	10	-149.69	478.468	-855.1	-232.89	573.3	
		Adjusted mean change (95% CI)		-123.44	(-1079.3, 832.37)				0.7885
Difference between groups	Week 26	Adjusted mean change (95% CI)		-669.86	(-2043.4, 703.73)				0.3179

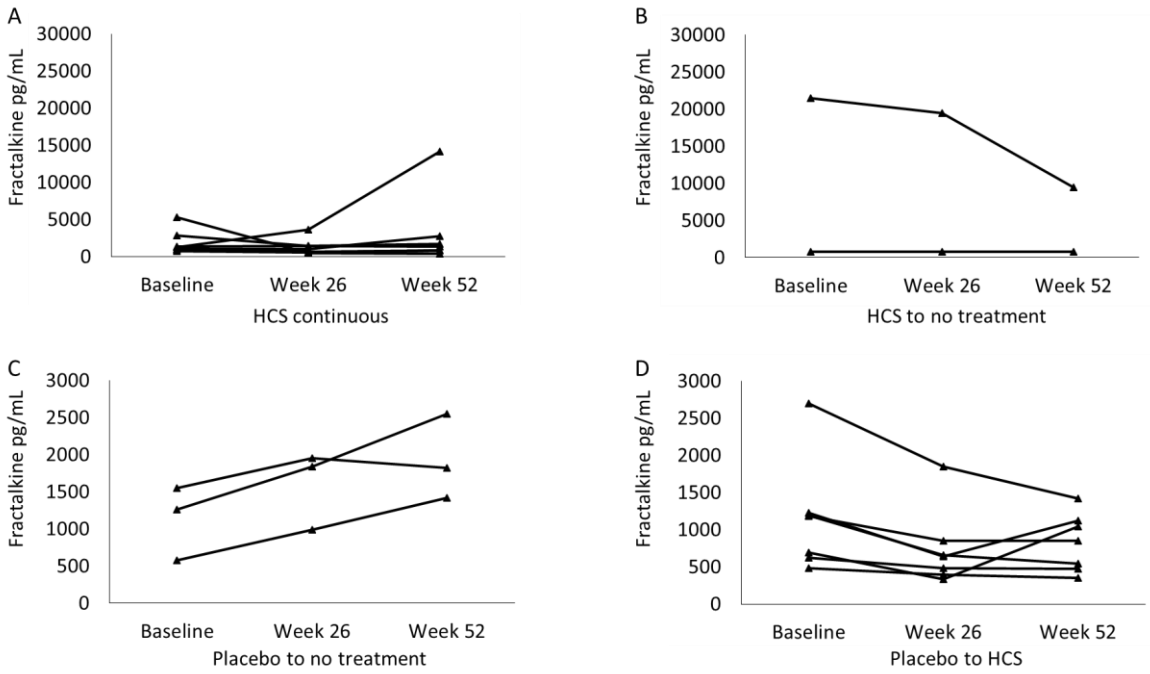


Figure 5.27: Graphic representation of Fractalkine levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.7 Cartilage Oligomeric Matrix Protein (COMP)

At 26 weeks, there was a decrease in COMP levels in HCS group and a slight increase in the placebo group, though the difference between groups was not statistically significant with p=0.2651, **Figure 5.28, Table 5.9.** The combined 26 weeks post hoc data showed a smaller difference between the groups, **Figure 5.29.**

At 52 weeks, there were no significant changes in any of the 4 groups compared to baseline or week 26, **Figure 5.30.**

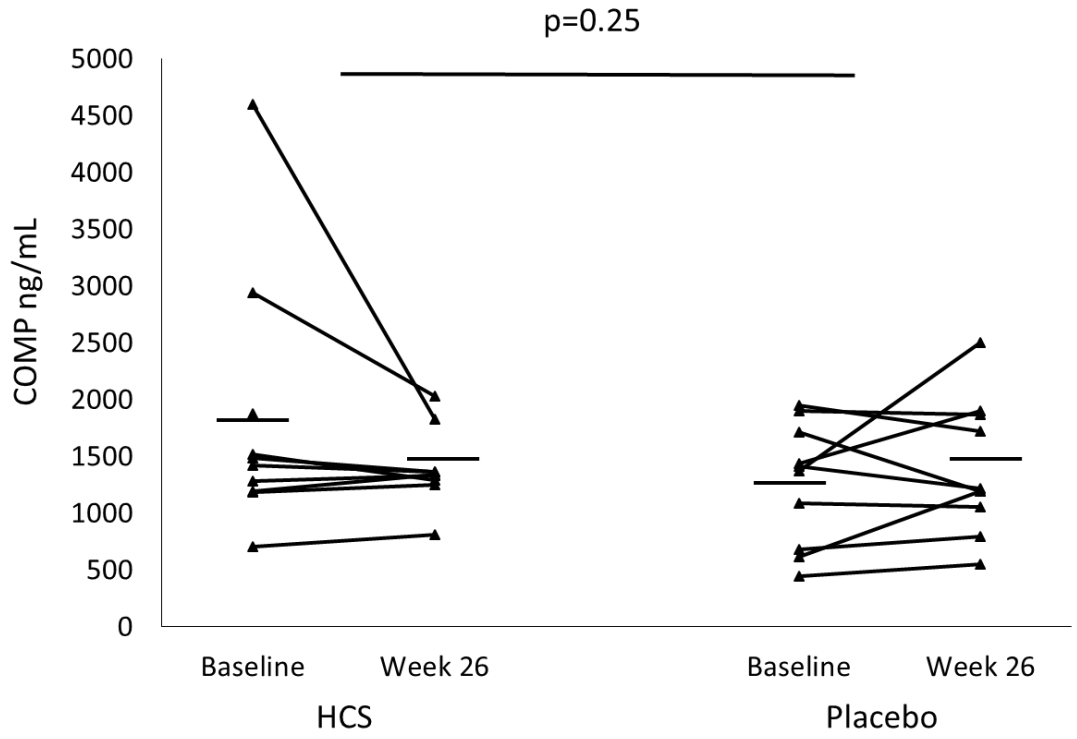


Figure 5.28: Graphic representation of COMP levels from baseline to week 26. Horizontal bars show mean values.

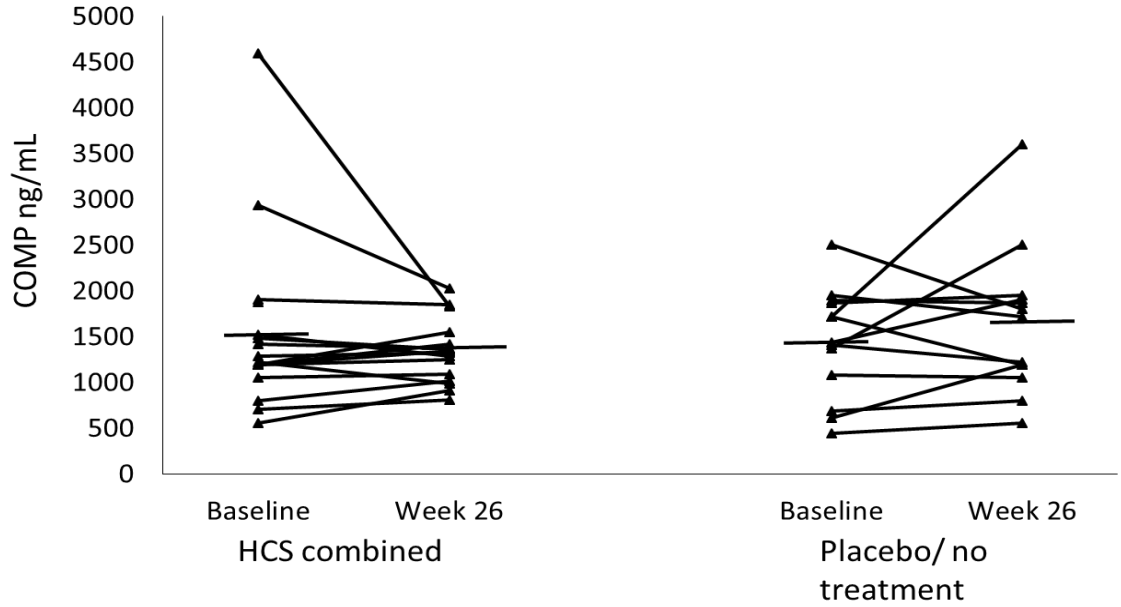


Figure 5.29: Graphic representation of combined 26 week post hoc data, COMP levels from baseline to week 26. Horizontal bars show mean values.

Table 5.9: Descriptive statistics for COMP (ng/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	1821	1138.11	707	1451	4598	
	Week 26	Visit value	10	1364.3	348	808	1333	2028	
		Change from baseline	10	-456.7	895.93	-2770	-84.5	144	
		Adjusted mean change (95% CI)		-267	(-540.8, 6.8)				0.0553
Placebo	Baseline	Visit value	10	1260.5	537.64	445	1389.5	1952	
	Week 26	Visit value	10	1400.1	588.1	553	1206.5	2501	
		Change from baseline	10	139.6	473.94	-521	40.5	1131	
		Adjusted mean change (95% CI)		-50.1	(-323.9, 223.7)				0.7043
Difference between groups	Week 26	Adjusted mean change (95% CI)		-216.9	(-614.1, 180.2)				0.2651

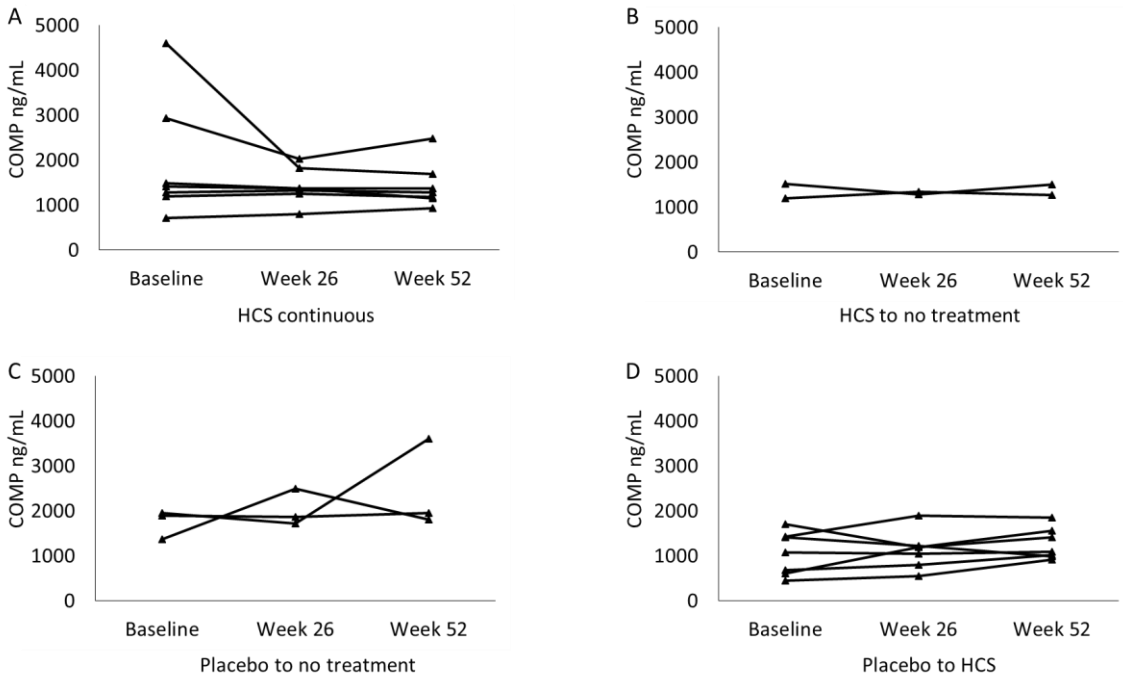


Figure 5.30: Graphic representation of COMP levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.8 Growth-Related Oncogene-α (GROα)

At 26 weeks, there was a decrease in GROα levels in HCS group and a slight increase in the placebo group, though the difference between groups was not statistically significant with p=0.2061, **Figure 5.31, Table 5.10.** The combined 26 weeks post hoc data showed a smaller difference between the groups, **Figure 5.32.**

At 52 weeks, there were no significant differences in any of the 4 groups compared to baseline. The HCS group who stopped treatment at 26 weeks (2 patients) had a significant increase GROα levels compared to week 26, **Figure 5.33.**

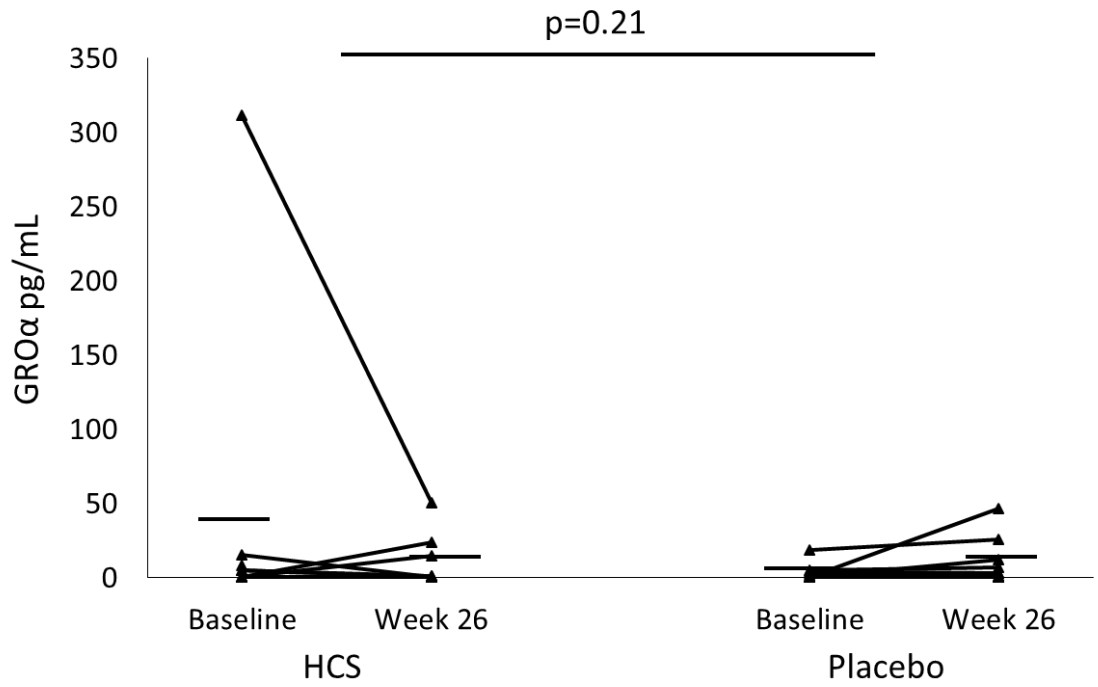


Figure 5.31: Graphic representation of GROα levels from baseline to week 26. Horizontal bars show mean values.

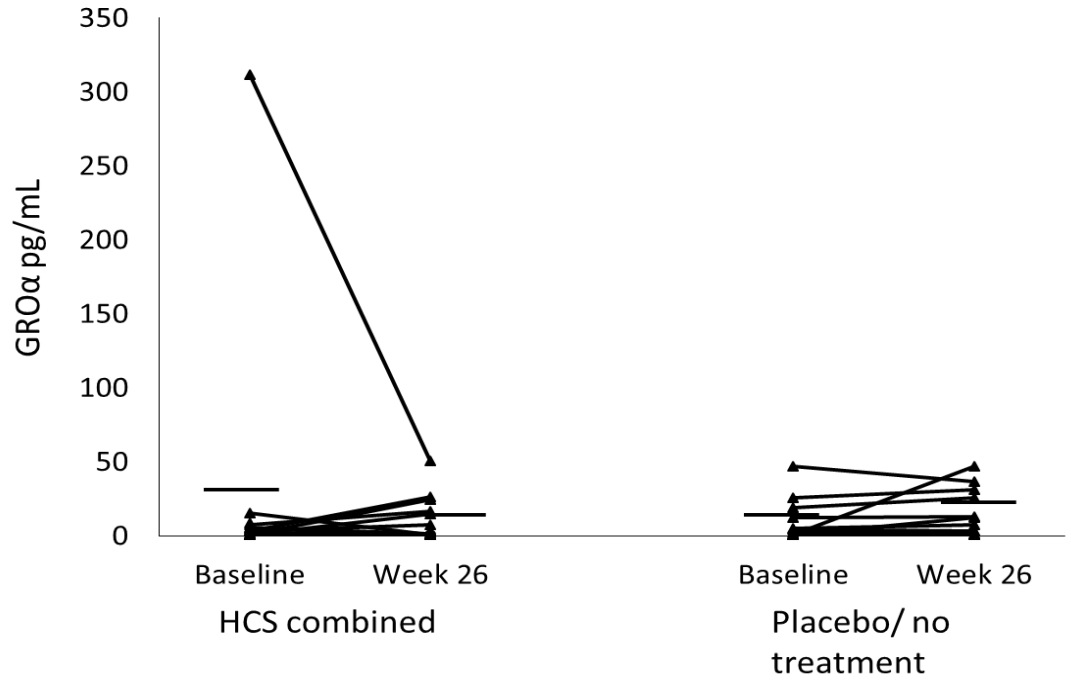


Figure 5.32: Graphic representation of combined 26 week post hoc data, GROα levels from baseline to week 26. Horizontal bars show mean values.

Table 5.10: Descriptive statistics for GROα (pg/ml) from baseline to week 26 in HCS and placebo groups.

Treatment group	Visit	Value	n	Mean	SD	Min	Median	Max	Prob
HCS	Baseline	Visit value	10	35.02	97.233	0.9	2.88	311.4	
	Week 26	Visit value	9	10.66	17.118	0.9	1.25	50.5	
		Change from baseline	9	-27.3	88.278	-260.9	0	23.3	
		Adjusted mean change (95% CI)		-19.27	(-40.82, 2.28)				0.0764
Placebo	Baseline	Visit value	10	3.62	5.549	0.9	0.9	18.7	
	Week 26	Visit value	10	10.19	15.088	0.9	2.96	47	
		Change from baseline	10	6.57	14.452	-1.4	0.92	46.1	
		Adjusted mean change (95% CI)		-0.76	(-21.20, 19.69)				0.9387
Difference between groups	Week 26	Adjusted mean change (95% CI)		-18.51	(-48.23, 11.20)				0.2061

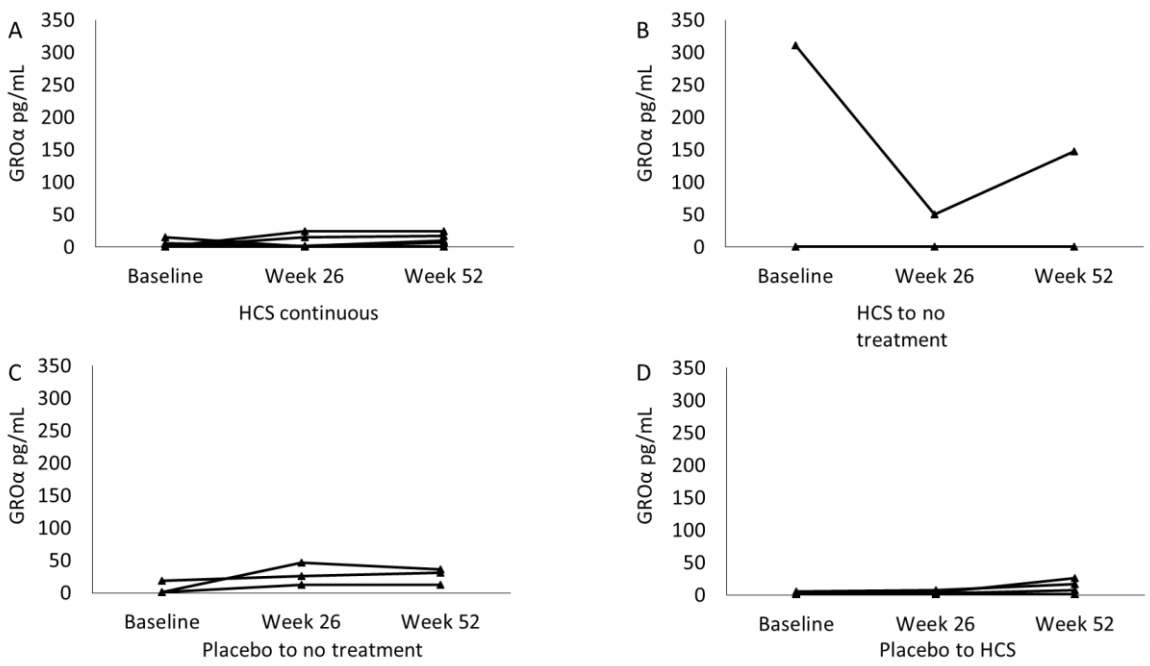


Figure 5.33: Graphic representation of GROα levels at baseline, week 26 and week 52; A) HCS continuing on compassionate HCS for a further 6 months, B) HCS treatment discontinued after 6 month double-blind period, C) Placebo patients who decided not to go on compassionate HCS, D) Placebo patients who started compassionate HCS for 6 months.

5.3.9 Cluster analysis

To strengthen and extend the analysis of individual serum analytes, cluster analysis was performed to better understand the changes that occurred in the patients during this study, focusing on the effects at 26 and 52 weeks compared with baseline. This approach is now well established for large datasets and was first developed and validated for analysis of microarray gene expression data. The advantage of this approach is that it takes account of the multiple factors that are analysed and permits analysis of normalized and scaled data so that different levels within the cohort and the range of change can be taken into account is defining patterns of change associated with treatment using HCS or placebo.

First, significance analysis of microarray testing (SAM®) was used to further interrogate the dataset and determine the significance of the findings observed in each analyte when examined separately as outlined earlier in this chapter.

Fold change >2 was taken as a cut-off point and the 4 most significant positive and 5 most significant negative serum factors were analysed separately. The results of this analysis are summarised in **Tables 5.11** and **5.12** included below.

Tale 5.11: SAM results for positive or upregulated serum factors; difference between baseline and 26 weeks for 10 HCS and 10 placebo patients. Top 4 values (in bold) were further defined individually.

Positive serum factors (20)					
Gene ID	**Score(d)**	**Numerator(r)**	**Denominator(s+s0)**	**Fold Change**	**q-value (%)**
PIIINP	2.640433	8.04325	3.046186311	**14.56365936**	0
FGF Basic	2.366466	19.35974445	8.180866024	**65.03317217**	0
MSH	1.950008	23.09005523	11.84100502	**298.5833202**	0
ACTH	1.755792	24.67471113	14.05332342	**-176.6136588**	20
PARC	1.623022	104588.3975	64440.52994	-3.103386906	43.07692308
VEGF	1.411976	72.34451238	51.23634762	4.767420819	46.89655172
I309	1.261012	8.5671673	6.793884919	5.508246003	46.89655172
IL12p70	1.239963	5.0243342	4.05200181	2.642028734	46.89655172
MMP 9	0.732223	34106.7405	46579.69077	-5.850080076	46.89655172
TIMP1	0.658503	1216.991525	1848.118263	-0.9899809	46.89655172
vWF	0.630152	10.4645	16.60630987	0.409519242	46.89655172
PDGFBB	0.530864	372.60309	701.8806505	-0.456093176	46.89655172
POMC	0.418754	0.424928775	1.014745138	-1.096768388	46.89655172
TNFβ	0.415387	0.317667	0.764749815	0	46.89655172
IL13	0.347142	1.30916645	3.771269521	-0.442608135	46.89655172
IL17	0.253017	0.132916625	0.525326243	Inf	46.89655172
CRH-CRF	0.23087	3.942164275	17.07523796	1.663474069	46.89655172
Cortisol	0.182868	1.07700025	5.88950338	0.1083206	46.89655172
RANTES	0.140456	1797.2911	12796.09594	1.205590518	46.89655172
IL2	0	0	0.40737444	NaN	46.89655172

Table 5.12: SAM results for negative or downregulated serum factors; difference between baseline and 26 weeks for 10 HCS and 10 placebo patients. Top 5 values (in bold) were further defined individually.

Negative serum factors (25)					
Gene ID	**Score(d)**	**Numerator(r)**	**Denominator(s+s0)**	**Fold Change**	**q-value (%)**
MCP1	-1.9592	-150.4625425	76.79810154	-0.516892076	36
COMP	-1.7311	-611.35	353.1576161	**-3.379297994**	36
Eotaxin	-1.47442	-45.661062	30.96874403	-0.893550124	36
sIL-2R	-1.46475	-175.825	120.0378606	-0.170216306	36
TARC	-1.39961	-122.7552213	87.70684383	-1.250242361	36
IL6	-1.29356	-14.15866368	10.94553866	-0.039524253	36
GROα	-1.26918	-19.73091858	15.54617371	**-2.299208472**	36
TNFα	-1.25196	-15.3312874	12.24578508	0.014255288	36
IL8	-1.23829	-17.85924148	14.42254707	-0.112771771	36
MMP 1	-1.16512	-2794.828925	2398.749522	-0.215739357	36
MCP2	-1.15495	-10.05505555	8.706025497	-0.228473599	36
IL15	-1.10331	-10.99097028	9.96183907	0.088592258	36
IL23	-1.02728	-279.0776533	271.6674578	-0.553160336	36
IL1α	-0.98516	-8.0595833	8.180948623	-0.035825212	36
IL4	-0.93619	-1.1429165	1.220819809	-0.636635387	36
HGF	-0.87733	-175.42056	199.9483156	-0.360281534	36
FRACT	-0.71827	-427.7533475	595.5317395	**6.103113065**	43.07692308
IL1β	-0.70907	-13.71445425	19.34133528	0.154455043	43.07692308
ANG2	-0.61767	-29.71100025	48.10206386	0.250561152	43.07692308
TIMP2	-0.58747	-817.65725	1391.817976	**12.68181916**	43.07692308
TGF-β1	-0.58689	-2726.425	4645.526243	**3.773011595**	43.07692308
IP10	-0.308	-6.67869775	21.684043	-0.124359133	56.77419355
IFNγ	-0.2878	-1.95020915	6.776181521	0.656905046	56.77419355
IL10	-0.26974	-0.9372499	3.474623409	0.718346281	56.77419355
IL5	-0.04987	-0.051832775	1.039392444	0.926058746	56.77419355

When SAM was used to interrogate the data for the combined dataset, different serum factors appeared to be up- or down-regulated compared to the original dataset, summarised in **Tables 5.13** and **5.14**.

Table 5.13: SAM results for positive or upregulated serum factors; difference between baseline and 26 weeks for 17 HCS and 13 placebo patients, post hoc combined analysis. The top 4 values (in bold) had a fold change > 2.

Positive serum factors (26)					
Gene ID	**Score(d)**	**Numerator(r)**	**Denominator(s+s0)**	**Fold Change**	**q-value (%)**
MCP1	1.832005807	98.93034282	54.00110768	-2.414793365	34.2
IL6	1.594572399	16.08315559	10.08618712	-1.777216259	34.2
IL15	1.540105361	13.64949101	8.862699501	-2.507476277	34.2
IL1α	1.242893284	8.412820513	6.768739219	-2.091054689	34. 2
IL1β	1.239217255	27.16116587	21.91800168	-0.921342864	34.2
TIMP2	1.220226809	1149.487974	942.0281258	-0.126599233	34.2
IL23	1.207957322	243.5651758	201.6339248	-1.305592763	34.2
TNFα	1.207041383	13.9251183	11.53657074	-5.085633024	34.2
IL8	1.172059278	12.57138449	10.72589478	**-27.31706687**	34.2
RANTES	1.094252363	14154.29901	12935.13222	5.138355352	34.2
COMP	0.915849974	262.9435897	287.1033434	-1.040431374	34.2
Eotaxin	0.853774696	22.98585421	26.92262293	**-7.03641438**	34.2
IL4	0.814276379	1.787897221	2.195688426	-0.565584165	34.2
GROα	0.787870405	8.892838528	11.28718438	-0.876170181	34.2
TGF-β1	0.771378672	2938.548718	3809.476234	-1.430695421	34.2
IL13	0.76105448	5.308623713	6.975353079	-0.098386497	34.2
TARC	0.75677734	49.04613026	64.8091951	-2.228194437	34.2
MMP-1	0.695902737	1220.623755	1754.014878	**7.543422795**	34.2
IL10	0.663175198	2.664394641	4.01763312	3.794170837	34.2
HGF	0.606078117	84.17718051	138.8883349	**5.93737534**	34.2
CRH/CRF	0.490188983	11.42492441	23.30718317	2.070046832	36.7677
MCP2	0.470832152	3.737037508	7.937090725	4.791922553	36.7677
IL12p70	0.424284411	3.097389831	7.300267817	3.14269436	36.7677
IFNγ	0.065101446	0.403957369	6.205044502	1.077264764	36.7677
IP10	0.055224539	0.966713077	17.50513626	1.575263514	36.7677
FRACT	0.036052922	14.36885487	398.548972	0.885803128	36.7677

Table 5.14: SAM results for negative or downregulated serum factors; difference between baseline and 26 weeks for 17 HCS and 13 placebo patients, posthoc combined analysis. The values in bold show fold change > 2.

Negative serum factors (19)					
Gene ID	**Score(d)**	**Numerator(r)**	**Denominator(s+s0)**	**Fold Change**	**q-value (%)**
PDGFBB	-1.629334901	-907.1397467	556.754628	-0.952471327	39.40741
VEGF	-1.486551442	-54.57591307	36.71310088	0.141891823	39.40741
PARC	-1.447486142	-75276.6	52005.05749	-0.229573828	39.40741
MMP-9	-1.22883471	-39991.07967	32543.90467	-1.160871013	39.40741
TIMP1	-1.149488678	-2084.084764	1813.053756	-0.262435512	39.40741
MSH	-1.111615105	-11.58038906	10.41762478	0.312294243	39.40741
ANG2	-1.099532478	-172.3082403	156.7104598	0.245481556	39.40741
ACTH	-1.090291169	-12.95268792	11.88002645	0.268928548	39.40741
vWF	-0.898412866	-13.66287154	15.20778704	**4.96886555**	41.48148
sIL-2R	-0.84630143	-147.9358974	174.8028446	0.277891812	41.48148
PIIINP	-0.823018236	-2.994358974	3.638265647	0.45623021	41.48148
Cortisol	-0.6719399	-4.502067439	6.700104338	**5.379335653**	43.92157
IL17	-0.610398442	-2.007110867	3.288197884	0	44.4
I309	-0.427784987	-2.707607103	6.329364479	0.662205925	44.4
bFGF	-0.309302754	-4.507010338	14.57151699	0.415828654	44.4
TNFβ	-0.24523127	-1.894325487	7.724649026	-0.129666371	44.4
IL2	-0.199250177	-0.3062222	1.536872913	0	44.4
POMC	-0.186659577	-0.319989169	1.714292799	0.220812678	44.4
IL5	-0.053787807	-0.195530631	3.635222235	0.830448463	44.4

Next, unsupervised cluster analysis was used to explore the patterns of co-ordinately up or down-regulated serum factors. The results of these analyses are included in **Figures 5.34-5.37** below. There were patterns that seem to confirm what was observed at the individual analyte level. As can be seen in **Figure 5.34**, most of the patients had similar serum profiles at baseline. It is interesting to note that the patients who had upregulated serum factors while most other patients were down regulated, were patients 2, 13 and 17. Patient 2 withdrew at week 2 due to a MCA infarction. Patient 13 had panenteric dysmotility and progression of disease with major GI and cardiac complications and patient 17 withdrew at week 14 due to worsening of lung disease.

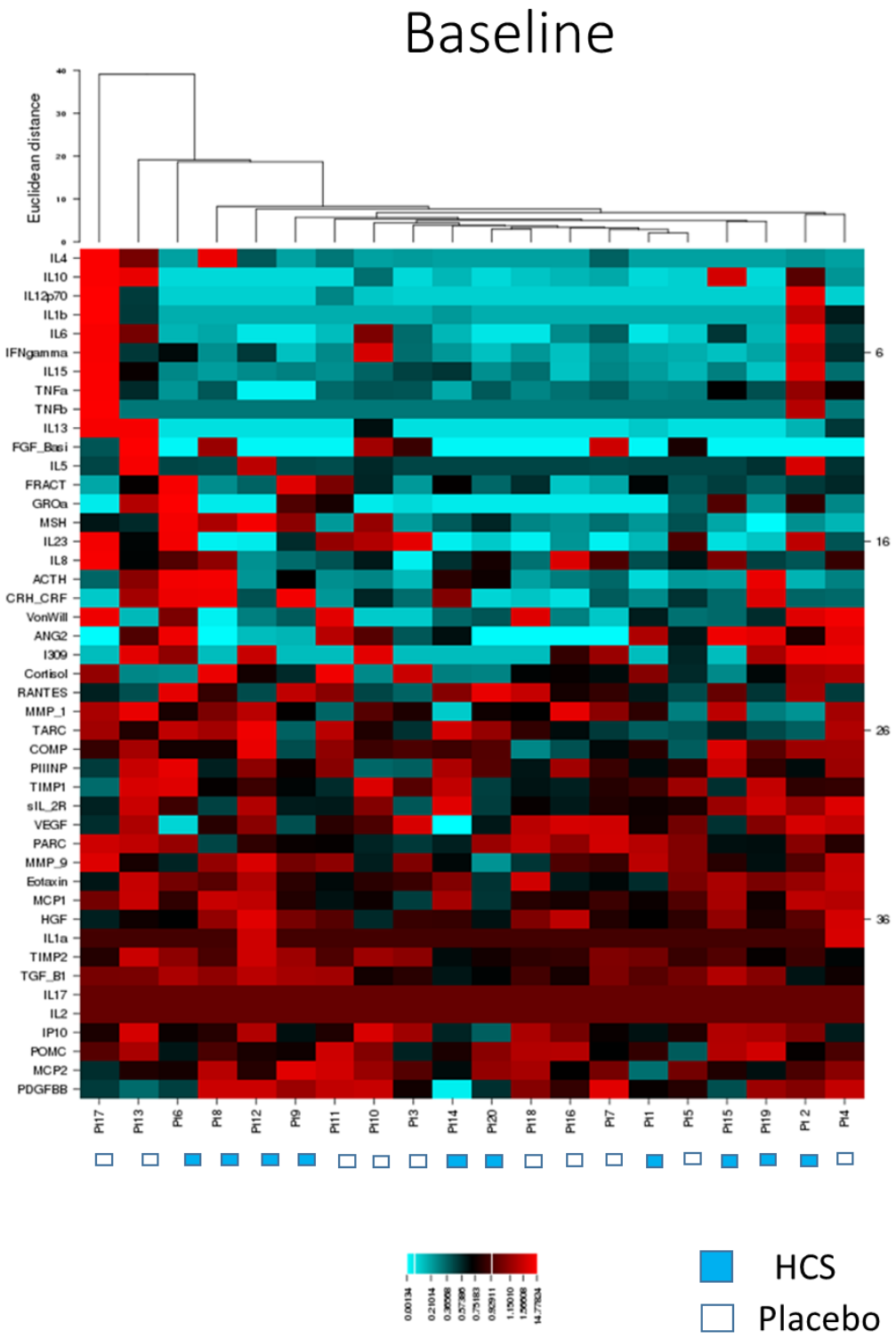


Figure 5.34: Unsupervised cluster analysis heat map, baseline, 10 HCS patients and 10 placebo patients.

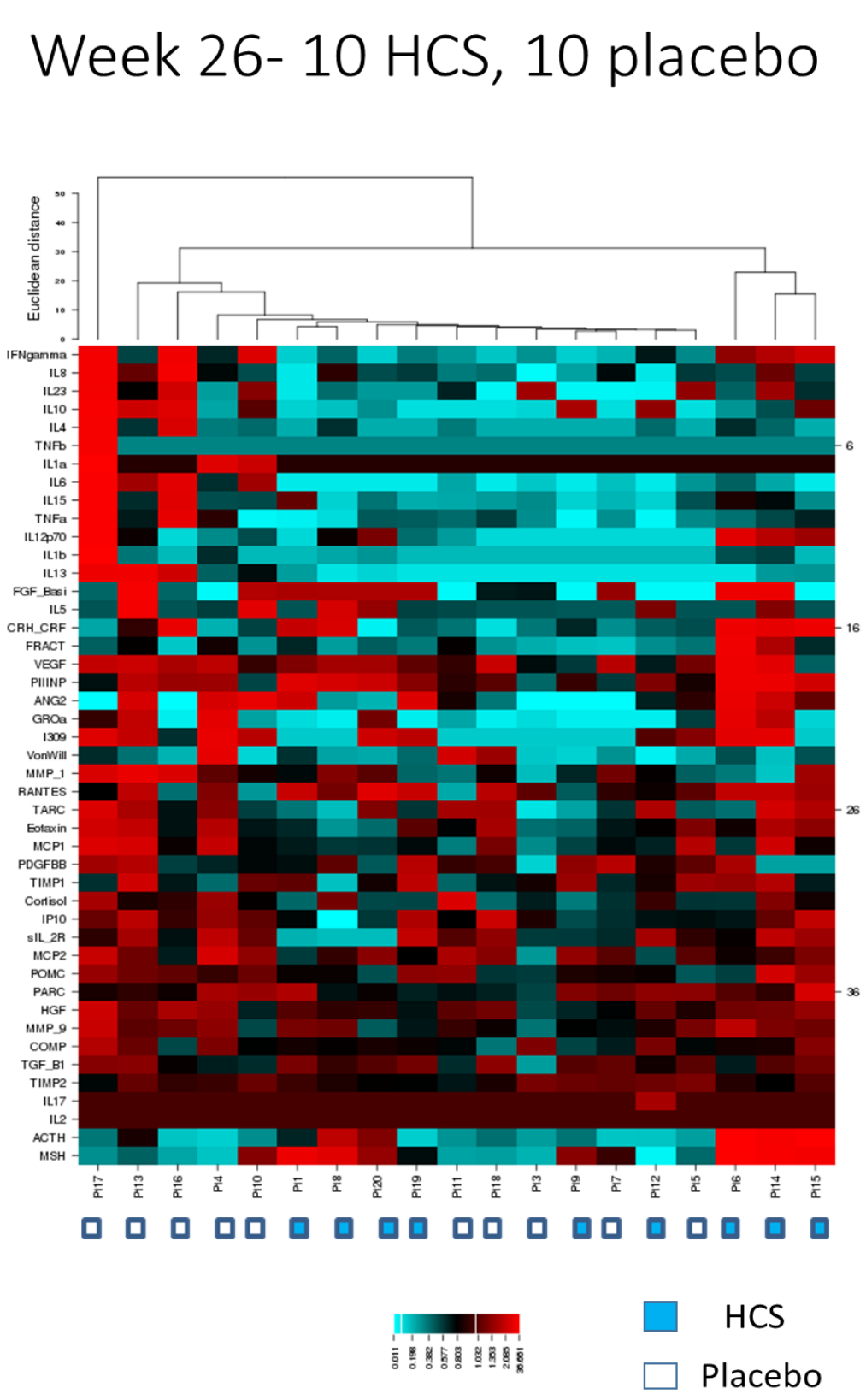


Figure 5.35: Unsupervised cluster analysis heat map, Week 26, 10 HCS patients and 10 placebo patients.

Figure 5.35 shows week 26 in the original cohort. Placebo patients align mainly on the left and HCS patients mainly on the right. The top half of the cohort shows the most change. **Figure 5.36** shows the difference between baseline and week 26 for the original cohort. In this figure, HCS patients align mainly on the left and placebo on the right. Many of the serum factors that are upregulated in the HCS patients, are downregulated in the placebo patients.

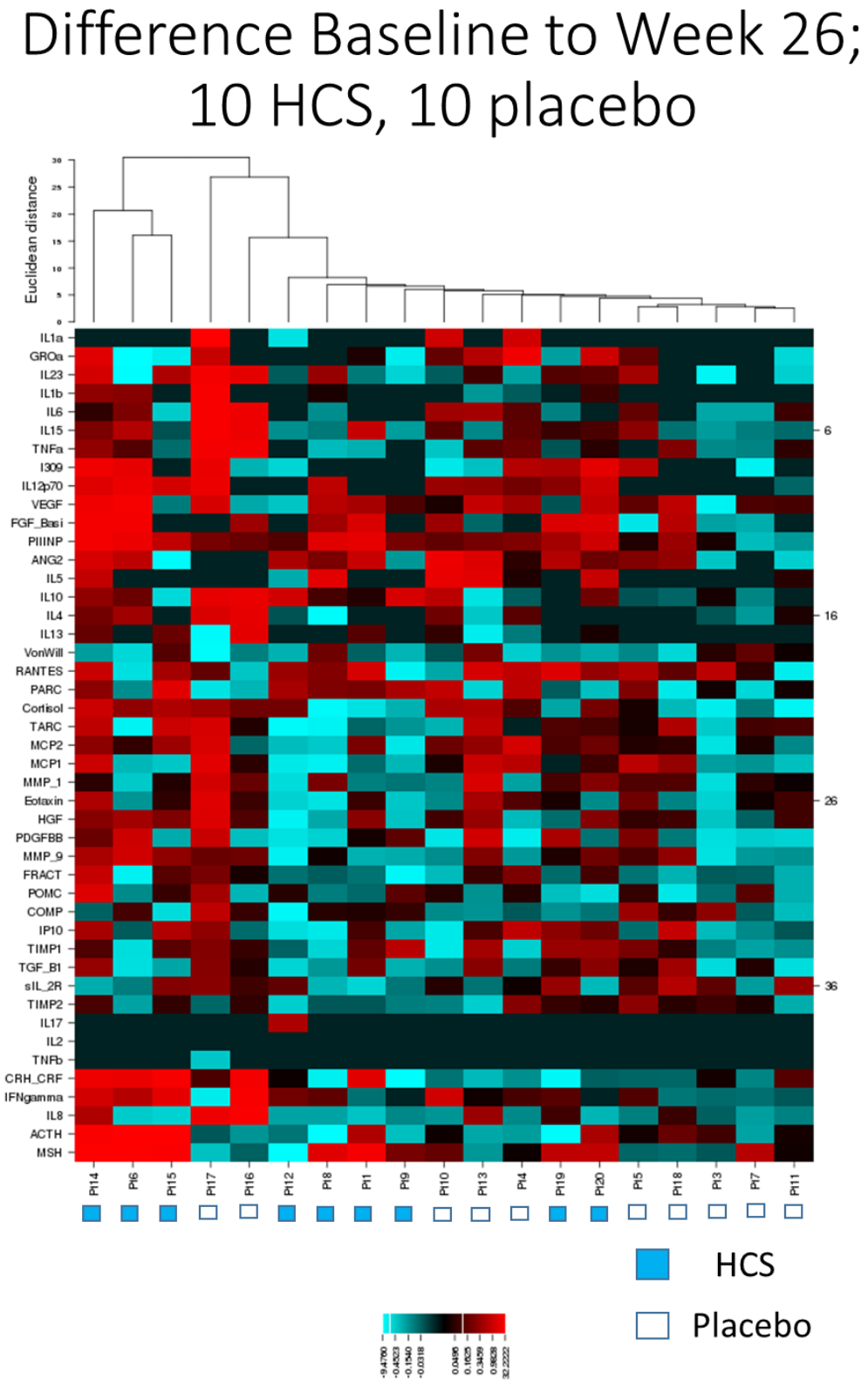


Figure 5.36: Unsupervised cluster analysis heat map, difference between baseline and Week 26, 10 HCS patients and 10 placebo patients.

Finally, supervised cluster analysis was used to better define the cluster of serum factors that could be defined as an HCS induced signature of change. This was a successful approach and the resulting annotated heat map is shown below, **Figure 5.37**.

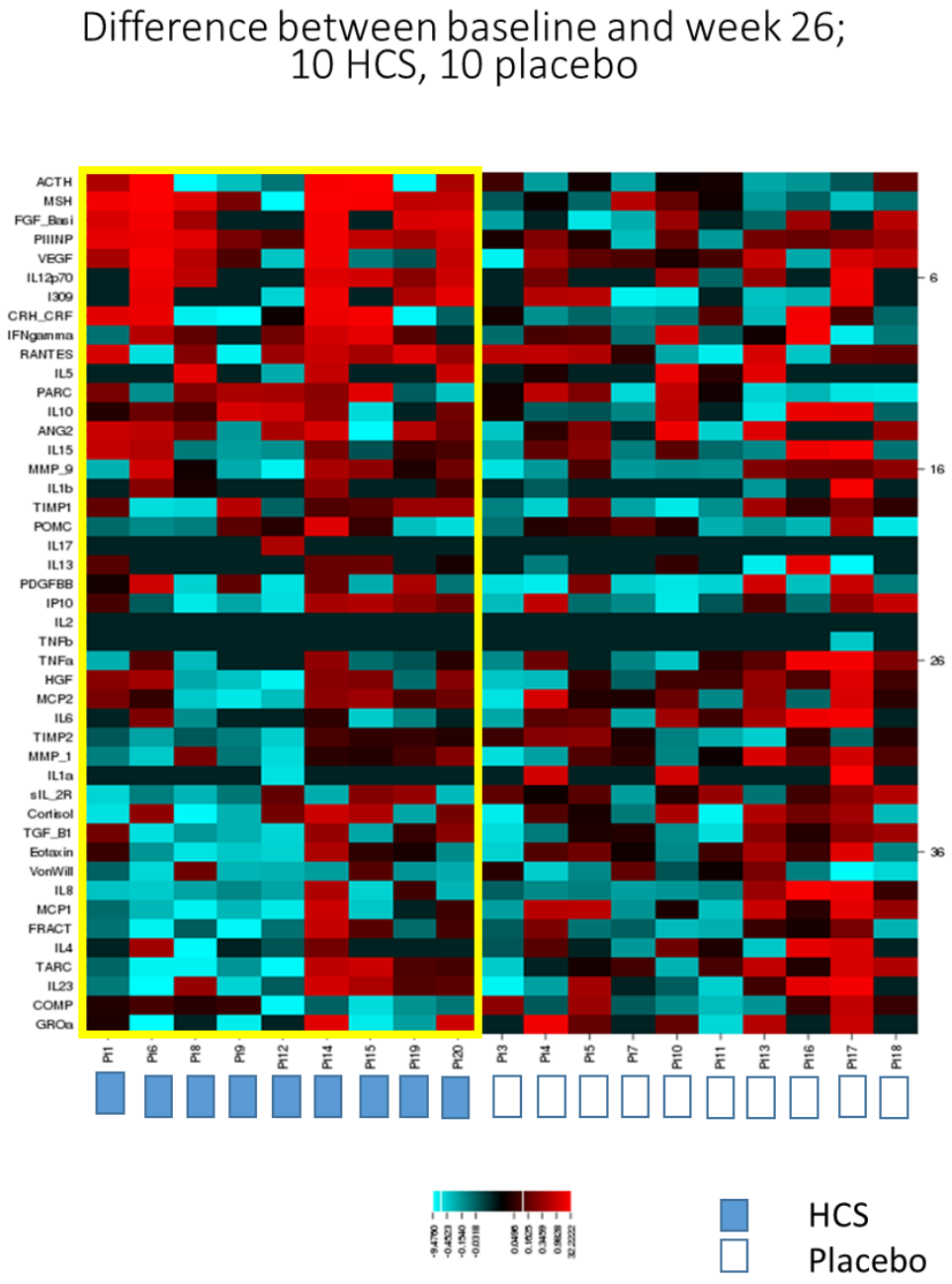


Figure 5.37: Supervised cluster analysis heat map, difference between baseline and Week 26, 10 HCS patients and 10 placebo patients. Yellow box highlights HCS patients.

In this figure, HCS patients align on the left and placebo on the right. A more consistent pattern is identifiable in HCS patients compared to placebo patients, with increasing serum factors at the top and decreasing factors at the bottom. This could be referred to as a "HCS induced signature" and the main serum factors that are increasing or decreasing extrapolated from the supervised cluster analysis are shown in **Table 5.15.** This echoes the SAM analysis tables shown earlier.

Table 5.15: This table shows the main increasing and decreasing serum factors extrapolated from the supervised cluster analysis.

"HCS induced signature"	
Increasing	ACTH αMSH bFGF PIIINP VEGF
Decreasing	TIMP-2 MMP-1 TGF-β1 Eotaxin IL-8 MCP-1 FRACT TARC IL-23 COMP GROα TNFα

The post-hoc combined cohort shows less change than the original cohort. This is also reflected in the analysis of separate serum factors seen in the preceding sections. **Figure 5.38** shows the difference between baseline and week 26 the post-hoc combined 26 week data in unsupervised cluster analysis. In this figure, HCS patients align mainly on the left and placebo on the right. The serum factors that are up/down regulated are different between this and the original cohort.

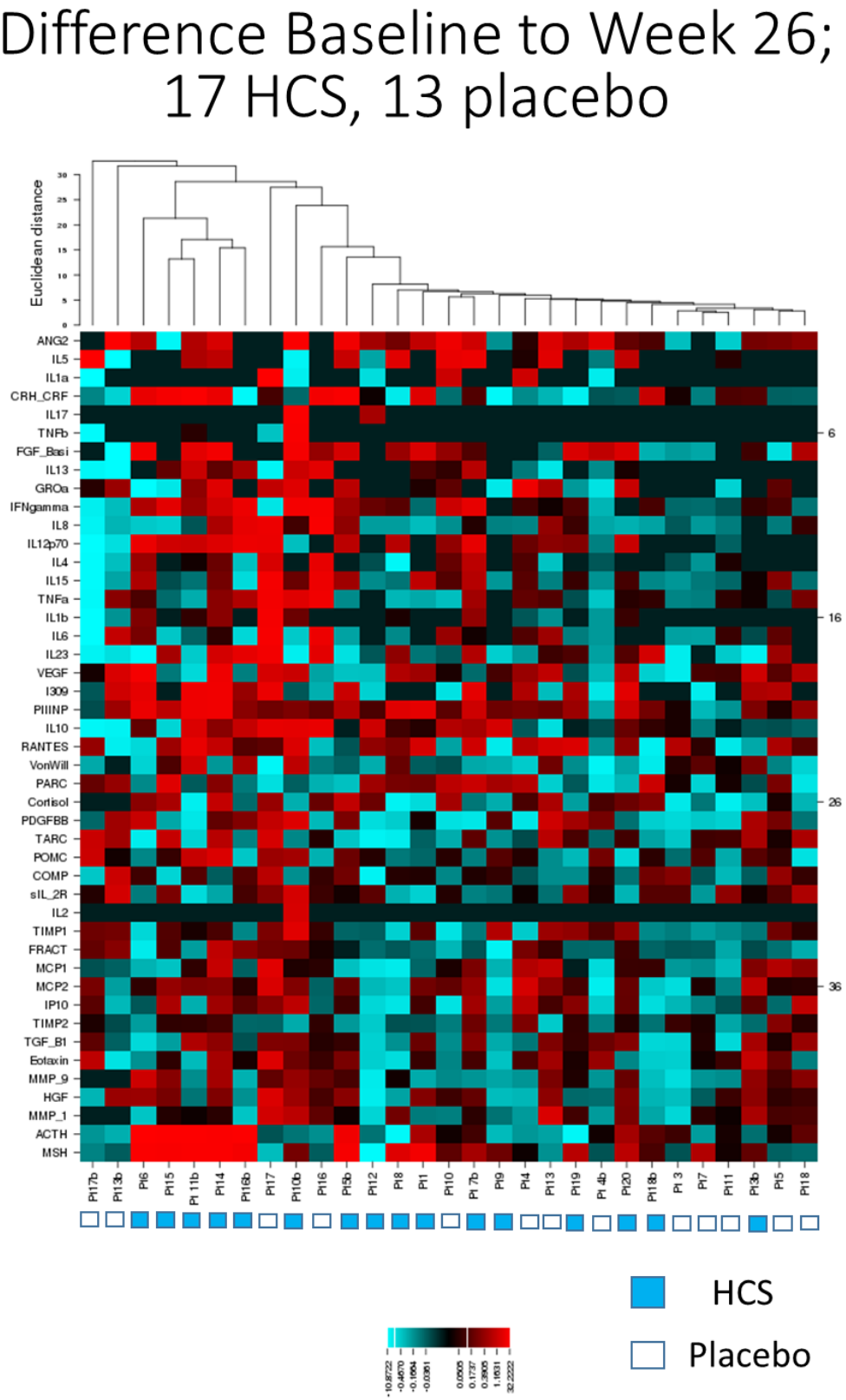


Figure 5.38: Unsupervised cluster analysis heat map, difference between baseline and Week 26, post-hoc combined data, 17 HCS patients and 13 placebo patients.

Supervised cluster analysis was used to better define the cluster of serum factors that could be defined as an HCS induced signature of change. The resulting annotated heat map for the post-hoc cohort is shown in **Figure 5.39,** below.

A more consistent pattern is identifiable in HCS patients compared to placebo patients, though again the post-hoc combined cohort show different changes than original cohort. An identifiable treatment signature is not as significant in the post-hoc cohort, although some increasing and decreasing serum factors are seen, extrapolated from the supervised analysis, **Table 5.16**.

Table 5.16: This table shows the main increasing and decreasing serum factors extrapolated from the supervised cluster analysis for the post-hoc cohort.

"HCS combined cohort signature"	
Increasing	ACTH αMSH PIIINP ANG2
Decreasing	TIMP-2 FRACT TARC IL-8 MCP-1

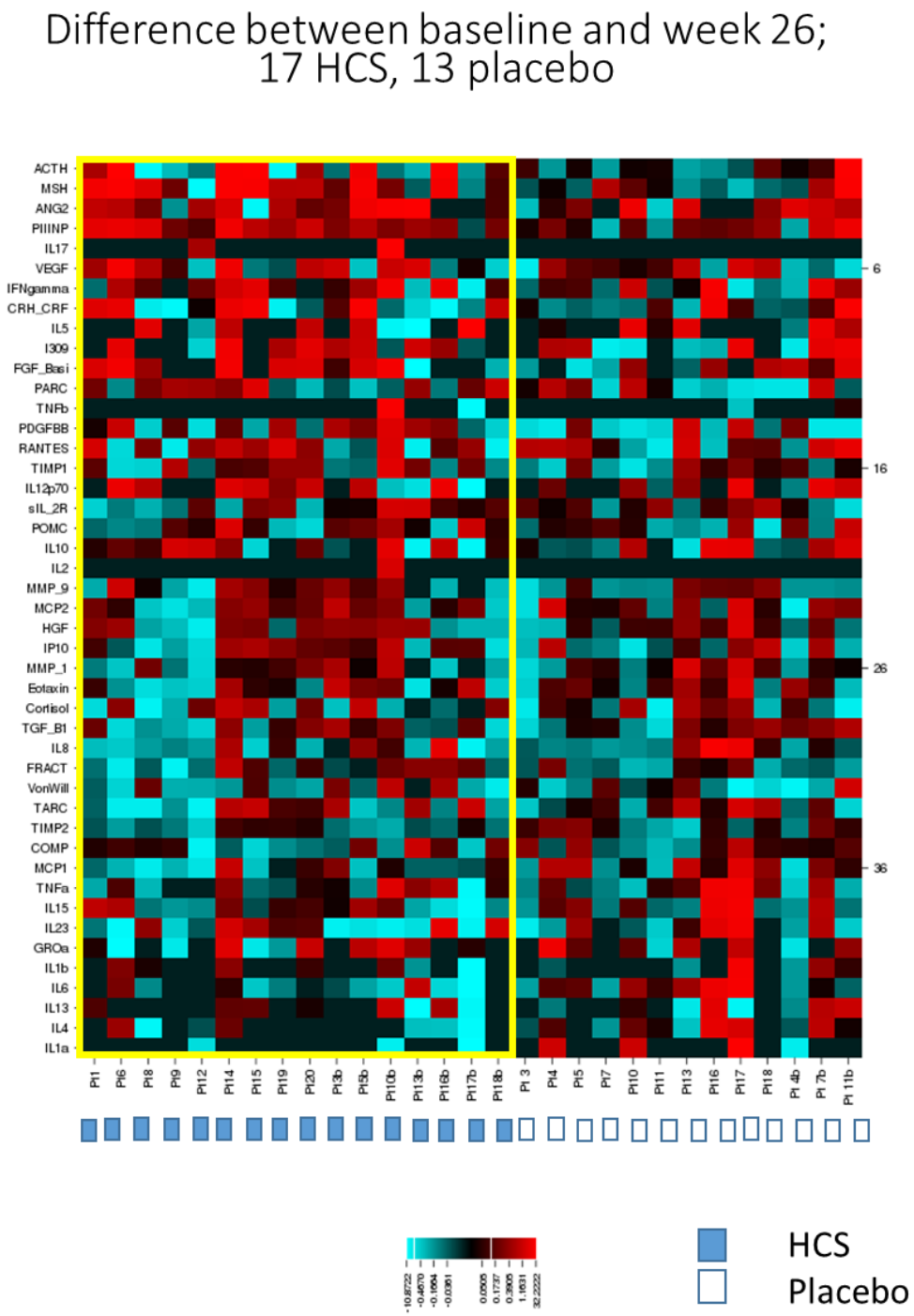


Figure 5.39: Supervised cluster analysis heat map, difference between baseline and Week 26, post-hoc combined data, 17 HCS patients and 13 placebo patients. Yellow box highlights HCS patients.

6 Discussion

This thesis describes the conduct and results of a clinical trial of a novel biological therapy in a cohort of established diffuse systemic sclerosis. The value of the study is that it explores treatment in a stage and subset of this disease with high unmet medical need and also that it provides valuable information about the safety and tolerability of this novel agent in a disease setting and within the framework of a placebo controlled clinical trial. Finally, the demonstration of treatment effect provides a powerful opportunity to better understand relevant aspects of disease biology. Central to this is the use of a contemporary trial design that reflects many aspects that have been discussed in expert groups. This is the first of a series of recent studies that are encouraging with evidence of effect of treatment on skin thickening. This study is unique in demonstrating effect in later stage disease and may inform future clinical studies. In this final discussion key aspects of the work described in the thesis are integrated and areas for future study are highlighted.

6.1 Primary endpoints and key secondary endpoints

6.1.1 Safety endpoints

Established dcSSc has a high morbidity, and as such, provides a unique “safety platform” to assess HCS, a novel immunomodulatory agent. There were frequent AEs in both groups, in keeping with the high morbidity associated with established SSc. Although not statistically significant, there were numerically more AEs and SAEs in the placebo group compared to the HCS group, supporting the conclusion that the study medication is safe and well tolerated.

The frequency of specific AEs were similar in both groups with the exception of injection site reactions which were much more common in the HCS group, both in the double-blind phase (9 of 10 patients) and also in the compassionate medication extension phase (5 of 7 patients). These reactions were self-limiting in the majority of patients, though a few patients with more severe reactions required a short course of anti-histamine and steroid to relieve symptoms. Injection site reactions had also previously been reported in other HCS studies

{Investigator Brochure, Daval International, personal communication}. None of the SAEs were considered to be treatment related.

Immunosuppression withdrawal prior to enrolment in a study is an important consideration. Once patients have established disease, it is thought that inflammation plays a less important role in the disease process, and therefore immunosuppression may also be less important. However, individual patients may still have complications, such as lung fibrosis, which require immunosuppressive agents.

All patients enrolled into the study were considered to have stable and established disease. Half of the study patients were required to stop immunosuppression prior to enrolment. Only 3 of these had to restart immunosuppression after the double-blind period and 1 patient started immunosuppression after having been off it for a number of years. All patients were in the placebo group and 2 of the 4 started immunosuppression for lung disease. This underlines the importance of regular monitoring for complications and may indicate a continuing role for inflammation even in established disease.

6.1.2 Efficacy endpoints

6.1.2.1 MRSS

Skin thickening usually peaks within the first 2 years of disease (163) and skin score declines overall during the course of a trial (164), but in one study patients enrolled with a disease duration <6 months had a small but significant increase in skin score before a subsequent decline and patients with a disease duration >2 years had a greater rate of skin score decline than the other groups (165).

Baseline characteristics show the mean disease duration between 10 and 11 years, no difference between the groups. Median disease duration was 8 years in the HCS group and 11 years in the placebo group. Mean MRSS at baseline was 16.9 in the HCS group and 13.2 in the placebo group, with median MRSS being almost identical at 12 and 12.5 in the HCS and placebo groups

respectively. Skin thickening was therefore mild to moderate in keeping with longstanding dcSSc, and there were no significant differences in baseline characteristics between the groups.

The HCS treated group had a slight improvement in skin score by mean 1.4 units and the placebo group worsened by mean 2.1 units (though the difference was not significant, p=0.18). We then looked at the number of responders in each group with the definition of a responder being MRSS improvement ≥4 units (minimum clinically important difference). There were more responders (50% vs 10%) at 26 weeks in the HCS group compared to the placebo group, responder frequency analysis with a z-pooled test, p=0.067. Furthermore we assessed an extended dataset; combining the original dataset with the results of placebo patients who started HCS at week 26 and using week 26 as baseline and week 52 as week 26. This shows a statistically significant improvement in MRSS in the HCS group (mean -2 units) compared to placebo (mean +2.39 units), p=0.025.

Furthermore, looking at the 4 groups separately, though the results are not statistically significant, the patients who continued on HCS for a further 6 months and the patients who had HCS in the first 6 months but chose not to continue treatment appear to have stabilisation or slight improvement of MRSS. The patients who had no treatment overall (placebo to no treatment) seemed to have a slight worsening of MRSS, while the patients who were on placebo and changed to HCS overall seemed to have a slight improvement in MRSS.

The study on tolerance to human Type 1 collagen (27) was used to generate a hypothesis and plan setup for our trial. It showed significant reduction in MRSS in late-stage dcSSc patients, but not in early-stage patients, suggesting that the processes that cause or promote skin sclerosis and inflammation may be different in early versus late-stage disease. In this study, there was a significant decrease in the MRSS from baseline to 15 months (p=0.0063) with a mean change in MRSS of -7.9 in the 20 active-treated patients with late-stage or established dcSSc compared with a mean change of -2.9 in the 30 placebo-treated patients with late-stage dcSSc. This difference became apparent after 8

months of treatment. It is, therefore, very interesting to note a similar improvement in MRSS in our cohort of patients with late-stage disease treated with HCS. Other possible reasons for this improvement are suggested below.

6.1.2.2 HAQ and HAQ-DI

There was no significant difference between the groups for HAQ-DI, though the placebo group was slightly worse. Mean ±SD at baseline was 1.2±0.07 for the HCS group and 1.6±0.63 for placebo group and at 26 weeks was 1.2±0.98 for HCS and 1.6±0.55 for placebo (p=0.47). In the eight functional activity category scores in the HAQ, no changes were seen in median values for any of these categories for patients in both groups at 26 weeks and no overall statistically significant difference was seen. The disease-specific VAS items showed no significant differences between HCS and placebo for 4 of 5 scales. The only significant difference found was in the scale for finger ulcers with the HCS group (percentage change) showing an improvement with treatment (p=0.0466). However there was large variability in a small sample size and the absolute change was not significant. Variability may be explained by the time of year the patients were seen as finger ulcers tend to be worse in colder months.

In one study, HAQ-DI shows a small improvement in early dcSSc in a meta-analysis of clinical trials, but the range of change was wide and HAQ-DI also rose slightly with longer disease duration (in keeping with morbidity associated with the disease). The authors' conclusion was the HAQ-DI in general remained stable throughout the course of a trial, with wide individual variation (164). The placebo patients in our study had slightly longer median disease duration than the HCS patients, which could account for the slightly higher HAQ-DI scores.

Minimal clinically important difference (MCID) for worsening of HAQ-DI in SSc was reported as 0.14 by Sekhon et al (209). A longitudinal study by a Canadian group (210) shows that in SSc patients HAQ-DI increases (worsens) over time, but only by a small amount. Their data shows an increase of 0.12 units over 3 years, by conservative estimates, almost at a clinically significant level. Considering that our study was only one year and with a small sample size, it is not surprising therefore, that there was no significant change in HAQ-DI.

Schnitzer et al (210) also reported the longitudinal results of the SSc-HAQ VAS scales. In comparison to the patients in that study, our patients had more severe disability in all the categories, particularly the HCS patients. Their patients had a mean disease duration of 11 years and 41% dcSSc patients in comparison to 100% patients in our study who had dcSSc, which may explain worse morbidity in our patients. A summary of comparison of the HAQ VAS scales is given in **Table 6.1.**

Table 6.1: A summary of HAQ VAS scales in HCS and Placebo patients at baseline and week 26, compared to the baseline results from Schnitzer et al (210).

	Mean score in cm (standard deviation)				
	HCS Baseline	HCS 26 weeks	Placebo Baseline	Placebo 26 weeks	Schnitzer et al Baseline
Intestinal VAS	3.83 (3.49)	4.67 (3.42)	2.96 (3.03)	3.28 (3.42)	1.92 (2.86)
Breathing VAS	3.35 (2.83)	3.98 (2.96)	3.75 (3.69)	2.27 (2.44)	2.08 (2.57)
Raynaud's VAS	4.74 (3.76)	4.42 (3.87)	3.43 (4.14)	4.19 (2.78)	3.01 (2.98)
Finger ulcers VAS	3.21 (4.11)	2.91 (4.21)	1.47 (3.20)	1.41 (2.54)	2.10 (3.03)
Pain VAS	5.56 (2.88)	5.00 (3.28)	3.49 (3.22)	4.94 (2.59)	3.71 (2.79)

6.1.2.3 SF-36

In the 8 domains of SF-36, the only domain to show some change was Role Physical, which showed a worsening in the placebo group and maintenance or stabilisation in the treatment group between baseline and week 26, with trend to significance between the groups (p=0.0685). For the SF-36 domain scales that mostly contribute to the scoring of the physical health summary outcome, patients in the HCS group showed no change from baseline median to week 26. Scores for patients in the placebo group declined (physical functioning, role-physical) or remained unchanged (bodily pain) from baseline to week 26. However there was a small improvement in median change from baseline for the general health domain. Overall for physical health there was no significant difference at week 6 or week 26 apart from role physical. There were no significant changes in the 4 mental health domains between the groups. The Health Transition Index showed improvement in the HCS group at week 6 and

week 26, while the placebo group showed initial improvement at week 6, with worsening at week 26.

In a study by Rannou et al (211), SF-36, SSc-HAQ and other quality of life outcome measures were assessed. They included 50 SSc patients (23 patients had dcSSc) with a mean disease duration of 9.1 years. The dcSSc patients in their study had a poor Role Physical score (mean 25.89) which correlated to the placebo patients score at week 26 in our study (mean 27.1). The placebo patients had a higher baseline score than the HCS patients (mean 48.8 vs 38.8 for HCS patients) but their scores declined whereas the HCS patients score improved at 26 weeks (mean 45.8).

In another study by Hudson et al (212) with 504 patients, mean disease duration 10.5 years, 44% dcSSc, the Role Physical mean score was 40.1, closer to the baseline scores for our patients. This study also compares results to US general female population norms and other chronic conditions. The results show significantly worse scores in SSc patients compared to US norms for most of the components and scores were comparable to or worse than other chronic conditions. The authors found the biggest impairments in the Physical Functioning, Role Physical and General Health domains in SSc patients. This is also reflected in the placebo patients of our study. The full results for the 8 categories of the SF-36 and comparisons with both the Rannou and Hudson studies are shown in **Table 6.2.**

Table 6.2: Comparison of the 8 categories of the SF-36 to the Rannou (211) and Hudson (212) study results for SSc patients.

	Mean scores (standard deviation)					
	HCS Baseline	**HCS Week 26**	**Placebo Baseline**	**Placebo Week 26**	**Rannou et al**	**Hudson et al**
Role Physical	38.8 (30.16)	45.8 (30.62)	48.8 (31.29)	27.1 (23.18)	25.89 (25.89)	40.1 (12.1)
Physical Functioning	39.5 (31.49)	40.6 (36.18)	33.5 (20.69)	26.9 (20.69)	55.43 (23.09)	36.4 (11.8)
Bodily pain	36.4 (28.12)	38.8 (31.27)	52.4 (32.38)	35.6 (23.17)	50.52 (23.57)	43 (10.0)
General Health	36.8 (19.76)	36.2 (26.51)	31 (18.47)	24 (16.12)	36.66 (15.14)	37.7 (10.7)
Vitality	35.5 (14.62)	38.3 (21.51)	34 (20.92)	32.2 (23.33)	38.45 (13.03)	45.5 (10.9)
Social Functioning	53.8 (28.9)	56.9 (27.32)	48.8 (33.57)	36.1 (34.49)	60.78 (19.69)	42.8 (11.8)
Role Emotional	45.8 (36.69)	59.3 (34.72)	70 (34.96)	57.4 (40.71)	42.26 (43.15)	44.9 (12.4)
Mental Health	58.8 (21.42)	58.2 (20.89)	62 (18.31)	57.3 (22.09)	51.86 (17.42)	47.6 (10.3)

6.1.2.4 SSc-FS

There was no significant change in the SSc-FS from baseline to week 26 in either group. However, the range of scores was large. The placebo group appeared to have slightly worse median scores than the HCS group, though mean scores were similar. Adjusted mean change from baseline was 1.56 in the HCS group and 0.46 in the HCS group. In a previous study a change of 3 units was deemed clinically relevant (184). Mean SSc-FS was between 10.7 and 11.4 at baseline and between 11.7 and 11.9 at week 26 for both groups. This is consistent with previously reported studies (184) (185), though disease duration was shorter in these studies. These studies also showed very good correlation between SSc-FS and HAQ-DI.

6.1.2.5 Other outcomes

Pain in SSc is often multifactorial and can be difficult to treat. Pain in SSc is more common than previously recognised. Between 60 and 83% of SSc patients report some element of pain (213) (214) (215) (216) and about 10% of patients report severe pain (213) (214). In one recent study by Perrot et al

(216), neuropathic pain was detected in almost half of SSc patients and just over one third of RA patients. Pain frequency and intensity were higher in dcSSc than lcSSc patients and dcSSc patients tended to have more joint, visceral and diffuse pain. Neuropathic pain has not been extensively studied in SSc.

For the neuropathic pain VAS, the HCS group were worse at baseline with a median score of 4.5cm compared to 0.6cm in the placebo group. The analysis of adjusted mean change from baseline in the neuropathic pain VAS indicates that there was significant difference between groups at week 26 with an improvement in the treatment group and no change in the placebo group, p=0.0461. Though the sample size is small, this is an interesting outcome as neuropathic pain seems to be more common than previously recognised and HCS may have the potential to improve neuropathic pain due to its sodium channel opening effect.

MRC sum score, a measure of muscle strength, did not change throughout the study apart from one patient with a middle cerebral artery infarct. All of the patients also had normal creatinine kinase levels, indicating that no patients had acute inflammatory myositis.

6.2 Physiological studies

6.2.1 Pulmonary function tests

Lung function indices showed a trend of benefit for the HCS group compared to the placebo group for those variables that reflect respiratory effort (FVC and FEV_1) for absolute values but not in % predicted values. At Week 26 FEV_1 had increased in the HCS group and decreased in the placebo group resulting in 5.83% difference between groups. A similar pattern was shown in FVC for a 7.37% difference between the groups. However when background disease was taken into account (pre-existing lung disease worsened in 1 patient in the placebo group), there was no significant difference between the two treatment groups. DLco and TLC did not change during the study.

This was an interesting positive trend in a small sample size. In most studies in lung function in SSc, % predicted values are used for comparison and these did not show a change. This study was not powered for lung function changes and while interesting, a bigger and properly powered trial would be needed to confirm lung function changes and benefit with treatment.

6.2.2 Exploratory physiological studies

Sniff nasal inspiratory pressure (SNIP) is a non-invasive method to assess respiratory muscle function and is used in patients with neuromuscular disease such as motor neurone disease and muscular dystrophies as well as chronic obstructive pulmonary disease. Theoretically, SSc patients may have respiratory muscle weakness due to myositis which can contribute to dyspnoea in some patients. As HCS has a potential sodium channel opening effect, and if there was respiratory muscle weakness, it may have been possible to record a therapeutic effect using SNIP pressures. SNIP has not been previously assessed in SSc patients in published literature. However, SNIP was normal in most of our patients and no treatment effect was detected. This confirms that there was no detectable respiratory muscle myositis in our group of patients. It may be interesting to study SNIP in SSc patients who have documented myositis or who have dyspnoea of unknown etiology as it is easy to perform and non-invasive.

Heart rate variability (HRV) is a normal physiological response governed by vagal tone which is controlled by the autonomic nervous system. Therefore, HRV can be a surrogate marker or biomarker of autonomic dysfunction. HRV dysfunction is well recognised in SSc and is characterised by parasympathetic dysfunction combined with sympathetic overactivity and depression of the circadian rhythm of heart rate (203) (204) (195). SDRR gives an overall impression of HRV and sinus node activity, while higher values for RMSSD indicate higher vagal tone. Sympathetic activation is also indicated by lower values in SDRR (204) (203). HRV was performed on our patients as a non-invasive test to assess HRV and to look for potential therapeutic effect from HCS via its sodium channel opening effect.

There were no clinically or statistically significant changes within or between the treatment groups before and after treatment for any of the 3 separate modes assessed (normal breathing, deep breathing and Valsalva manoeuvre). The Valsalva results appear normal in comparison to controls in one study and there were no changes with HCS. The Deep Breathing results again show no change with treatment. However, our results in the Normal Breathing mode, when compared to controls in other published studies, show reduction in both SDRR and RMSSD, indicating sympathetic overdrive and parasympathetic dysfunction, in keeping with other published data in SSc.

6.3 Laboratory studies and candidate biomarker analysis

The pathogenesis of SSc is a complex interplay between vasculopathy, inflammation and fibrosis. In order to assess these three areas, potential biomarkers representative of these areas were studied. PIIINP was used to assess fibrosis, vWF for endothelial damage and vasculopathy and sIL-2R for lymphocyte activation and inflammation.

6.3.1 Trial specified candidate biomarkers

6.3.1.1 PIIINP

PIIINP is a marker for collagen synthesis, and therefore fibrosis and there has been interest in it since the 1980's as a possible surrogate biomarker for SSc. Many studies have shown that PIIINP increases in SSc and is higher in dcSSc than lcSSc (217) (218) (219) (220). Some studies have shown that PIIINP is a marker for disease activity (219) (221) and is responsive to change in SSc (218). Studies have also shown that PIIINP is higher in early disease compared to late disease and predicts mortality (222) (223) and that treatment reduces PIIINP levels (224). PIIINP also appears to correlate with lung involvement (225) (221).

In our study, at week 26, PIIINP was significantly increased from baseline in the HCS group by 8.080 µg/L, (p=0.0002), and relatively unchanged in the placebo group, 1.104 µg/mL, (p=0.5301). The difference between the groups at Week 26 was significant (p=0.0118). The post hoc combined baseline to week 26

showed an even more significant result with p=0.0085. In the group that switched from placebo to HCS at 26 weeks, significant increases from baseline (and from 26 weeks) were seen in PIIINP at 52 weeks (p=0.0008 compared to baseline). The HCS group who continued HCS had a significant reduction in PIIINP from 26 weeks to 52 weeks (p=0.0078) though not back to baseline levels.

These data are intriguing considering that MRSS improved on treatment with HCS and PIIINP correlates with MRSS in most previously published studies, as seen above. There were no statistical differences between the groups for AEs and SAEs and lung involvement appeared to be worse in the placebo group, therefore PIIINP does not appear to be associated with adverse events or worse prognosis in this group. It is also interesting that after 6 months on HCS, the group who continued HCS had a reduction in PIIINP, though not back to baseline levels. As the HCS group did not have evidence of worsening fibrosis, it is unknown why the PIIINP levels increased in this group.

6.3.1.2 vWF

vWF is a marker for endothelial damage and vasculopathy. A number of studies report increased levels of vWF in SSc (226) (227) (228) (229) (230) and higher levels of vWF correlate with more extensive disease (230) (228) or visceral complications (227) (231). Raised vWF levels have also been associated with the development of PAH (222) (232).

In our study, at week 26, overall there was no significant difference between the groups for vWF, p=0.6875 and post hoc combined 26 week data for vWF also revealed no significant difference, p=0.35. At week 52, there was a significant decrease in vWF in the placebo patients who did not take HCS, p=0.003; however, there were only 2 patients in this group. These 2 patients were unwell throughout the study and withdrew at week 14 due to disease progression and/or worsening lung fibrosis. Both went on immunosuppressive agents after week 14, and their disease stabilised which could explain the decrease in vWF levels.

6.3.1.3 sIL-2R

sIL-2R is a marker for lymphocyte activation. sIL-2R levels are increased in SSc and higher levels are associated with mortality, more extensive disease and earlier disease (233) (234) (235) (221) (236) (237). Some studies have shown a reduction in sIL-2R with treatment (88) while others haven't (234) (238).

In our study, at week 26, there was no significant difference between the groups for sIL-2R, p=0.7862. The combined 26 week data from the post hoc analysis was also not significant, p=0.2218. At week 52, there was no significant change in the HCS group who continued a further 26 weeks of treatment or in the HCS group who discontinued treatment. In the placebo group, there was a trend to significant increase in the patients who did not take HCS (p=0.053). There were only 3 patients in this group and 2 of these did not show a change; the third showed an increase in sIL-2R levels. The patient who had an increase in sIL-2R was extremely unwell throughout the study, particularly between week 26 and week 52, when he was an inpatient. He died soon after week 52 as a result of progression of his disease and sIL-2R is associated with mortality, therefore this explains the increased levels in this patient.

There was a significant increase in sIL-2R in the placebo patients who opted for HCS compassionate treatment for 26 weeks (p=0.0311). This change was not seen in the double-blind HCS patients, mentioned above and the difference may be explained by fewer patients in the group (7 patients versus 10 patients in the original group) with a wider range of results. There was one patient who had a particularly high level a week 52, which skewed the results somewhat. This patient had significant issues with finger ulcers, which may have increased her levels.

6.3.2 Multiplex cytokine analysis

6.3.2.1 Cluster analysis

Multiplex cytokine analysis is increasingly being reported in inflammatory diseases, including SSc, and is being used as a tool to improve understanding of pathophysiology of disease as well as identifying potential biomarkers and

assessing response to therapy. Some examples of multiplex cytokine analysis in SSc are given below.

In a report by Beirne et al (239) looking at sarcoidosis and SSc patients, the authors found increased levels of IP-10, MIG, MIP-1α, MIP-1β, MCP-1, eotaxin and IL-17 in SSc patients but there was considerable overlap with cytokines that were elevated in sarcoidosis patients. When the authors looked at complications, they observed elevated levels in EGF in SSc patients with anti-topoisomerase antibody compared to those negative for the antibody. bFGF was higher in untreated SSc patients than treated patients and eotaxin was elevated in untreated SSc patients compared to controls. They also performed a correlation analysis to assess correlations between cytokines and found that the most complex inflammatory network was in SSc patients. They identified a group of 17 mediators that could be used to group cases into controls, sarcoidosis or SSc correctly in 89.5% cases and even with reduction to 6 core analytes, the model showed 78.9% accuracy. Using another group of analytes, they could also correctly identify patients with SSc with or without lung fibrosis.

In another study, Vettori et al (240) looked at cytokine profiles in early SSc and showed increased sICAM-1, CCL2, CXCL8 and IL-13 in early SSc patients compared to controls and lower levels than in definite SSc patients. IL-33 levels were highest in early SSc patients and lowest in lcSSc patients. sVCAM-1 and TGF-β were elevated in dcSSc and lcSSc but not in early SSc or controls.

Gourh et al (241) looked at plasma multiplex cytokine profiles in SSc and found that after adjusting for age and gender, TNFα, IL-6 and IFN-γ levels were raised in SSc patients compared to controls and IL-17 and IL-23 were reduced compared to controls. When looking at disease duration, TNFα and IL-6 were increased in SSc patients with disease duration 0-5 years and 5-10 years compared to controls and IL-5, IL-10 and IFN-γ were increased in patients with disease duration >10 years. IL-13 was increased in patients with disease duration <5 years and IL-17 was decreased in all SSc patients independent of disease duration. IL-23 was reduced in patients with disease duration 0-5 years and 5-10 years but not >10 years. Comparing autoantibody subsets, increased

TNFα and reduced IL-23 were found in all autoantibody subsets. IL-6 was increased in all subsets apart from ACA positive and IL-17 was reduced in all but the autoantibody negative group. They also noted associations with complications; increased IL-6 was associated with ILD and PAH patients were more likely to have high IL-6. IL-6 was also correlated to MRSS. The authors conclude that many factors influence cytokine profiles in SSc, particularly disease duration and autoantibody profile. They didn't, however, comment on dcSSc versus lcSSc.

Schiopu et al (242) looked at subclinical atherosclerosis in SSc patients and assessed carotid intima media thickness (CIMT) and plaque formation with ultrasound as well as a multiplex serum profile. They found 8 cytokines associated with plaque and 5 different cytokines associated with CIMT. Only 2 proteins were associated with both, NT-proBNP and IL-6. They looked at a composite score of the 8 proteins associated with plaque and found that 5 or more had a high sensitivity and specificity, suggesting that it could be used in clinical practise to pick up patients with subclinical atherosclerosis.

Clark et al (243) compared cytokine profiles in dermal blister fluid to plasma multiplex cytokine profiles. In dermal blister fluid, the authors found increased levels of IL-6, IL-15, MCP-3, FGF-2, and PDGF-AA in SSc patients compared to controls and IL-17 was only detected in dcSSc blisters and not in lcSSc or controls. IL-6 and MCP-3 were higher but not significantly higher in dcSSc compared to lcSSc in blister fluid samples. In plasma multiplex samples, the authors found increased levels of IL-1RA, TNFα, RANTES and GMCSF in SSc patients compared to controls. They also found greater than 1.5 fold higher levels of MCP-3, IL-12p40, VEGF, IL-10, IL-4, IL-2 and IL-1α in SSc samples compared to controls using significance analysis of microarrays (SAM®) software. IL-4, IL-5, IL-6 and IL-13 were only detectable in SSc plasma but not at significant levels. Comparing blister fluid and plasma samples in SSc, there were no significant correlations, reflecting local inflammatory processes in the blister fluid samples and not leakage from serum. However, the health control samples did show some correlations between blister fluid and plasma samples. Using hierarchical clustering on the blister fluid, the authors identified 3 groups;

an inflammatory group (group 1) with high IL-6, IL-10, TNFα and IL-1α, an IFN-γ group (group2) with high IFN-γ, IL-2, IL-4, IL-5, MCP-3, IL-12p40 and IL-12p70 and a quiescent group (group 3) with low levels of cytokines and chemokines. Group 1 was early dcSSc, group 2 late dcSSc and group 3 lcSSc or dcSSc with low skin score.

In our study, using SAM® we identified 4 cytokines in the original dataset (6 month double-blind cohort) that were increased by more than 2 fold in the HCS treated patients compared to placebo treated patients. These were α-MSH, ACTH, bFGF and PIIINP. PIIINP is already mentioned above. We also identified 5 cytokines that were reduced more than 2 fold in HCS treated patients compared to controls; TGF-β1, TIMP-2, Fractalkine, COMP and GROα. Apart from α-MSH, ACTH and PIIINP, the differences between HCS and placebo patients for individual cytokines are not statistically significant. However, analysing the data using this method gives us very interesting and useful data.

Interestingly, all of the cytokines that were more than 2 fold reduced with HCS treatment have been reported to be elevated in SSc patients versus controls with TGF-β1 being one of the most important cytokines involved in the pathophysiology of SSc. Increased levels of α-MSH and ACTH with treatment point to a possible mechanism of action for HCS treatment. However, it is difficult to explain increased levels of bFGF and PIIINP with HCS treatment. Different cytokines were increased or decreased in the post hoc 26 week combined cohort and results were less significant. Each of the cytokines identified by SAM® are discussed separately below.

6.3.2.2 α-MSH and ACTH

α-MSH is a 13-amino acid peptide hormone produced from the processing of pro-opiomelanocortin (POMC). POMC is also a precursor for at least 4 other peptides including β-MSH, γ-MSH, ACTH and β-endorphin. They bind to melanocortin receptors, of which 5 have been identified, (MC1-5R). α-MSH is known to have a number of anti-inflammatory and anti-microbial properties. It suppresses TNF production and inhibits activation of I- and E-selectin, as well as NF-κB (244). POMC and ACTH are produced from the pituitary gland in

response to CRH stimulation. CRH has also been found to stimulate POMC activity and ACTH production and release in human dermal fibroblasts (HDF). CRH and ACTH stimulate the production of corticosterone in fibroblasts, with ACTH being more potent (245).

Previous reports have looked into melanocortins, primarily β-MSH (246) and adrenal deficiency (247) as a cause for abnormal pigmentation in SSc, but found no evidence for either theory. In an interesting series of studies, Bohm et al described that HDF express the MC1 receptor (MC1R) that binds α-MSH with high affinity and they found that α-MSH suppressed TGF-β induced collagen synthesis in HDF in vitro (248) (249).

Furthermore, the authors used a bleomycin mouse model (systemic sclerosis mouse model) to investigate the effects of α-MSH on skin fibrosis and found that simultaneous administration of α-MSH with bleomycin supressed the effects of bleomycin on HDF. ACTH was also found to have similar suppressive effects. α-MSH exerts its effects via a cAMP driven pathway and not via Smad 2/3. α-MSH upregulates superoxide dismutase 2 and hemeoxygenase 1 which is protective against the effects of bleomycin on reactive oxygen species. They also confirmed the presence of POMC and the MC1R in affected skin from patients with SSc and that HDF from these patients strongly expressed both POMC and MC1R (as well as in normal skin), making α-MSH a potential therapeutic target in the future according to the authors (250). In a recent study, the same group also conclude that MC1-signalling deficient mice with a C57BL/J6 background exhibit experimentally induced fibrosis in response to bleomycin, whereas wildtype animals with the same genetic background do not (251). The authors conclude that it would be fascinating to investigate expression and function of MC1R in patients with fibrotic skin disorders such as SSc.

There is one interesting case report on one patient (252), who was enrolled in the CAT-192 clinical trial (253), which shows that before treatment, the patient had increased TGF-β mRNA expression and suppressed POMC mRNA expression and MCR 1-3 and 5 receptor subtypes in the skin lesion compared

to controls. After treatment there was rebound expression of POMC, MCR 2, 3 and 5 receptors, which may indicate a role for the melanocortin system in SSc.

As mentioned in the introduction, HCS contains a multi-protein complex which includes CRH. It is not surprising, therefore, that both α-MSH and ACTH were significantly increased in the HCS treated patients compared to the placebo patients during the double-blind part of the trial. α-MSH and ACTH decreased again between 26 weeks and 52 weeks, though not to baseline levels. This may have been, in part, due to poorer compliance in the compassionate use part of the trial where patients were not monitored as frequently. The action of α-MSH and ACTH on the MC1R is one possible mechanism by which the MRSS in HCS treated patients may have improved. As mentioned, CRH also stimulates the production of corticosterone in HDF, so this may also have some relevance due to an additional anti-inflammatory effect.

6.3.2.3 bFGF

Basic FGF (bFGF, also called FGF-2) is a growth factor and chemotactic factor for fibroblasts and endothelial cells. It is a key molecule in the induction of angiogenesis. It stimulates proliferation, migration and differentiation of endothelial cells and synergises with vascular endothelial growth factor (VEGF) in its angiogenic actions (254) (255). In SSc, data on serum levels of bFGF are conflicting with some groups finding elevated levels in SSc but not in controls (256) (257) while others did not find any difference in serum levels between SSc and controls (258) (259). Lawrence et al did find increased expression on bFGF in the skin of SSc patients however they found only a few patients with SSc had high serum levels of bFGF (259).

In our study, at 26 weeks bFGF levels showed an increase from baseline in both groups but a larger increase in the HCS group. The difference was not statistically significant, p=0.148. Post hoc combined 26 week data for FGF also showed an increase in the HCS group and slight decrease in the placebo group, but again the difference was not statistically significant. The separate groups showed that the HCS group continuing HCS and the placebo group who started on HCS had some increase in bFGF levels but again it was not significant. The

effect of treatment on serum bFGF in SSc has not yet been studied, but it is interesting but difficult to explain increase in bFGF with HCS treatment.

6.3.2.4 TGF-β1

TGF-β is the pre-eminent signal for connective tissue synthesis and is considered the core pathway in wound healing and pathological fibrosis. TGF-β promotes fibroblast proliferation, differentiation, migration, adhesion and survival, induces cytokine secretion and upregulates synthesis of collagen and extracellular matrix (260). TGF-β is secreted from monocytes, lymphocytes and fibroblasts in an inactive form and sequestered in the ECM. Data on serum TGF-β1 levels in SSc are conflicting with some groups find elevated levels (261) (262) and some no difference from controls (263) (264) (265). One group found a reduction in active TGF-β1 in dcSSc compared to lcSSc and controls and levels correlated inversely with MRSS (266). These conflicting results may be due to a number of issues including type of SSc (dcSSc or lcSSc), disease duration, heterogenous population, the assays used and/or the form of TGF-β studied (active versus latent).

In this study, at 26 weeks, both groups showed a decrease in TGF-β1 levels while the HCS group showed a slightly bigger decrease but this was not significant with p=0.6009. The post-hoc combined 26 week data showed a very slight increase in the HCS group but no change in the placebo group and the difference again was not significant. At 52 weeks, the group of placebo patients who were followed up only with no other treatment showed an increase in TGF-β1 levels but there were only 3 patients in this group, p=0.0118 compared to baseline. None of the other 3 groups showed statistically significant changes compared to baseline or week 26. Though not significant, it is interesting nonetheless, that there was a slight decrease in TGF-β1 with HCS treatment that may coincide with decrease in MRSS.

6.3.2.5 TIMP-2

Fibroblasts produce matrix metalloproteinases (MMPs) which digest all the ECM products. They also produce tissue inhibitors of MMPs (TIMPs) and the quantity of ECM is determined by the balance between the MMPs and TIMPs.

Previous studies report contradictory results about serum TIMP-2 levels in SSc. Yazawa et al found elevated levels of TIMP-2 in 22.7% patients with SSc. They also found that TIMP-2 levels were significantly correlated with skin score and significantly higher in active disease (267).

Others found no difference in TIMP-2 levels in SSc compared to controls (268) (269). Dziankowska-Bartkowiak et al however, did note that patients with lcSSc and a restrictive lung defect on PFTs had high or borderline high levels of TIMP-2. In a follow-up study, Dziankowska-Bartkowiak et al again found that TIMP-2 levels were not significantly different in patients compared to controls in the group as a whole, but did find elevated levels in SSc patients who had cardiovascular disease (270). Shahin et al found that in patients with disease duration of more 2 years, TIMP-2 levels were higher in SSc, particularly dcSSc, than in controls. They also found a correlation between TIMP-2 levels in dcSSc patients and CT scoring for fibrosis and also between TIMP-2 levels in lcSSc patients and cardiovascular problems, in agreement with the studies by Dziankowska-Bartkowiak et al (271).

In our study, at 26 weeks there was a slight decrease in TIMP-2 levels compared to baseline in the HCS group and a slight increase in the placebo group. However, the difference was not statistically significant, p=0.1044, and the combined 26 week post hoc analysis showed a less significant difference. At 52 weeks, there were no statistically significant changes in any of the 4 groups compared to baseline or week 26.

6.3.2.6 Fractalkine

Fractalkine is a member of the CX3C chemokine family and is found on TNFα and IL-1 stimulated endothelial cells (272) (273). It has a soluble form (consisting of the extracellular domain and a mucin-like stalk) and a membrane bound form and it binds to its receptor CX3CR1. The soluble form is generated by enzymatic cleavage of the extracellular part of the membrane bound form by TNFα converting enzyme (TACE/ ADAM-17). The membrane bound form promotes leucocyte activation and can mediate in each step in the leucocyte adhesion cascade and the soluble form is a powerful chemoattractant for

monocytes, macrophages, NK cells and T cells expressing its receptor (274) (272) (275) (276).

Hasegawa et al found an increase in CXCR1 in skin and lung tissue samples compared to controls, with higher number in dcSSc compared to lcSSc as well as increased levels of CX3CR1 on peripheral macrophages/monocytes and T cells in dcSSc. Fractalkine was strongly expressed in endothelial cells in affected skin and lung tissues. Serum fractalkine was 4 times higher in SSc patients than controls and patients who had pulmonary fibrosis had levels 4 times higher than patients without. Raised serum fractalkine levels also reduced after treatment with immunosuppressants (277). In another study, fractalkine was again found to be higher in SSc than controls and higher in dcSSc than lcSSc. After treatment with prostaglandin E1, fractalkine levels dropped at day 3 and remain reduced after a month (278). Increased susceptibility to SSc has also been shown to be associated with a polymorphism in the fractalkine receptor and is associated with PAH (279).

In our study, at 26 weeks, there was a decrease in fractalkine levels in both groups. The HCS group had a bigger decrease, however the difference was not statistically significant with p=0.3179 and the combined 26 week post hoc analysis showed an even smaller difference. At week 52, there were no significant changes in any of the 4 groups compared to baseline or week 26. As previous studies have shown reduction of fractalkine with immunosuppressants, it is likely that there is some treatment effect with HCS treatment but not big enough for a significant result.

6.3.2.7 COMP

Cartilage oligomeric matrix protein (COMP, Thrombospondin 5) is a large disulphide linked pentameric extracellular glycoprotein member of the thrombospondin family found mainly in cartilage and tendon extracellular matrix (280) (281). It can also be produced by dermal and synovial fibroblasts (282) and can bind to several extracellular proteins including type I, type II and type IX collagen (283) (284) (285).

COMP accumulates in SSc skin but not normal skin and cultured fibroblasts from SSc skin demonstrate more staining for COMP than normal controls (286). Serum COMP levels are increased in SSc patients compared to controls and higher in dcSSc than lcSSc (287) (288). Serum COMP correlates with skin involvement measure by MRSS (289) and is higher in SSc patients with arthritis (288). High serum COMP levels in early disease are associated with an increased risk of mortality (290). Complexes between COMP and complement C3b in the serum are elevated in SSc but no co-localisation was found in skin biopsies indicating that complexes are formed after release of COMP into circulation and COMP does not drive complement activation in SSc (291).

In our study, at 26 weeks, there was a decrease in COMP levels in HCS group and a slight increase in the placebo group, though the difference between groups was not statistically significant with p=0.2651 and the combined 26 weeks post hoc data showed a smaller difference between the groups. At 52 weeks, there were no significant changes in any of the 4 groups compared to baseline or week 26.

6.3.2.8 GROα

Growth-related oncogene α (GROα), also called CXCL1, is related to IL-8 and attracts and activates neutrophil and basophil leucocytes (292) (293). GROα is produced by macrophages, neutrophils, epithelial cells, endothelial cells and fibroblasts (294). GROα levels are elevated in SSc patients compared to controls, SLE patients and dermatomyositis patients. GROα levels were similar in dcSSc and lcSSc patients. There is a correlation between GROα levels and IL-8 levels. When elevated GROα levels were defined as the mean plus 2 standard deviations of control serum samples, patients with elevated GROα levels had increased frequency of decreased lung function indices, kidney involvement, muscle involvement and anti-topoisomerase 1 antibody (295).

In our study, at 26 weeks, there was a decrease in GROα levels in HCS group and a slight increase in the placebo group, though the difference between groups was not statistically significant with p=0.2061. The combined 26 weeks post hoc data showed a smaller difference between the groups. At 52 weeks,

there were no significant differences in any of the 4 groups compared to baseline. The HCS group who stopped treatment at 26 weeks (2 patients) had a significant increase GROα levels compared to week 26, though this was due to one patient of the two and she had significant digital ulcers with infection and severe vasculopathy.

6.4 Conclusions

Systemic sclerosis is a complex multisystem autoimmune disorder that has a very high morbidity and the highest case-specific mortality of any rheumatic disorder with 50% of patients dying or developing major internal organ complications within 3 years of diagnosis (1). Currently, no treatment is proven to be effective in preventing progression of disease, reversing fibrosis or improving long-term outcome. Therefore there is a huge unmet clinical need for targeted and effective novel therapies.

In this study, we found that HCS is safe and well tolerated with few side effects. MRSS improved in the HCS group and worsened in the placebo group, with more responders in the HCS group at 26 weeks. Neuropathic pain, which is more common in SSc than previously recognised, also improved in the HCS group compared to placebo. We found a trend to benefit for lung function indices but as the study was not powered to look at lung outcomes, a larger study would be needed to confirm. SNIP testing may be useful in SSc patients with myositis or in patients who have dyspnoea of unknown origin. HRV testing confirmed autonomic dysfunction in our cohort of SSc patients but did not show a treatment effect.

A key strength of this study is the opportunity to analyse serum levels of candidate biomarkers that can be correlated with clinical change and also with administration of HCS. Although the approach taken in this study is essentially hypothesis generating it provides information that may lead to further exploration of the potential mechanisms of action of this novel agent and also help to define the serological changes that accompany clinically meaningful change in skin thickness in SSc. This has implications for future clinical trials but the findings described in this thesis will need further confirmation and

validation in SSc and also in different stages and subsets of disease. The most interesting results are the cytokine analyses. Using SAM® we identified 4 cytokines and proteins that were more than 2 fold increased in HCS patients compared to placebo patients and 5 that were more than 2 fold reduced. We found that α-MSH and ACTH were significantly increased with HCS treatment. The action of α-MSH and ACTH on the MC1R is one possible mechanism by which the MRSS in HCS treated patients may have improved as well as a local anti-inflammatory effect. MC1R is a potentially important novel target recently identified in SSc. PIIINP and bFGF were increased with HCS treatment and TGF-β1, TIMP-2, fractalkine, COMP and GROα were reduced with HCS treatment indicating that there may be different processes contributing to ongoing inflammation in established SSc compared to early SSc.

7 Concluding comments

In this chapter the overall implications of the work described in the thesis is considered and put into the context of other potential clinical strategies for HCS and also the emerging landscape of possible targets for therapy in systemic sclerosis. Future direction for study of HCS in systemic sclerosis is also considered.

7.1 Clinical implications

On reflection, a number of points can be made about the results of the trial. Firstly, the medication is relatively safe. Secondly, there is some signal of efficacy both clinically with change in MRSS and neuropathic pain score and in parallel, changes in serum proteins which is evident even with the small sample size. Thirdly, we hypothesize that we may have found a potential novel mechanism of action of HCS through the melanocortin system and that this system may explain the improvement in MRSS and neuropathic pain and the changes in the serum proteins in these patients.

Another clinical trial in secondary progressive MS, **Chapter 1, Section 1.2.5.3** (conducted at the same time as our trial but the results were reported later) also confirmed safety and reported some efficacy in MS patients. Serum cytokines have not been reported as yet, but it would be interesting to correlate our findings with the serum protein findings in that trial, in spite of different disease processes.

Animal studies performed in the scleroderma bleomycin mouse model after the completion of our trial (**Chapter 1, Sections 1.2.4.2-4**) show a non-significant increase in serum α-MSH and a non-significant decrease in serum MMP-1 and MMP-13, while a significant decrease was found in serum MMP-9, BAL fluid IL-12p70, MCP-1 and TNFα in the HCS treated group. Lung function improved in the HCS treated bleomycin lung model. In the bleomycin mouse skin model, α-MSH levels were increased or maintained in the HCS treated group. MC1R

expression did not change but MC4R expression decreased in the HCS treated group. Hydroxyproline levels and expression was lower in the HCS treated group indicating less fibrosis. PIIINP and TGFβ showed no significant change and TIMP-1 analysis revealed a strong trend in reduction in expression in the HCS treated group. These results are in keeping with some of the changes described in our study, though the bleomycin mouse model does not have all the features seen in human SSc, and therefore cannot be compared directly.

7.2 Suggestions for future studies

Some questions remain after analysing the results of the study. Future clinical trials in a larger cohort of SSc patients will be needed to 1) confirm the efficacy of HCS in reducing MRSS, 2) confirm the cytokine changes, 3) investigate the mechanism of action of HCS and/or confirm that the melanocortin system is involved by assessing melanocortin receptor expression and function in skin and serum samples, 4) investigate lung function changes in a cohort powered for assessment of lung function outcomes, and 5) consideration of a dose finding study as higher doses may improve MRSS outcomes . Our current study was in late-stage SSc patients. Other studies could potentially look at other stages or subsets of disease and other diseases including inflammatory diseases and diseases where the melanocortin system is implicated. Other scientific research could include experiments using HCS treatment on human dermal fibroblasts and looking at changes in fibrosis markers and melanocortin receptors. Other animal models of SSc could also be investigated.

In the first instance, a phase III double-blind clinical trial with a larger cohort of dcSSc patients should be considered. This would probably need to be a multicentre clinical trial due to challenges involved in recruiting the required number of patients. Some of these challenges include: the number of patients seen in a single centre, competing studies, eligibility criteria, patient consent and logistic challenges such as travel and childcare. There are now many studies recruiting or in planning stages because of the identification of new targets, as mentioned in the section below, and as the number of patients seen each year in a single centre is relatively constant, patients can be eligible for

more than one study but usually can be recruited into only one at a time causing competition for clinical trials. However, recruitment is possible with a well-designed study with clear objectives and outcomes.

Systemic sclerosis is considered a rare disease and to date, there is no effective treatment approved by the European Medicines Agency (EMA). Therefore SSc is designated an orphan disease and medications under development for treatment of SSc could potentially be eligible for orphan medicinal product designation. An orphan designation application may be submitted at any time in the development of a drug but has to be submitted before marketing authorisation. In the EU, this is submitted to the Committee for Orphan Medicinal Products in the EMA and the process can take up to 6 months. In the US, the corresponding office is the Food and Drugs Administration (FDA). There are a number of incentives in applying for orphan drug status, namely protocol assistance and follow-up, reduced or waived regulatory fees, tax credits or subsidies on clinical trials and of course, market exclusivity, which is granted only after marketing authorisation (296). An application for orphan medicinal product status should be submitted for HCS as the next step. If approved, this would provide assistance with other trials and would fast-track development of this novel medication.

7.3 Targeted therapies and future clinical development of HCS

Systemic sclerosis is a multisystem autoimmune rheumatic disorder with a high morbidity and case specific mortality. To date, no treatment has been proven to be unequivocally effective. In the past, immunosuppressive treatments have been borrowed from other diseases, but these treatments are broadly immunosuppressant with many unwanted side effects and toxicities. In recent years, as our understanding of the pathophysiological processes that cause SSc grows, there has been much interest in developing more targeted treatments in an attempt to modify one or more pathological processes in disease while restricting toxicity. There are now a huge number of possible targets of interest and many of these have specific medications that are currently in ongoing clinical trials, being considered for trials or could be considered in future trials, **Table 7.1,** (129) (297).

Table 7.1: Potential therapeutic targets in SSc.

Targets	Existing or potential drugs
TGF-β	CAT-192, Fresolimumab, GC1008, LY2382770
IL-1	Rilonacept
IL-6	Tocilizumab
IL-13	Tralokinumab, QAX576
IL-17	Ixekizumab, Brodalumab
CCL2	NOX-E36, CNTO 888
MCP-1/CCR2	PF-04136309, BMS-741672, MLN1202
CXCL12/CRCR4	AMD3100
PPARγ	Rosiglitazone, Pioglitazone
Endothelin	Bosentan, Ambrisentan, Macitentan
LPA	AM966 and AM095, SAR100842
Serotonin	Terguride, Cyproheptadine, SB 204741
Adenosine	Targeting the relevant adenosine receptor might be a novel therapeutic option in SSc, No current treatment.
Phosphodiesterases	Sildenafil, Tadalafil
Prostanoids	Iloprost, Treprostinil
Leukotrienes	Montelukast, Zileuton
Cannabinoids	Synthetic analogues of tetrahydrocannabinol such as ajulemic acid
Morphogen (Wnt, Notch, Hedgehog)	PRI-724, CWP232291, Resveratrol, DAPT, Vismodegib
PDGF	Imatinib, Nilotinib, Dasatinib, SU6656
CTGF	FG3019
Th17	Halofuginone
T-cell costimulation	Abatacept
Immunomodulatory	Pomalidomide, HSCT
Antioxidant	N-acetylcysteine
IL-2 receptor	Basiliximab
mTOR	Rapamycin
B cell	Rituximab, anti-CD19
BAFF/BlyS	Belimumab
STAT4	Statins
FRA-2 (AP-1 family)	T5224, small-molecule inhibitor of AP-1
Epigenetic pathways	HDAC inhibitors include trichostatin A, SAHA; DNA methyltransferase inhibitors such as 5-aza-2-deoxycytidine
Integrin signalling	Monoclonal antibody therapies against α5β6, α1β1 and α2β1 integrins

Although not in this list, the melanocortin pathway is an emerging pathway that should be explored further as a potential target for treatment in SSc. Recent evidence suggests that the melanocortin pathway and MC1R may be important in SSc (250) (251) (252).

The melanocortin pathway is an emerging pathway in a number of other diseases and there is renewed interest in using ACTH and other melanocortin peptides for new indications, based on recent discoveries about the melanocortin receptors, their functions and mechanisms of action. Unlike targeted therapies specifying one pathway, α-MSH could be considered a pro-resolving therapy, modulating a number of different pathways such as TNFα, IL-1β, prostaglandins, adhesion molecules, neutrophils, monocytes and phagocytosis (298).

Melanocortins have adrenal and extra-adrenal actions. Adrenal-based actions are mediated only by the action of ACTH on the MC2R receptor in the adrenals. This mechanism is also responsible for the unwanted side-effects of glucocorticoids and ACTH. Extra-adrenal actions are mediated by melanocortins (including ACTH) on the remaining 4 receptors. The receptors and their functions and agonist profiles are further explained in **Table 7.2**, (298) (299) (300).

Apart from α-MSH and ACTH, over the past decade a number of agonists, selective antagonists and small molecules have been developed, some of which may have therapeutic benefits (299). Melanocortins have been shown to be involved in many diseases, and therefore medications modulating the melanocortin pathway may have implications for some or all of these diseases. Besides SSc mentioned above, other rheumatological conditions such as RA and gout have previously benefited from α-MSH and ACTH treatment and improvement of gout has been associated with MC3R separate to the effect on adrenal MC2R. All of the following diseases have been associated with the

melanocortin pathway in recent published literature; bronchial asthma, inflammatory bowel disease, cardiac reperfusion injury, erectile dysfunction (associated with MC4R), infections/antimicrobial action, melanoma (associated with MC1R) (301), inflammatory brain disorders such as MS, meningitis, brain reperfusion injury (associated with MC3 and 4R), septic shock, allergic inflammation, obesity (associated with MC4R), neuropathic pain syndromes (associated with MC4R in rat model) (302) (303) and some degenerative brain disorders such as Alzheimer's disease (298) (299) (300).

Table 7.2: Melanocortin receptors, distribution, function and agonists

Receptor	Distribution and cell receptors	Function	Agonist profile
MC1R	Macrophages, neutrophils, endothelial cells, fibroblasts, chondrocytes, osteoblasts, osteoclasts, lymphocytes	Pigmentation, anti-inflammatory, anti-pyretic, pain modulation, regulation of skin physiology	α-MSH > ACTH >> γ-MSH
MC2R	Adrenals, skin, melanoma cells, osteoblasts, chondrocytes	Steroidogenesis	ACTH
MC3R	CNS, stomach, kidneys, heart, gut, thymus, placenta, macrophages, chondrocytes, osteoclasts	Cardiovascular, anti-inflammatory, energy homeostasis, feeding	γ-MSH = ACTH = α-MSH
MC4R	CNS, osteoblast	Feeding control, energy homeostasis, sexual function, anti-pyretic, neuropathic pain	α-MSH = ACTH >> γ-MSH
MC5R	Many peripheral tissues including exocrine glands, spleen, adipose tissue, skin, muscle, gut, lung, sexual organs, macrophages, T and B cells, chondrocytes	Exocrine secretion, lipolysis, sebaceous secretion, immunoregulatory functions	α-MSH > ACTH > γ-MSH

HCS increases α-MSH and ACTH and can potentially modulate the melanocortin system. HCS is already approved for motor neurone disease and

a number of other neurological diseases. Potentially, HCS could be used to treat some of the diseases listed above, depending on outcome of future studies and confirmation of mechanism of action/ which receptor it modulates and is a possible novel therapeutic agent for late-stage SSc and for neuropathic pain.

In conclusion, HCS is a potentially exciting novel therapeutic agent. In this thesis we have explored safety and possible efficacy of this agent as well as forming a hypothesis on a potential new mechanism of action and on an emerging exciting novel therapeutic target in SSc.

Appendix

8.1 Questionnaires

8.1.1 Scleroderma Health Assessment Questionnaire

In this section we are interested in learning how your illness affects your ability to function in daily life. Please feel free to add comments.

Please tick the one response that best describes your usual abilities **IN THE PAST SEVEN DAYS:**

	Without **ANY** difficulty	With **SOME** difficulty	With **MUCH** difficulty	**UNABLE** to do
DRESSING & GROOMING				
Are you able to:				
• Dress yourself, including tying shoelaces and doing buttons?	_____	_____	_____	_____
• Shampoo your hair?	_____	_____	_____	_____
ARISING				
Are you able to:				
• Stand up from an armless straight chair?	_____	_____	_____	_____
• Get in and out of bed?	_____	_____	_____	_____
EATING				
Are you able to:				
• Cut your meat?	_____	_____	_____	_____
• Lift a full glass to your mouth?	_____	_____	_____	_____
• Open a new milk carton?	_____	_____	_____	_____
WALKING				
Are you able to:				
• Walk outdoors on flat ground?	_____	_____	_____	_____
• Climb up five stairs?	_____	_____	_____	_____

Please tick any **AIDS or DEVICES** that you usually use for any of these activities:

___	Cane	___	Devices for dressing (button hook, zipper pull, long-handled shoe horn, etc.)
___	Walker	___	Special Utensils
___	Crutches	___	Special or built-up chair
___	Wheelchair	___	Other (specify: _____)

Please tick any categories for which you usually need **ASSISTANCE FROM ANOTHER PERSON**

___	Dressing & Grooming	___	Eating
___	Arising	___	Walking

Please tick the one response which best describes your usual abilities **IN THE PAST SEVEN DAYS:**

	Without **ANY** difficulty	With **SOME** difficulty	With **MUCH** difficulty	**UNABLE** to do
HYGIENE				
Are you able to:				
• Wash and dry your entire body?	_____	_____	_____	_____
• Take a tub bath?	_____	_____	_____	_____
• Get on and off the toilet?				
REACH				
Are you able to:				
• Reach and get down a 2 kilo object (such as a bag of sugar) from just over your head?	_____	_____	_____	_____
• Bend down and pick up clothing off the floor?	_____	_____	_____	_____
GRIP				
Are you able to:				
• Open car doors?	_____	_____	_____	_____
• Open jars that have been previously opened?	_____	_____	_____	_____
• Turn taps on and off?	_____	_____	_____	_____
ACTIVITIES				
Are you able to:				
• Run errands and shop?	_____	_____	_____	_____
• Get in and out of a car?	_____	_____	_____	_____
• Do everyday household cleaning?	_____	_____	_____	_____

Please tick any **AIDS or DEVICES** that you usually use for any of these activities:

___ Raised Toilet Seats

___ Long-handled appliances for reach

___ Long-handled appliances in bathroom

___ Bath tub bar

___ Bathtub Seat

___ Jar Opener (for jars previously opened)

Other (specify: _____)

Please tick any categories for which you usually need **ASSISTANCE FROM ANOTHER PERSON**

___ Hygiene

___ Reach

___ Gripping and opening things

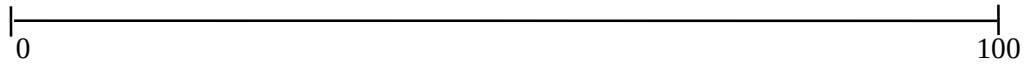
___ Errands and chores

We are also interested in learning whether or not you are affected by pain because of your illness.

How much pain have you had because of your illness **IN THE PAST WEEK?**

PLACE A MARK ON THE LINE TO INDICATE THE SEVERITY OF THE PAIN.

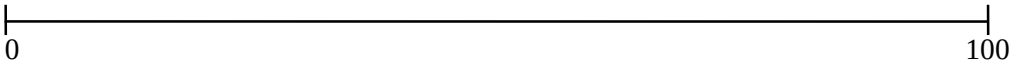
NO PAIN — VERY SEVERE PAIN



IN THE PAST WEEK how much have your intestinal problems interfered with your daily activities?

PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

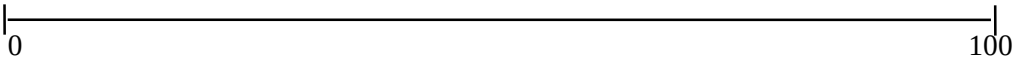
INTESTINAL PROBLEMS DO NOT LIMIT ACTIVITIES — VERY SEVERE LIMITATION



IN THE PAST WEEK how much have your breathing problems interfered with your daily activities?

PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

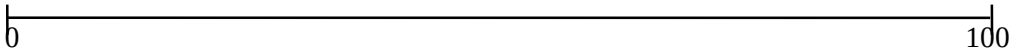
BREATHING PROBLEMS DO NOT LIMIT ACTIVITIES — VERY SEVERE LIMITATION



IN THE PAST WEEK how much has your Raynaud's interfered with your daily activities?

PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

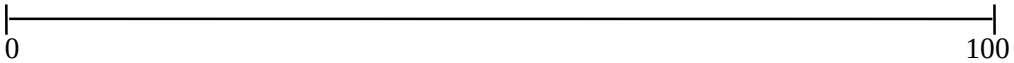
RAYNAUD'S DOES NOT LIMIT ACTIVITIES — VERY SEVERE LIMITATION



IN THE PAST WEEK how much have your finger ulcers interfered with your daily activities?

PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

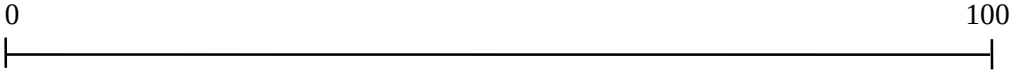
FINGER ULCERS DO NOT LIMIT ACTIVITIES — VERY SEVERE LIMITATION



Overall, considering how much pain, discomfort, limitations in your daily life and other changes in your body and life, how severe would you rate your disease today?

PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

NO DISEASE — VERY SEVERE LIMITATION



8.1.2 Short Form-36, version 2

For each of the questions below, please mark only one response in the box that best describes your answer.

1. In general, would you say your health is:

(1) Excellent	(2) Very good	(3) Good	(4) Fair	(5) Poor
❒	❒	❒	❒	❒

2. Compared to one year ago, how would you rate your health in general now?

(1) Much better now than one year ago	(2) Somewhat better now than one year ago	(3) About the same as a year ago	(4) Somewhat worse now than one year ago	(5) Much worse now than one year ago
❒	❒	❒	❒	❒

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

ACTIVITIES	(1) Yes, Limited a lot	(2) Yes, Limited a little	(3) No, not limited at all
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	❒	❒	❒
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	❒	❒	❒
c. Lifting or carrying groceries	❒	❒	❒
d. Climbing several flights of stairs	❒	❒	❒
e. Climbing one flight of stairs	❒	❒	❒
f. Bending, kneeling, or stooping	❒	❒	❒
g. Walking more than one kilometre	❒	❒	❒
h. Walking half a kilometre	❒	❒	❒
i. Walking one hundred metres	❒	❒	❒
j. Bathing or dressing yourself	❒	❒	❒

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	(1) All of the time	(2) Most of the time	(3) Some of the time	(4) A little of the time	(5) None of the time
a. Cut down the amount of time you spent on work or other activities	❒	❒	❒	❒	❒
b. Accomplished less than you would like	❒	❒	❒	❒	❒
c. Were limited in the kind of work or other activities	❒	❒	❒	❒	❒
d. Had difficulty performing the work or other activities (for example, it took extra effort)	❒	❒	❒	❒	❒

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	(1) All of the time	(2) Most of the time	(3) Some of the time	(4) A little of the time	(5) None of the time
a. Cut down the amount of time you spent on work or other activities	❒	❒	❒	❒	❒
b. Accomplished less than you would like	❒	❒	❒	❒	❒
c. Did work or other activities less carefully than usual	❒	❒	❒	❒	❒

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, neighbours, or groups?

(1) Not at all	(2) Slightly	(3) Moderately	(4) Quite a bit	(5) Extremely
❒	❒	❒	❒	❒

7. How much bodily pain have you had during the past 4 weeks?

(1) None	(2) Very Mild	(3) Mild	(4) Moderate	(5) Severe	(6) Very Severe
❒	❒	❒	❒	❒	❒

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

(1) Not at all	(2) A little bit	(3) Moderately	(4) Quite a bit	(5) Extremely
❒	❒	❒	❒	❒

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks...

	(1) All of the time	(2) Most of the time	(3) Some of the time	(4) A little of the time	(5) None of the time
a. Did you feel full of life?	❒	❒	❒	❒	❒
b. Have you been very nervous?	❒	❒	❒	❒	❒
c. Have you felt so down in the dumps that nothing could cheer you up?	❒	❒	❒	❒	❒
d. Have you felt calm and peaceful?	❒	❒	❒	❒	❒
e. Did you have a lot of energy?	❒	❒	❒	❒	❒
f. Have you felt downhearted and depressed?	❒	❒	❒	❒	❒
g. Did you feel worn out?	❒	❒	❒	❒	❒
h. Have you been happy?	❒	❒	❒	❒	❒
i. Did you feel tired?	❒	❒	❒	❒	❒

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

(1) All of the time	(2) Most of the time	(3) Some of the time	(4) A little of the time	(5) None of the time
❒	❒	❒	❒	❒

11. How TRUE or FALSE is each of the following statements for you?

	(1) Definitely True	(2) Mostly True	(3) Don't Know	(4) Mostly False	(5) Definitely False
a. I seem to get sick a little easier than other people	❒	❒	❒	❒	❒
b. I am as healthy as anybody I know	❒	❒	❒	❒	❒
c. I expect my health get worse	❒	❒	❒	❒	❒
d. My health is excellent	❒	❒	❒	❒	❒

8.1.3 Systemic Sclerosis Functional Score

ITEM		**Please tick one (please see key below)**			
		0	1	2	3
1	Can you lift and pour water (about **3 pints**) from a saucepan?				
2	Can you unscrew a jam-jar lid from a jar which has been opened?				
3	Can you take money (20p and £1) out of a purse with a thumb and second digit?				
4	Can you hold a pen and write your name?				
5	Can you hold a pen and write half a sheet of typing paper **(A4)**?				
6	Can you do and undo shirt buttons?				
7	Can you tuck your shirt or blouse into the waistband?				
8	Can you comb the back of your hair?				
9	Can you wash your hair?				
10	Can you get up from the toilet without using your hands?				
11	Can you walk up to 20 steps without using a banister?				

0 = Able to perform in normal manner
1 = Able to perform with alteration in style
2 = Can only manage with difficulty
3 = Impossible to achieve

8.2 HAQ and HAQ-DI additional results

There are eight functional activity category scores in the HAQ; dress and groom, arise, eating, walking, hygiene, reach, grip and activity. The descriptive statistics of each category are illustrated in the following tables, **Tables 8.1-8.**

At 26 weeks, no changes were seen in median values for any of these categories for patients in both groups. Statistically there was no overall significant difference between the groups in any of the eight categories: groom and dress, arise, eating, walking, hygiene, reach, grip and activity (p=0.6139, p=0.6560, p=0.3927, p=0.8015, p=0.2506, p=0.5363, p=0.5628, p=0.8133 respectively).

Table 8.1: Summary statistics for HAQ Dress and Groom scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	1	0.67	0	1	2	
	Week 6	Visit value	9	1	0.87	0	1	2	
		Change from baseline	9	0	0.5	-1	0	1	
		Adjusted mean change		-0.1	(-0.7, 0.4)				0.5982
	Week 26	Visit value	9	1.3	0.87	0	2	2	
		Change from baseline	9	0.3	0.5	0	0	1	
		Adjusted mean change		0.2	(-0.2, 0.6)				0.3174
Placebo	Baseline	Visit value	10	1.7	0.95	0	2	3	
	Week 6	Visit value	10	1.2	0.92	0	1	3	
		Change from baseline	10	-0.5	0.97	-3	0	0	
		Adjusted mean change		-0.4	(-0.9, 0.1)				0.1236
	Week 26	Visit value	9	1.8	0.67	1	2	3	
		Change from baseline	9	0	0.71	-1	0	1	
		Adjusted mean change		0.2	(-0.2, 0.6)				0.4251
Difference between groups	Week 6	Adjusted mean change		0.3	(-0.5, 1.0)				0.4898
	Week 26	Adjusted mean change		0	(-0.6, 0.6)				0.8782
Treatment groups were not significantly different (p = 0.6139) overall.									

Table 8.2: Summary statistics for HAQ Arise scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	0.7	0.67	0	1	2	
	Week 6	Visit value	9	0.9	0.93	0	1	2	
		Change from baseline	9	0.2	0.67	-1	0	1	
		Adjusted mean change		0.1	(-0.4, 0.7)				0.5674
	Week 26	Visit value	9	1	1.12	0	1	3	
		Change from baseline	9	0.3	0.87	-1	0	2	
		Adjusted mean change		0.3	(-0.3, 0.8)				0.3806
Placebo	Baseline	Visit value	10	0.9	0.99	0	1	3	
	Week 6	Visit value	10	1	0.67	0	1	2	
		Change from baseline	10	0.1	0.99	-2	0	1	
		Adjusted mean change		0.1	(-0.3, 0.6)				0.5239
	Week 26	Visit value	9	0.9	0.6	0	1	2	
		Change from baseline	9	-0.1	1.05	-2	0	1	
		Adjusted mean change		-0.1	(-0.6, 0.5)				0.8104
Difference between groups	Week 6	Adjusted mean change		0	(-0.7, 0.7)				0.982
	Week 26	Adjusted mean change		0.3	(-0.5, 1.1)				0.4271
Treatment groups were not significantly different (p = 0.6560) overall.									

Table 8.3: Summary statistics for HAQ Eating scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	0.9	0.88	0	1	2	
	Week 6	Visit value	9	1.1	1.05	0	1	3	
		Change from baseline	9	0.1	0.6	-1	0	1	
		Adjusted mean change		0.1	(-0.4, 0.6)				0.7015
	Week 26	Visit value	9	1.1	1.05	0	1	3	
		Change from baseline	9	0.1	0.6	-1	0	1	
		Adjusted mean change		0.1	(-0.3, 0.5)				0.6031
Placebo	Baseline	Visit value	10	1.2	1.03	0	1	3	
	Week 6	Visit value	10	0.9	0.88	0	1	2	
		Change from baseline	10	-0.3	0.82	-2	0	1	
		Adjusted mean change		-0.3	(-0.8, 0.2)				0.2073
	Week 26	Visit value	9	1.2	0.83	0	1	2	
		Change from baseline	9	-0.1	0.33	-1	0	0	
		Adjusted mean change		0	(-0.4, 0.3)				0.9867
Difference between groups	Week 6	Adjusted mean change		0.4	(-0.3, 1.1)				0.2532
	Week 26	Adjusted mean change		0.1	(-0.4, 0.6)				0.6999
Treatment groups were not significantly different (p = 0.3927) overall.									

Table 8.4: Summary statistics for HAQ Walking scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	0.7	0.95	0	0	2	
	Week 6	Visit value	9	0.8	0.97	0	0	2	
		Change from baseline	9	0	0	0	0	0	
		Adjusted mean change		0	(-0.4, 0.4)				0.9689
	Week 26	Visit value	9	1	1.22	0	0	3	
		Change from baseline	9	0.2	0.44	0	0	1	
		Adjusted mean change		0.2	(-0.3, 0.7)				0.4044
Placebo	Baseline	Visit value	10	0.9	0.88	0	1	2	
	Week 6	Visit value	10	1	0.82	0	1	2	
		Change from baseline	10	0.1	0.88	-1	0	2	
		Adjusted mean change		0.1	(-0.3, 0.5)				0.5345
	Week 26	Visit value	9	1	0.87	0	1	2	
		Change from baseline	9	0.2	0.97	-2	0	1	
		Adjusted mean change		0.2	(-0.3, 0.7)				0.3688
Difference between groups	Week 6	Adjusted mean change		-0.1	(-0.7, 0.5)				0.6477
	Week 26	Adjusted mean change		0	(-0.8, 0.7)				0.9703
Treatment groups were not significantly different (p = 0.8015) overall.									

Table 8.5: Summary statistics for HAQ Hygiene scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	1.8	1.23	0	2	3	
	Week 6	Visit value	9	1.4	1.01	0	2	3	
		Change from baseline	9	-0.2	0.44	-1	0	0	
		Adjusted mean change		-0.3	(-0.7, 0.2)				0.2828
	Week 26	Visit value	9	1.6	1.13	0	2	3	
		Change from baseline	9	-0.1	0.33	-1	0	0	
		Adjusted mean change		-0.1	(-0.5, 0.2)				0.4067
Placebo	Baseline	Visit value	10	2	1.05	0	2	3	
	Week 6	Visit value	10	1.9	1.2	0	2	3	
		Change from baseline	10	-0.1	0.88	-1	0	2	
		Adjusted mean change		-0.1	(-0.5, 0.4)				0.7706
	Week 26	Visit value	9	2.1	0.93	1	2	3	
		Change from baseline	9	0.2	0.67	-1	0	1	
		Adjusted mean change		0.3	(-0.1, 0.6)				0.1356
Difference between groups	Week 6	Adjusted mean change		-0.2	(-0.8, 0.5)				0.5573
	Week 26	Adjusted mean change		-0.4	(-0.9, 0.1)				0.108
Treatment groups were not significantly different (p = 0.2506) overall.									

Table 8.6: Summary statistics for HAQ Reach scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	1.3	0.95	0	2	2	
	Week 6	Visit value	9	1.4	1.13	0	2	3	
		Change from baseline	9	0.2	0.83	-1	0	2	
		Adjusted mean change		0.1	(-0.4, 0.6)				0.5809
	Week 26	Visit value	9	1.2	1.2	0	2	3	
		Change from baseline	9	0	1.12	-2	0	2	
		Adjusted mean change		-0.1	(-0.7, 0.6)				0.7845
Placebo	Baseline	Visit value	10	2	0.67	1	2	3	
	Week 6	Visit value	10	1.6	0.7	1	1.5	3	
		Change from baseline	10	-0.4	0.52	-1	0	0	
		Adjusted mean change		-0.3	(-0.8, 0.2)				0.1828
	Week 26	Visit value	9	1.8	0.97	0	2	3	
		Change from baseline	9	-0.2	0.67	-2	0	0	
		Adjusted mean change		-0.1	(-0.7, 0.5)				0.7451
Difference between groups	Week 6	Adjusted mean change		0.5	(-0.3, 1.2)				0.2126
	Week 26	Adjusted mean change		0	(-0.9, 1.0)				0.9758
Treatment groups were not significantly different (p = 0.5363) overall.									

Table 8.7: Summary statistics for HAQ Grip scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	1.7	0.67	0	2	2	
	Week 6	Visit value	9	1.3	1	0	2	2	
		Change from baseline	9	-0.3	0.71	-2	0	0	
		Adjusted mean change		-0.3	(-0.9, 0.2)				0.246
	Week 26	Visit value	9	1.3	1	0	2	2	
		Change from baseline	9	-0.3	0.71	-2	0	0	
		Adjusted mean change		-0.3	(-0.9, 0.2)				0.2073
Placebo	Baseline	Visit value	10	2.1	0.32	2	2	3	
	Week 6	Visit value	10	1.4	0.84	0	2	2	
		Change from baseline	10	-0.7	0.82	-2	-0.5	0	
		Adjusted mean change		-0.7	(-1.3, -0.2)				0.0147
	Week 26	Visit value	9	1.8	0.83	0	2	3	
		Change from baseline	9	-0.3	0.71	-2	0	0	
		Adjusted mean change		-0.4	(-0.9, 0.2)				0.1587
Difference between groups	Week 6	Adjusted mean change		0.4	(-0.4, 1.2)				0.3435
	Week 26	Adjusted mean change		0	(-0.7,	0.8)			0.9318
Treatment groups were not significantly different (p = 0.5628) overall.									

Table 8.8: Summary statistics for HAQ Activity scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	1.1	0.99	0	1.5	2	
	Week 6	Visit value	9	1.1	0.93	0	1	2	
		Change from baseline	9	0.1	0.33	0	0	1	
		Adjusted mean change		0	(-0.4, 0.4)				0.9259
	Week 26	Visit value	9	1.3	1.12	0	2	3	
		Change from baseline	9	0.3	0.71	0	0	2	
		Adjusted mean change		0.2	(-0.3, 0.8)				0.3381
Placebo	Baseline	Visit value	10	1.9	0.57	1	2	3	
	Week 6	Visit value	10	1.7	0.67	1	2	3	
		Change from baseline	10	-0.2	0.63	-1	0	1	
		Adjusted mean change		-0.1	(-0.5, 0.3)				0.5332
	Week 26	Visit value	9	1.9	0.78	1	2	3	
		Change from baseline	9	0	0.5	-1	0	1	
		Adjusted mean change		0.2	(-0.3, 0.7)				0.3514
Difference between groups	Week 6	Adjusted mean change		0.1	(-0.4, 0.7)				0.6426
	Week 26	Adjusted mean change		0	(-0.7, 0.8)				0.9701
Treatment groups were not significantly different (p = 0.8133) overall.									

8.3 SF- 36 additional results

The SF-36 is split into 8 domains, 4 for physical health (Physical functioning, Role Physical, Bodily pain and General Health) and 4 for mental health (Vitality, Social Functioning, Role Emotional and Mental Health). Role Physical has already been discussed in Chapter 3. The descriptive statistics of each of the other categories are illustrated in the following tables, **Tables 8.9-15.**

For the SF-36 domain scales that mostly contribute to the scoring of the physical health summary outcome, patients in the HCS group reported improvement between baseline and Week 6 in role-physical and general health, however this was not maintained at Week 26 as results indicate that there had been no change from baseline median in role-physical and a worsening in general health. For the two other domain scales, physical functioning and bodily pain in this category there were no changes in median results between baseline and Week 6 or Week 26. Scores for patients in the placebo group at Week 6 mostly declined in all but one (general health) of the domain scales that contribute mostly to the physical health summary outcome. At Week 26 patients in the placebo group either reported continued decline (physical functioning, role-physical) or remained unchanged (bodily pain). However there was a small improvement in median change from baseline for the general health domain. Overall for physical health there was no significant difference at week 6 or week 26 apart from role physical mentioned above. There were no significant changes in the 4 mental health domains between the groups.

Table 8.9: Summary statistics for SF-36 Physical Functioning scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	39.5	31.49	0	32.5	90	
	Week 6	Visit value	9	45.6	33.21	0	50	95	
		Change from baseline	9	5.6	12.36	-10	0	30	
		Adjusted mean change		5.9	(-6.3, 18.1)				0.3209
	Week 26	Visit value	9	40.6	36.18	0	35	100	
		Change from baseline	9	0.6	13.79	-20	0	20	
		Adjusted mean change		0.9	(-8.9, 10.7)				0.8509
Placebo	Baseline	Visit value	10	33.5	20.69	5	32.5	85	
	Week 6	Visit value	10	37	20.3	10	30	70	
		Change from baseline	10	3.5	21.35	-15	-7.5	45	
		Adjusted mean change		3.1	(-8.5, 14.7)				0.5782
	Week 26	Visit value	8	26.9	20.69	0	20	70	
		Change from baseline	8	-8.1	13.35	-25	-12.5	10	
		Adjusted mean change		-9.7	(-19.9, 0.4)				0.0596
Difference between groups	Week 6	Adjusted mean change		2.8	(-14.0, 19.6)				0.7301
	Week 26	Adjusted mean change		10.6	(-3.6, 24.8)				0.1319
Higher values indicate better functioning. Treatment groups were not significantly different (p = 0.3003) overall.									

Table 8.10: Summary statistics for SF-36 Pain scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	36.4	28.12	0	32	100	
	Week 6	Visit value	9	42.2	29.65	0	41	100	
		Change from baseline	9	4.2	29.76	-49	0	50	
		Adjusted mean change		1.3	(-17.1, 19.7)				0.8821
	Week 26	Visit value	9	38.8	31.27	0	41	100	
		Change from baseline	9	0.8	14.92	-32	0	19	
		Adjusted mean change		-2.1	(-17.5, 13.3)				0.7728
Placebo	Baseline	Visit value	10	52.4	32.38	0	46.5	100	
	Week 6	Visit value	10	45.6	25.13	12	41	80	
		Change from baseline	10	-6.8	33.52	-50	-6	72	
		Adjusted mean change		-2.8	(-20.4, 14.8)				0.7362
	Week 26	Visit value	9	35.6	23.17	12	22	74	
		Change from baseline	9	-11.6	30.59	-69	0	22	
		Adjusted mean change		-9.7	(-24.9, 5.5)				0.1944
Difference between groups	Week 6	Adjusted mean change		4.2	(-21.6, 29.9)				0.7363
	Week 26	Adjusted mean change		7.6	(-14.2, 29.4)				0.4722
Higher values indicate better functioning. Treatment groups were not significantly different (p = 0.5522) overall.									

Table 8.11: Summary statistics for SF-36 General Health scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	36.8	19.76	5	35	67	
	Week 6	Visit value	9	40.1	27.31	5	30	77	
		Change from baseline	9	4.8	10.39	-12	5	25	
		Adjusted mean change		5.9	(-7.6, 19.3)				0.3707
	Week 26	Visit value	9	36.2	26.51	10	25	77	
		Change from baseline	9	0.9	11.27	-10	-5	15	
		Adjusted mean change		2	(-10.4, 14.4)				0.7408
Placebo	Baseline	Visit value	10	31	18.47	0	26	62	
	Week 6	Visit value	10	27.3	14.41	5	26	52	
		Change from baseline	10	-3.7	26.03	-47	0	47	
		Adjusted mean change		-4.1	(-16.9, 8.6)				0.4996
	Week 26	Visit value	9	24	16.12	0	20	57	
		Change from baseline	9	-3.6	19.53	-42	5	20	
		Adjusted mean change		-7.9	(-19.8, 4.1)				0.1813
Difference between groups	Week 6	Adjusted mean change		10	(-8.6, 28.6)				0.2706
	Week 26	Adjusted mean change		9.8	(-7.4, 27.1)				0.2451
Higher values indicate better functioning. Treatment groups were not significantly different (p = 0.2342) overall.									

Table 8.12: Summary statistics for SF-36 Vitality scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	35.5	14.62	15	37.5	60	
	Week 6	Visit value	9	38.9	21.91	10	30	70	
		Change from baseline	9	2.2	23.33	-25	-5	50	
		Adjusted mean change		2.6	(-10.2, 15.5)				0.6678
	Week 26	Visit value	9	38.3	21.51	15	30	75	
		Change from baseline	9	1.7	21.65	-25	0	35	
		Adjusted mean change		2.1	(-10.1, 14.3)				0.7221
Placebo	Baseline	Visit value	10	34	20.92	10	27.5	70	
	Week 6	Visit value	10	33.5	15.82	15	35	60	
		Change from baseline	10	-0.5	17.23	-20	0	40	
		Adjusted mean change		-1.1	(-13.2, 11.1)				0.8537
	Week 26	Visit value	9	32.2	23.33	10	20	65	
		Change from baseline	9	-2.8	10.93	-20	0	15	
		Adjusted mean change		-2.8	(-14.9, 9.2)				0.6222
Difference between groups	Week 6	Adjusted mean change		3.7	(-14.0, 21.4)				0.6619
	Week 26	Adjusted mean change		4.9	(-12.2, 22.1)				0.5505
Higher values indicate better functioning. Treatment groups were not significantly different (p = 0.5617) overall.									

Table 8.13: Summary statistics for SF-36 Social Functioning scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	53.8	28.9	0	50	100	
	Week 6	Visit value	9	61.1	32.74	0	50	100	
		Change from baseline	9	9.7	27.08	-13	0	75	
		Adjusted mean change		11.1	(-9.1, 31.2)				0.2609
	Week 26	Visit value	9	56.9	27.32	25	37.5	100	
		Change from baseline	9	5.6	24.3	-25	0	50	
		Adjusted mean change		6.9	(-12.4, 26.2)				0.4588
Placebo	Baseline	Visit value	10	48.8	33.57	0	56.3	100	
	Week 6	Visit value	10	51.3	29.14	0	50	100	
		Change from baseline	10	2.5	37.64	-38	0	88	
		Adjusted mean change		2.5	(-16.5, 21.6)				0.7805
	Week 26	Visit value	9	36.1	34.49	0	25	100	
		Change from baseline	9	-6.9	34.86	-63	0	50	
		Adjusted mean change		-8.4	(-27.4, 10.6)				0.3629
Difference between groups	Week 6	Adjusted mean change		8.5	(-19.2, 36.3)				0.524
	Week 26	Adjusted mean change		15.3	(-11.8, 42.4)				0.2491

Higher values indicate better functioning.

Treatment groups were not significantly different (p = 0.3082) overall.

Table 8.14: Summary statistics for SF-36 Role Emotional scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	45.8	36.69	0	41.7	100	
	Week 6	Visit value	9	72.2	33.59	0	83.3	100	
		Change from baseline	9	24.1	28.7	0	25	83	
		Adjusted mean change		17.8	(-3.6, 39.1)				0.0973
	Week 26	Visit value	9	59.3	34.72	0	66.7	100	
		Change from baseline	9	11.1	34.86	-25	0	83	
		Adjusted mean change		4.8	(-19.7, 29.3)				0.6836
Placebo	Baseline	Visit value	10	70	34.96	25	87.5	100	
	Week 6	Visit value	10	61.7	30.23	25	62.5	100	
		Change from baseline	10	-8.3	42.67	-75	-8.3	67	
		Adjusted mean change		-1.4	(-21.8, 19.0)				0.8862
	Week 26	Visit value	9	57.4	40.71	0	50	100	
		Change from baseline	9	-9.3	43.39	-100	0	50	
		Adjusted mean change		-4.2	(-28.1, 19.7)				0.7144
Difference between groups	Week 6	Adjusted mean change		19.2	(-11.1, 49.4)				0.1977
	Week 26	Adjusted mean change		9	(-25.8, 43.8)				0.591
Higher values indicate better functioning. Treatment groups were not significantly different (p = 0.3391) overall.									

Table 8.15: Summary statistics for SF-36 Mental Health scale, baseline to week 6 and week 26.

			Summary statistics						
Treatment group	**Visit**	**Value**	**n**	**Mean**	**SD**	**Min**	**Median**	**Max**	**Prob**
HCS	Baseline	Visit value	10	58.8	21.42	20	68	80	
	Week 6	Visit value	9	61.8	21.83	20	68	88	
		Change from baseline	9	5.3	8.94	-8	0	20	
		Adjusted mean change		4.5	(-4.9, 14.0)				0.3225
	Week 26	Visit value	9	58.2	20.89	24	68	88	
		Change from baseline	9	1.8	14.16	-28	4	20	
		Adjusted mean change		1	(-11.4, 13.4)				0.868
Placebo	Baseline	Visit value	10	62	18.31	28	62	80	
	Week 6	Visit value	10	66.4	14.87	40	62	84	
		Change from baseline	10	4.4	18.03	-24	4	32	
		Adjusted mean change		5.5	(-3.5, 14.4)				0.2157
	Week 26	Visit value	9	57.3	22.09	20	64	88	
		Change from baseline	9	-2.7	22.54	-40	0	32	
		Adjusted mean change		-1.7	(-13.9, 10.4)				0.7675
Difference between groups	Week 6	Adjusted mean change		-0.9	(-14.0, 12.2)				0.8835
	Week 26	Adjusted mean change		2.7	(-14.7, 20.1)				0.7456
Higher values indicate better functioning. Treatment groups were not significantly different (p = 0.8934) overall.									

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