| | |
|---|---|
| **Study Title:** | Sclero XIII: a phase II, double-blind, randomised, placebo-controlled study to investigate the pharmacokinetics, safety and efficacy of intravenous factor XIII treatment in patients with systemic sclerosis |
| **Investigational medicinal product:** | Purified Factor XIII (Fibrogammin®) |
| **Protocol number:** | 13/0417 |
| **Phase:** | II |
| **Indication:** | Systemic sclerosis (scleroderma, SSc) |
| **Study design:** | Single-centred, randomised, double-blind, placebo controlled study |
| **Study start date:** | PK phase: 1st February 2016<br>Treatment phase: 5th September 2016 |
| **Study completion date:** | PK phase: 19th May 2016<br>Treatment phase: 23rd August 2018 |
| **Principal Investigator:** | Professor C. P. Denton<br>The Royal Free Hospital<br>Pond Street<br>London<br>NW3 2QG |
| **Study Sponsor:** | Joint Research Office<br>UCL Gower Street<br>London WC1E 6BT |
| **Supplier of investigational medicinal product:** | CSL Behring<br>Emil-von-Behring-Strasse 76<br>35041<br>Marburg<br>Germany |
| **Statisticians:** | Dr Tao Ding<br>Dr Gareth Ambler<br>University College London |
| **Report prepared by:** | Dr Anna Leslie |
| **Date of report:** | September 2019 |

**Synopsis**

<table>
<tr><td colspan="2">Study Title<br>Sclero XIII: a phase II, double-blind, randomised, placebo-controlled study to investigate the pharmacokinetics, safety and efficacy of intravenous factor XIII treatment in patients with systemic sclerosis</td></tr>
<tr><td>Investigational medicinal product:<br>Purified Factor XIII (Fibrogammin®)</td><td rowspan="2">Studied period<br>Date of first enrolment: 1st February 2016<br>Date of last follow up: 23rd August 2018</td></tr>
<tr><td>Phase of study: II</td></tr>
<tr><td>Study centre:<br>The Royal Free Hospital<br>Pond Street<br>London<br>NW3 2QG</td><td>Principal Investigator:<br>Professor C. P. Denton</td></tr>
<tr><td colspan="2">Number of patients (planned and analysed): Planned: 26, Actual: 26</td></tr>
<tr><td colspan="2">Objectives:<br>1. To identify any effects of factor XIII treatment on clinical manifestations of SSc<br>2. To investigate factor XIII safety<br>3. To measure individual factor XIII levels in patients with SSc<br>4. To measure the effects of factor XIII treatment on factor XIII PK parameters.<br>5. To explore effects of factor XIII on thrombospondin expression</td></tr>
<tr><td colspan="2">Methodology:<br>Pharmacokinetic (PK) phase: 4 patients with limited and 4 patients with diffuse systemic sclerosis received a single dose of factor XIII and levels were monitored over a six-week period.<br><br>Treatment phase: 9 patients with limited and 9 patients with diffuse systemic sclerosis were randomised 2:1 to receive Factor XIII or placebo by intravenous infusion weekly for 24 weeks.</td></tr>
<tr><td colspan="2">Main criteria for inclusion:<br>• Male and female adults aged ≥ 18 years<br>• Subjects with a diagnosis of limited or diffuse SSc<br>• Females of childbearing potential must be willing to use a reliable form of medically acceptable contraception and have a negative pregnancy test<br>• Patients who have given their free and informed consent</td></tr>
<tr><td colspan="2">Test product, mode of administration and dose: intravenous purified factor XIII (Fibrogammin®) at a concentration of 62.5 IU/ml, dose based on patient weight and endogenous factor XIII level</td></tr>
<tr><td colspan="2">Reference therapy, mode of administration and dose: intravenous 0.9% sodium chloride, volume based on patient's weight and endogenous factor XIII level</td></tr>
<tr><td colspan="2">Duration of Treatment: PK phase – single dose, Treatment phase – 24 weeks</td></tr>
<tr><td colspan="2">Evaluation Criteria – Safety:<br>• Physical examination (including height, weight, BMI, digital ulcer (DU) characterization)<br>• Adverse events<br>• Serious adverse events<br>• ECG</td></tr>
</table>

- Vital signs
- Clinical laboratory parameters
- Pregnancy
- Adverse events of special interest: thromboembolic events

**Evaluation Criteria – Efficacy:**

**Primary endpoints:**

- Skin involvement measured with modified Rodnan skin score
- Raynaud condition score

**Secondary endpoints:**

- Pulmonary function
- Hand function measured with Cochin hand function
- Quality of life measured with SF36 quality of life questionnaire
- Prevention of new DU: Number of new DU developed during a 24-week period of treatment
- Healing of DU: Complete healing of DUs present at baseline; each DU is considered as an entity
- DU Pain assessment at 4, 8, 12, 16, 24 weeks of treatment: Pain will be assessed by analogue scale for pain (VAS) and Raynaud's severity (Raynaud's condition score)
- DU worsening, defined as:
    - Overnight Hospitalization for digital ulcers
    - Addition surgical treatment for digital ulcer in outpatient clinic
    - Digital ulcer infection
    - Gangrene and/or amputation
    - Need of local sympathectomy
    - Need of toxin botulinum A)
    - Need of oral or parenteral antibiotic
    - Need of IV Iloprost: this is considered treatment failure

**Statistical Methods:**

**Summary of baseline data and flow of patients**

Clinical and demographic data were used to assess baseline comparability of the randomised groups including all the variables that were measured as primary or secondary outcome assessments.

**Primary outcome analysis**

This is an early stage exploratory study not looking for statistical significance. It included a small number of subjects to assess safety and feasibility of the study intervention in this patient population. The outcome analysis is descriptive with summary statistics and confidence interval being determined. For the primary outcomes we also assessed the possible effect size by comparing baseline with week 26 values. These changes were compared with the recognised minimal clinically important difference (MCID).

**Safety analysis and results**

Safety was assessed by summarising the incidence and type of adverse events in both phases of the trial, through to the end of follow-up. Laboratory safety parameters were assessed by tabulation of results and comparison throughout the duration of the trial. There were no safety concerns raised during or after the trial.

**Conclusions**

The study was fully recruited. There were no significant safety concerns. As this is a small trial all analysis is descriptive and no robust conclusion about efficacy can be drawn from the data although there was observation of improvement in some variables for both active treatment and placebo treated patients. Therefore, this study confirms feasibility of recruitment to the designed trial and provides a platform for future studies including any further evaluation of Factor XIII in systemic sclerosis. The justification for this is likely to depend upon work outside the present trial including possible mechanistic and preclinical scientific experiments.

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Figure A.2: Thombospondin-1 level change over time: FXIII or placebo (dcSSc only)

**Ethics**

The study (Protocol version 1.1 dated 31-12-2014) was initially approved by the Royal Free Hospital NHS Trust Research Ethics committee on 16th February 2015. Approval from the Medicines and Healthcare products Regulatory Agency (MHRA) was granted on 24th February 2015.

**Patient Information and Consent**

The process of informed consent was initiated by the investigator prior to any study procedures being carried out. Patients were provided with extensive verbal and written information regarding the possible risks and proposed benefits of the study. Information was provided to patients' friends and family at the patient's request.

The informed consent form (ICF) was provided to the patient a minimum of 24 hours in advance of the proposed screening visit, together with the patient information sheet (PIS). Patients were encouraged to share the information with their friends and family, as well as their General Practitioner (GP) if they chose to do so. If the patient chose to attend the proposed screening visit, the PIS and ICF were discussed by the investigator with the patient, and any question the patient had were answered. If the patient was satisfied, they then signed the ICF prior to any study-related procedure being carried out. A copy was provided to the patient for their records.

Patients were made aware that informed consent could be withdrawn at any time without a reason needing to be provided. They were also informed that their right to high quality medical care would not be compromised if they decide not to partake in the study.

Once informed consent as gained, the patient's history was reviewed by the investigator to ensure they met the eligibility criteria as defined by the protocol.

Patients were informed that their medical notes would be reviewed by the Sponsor as part of routine study monitoring. They were also informed that data would be gathered into an anonymised database and this would be shared with the wider study team, including the drug manufacturers CSL Behring for the purposes of generating dosing information, and the study statisticians for the statistical interpretation of the study.

The role of the principal and sub investigators on the study were:

1. To ensure that written informed consent was gained prior to the initiation of any study related procedure.
2. To ensure patients met the eligibility criteria before entering the study.
3. To ensure appropriate monitoring took place.
4. To ensure the rights of the patients were protected.
5. To ensure that the study data were accurate, complete and verifiable from source documents.
6. To ensure that the study ran in compliance with the protocol and according to the tenants of Good Clinical Practice (GCP).

## 1. Introduction

## 1.1 Systemic Sclerosis

### 1.1.1 Overview

Systemic sclerosis (SSc), also known as scleroderma, is a rare multi-system auto-immune connective tissue disease with significant morbidity and mortality. It leads to the accumulation of collagen and other extra-cellular matrix components in the skin and internal organs, and vascular damage. It is associated with the development of specific autoantibodies, which include anti-nuclear antibody (ANA), anti-Scl70 antibody (also known as anti-topoisomerase 1 antibody), and anti-centromere antibody (ACA). There are many signs and symptoms seen in systemic sclerosis. The eponymous symptom is skin thickening, but the disease also leads to painful tri-fasic colour changes in the fingers and toes (Raynaud's phenomenon), arthralgia, dysphagia, dyspepsia, digital ulcers, bowel disturbance and shortness of breath.

### 1.1.2 Classification

The initial classification criteria for SSc were developed in 1980 by Masi et al; and formed the preliminary American College of Rheumatology (ACR) classification criteria for SSc (table 1.0). They were not designed as a diagnostic tool and had a high specificity, therefore there was a degree of under-diagnosis using these criteria, particularly affecting patients with mild or no skin involvement (Masi et al., 1980). The preliminary ACR criteria also did not take into account the auto-antibodies which have been shown to correlate with systemic sclerosis (Reveille and Solomon, 2003).

***Table 1.0: 1980 American College of Rheumatology preliminary classification criteria for systemic sclerosis (Masi et al., 1980)***

| | |
|---|---|
| Major Criterion | 1. Proximal scleroderma |
| Minor Criteria | 1. Sclerodactyly |
| | 2. Digital pitting scars of fingertips or loss of substance of the distal finger pad |
| | 3. Bibasilar pulmonary fibrosis |
| One major or two or more minor criteria were found to confer a high specificity for systemic sclerosis | |

A set of diagnostic criteria were developed by LeRoy and Medsger in 2001 (table 1.1) (LeRoy and Medsger Jr., 2001). These criteria aimed to encompass systemic sclerosis-associated autoantibodies and also consider the vascular effects of the disease.

***Table 1.1: 2001 criteria for the diagnosis of Systemic Sclerosis (LeRoy and Medsger Jr., 2001)***

| | | |
|---|---|---|
| Raynaud's phenomenon objectively documented by: | 1. Direct observation of any 2 of: | A. Pallor (well demarcated whitening of acral skin) |
| | | B. Cyanosis (dusky blueness, which disappears on rewarming) |
| | | C. Suffusion (well demarcated redness) |
| OR | 2. Direct measurement of response to cold by: | A. Objective evidence of delayed recovery after cold challenge |
| | | B. Nielsen test or equivalent |

| | |
|---|---|
| PLUS | 1. Abnormal wide-field nail fold capillaroscopy, consisting of dilation and/or avascular areas |
| OR | SSc selective autoantibodies (anti-centromere, anti-topoisomerase I, anti-fibrillarin, anti-PM-Scl, anti-fibrillin or anti-RNA polymerase I or III in a titre of 1:100 or higher) |
| *If RP is subjective only, both SSc capillary pattern and SSc selective autoantibodies (in titre > 1:100) are required to define LSSc. LSSc can overlap with any other disease.* | |

The LeRoy criteria from 2001 were followed in 2013 by the ACR/EULAR classification criteria for systemic sclerosis (table 1.2) (Hoogen, 2013). These were developed by an international panel of physicians specialising in systemic sclerosis.

***Table 1.2: Preliminary ACR/EULAR criteria for systemic sclerosis (Hoogen, 2013)***

| Items | | Weight |
|---|---|---|
| Skin thickening of the fingers of both hands, extending proximally to the metacarpophalangeal (MCP) joints | | 9 |
| Skin thickening of the fingers *(only count the highest score)* | Puffy fingers | 2 |
| | Whole Finger, distal to MCP | 4 |
| Fingertip lesions *(only count the highest score)* | Digital Tip Ulcers | 2 |
| | Pitting Scars | 3 |
| Telangiectasia | | 2 |
| Abnormal nail fold capillaries | | 2 |
| Pulmonary arterial hypertension and/or Interstitial lung Disease | | 2 |
| Raynaud's phenomenon | | 3 |
| Scleroderma related antibodies (any of anti-centromere, anti-topoisomerase I or anti-RNA polymerase III) | | 3 |
| *Patients having a total score of 9 or more are being classified as having definite systemic sclerosis* | | |

Systemic sclerosis with skin involvement is classified into two subsets: diffuse cutaneous systemic sclerosis and limited cutaneous systemic sclerosis. These are defined by the extent of skin involvement and diagnostic criteria have also been established by LeRoy and Medsger (LeRoy and Medsger Jr., 2001).

***Table 1.3: Criteria for Diagnosis of Subsets of SSC, adapted from (LeRoy and Medsger Jr., 2001)***

| *Disease subset* | *Criteria* |
|---|---|
| LcSSc | Criteria for SSc plus distal cutaneous changes |
| DcSSc | Criteria for SSc plus proximal cutaneous changes |

### 1.1.3 Epidemiology

The incidence and prevalence of systemic sclerosis has been difficult to estimate historically due to different methods of diagnosis and classification, as described above. Even with the 2013 ACR/EULAR classification criteria now widely accepted by the international community, estimates of incidence and prevalence vary geographically. This may be partly due to differing reporting rates related to different

health care systems, reporting methods and disease definitions. The variation may also be partly due to true variation between populations due to genetic or environmental factors.

Many data used to estimate incidence and prevalence rates are from the latter half of the 20th century. Reviewing more recent data from the 21st century shows a wide variation in published incidence and prevalence rates of systemic sclerosis. Diagnostic coding data from the USA from 2003-2008 produces an incidence rate of 46 cases per million per year and a prevalence of 135-184 cases per million (Furst et al., 2012). Diagnostic coding data gathered Canada in 2003 gives a prevalence rate of 443 cases per million (Bernatsky et al., 2009). Data gathered in England in 2000 generates a prevalence of 88 cases per million (Allcock et al., 2004), whereas data gathered in France in 2006 gives a prevalence of 132.2 cases per million (El Adssi et al., 2013). The variation is likely to be due in part to different methods of data collection, data analysis and the use of different classification criteria to define the disease.

It has been suggested that the incidence rate of systemic sclerosis is increasing over time. In 1971 Medsger and Masi published data reporting that the incidence rate of systemic sclerosis in Tennessee increased from 0.6 cases per million per year between 1947 and 1952; to 4.5 cases per million per year between 1953 and 1968 (Medsger and Masi, 1971). Similar increases have been reported in Pennsylvania where reported incidence of systemic sclerosis rose from 9.6 cases per million per year between 1963 and 1972, to 18.7 cases per million per year between 1973 and 1982 (Steen et al., 1997) However during the periods that have been reviewed, healthcare has improved significantly on a global level, perhaps leading to better diagnosis and increased reporting. In addition, the classification criteria discussed above have been published and used widely in clinical practice, so patients presenting now might receive a diagnosis of systemic sclerosis, where physicians may previously have assigned an alternative diagnosis. In 2014, Andreasson *et al* compared incidence and prevalence rates in Sweden using both the 1980 and 2013 ACR/EULAR classification criteria for systemic sclerosis. They found that applying the 2013 criteria to the patient population generates incidence and prevalence rates which are 30-40% higher than those determined using the 1980 criteria (Andreasson et al., 2014).

Geographic variations in the incidence of SSc lead to speculation that genetic factors may play a significant role. As discussed above, incidence rates for North America tend to be higher than those for Europe, even when different data collection methods are taken into account. In 1996, Arnett et al. reported a high incidence of SSc among a native American population in Oklahoma, and interestingly the patients from this population mostly displayed a similar phenotype of SSc, suggesting that a genetic factor relating to a specific human leukocyte antigen (HLA) might be contributing to the high incidence (Arnett et al., 1996). Other racial variations have been noted including a higher prevalence among black patients compared to white, and a higher rate of diffuse SSc and interstitial lung disease (ILD) among black patients (Mayes et al., 2003, Le Guern et al., 2004).
Additionally, high incidences in geographic locations where there is significant genetic variation in the population could suggest environmental factors are present that predispose patients to developing SSc. Examples of such areas include higher than expected rates in Ontario, Canada, compared to other local areas (Thompson and Pope, 2002); and higher rates around London airports compared to the rest of the city (Silman et al., 1990).

All epidemiological studies of SSc note that it is much more common in females, and this is true across different geographical locations. Estimated female to male ratio of disease incidence varies from 1.5:1 (Medsger and Masi, 1971) to 14:1 (Tamaki et al., 1991).

### 1.1.4 Aetiology

The aetiology of the disease remains unknown, but it is thought to be due to both genetic and environmental factors.

Due to the rare nature of the condition, only one twin study has been published, comparing 42 twin pairs where at least one twin had SSc. This study found a concordance rate of 4.7% with no variation between monozygotic and dizygotic twins (Feghali-Bostwick et al., 2003). There are also limited numbers of studies that look at heritability in a large cohort. Published data suggest an increased relative risk of between 3.07 and 14.3 when a first degree relative has a diagnosis of SSc, and other data suggest that the presence of SSc in a first degree relative increases the relative risk of other autoimmune conditions (Frech et al., 2010, Chandran et al., 1995, Arnett et al., 2001, Hudson et al., 2008).
Though the epidemiological data show that SSc is not inherited in a Mendelian fashion, genetics are still believed to be relevant in the aetiology of the disease. It is most likely that multiple genes are involved in predisposing a person to the development of SSc. Candidate genes are involved in innate and adaptive immunity, apoptosis and fibrosis.

There is a well-established link between the presence of certain HLA sub-types and the development of SSc; in particular SSc has been shown to correlate with HLA DQB*0301, DRB1*1104 and DQA1*0501 (Arnett et al., 2010) . Furthermore, the different SSc auto-antibodies have been shown to be associated with different HLA sub-types. For example, HLA DRB1*11 correlates with the presence of the Scl-70 antibody whereas HLA-DRB1*01, DRB01*04 and DQB1*0501 correlate with the presence of the anti-centromere antibody (Reveille et al., 2001). Other immune system genetic variants linked with the development of SSc include interferon 5 and 8 (IL-5, IL-8), and toll-like receptor 2 (TLR-2) (Dieude et al., 2009, Gorlova et al., 2011, Broen et al., 2012).

Exposure to some environmental triggers has been linked to the development of SSc. The most well reported environmental triggers are solvents and silica. There are multiple reports suggesting that exposure to chemical solvents can predispose people to the development of SSc. A meta-analysis of 11 case control series by Kettaneh et al. has confirmed an increased risk in those exposed to chemical solvents, although it was impossible to determine which solvents particularly may increase risk due to the wide variety of solvent types and the limited number of reports available (Kettaneh et al., 2007). A further meta-analysis by Mc Cormic et al. indicates that the relative risk of development of SSc is increased in men who have been exposed to silica (McCormic et al., 2010).

Infectious agents have long been considered relevant to the aetiology of autoimmune diseases. The hypothesis is that the presence of specific infectious agents causes the immune system to develop antigens against self via molecular mimicry. Various pathogens have been implicated in the development of SSc, particularly viral infections including Epstein-Barr virus, cytomegalovirus and parvovirus B19 (Farina et al., 2014, Halenius and Hengel, 2014, Magro et al., 2004). However, it is difficult to establish a causal link due to the high rates of infection with these common viral illnesses in the general population.

### 1.1.5 Pathogenesis

The pathogenesis of SSc is incompletely understood and appears to involve the interaction of multiple different factors. It is generally believed that vascular dysfunction helps to contribute to immune activation, the release of soluble mediators and a dysfunctional extracellular matrix environment. This

then results in inappropriate fibroblast activation and the deposition of excess collagen (1996, Sollberg et al., 1994).

*Vascular dysfunction*

Vascular changes occur early in the disease process of SSc and include perivascular infiltration, capillary leakage and disorganized, abnormal capillary network structure (Cutolo et al., 2003). This manifests clinically as Raynaud's phenomenon and abnormal nail fold capillaroscopy, which often pre-date other symptoms and signs.

Multiple vascular processes have been implicated in the pathogenesis of SSc, including those involved in vasoconstriction, vasodilation, angiogenesis, vasculogenesis and vascular endothelial cell function. Endothelin is a vasoconstrictor and fibrogenic molecule which may be involved in the pathogenesis of SSc by contributing to early vascular dysfunction (Zamora et al., 1990). Levels of endothelin are raised in patients with predominantly vascular symptoms of SSc; and in patients with a more fibrotic phenotype, suggesting that it may also contribute to fibrosis (Yamane et al., 1992, Vancheeswaran et al., 1994). This is supported by data that show a dose-dependent collagen-synthesising effect for endothelin when applied to fibroblasts (Kahaleh, 1991) and that endothelin is upregulated in the fibroblasts of SSc patients (Kawaguchi et al., 1994).

Nitric oxide is a vasodilator, which in normal vessels counterbalances the effects of endothelin. Studies have shown that nitric oxide-producing compounds are overall reduced in the plasma of patients with SSc, and also the normal production of nitric oxide in response to a cold challenge is reduced in these patients (Kahaleh and LeRoy, 1999). However the input of nitric oxide is not clear, because a further study has shown elevated nitric oxide levels in the plasma of SSc patients (Andersen et al., 2000).
A study by Flavahan et al has shown that the arterioles in SSc patients are abnormally sensitive to selective alpha-2 adrenergic receptor agonists. This raises the suggestion that although the vasoconstrictor/vasodilator balance may be disrupted in SSc, the vessel response to these mediators may also be abnormal (Flavahan et al., 2000).

In the healthy population, tissue hypoxia leads to angiogenesis in response. Angiogenesis is the formation of new blood vessels as a branch from pre-existing vessels whereas vasculogenesis is the formation of new vessels independent of pre-existing vessels. Vessels formed by vasculogenesis are formed from circulating endothelial progenitor cells. These two processes are necessary to provide oxygen and nutrients to growing tissues, such as during gestation and childhood. They involve multiple signalling pathways and an interplay between pro-angiogenic and anti-angiogenic molecules.

Angiogenesis is triggered by hypoxia inducible factor (HIF), a transcription factor whose expression increases in hypoxic conditions. It binds to the hypoxia response element (HRE) located in the 3'-flanking region of the EPO gene which encodes erythropoietin (Semenza and Wang, 1992). It also has binding sites in the genes for vascular endothelial growth factor (VEGF) (Forsythe et al., 1996), transforming growth factor $\beta_3$ ($TGF\beta_3$) (Caniggia et al., 2000), membrane type 1 matrix metalloproteinase (Petrella et al., 2005) and overall activates over 40 genes, many of which are involved in angiogenesis (Hirota and Semenza, 2006). VEGF is known to be an important pro-angiogenic factor to the extent that embryos with mutations in VEGF are unviable (Ferrara, 2001).

It has been hypothesised that patients with SSc are deficient in part of the angiogenesis pathway, so tissue hypoxia does not trigger appropriate angiogenesis, resulting in ischaemic damage such as digital

ulceration (Distler et al., 2002, LeRoy, 1996). As VEGF is a key player in angiogenesis, its role in SSc has been investigated.

Patients with SSc actually exhibit higher levels of VEGF than their healthy counterparts. In skin biopsies taken from 15 patients, the mean percentage of keratinocytes expressing VEGF was 14% in healthy controls and 50% in SSc patients. However, the levels of HIF were not consistent with this upregulation, suggesting that a HIF-independent mechanism may be the cause of the VEGF upregulation (Distler et al., 2004). It seems that the above-average VEGF levels might actually have a negative effect on blood vessels, rather than a pro-angiogenic one. Sustained high levels of VEGF cause vessels to form in an uncontrolled manner and with increased permeability, which has been noted to resemble the vascular changes seen in SSc (Dor et al., 2002).

VEGF levels correlate well with the development of digital ulcers. The link between VEGF and digital ulceration was studied by Distler et al in 2002. The highest levels of VEGF were seen in SSc patients without digital ulcers. SSc patients with digital ulcers also had raised levels, but lower than their ulcer-free counterparts. Healthy controls had lower levels of VEGF than SSc patients (Distler et al., 2002). This suggests that high levels of VEGF are protective against digital ulceration in SSc.
Platelet derived growth factor (PDGF) is a growth factor from the same family as VEGF, which is implicated in angiogenesis and tissue remodelling. It has been found in the endothelial cells of patients with SSc (Gay et al., 1989).

Thrombospondin-1 (TSP-1) is a known anti-angiogenic modulator. It works via both direct and indirect mechanisms. Its direct effect is to inhibit the migration of endothelial cells, and to induce apoptosis in these cells, therefore preventing them from contributing to new vessel formation (Lawler, 2002). Its indirect effect is to cause the activation and chemotaxis of inflammatory cells (BenEzra et al., 1993), and the growth and migration of myofibroblasts (Nicosia and Tuszynski, 1994). It is also known that TSP-1 inhibits pro-angiogenic factors such as metalloproteinases (Rodriguez-Manzaneque et al., 2001).

TSP-1 is thought to be directly involved in the pathogenesis of systemic sclerosis, as upregulated mRNA (Mimura et al., 2005) and increased protein synthesis of TSP-1 have been demonstrated in skin and cultured fibroblasts from patients with systemic sclerosis (Macko et al., 2002). Levels of TSP-1 have also been shown to correspond clinically with the modified Rodnan skin score, a validated method of assessing the severity of skin fibrosis in systemic sclerosis (Farina et al., 2010, Delbarre et al., 1981).
Vasculogenesis is also thought to be impaired in SSc patients, who have significantly reduced numbers of circulating endothelial progenitor cells from which vasculogenesis can be generated. The endothelial progenitor cells that do exist are also less capable of differentiating into endothelial cells *in vitro* (Kuwana et al., 2004).
Vascular endothelial cells behave abnormally in SSc. This is due to multiple factors. Approximately 25% of patients with SSc produce antibodies directed against endothelial cells. These antibodies lead to the upregulation of adhesion molecules on the surfaces of endothelial cells, and can also initiate apoptotic pathways in endothelial cells (Carvalho et al., 1996, Sgonc et al., 2000, Worda et al., 2003, Ahmed et al., 2006). Endothelial cell apoptosis may also be triggered by generation of the complement-derived membrane attack complex, which has been detected in the vasculature of SSc patients (Sprott et al., 2000). This is thought to be secondary to chronic endothelial cell activation which eventually leads to endothelial cell damage (McHugh et al., 2003).

An interaction between endothelial cells and immune cells via adhesion molecules may contribute to the recruitment of inflammatory cells in the pathogenesis of SSc. Adhesion molecules such as

intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) are usually displayed by endothelial cells following trauma, in order to recruit inflammatory cells into the extracellular matrix for tissue repair. In SSc, the endothelial cells constitutively display higher than expected levels of the adhesion markers ICAM-1 and E-selectin (Claman et al., 1991, Sollberg et al., 1992, Gruschwitz et al., 1992). The soluble forms of the markers ICAM-1, VCAM-1 and E-selectin have been isolated from the blood of SSc patients. High levels of these markers have also been linked to more aggressive disease (Andersen et al., 2000, Sfikakis et al., 1993). Subtypes of T-cells from patients with SSc have an increased ability to adhere to the endothelium compared to non-SSc T-cells (Rudnicka et al., 1992).

*Immune activation*

A variety of immune cells behave abnormally in the SSc phenotype and may be related to its pathogenesis. The innate immune system is implicated in the pathogenesis of SSc, with potential roles for TLRs PRRs and dendritic cell activation (York, 2011, Bhattacharyya et al., 2011).
CD4+ T-cells are more likely to migrate across the vascular endothelium in SSc compared to controls, leading to a higher than expected CD4:CD8 ratio for the migrated cells. The migrated CD4+ cells also display an activated phenotype including expressing adhesion proteins (Stummvoll et al., 2004). The types of mononuclear cells that are recruited into the extracellular matrix in SSc are also unusual in that they display T-cell markers and multiple integrins at their surface (Prescott et al., 1992).
Antibodies directed against self-antigen (autoantibodies) are frequent in SSc and form part of the 2013 classification criteria (see above). The most common autoantibodies are anti-centromere antibody and anti-Scl-70 antibody (also known as anti-topoisomerase-I antibody). Other less common antibodies include anti-RNA polymerase antibody, anti-fibrillarin antibody (also called anti-U3 RNP antibody), anti PM-Scl antibody, anti U1-RNP antibody, anti Th/To antibody, anti U11/U12 antibody and anti-Ku antibody. Autoantibodies more commonly associated with other autoimmune diseases may co-exist in patients with overlap conditions, for example a positive anti-Ro antibody might be seen in a patient with SSc/myositis overlap syndrome.

The SSc autoantibodies are generally regarded as markers of disease rather than key mediators in the disease pathogenesis. However some data have shown that anti-Scl-70 antibody binds specifically to topoisomerase-1 on the fibroblast surface, which in turn stimulates monocyte adhesion and activation (Henault et al., 2006).

Other less well recognized autoantibodies may have a pathogenic role in SSc. A study by Chizzolini et al showed that 58% of serum samples from SS patients displayed anti-fibroblast antibodies (AFA), in contrast none of the healthy control serum samples tested were positive for AFA. The serum from AFA positive patients also induced upregulation of interleukin-6 (IL-6) and ICAM-1, suggesting that the presence of this antibody might be involved in pathogenesis (Chizzolini et al., 2002).

*Soluble mediators*

Multiple soluble mediators in the form of growth factors and cytokines have been implicated in the pathogenesis of SSc. Mediators are produced by inflammatory cells as well as fibroblasts. They are produced and persist in both autocrine and paracrine feedback cycles, which may explain the persistence of the SSc fibroblast phenotype *in vitro* even after several cell passages (Leroy, 1972, Uitto et al., 1979).

The importance of endothelin, nitric oxide, VEGF, PDGF and TSP-1 are discussed above. Transforming growth factor beta (TGFβ) is discussed below.

IL-6 has been implicated in SSc pathogenesis, since levels have been shown to be elevated in the serum and skin of SSc, particularly in patients with DcSSc (Khan et al., 2012). Other interleukins of interest include interleukin-1 (IL-1) and interleukin-4 (IL-4). IL-1 is overproduced in SSc, and may be linked to excess IL-6 production (Kawaguchi et al., 1999, Kawaguchi et al., 2004). IL-4 may play a role in inducing the pro-fibrotic SSc fibroblast phenotype (Distler et al., 2006).

CXCL4 is an anti-angiogenic chemokine, levels of which have been found to be elevated in the plasma of SSc patients (van Bon et al., 2014) and this may therefore play a role in the pathogenesis of the disease. Connective tissue growth factor (CTGF) is a growth factor induced by TGFβ which is implicated in fibrotic conditions including SSc. Levels of the N-terminal portion of CTGF have been shown to be elevated in SSc, and the levels correlate with the extent of skin fibrosis (Dziadzio et al., 2005).

*Fibroblasts*

Fibroblasts are a cell type that is common in the connective tissue. Fibroblasts synthesise extracellular matrix proteins including collagen. They therefore have a key role in maintaining the function and integrity of connective tissue. There are multiple sub-types of fibroblasts whose function is specialised to meet the needs of their location.

Abnormal fibroblast functioning is thought to play a key role in SSc, and is the end-point of the other pathogenic processes described above. Though not all the mechanisms are know, we know that the pathogenic processes underlying SSc result in an inappropriate population of myofibroblasts – fully differentiated fibroblasts which lay down collagen.

In normal wound healing mechanisms, tissue injury results in fibroblast activation. Quiescent fibroblasts express endothelin-1 (ET-1) and intracellular adhesion molecules. Once activated by mechanical stress, fibroblasts transform into myofibroblasts and produce alpha-smooth muscle actin (α-SMA) and TGFβ. Myofibroblasts contribute to granulation tissue formation, which eventually leads to scar formation as part of normal wound healing. In SSc the myofibroblast transformation occurs inappropriately, without a mechanical trigger being present. Other disease processes exist in which fibroblast functioning is abnormal, such as keloid scar formation.

SSc fibroblasts dedicate 4 times more protein synthesis to collagen production than non-SSc fibroblasts. This leads to a greater volume of collagen being produced and more quickly than normal (LeRoy, 1974, Jimenez et al., 1986). This is due to the upregulation of genes expressing type I, III, VI and VII collagen (Buckingham et al., 1978, Peltonen et al., 1990, Rudnicka et al., 1994). This fibrogenic phenotype is common to both dermal and pulmonary SSc fibroblasts, suggesting that excess collagen deposition is also part of the pathogenesis of SSc-related pulmonary fibrosis (Shi-Wen et al., 1997). However, not all populations of fibroblasts in SSc patients display the excess collagen-producing phenotype. The phenotype appears to be isolated in specific subpopulations, particularly those close to mononuclear cells and proximal to blood vessels (Kahari et al., 1988, Scharffetter et al., 1988).

There are multiple non-collagen extracellular matrix molecules produced excessively by scleroderma fibroblasts which are also thought to contribute to the pathogenesis of SSc. These include elevated levels of fibronectin (Xu et al., 1991), increased synthesis of glycosaminoglycans (Falanga et al., 1987),

increased expression of tissue inhibitor of metalloproteinase (TIMP-1) (Bou-Gharios et al., 1994, Kirk et al., 1995), increased expression of intercellular adhesion molecule (Abraham et al., 1991) and constitutive secretion of IL-6 (Feghali et al., 1994).

Much of the abnormal protein production seen in scleroderma fibroblasts mimics a normal fibroblast response to exposure to TGFβ (Massague, 1990). Dysregulated TGFβ production and signalling has been proposed as a key mechanism in the development of the fibrogenic SSc myofibroblast phenotype (Gilbane et al., 2013). TGFβ signalling pathways are dysregulated in SSc partly because there are high levels of autocrine TGFβ produced by scleroderma myofibroblasts, and partly due to increased expression of TGFβ receptors on scleroderma fibroblasts compared to normal. Excessive TGFβ signalling via these receptors leads to downstream dysregulation of many of the genes regulated by TGFβ including collagen (Kawakami et al., 1998). Furthermore, excessive TGFβ expression seems to dysregulate its own feedback processes that would allow a return to homeostasis. Persistent activation of the TGFβ signalling pathways results in inhibition of NR4A1, an endogenous inhibitor of TGFβ signalling (Palumbo-Zerr et al., 2015). The importance of this dysregulated TGFβ signalling can be seen in mouse models, where inhibition of the TGFβ receptor in fibroblasts reduced the amount of fibrosis occurring as a response to injury (Hoyles et al., 2011).

### 1.1.6 Clinical Features

Clinical features are systemic and variable between patients. They are commonly sub-divided by body system as below.

Skin thickening is the hallmark of SSc and informs the disease classification. Patients often experience an initial phase of “puffy” skin, during which time they might state that their hands became swollen or that they could no longer remove rings. After this initial phase, the skin involvement progresses to cause skin thickening. Patients will commonly describe this as “tight”, “hard” or “restricted” rather than thickened. The skin involvement most commonly affects the hands and face but can spread to involve the entire dermis. Patients may develop a classic SSc facial appearance including thinning of the lips and nose, microstomia, and loss of tissue from the cheeks leading to a “hollowed” appearance. In late disease the skin thickening can recede and leave patients with loss of subcutaneous fat, leading to a “bony” appearance in the fingers and toes.

Skin involvement can be functionally very disabling for patients as it restricts the motion of the joints and structures underlying the thickened skin. This is a particular issue in the hands where thickened skin can lead to contractures and result in loss of function. Patients will often describe limitations caused by skin involvement of the hands, including limitations to personal care and activities of daily living. When affecting the mouth, restricted skin results in microstomia which can affect the patient’s ability to eat and can lead to weight loss. Skin involvement can also cause a neuropathic pain.

SSc can affect the entire GI tract. In the oesophagus SSc commonly causes gastro-oesophageal reflux disease, dysphagia and regurgitation of food. In the stomach SSC causes early satiety and gastric antral vascular ectasia, also known as watermelon stomach, which can case chronic bleeding and anaemia. In the lower gastro-intestinal tract SSc can cause bloating, diarrhoea, abdominal pain, small bowel bacterial overgrowth (SBBO), constipation, faecal urgency and faecal incontinence.

In common with other chronic diseases, living with systemic sclerosis can cause depression and anxiety (Thombs et al., 2007).

Systemic sclerosis causes widespread fatigue that can limit the patient's ability to work and even to perform daily activities of living (Thombs et al., 2008).

Arthralgia is a common complaint, seen in up to 80% of patients (Di Franco et al., 2015). Patients may also have an overlap syndrome, where their SSc co-exists with an inflammatory arthritis, leading to joint pain and swelling, secondary to synovitis; or with an inflammatory myositis causing muscle pain and weakness.

Vascular manifestations are among the most common symptoms of the disease and can cause significant pain, morbidity and loss of function. Systemic sclerosis leads to Raynaud's phenomenon, a vasculopathy where digits pass through a tri-phasic colour change when exposed to cold. Patients can also develop digital ulcers which are painful and slow to heal. In severe cases peripheral vascular damage secondary to systemic sclerosis can result in auto-amputation of the digits, or gangrene requiring surgical amputation.

Systemic sclerosis can lead to pulmonary artery hypertension (PAH) which manifests as progressive breathlessness that can limit the patient's ability to walk, speak and perform activities of daily living.

Patients with systemic sclerosis can develop scleroderma renal crisis (SRC). This is associated with steroid use therefore high dose steroids are not recommended in patients with SSc (Steen and Medsger, 1998).

Systemic sclerosis can lead to interstitial lung disease (ILD), which causes breathlessness, cough and reduced exercise capacity. Typically the ILD is non-specific interstitial pneumonia (NSIP) pattern, but it is also possible to find usual interstitial pneumonia (UIP) and organising pneumonia (OP) related to SSc. Additionally patients can develop uncommon forms of SSc-related lung disease including bronchiectasis. Respiratory involvement can lead to significant morbidity and affect a patient's independence and ability to self-care.

### 1.1.7 Treatment

Management guidance is produced by the EULAR Scleroderma Trials and Research Group (EUSTAR) in 2009 (Kowal-Bielecka et al., 2009). Management depends on the body systems affected.

Conservative treatments for skin thickening include emollients and hand waxing to improve skin flexibility. Hand and face exercises can help maintain mobility. Neuropathic pain due to skin involvement is managed using gabapentin, pregabalin or amitriptyline.

There are many options for the treatment of Raynaud's phenomenon. Hand warmers and lined gloves are commonly used to treat and prevent attacks. Medical therapies focus on vasodilators to improve arterial supply to the digits and reduce ischemia. Di-hydropyridine calcium channel blockers are recommended to treat Raynaud's phenomenon (Thompson et al., 2001). Additionally prostacyclin analogues such as iloprost are effective but must be administered intravenously, therefore are recommended for severe Raynaud's phenomenon (Pope et al., 2000) or digital ulceration (Wigley et al., 1994). Treatment with prostacyclin analogues is commonly associated with adverse effects including nausea and vomiting, headaches and fatigue. Some patients are not able to tolerate the treatment due

to adverse effects. Bosentan is effective in preventing digital ulceration but does not speed up the healing time of active ulcers (Korn et al., 2004).

Musculoskeletal pain can be treated with simple analgesia, such as paracetamol and ibuprofen. Patients may also be benefit from treatment with codeine. Non-steroidal anti-inflammatory drugs (NSAIDs) can exacerbate GORD so should be used cautiously. Musculoskeletal pain in the context of an overlap syndrome may be related to active myositis or synovitis, in which case it should be treated with immune suppression.

Proton-pump inhibitors are widely used to treat acid reflux in SSc. H2-receptor antagonists may also be used as a second line. Prokinetics such as domperidone and metoclopramide are recommended for symptoms related to motility disturbance such as dysphagia, GORD, early satiety and bloating (van Pinxteren et al., 2006). Diarrhoea and constipation are managed using loperamide and laxatives such as movicol and senna. Prucalopride can be helpful as a second line agent for constipation. Antibiotics are given for SBBO, and courses are often required on a cyclical basis. Frequently used antibiotics include ciprofloxacin, co-amoxiclav and doxycycline. Faecal incontinence is difficult to treat but there have been some successes using posterior tibial nerve stimulation (van der Wilt et al., 2017).

Mental health problems in SSc are treated a similar manner to non-SSc related mental health disorders. First line therapy for anxiety and depression usually takes the form of selective serotonin reuptake inhibitors (SSRIs). Amitriptyline should be used with caution as its anti-muscarinic properties can exacerbate sicca symptoms. Psychotherapy and counselling can also be useful.

It is recommended that SRC is treated with angiotensin converting enzyme (ACE) inhibitors, often at high doses. Concurrent treatment with angiotensin receptor blockers and ACE inhibitors can increase the risk of hyperkalaemia (Makani et al., 2013). Intravenous iloprost infusion can be used as a renal vasodilator, although the benefits remain unclear (Scorza et al., 1997). Renal replacement may be needed, often temporarily, and therefore these patients should be under the care of a renal physician.

Treatment options for PAH include sildenafil, bosentan and sitaxsentan which have all been shown to improve exercise tolerance, functional class and some haemodynamic measures in PAH (Galie et al., 2005) (Channick et al., 2001) (Barst et al., 2006). Epoprostenol given continuously has also been shown to be beneficial in PAH and can be considered for severe PAH in SSc (Badesch et al., 2000).

Systemic immune suppression is indicated in SSc where there is progressive skin thickening in a diffuse distribution, or interstitial lung disease. This takes the form of methotrexate (van den Hoogen et al., 1996) (Pope et al., 2001) or mycophenolate mofetil (Volkmann et al., 2017, Omair et al., 2015).
In progressive SSc-related interstitial lung disease that does not respond to mycophenolate mofetil, cyclophosphamide may be used despite the known toxicity of the drug (Tashkin et al., 2006, Hoyles et al., 2006).

## 1.2 Factor XIII

### 1.2.1 Coagulation

Coagulation, also known as clotting or haemostasis, is the process by which liquid blood becomes solid blood clot or thrombus. This is a mechanism employed by the human body to reduce blood loss. It is a complex process involving multiple serine proteases known as clotting factors. The series of molecular

events which lead to coagulation occur via two pathways which are known as the intrinsic and extrinsic pathways, and finally combine into a common end-point pathway (figure 1).

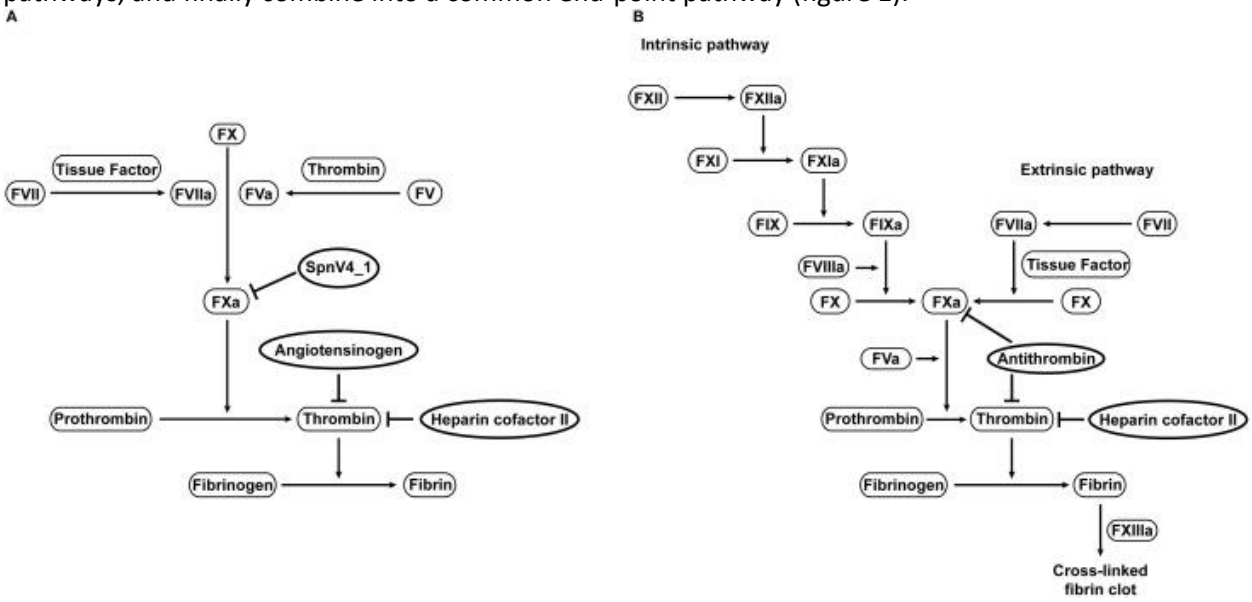



***Figure 1: The coagulation cascade (Wang et al., 2014). Section A represents the common end-point pathway and section B represents the entire coagulation cascade.***

The extrinsic pathway is also known as the Tissue Factor (TF) pathway. It is triggered by trauma which causes activation of TF. Activated TF forms a complex with Factor VII (TF-FVIIa) which then activates Factor IX and Factor X. Once activated, Factor X feeds into the common end-point pathway which is described below.

The intrinsic pathway is also known as the Contact Activation Pathway. It is triggered by the exposure of vascular collagen following a trauma to the blood vessel, which leads to the activation of Factor XII. Activated Factor XII results in the downstream activation of Factors XI, IX and VIII. Activated Factors VIII and IX then activate Factor X, which feeds into the common end-point pathway.

The common end point pathway leads to the conversion of prothrombin to thrombin by activated Factor X. Thrombin has two roles in the coagulation cascade: it activates fibrinogen to form fibrin, and it activates Factor XIII to form activated Factor XIII (Factor XIIIa). Fibrin forms the basis of the blood clot and is further altered into a more stable cross-linked fibrin clot by activated Factor XIII.

### 1.2.2 Factor XIII and coagulation

Factor XIII is a plasma clotting factor and a pro-transglutaminase enzyme. It consists of 2 A subunits (FXIII-A) and 2 B subunits (FXIII-B). It was discovered by Robbins in 1944 (Robbins, 1944). The B subunit is produced in the liver (Nagy et al., 1986) whereas the A subunit is produced by haematopoetic cells (Poon et al., 1989). Activated Factor XIII catalyses an acyl-transfer reaction; resulting in the formation of covalent lysine cross-links between fibrin α chains, leading to α polymers; and between fibrin γ chains, leading to γ dimers (Standeven et al., 2007, Siebenlist et al., 2001). This reaction, when amplified, results in cross-linked fibrin clots (Greenberg et al., 1991). The α and γ cross-linking processes result in increased blood clot stability and strength (Shen et al., 1974, Standeven et al., 2007).

Multiple lysine donor sites are present on the fibrin α chain, creating the potential for fibrin chains to cross-link, whilst also recruiting other plasma proteins, such as thrombospondin-1 (TSP-1) (Sobel and Gawinowicz, 1996). The recruitment of TSP-1 and alpha 2-antiplasmin to the fibrin clot is catalysed by FXIII.

TSP-1 is a glycoprotein involved in platelet aggregation (Roberts et al., 2010). TSP-1 cross-links with itself in the presence of activated FXIII, forming homopolymers (Bale and Mosher, 1986, Lynch et al., 1987). Radioactive labelling has shown that these homopolymers are integrated into fibrin clots, and again this is increased in the presence of FXIIIa. The integration of TSP-1 homopolymers into the fibrin clot increases the clot's density in a dose dependent manner (Bale et al., 1985). The recruitment of TSP-1 by FXIII therefore further strengthens the fibrin clot.

Alpha 2-antiplasmin is a serine protease inhibitor that inhibits plasmin, and therefore prevents plasmin from breaking down fibrin. Alpha 2-antiplasmin is integrated into the fibrin clot during its cross-linking, a process facilitated by FXIII (Lee et al., 2004). Factor XIII therefore helps to stabilise the fibrin clot whilst also recruiting a protein to protect it from being broken down. This is physiologically important, as clots deficient in activated FXIII or α2-antiplasmin lyse more quickly than clots containing both of these proteins (Fraser et al., 2011).

### 1.2.3 Other roles for Factor XIII

Beyond its extracellular function in coagulation, the Factor XIII A subunit is found intracellularly, where it is known as cellular Factor XIII A (cFXIII-A). A variety of cell types express cFXIII-A including platelets, megakaryocytes, monocytes, macrophages, fibroblasts, mast cells and dendritic cells (Poon et al., 1989, Adany and Bardos, 2003, Collin et al., 2013, Nemeth and Penneys, 1989, Shubin et al., 2017).
Factor XIII has various effects on platelets. The platelet cytoskeleton components actin and myosin are both substrates for FXIII-A. Polymerisation of actin and myosin formation is enhanced in the presence of FXIII-A (Cohen et al., 1979, Cohen et al., 1980). This suggests that FXIII-A may have a role in platelet cytoskeleton strength and stability prior to clot retraction. This is supported by evidence from FXIII-A deficient knockout mice, in which clot retraction is impaired (Kasahara et al., 2010).
Factor XIII and Factor XIII-A facilitate cross-link formation between endothelial and platelet integrins, helping to mediate the interaction between platelets and endothelial cells (Dardik et al., 2002).
A further action of Factor XIII on platelets may involve stimulating transformation to a pro-thrombotic platelet subset known as collagen and thrombin activated (COAT) platelets. COAT platelets express fibrinogen, von-Willebrand factor (vWF), fibronectin, α2-antiplasmin and TSP-1 at their surface. These are pro-thrombotic proteins released from platelet α-granules which make COAT platelets more likely to engage in thrombosis than non-COAT platelets (Richardson et al., 2013). As discussed above, α2-antiplasmin and TSP-1 are known FXIII substrates. Inhibiting FXIII-A activity reduces the appearance of the specific COAT protein complexes on the surface of COAT platelets (Dale et al., 2002). It has been suggested that coordinated activity between the platelet α-granules, FXIII, phosphatidyl and platelet glycoprotein receptor activation must occur for the COAT platelet subset to develop (Szasz and Dale, 2002, Dale, 2005).

Factor XIII-A acts on fibronectin, which is cross-linked to the fibrin chain by FXIII (Matsuka et al., 1997). Fibronectin is a protein which binds integrins, fibrin, collagen and heparin. It has key roles in cell adhesion, migration and tissue repair (Pankov and Yamada, 2002, Brotchie and Wakefield, 1990).

Fibronectin cross-linking to the fibrin chain appears to improve platelet recruitment into a clot (Cho and Mosher, 2006). In the cell-cell matrix setting, fibronectin cross-linking to fibrin aids the adhesion and spread of fibroblasts on fibronectin and the spread of megakaryocytes on type-1 collagen (Corbett et al., 1997, Malara et al., 2011). Therefor the FXIII-dependent cross-linking of fibronectin to fibrin has important roles in the recruitment and spread of various cell types within the cell-cell matrix.
The extracellular matrix is also affected by FXIII. In addition to cross-linking fibronectin to fibrin, FXIII also cross-links type I, II, III and IV collagen to fibronectin. The formation of collagen-fibronectin connections affects extracellular matrix remodelling, cell proliferation and cell migration *in vitro* (Sevilla et al., 2013, Sottile et al., 2007) suggesting that these FXIII-dependent interactions may play a role in wound healing. FXIII also has a role in cross-linking vWF to the collagen in the extracellular matrix (Bockenstedt et al., 1986). vWF is a key protein in the coagulation cascade, as detailed above, particularly in terms of recruiting platelets into the clot. The FXIII-mediated role of vWF in binding to matrix collagen could be related to improving clot adhesion to the vessel wall at the site of trauma, but this remains unclear.

FXIII-A accelerates cross-linking of plasminogen molecules, as well as cross-linking plasminogen to fibronectin (Bendixen et al., 1993). Once activated, plasminogen becomes plasmin which is involved in clot fibrinolysis. This suggests that although Factor XIII is a key player in the coagulation process, it may also facilitate timely clot degradation, which is necessary for complete wound healing. Furthermore, plasmin activates matrix metalloproteinases (MMPs) which are essential for tissue repair, so Factor XIII indirectly activates MMPs and assists in tissue repair by this mechanism (Castellino and Ploplis, 2005, Pepper, 2001).

Together with tissue transglutaminase 2 (TG2), Factor XIII-A is one of the most abundant transglutaminases expressed in the cartilage and bone and is produced by chondrocytes and osteoblasts (Nurminskaya et al., 2002, Piercy-Kotb et al., 2012, Aeschlimann et al., 1996). FXIII-A may have a role in the maturation of both chondrocytes and osteoblasts, as well as the maturation of pre-osteoblasts into osteoblasts (Nurminskaya et al., 1998, Al-Jallad et al., 2006, Nakano et al., 2007). It also may play a role in the generation and maintenance of the extracellular matrix in cartilage and bone. We can see that FXIII-A has cross-linking activity in the cartilage and bone matrix (Nurminskaya et al., 2002, Nurminskaya et al., 1998, Sorensen et al., 1994, Kaartinen et al., 2002). Together with TG2, FXIII-A helps stabilise the osteoblast microtubule system, allowing enhanced secretion of type 1 collagen and fibronectin, which contribute to the extracellular matrix (Piercy-Kotb et al., 2012, Al-Jallad et al., 2011). By inhibiting FXIII-A we can see that its microtubule-stabilising role is important for effective deposition and mineralisation of the bone matrix (Al-Jallad et al., 2006, Al-Jallad et al., 2011) however when FXIII-A is not present its role appears to be compensated for by other transglutaminases (Nurminskaya and Kaartinen, 2006, Tarantino et al., 2009).

FXIII also has a role in wound healing. This is via various mechanisms. FXIII creates bridges between endothelial αvβ3 and platelet glycoprotein IIb/IIIa integrins which are involved in the interaction between platelets and endothelial cells during wound healing (Dallabrida et al., 2000). Furthermore, activated FXIII improves endothelial tube formation, increases endothelial cell migration and decreases apoptotic cell numbers in a dose-dependent manner (Dardik et al., 2002). Further cell types affected by Factor XIII are fibroblasts and vascular smooth muscle cells. Factor XIII is known to influence the migration of fibroblasts and vascular smooth muscle cells (Brown et al., 1993, Naito et al., 1998) as well as to increase fibroblast proliferation and adhesion of fibroblasts to the extracellular matrix (Barry and Mosher, 1990, Muszbek et al., 1996). Endothelial cells adhere to activated FXIII in an integrin-dependent manner (Dallabrida et al., 2000).

A key function of Factor XIII in wound healing is thought to be due to downregulation of the anti-angiogenic protein TSP-1 (Dardik et al., 2003). TSP-1 is known to cause endothelial cell apoptosis via upregulation of the pro-apoptotic protein *Bax*, reduction of the anti-apoptotic Bcl-2, and activation of the caspase death pathway (Mirochnik et al., 2008, Nor et al., 2000). When TSP-1 is overexpressed in mice, this is associated with poor levels of cutaneous healing, granulation tissue formation and wound-related angiogenesis, suggesting that inhibition of TSP-1 by Factor XIII might be a mechanism by which FXIII assists in wound healing (Streit et al., 2000).

The downregulation of TSP-1 by Factor XIII appears to be mediated through the VEGF pathway. Factor XIII cross-links vascular endothelial growth factor receptor 2 (VEGFR-2) to αvβ3 integrin, which may trigger a downstream intracellular pathway leading to the downregulation of TSP-1, a model of which has been proposed by Dardik et al (Dardik et al., 2006).

Clinical support for a role for Factor XIII in wound healing comes from evidence that congenital Factor XIII deficiency is associated with impaired wound healing (Duckert et al., 1960, Muszbek et al., 1996). Excisional wounds in mice with congenital Factor XIII deficiency take longer to heal when compared to a second group of mice with congenital Factor XIII deficiency, in which Factor XIII has been replaced. Histology of the lesions shows delayed re-epithelialisation and necrosis in the factor XIII deficient mice, but normal healing histology in mice where Factor XIII was replaced, therefore confirming that administration of Factor XIII can reverse the abnormalities in wound healing (Inbal et al., 2005).

Injection of thrombin-activated FXIII into the graft tissue in a heterotopic mouse heart allograft model produces increased numbers of new vessels and improved cardiac contractile performance. The development and infiltration of new blood vessels into a Matrigel™ graft (an artificial mixture that replicates the extracellular environment) is reduced in FXIII-knockout mice. This effect was almost entirely reversed by the addition of activated Factor XIII (Dardik et al., 2003). This concept has also been applied in rats, where the development of blood vessels into a hydroxyapatite bone implant is stimulated by the addition of FXIII (Kilian et al., 2005). Administration of FXIII-A in rabbits results in corneal neo-vascularisation associated with inhibition of TSP-1 at 48 hours, an effect not seen in the placebo group (Dardik et al., 2003).
A review of patients with unexplained peri-operative bleeding has suggested that some cases may be related to increased levels of soluble fibrin monomers and rapid consumption of FXIII and fibrinogen (Wettstein et al., 2004). A double-blinded, randomised, controlled trial using Factor XIII vs. placebo in patients with an increased risk of peri-operative bleeding due to gastric cancer was carried out in 2009 (Korte et al., 2009). Patients were randomised to receive either Factor XIII or placebo, which was administered intravenously 15 minutes after the start of surgery for gastric cancer. Clot firmness was measured using non-activated plasma thromboelastography. They showed a statistical significant difference in the rate of reduction of blood clot firmness over time. Using a linear model, the reduction in clot firmness in the placebo group was 38% at 195 minutes, whereas in the FXIII group the reduction was 7%.

A further study in 2006 showed reduced post-operative blood loss in patients undergoing coronary surgery who received peri-operative Factor XIII, but this effect was only seen in patients who had a low plasma Factor XIII level before the operation (Godje et al., 2006) .

### 1.2.4 Factor XIII deficiency

In factor XIII deficiency, tests of the clotting pathways (activated partial thromboplastin time, bleeding time and prothrombin time) are normal as these tests assess the formation of the clotting end point, fibrin, which is unaffected by the deficiency. It is the stability of the fibrin clot which is abnormal in these patients.

Clinically significant factor XIII deficiency was first described in 1960 in a case report describing a boy with a severe bleeding diathesis and normal standard clotting tests. The only abnormality that could be found was that his blood clots were soluble in urea (Duckert et al., 1960). The urea clot lysis test has since been found to correlate directly with undetectable Factor XIII levels on Factor XIII assay (Al-Sharif et al., 2002).

Inherited Factor XIII deficiency is now known to be a rare, autosomal recessive disorder (Board et al., 1993) that affects approximately 1 in every 2 to 5 million people (Lorand et al., 1980). It can be divided into deficiencies of subunit A and subunit B.

In type 1 Factor XIII deficiency both A and B subunits are physiologically absent (Girolami et al., 1977). Genomic studies have found that the genetic mutation in this disorder is in the gene for the B subunit alone. Subunit B is not detected in plasma of these patients. It is proposed that the B subunit is required for the stability of the A subunit in plasma (Saito et al., 1990), hence patients with type 1 deficiency have no appreciable subunit A activity , although their subunit A gene is normal (Izumi et al., 1996). This condition is rare and causes less than 5% of all reported cases of factor XIII deficiency.

The classical deficiency is type 2, in which subunit A is absent. There are 69 reported mutations causing this phenotype, which are recorded online on the Factor XIII Registry Database. Of these 34 are missense mutation, 21 are deletions and insertions, 9 are splice site mutations and 5 are nonsense mutations (14) (www.f13-database.de) .

The subtype of disease affects the clinical picture: type 1 deficiency is often asymptomatic other than during delivery where is causes post-partum haemorrhage (Saito et al., 1990) whereas type 2 deficiency is symptomatic and causes high proportions of umbilical cord bleeding and intra-cranial haemorrhage (Duckert, 1972).

***Table 1.4: Common sites of bleeding in Factor XIII deficiency (Karimi et al., 2009)***

| Site of bleeding | Percentage of patients affected (%) |
|---|---|
| Umbilical | 80 |
| Superficial bruising | 60 |
| Subcutaneous haematoma | 55 |
| Oral | 30 |
| Intracranial haemorrhage | 30 |
| Muscles | 27 |
| Laceration | 26 |
| Joints | 24 |
| Post-operative | 17 |
| Peritoneal | 14 |

| | |
|---|---|
| Epistaxis | 10 |

A knockout mouse model for type 2 Factor XIII deficiency has been established by targeted deletion of exon 7 of the FXIII-A gene. Mice which are homozygous for the mutant allele have no detectable FXIII-A activity and exhibit increased bleeding time, spontaneous subcutaneous, intra-thoracic and intra-peritoneal bleeding, and reduced survival. Bleeding time is restored to normal in the mouse model by administration of Factor XIII concentrate (Lauer et al., 2002).

### 1.2.5 Factor XIII replacement therapy

Fibrogammin® is purified, pasteurised, plasma-derived human FXIII that has been available for medicinal use in Europe since 1993. It is produced by CSL Behring as a white powder and reconstituted in normal saline (0.9% sodium chloride) for intravenous administration. It is supplied in vials containing 250 or 1250 IU of Factor XIII.

Fibrogammin® is used in prophylactic treatment of patients with FXIII deficiency and is known to be effective and well tolerated with a low risk of viral transmission (Nugent, 2012). Post-marketing data has shown that adverse events resulting from treatment with Fibrogammin® are rare. The development of inhibitors to Factor XIII occurs in < 1/10,000 people treated, anaphylactoid-type reactions occur in <1/1000 people treated and a rise in temperature occurs in <1/1000 people treated (www.medicines.org.uk/emc/medicine/405).

Fibrogammin® is known as Corifact™ in the USA.

### 1.2.6 Factor XIII and systemic sclerosis

Multiple studies have already shown promising results regarding Factor XIII treatment in patients with systemic sclerosis. The first was undertaken in 1975 and was a case report series of 20 patients, who received. Of these 12 showed an improvement in the systemic sclerosis symptoms after treatment with Factor XIII (Thivolet et al., 1975).

A double-blind, randomised, placebo-controlled, cross-over study analysing Factor XIII efficacy in systemic sclerosis compared to placebo was undertaken in 1982. 25 patients received intravenous Factor XIII or placebo twice each day for 3 weeks. Patients then had a 6 week wash-out period after which they were crossed-over into the other group (Factor XIII or placebo) for 3 weeks. Symptoms and signs of systemic sclerosis were assessed by the patients and the physicians at the end of each 3-week treatment period. These assessments showed significant improvement in skin involvement and functionality in patients treated with Factor XIII compared to placebo (Guillevin et al., 1982). In a second report on the same study the author reported that there was a high concordance between patient and physician-selected outcomes for the Factor XIII treatment (Guillevin et al., 1985a).

Guillevin performed a second study in 1985 looking at the long term efficacy and safety of Factor XIII in systemic sclerosis. Eighty-six systemic sclerosis patients received different dosing regimens of intravenous Factor XIII for a mean duration of 19 months. The patients were followed up for a mean period of 22.9 months. Guillevin and his team found that 44 out of the 86 patients had improvements in their skin disease, and the Factor XIII was well tolerated. The greatest response was seen in patients treated more intensively, with 500 units of Factor XIII per day for 21 days, cycle repeated 2-4 times. The lowest response was seen in patients who received 1 cycle of intensive treatment (500 units of factor

XIII per day for 21 days) followed by a maintenance dose of 500 units of Factor XIII every 10 days (Guillevin et al., 1985b).

Dosing efficacy was also tested by Marzano et al in 1995. In this study 12 patients received 500 units of Factor XIII on alternate days for 21 days, then entered a maintenance phase where they received 500 units every 10 days. This cycle was repeated every 6 months for a mean duration of 35.5 months. The study found that after an average treatment period of 10 months, 75% of patients noticed improved skin symptoms. Smaller numbers reported improvements in Raynaud's syndrome, gastrointestinal symptoms, joint mobility and mouth opening. (Marzano et al., 1995).

Once Factor XIII treatment began to show benefit in systemic sclerosis, it was hypothesised that a relative Factor XIII deficiency might be contributing to the aetiology of the disease. Endogenous Factor XIII levels were measured in 22 patients with systemic sclerosis by Marzano and colleagues in 2000 (Marzano et al., 2000). Levels were found to be normal in 19 out of 22 patients therefore excluding any deficiency of Factor XIII as related to the aetiology of systemic sclerosis.

Additionally, despite reductions in collagen synthesis seen when Factor XIII was administered to SSc fibroblasts in vitro, this result was not replicated in vivo. Patients who had received IV Factor XIII treatment in two courses, 1250 U once daily for 15 days, followed by 1250 U once daily for 16 or 21 days; had skin biopsies analysed before and after treatment. Fibroblasts extracted from biopsies before and after treatment did not vary in their degree of collagen synthesis (Paye et al., 1990).

### 1.3 Study Objectives

1. To identify any effects of factor XIII treatment on clinical manifestations of SSc.
2. To investigate factor XIII safety.
3. To measure individual factor XIII levels in patients with SSc.
4. To measure the effects of factor XIII treatment on factor XIII PK parameters.
5. To explore effects of factor XIII on thrombospondin expression.

## 2. Methodology

### 2.1 Sclero XIII Clinical Trial – Pharmacokinetic Phase

The Pharmacokinetic (PK) phase involved administration of a single dose of IMP in 8 subjects followed by close review to assess the safety and tolerability of the IMP, and its pharmacokinetics.
Patient visits took place in the Institute Unit (Institute of Immunity and Transplantation; IIT), an Outpatient Clinic at the Royal Free Hospital, Pond Street, London. All data relating to patient visits were gathered at this location. Capillaroscopy, pulmonary function test and echocardiography results were gathered from records at the Royal Free Hospital. All tests performed as part of the trial took place at the Royal Free Hospital.

***Table 2.0: PK Phase Participant Flow***

| Week | -4 | -3 | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| All patients | Screening | | | | Fibrogammin® | Follow-up | | | | | | |

The Pharmacokinetic (PK) phase of the trial aimed to assess the safety and tolerability of the IMP in the SSc patient. It also aimed to generate data to produce a dosing algorithm that could be used in the Treatment Phase. 8 patients were recruited: 4 with limited cutaneous systemic sclerosis and 4 with diffuse cutaneous systemic sclerosis.

The 8 subjects received a single dose of Investigational Medicinal product (IMP) Fibrogammin®. The treatment allocation was therefore unblinded to participants and investigators. The dose of IMP was calculated based on the patient's weight and their endogenous Factor XIII level, both of which were assessed at the Screening Visit.

Participants with endogenous Factor XIII level greater than or equal to 90 IU received 20 IU/kg. Participants with endogenous levels less than 90 IU received 40 IU/kg. The dosing protocol aimed to maintain Factor XIII levels below 220 IU. Infusions were administered at a maximum rate of 4ml/minute by investigators.

Bloods for Factor XIII level were drawn at Screening, at Baseline prior to IMP infusion, and again 1 hour after the infusion commenced. Patients were then reviewed weekly for 4 weeks. Blood draws were performed at each visit therefore generating results for Factor XIII levels 1 hour, and 7, 14, 21, and 28 days after infusion.

The Baseline Visit took place within 28 days of the Screening Visit. Other visits occurred at the specified timepoint +/- 3 days.

A tabulated view of the assessments performed during the PK phase can be viewed in the Schedule of Assessments (Table 2.1).

### 2.1.1 Screening Visit

The patients received the Patient Information Sheet a minimum of 24 hours in advance of the Screening Visit. During the Screening Visit, the study was explained to the patients and they were given the opportunity to ask any questions. Once the patient was happy, they were asked to sign the Informed Consent Form (ICF). This was signed prior to starting any study procedures.
The rest of the Screening Visit aimed to establish a complete medical history and examination, in order to determine a patient's eligibility to participate in the trial; as well as drawing blood to establish their endogenous Factor XIII level.

Study procedures were then competed as follows:

- Review of inclusion and exclusion criteria
- Demographics
- Medical history
- Physical examination

- Modified Rodnan Skin Score (mRSS).
- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Urine pregnancy test for women of child-bearing potential
- Concomitant medication review
- Blood draw for Factor XIII level

### 2.1.2 Baseline Visit

The purpose of the Baseline Visit was to review eligibility for entry into the trial and establish baseline findings for the trial endpoints; including mRSS, Patient Reported Outcomes (PROs) and physician-led assessments. Once this was completed, suitable patients were randomised to IMP or placebo and the IMP infusion was given.

During the Baseline Visit, study procedures were completed as follows:

- Review of inclusion and exclusion criteria
- Capillaroscopy was performed if it had not been done previously
- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Physician assessments (Physician global visual analogue score (VAS), digital ulcer count)
- Patient reported outcomes (PROs) (Scleroderma Health Assessment Questionnaire (SHAQ), Cochin Hand Function Score, Raynaud's Condition Score and 36 item Short Form Health Survey (SF 36))
- 12 lead ECG
- Modified Rodnan Skin Score
- Physical examination
- Serum pregnancy test
- Blood draw for Factor XIII level (pre-dose and 1-hour post-dose), Thrombospondin I level, haematology and biochemistry
- Urinalysis
- Pulmonary function test, if not performed in the 12 months preceding baseline visit
- Serious Adverse Events and Adverse Events review
- Concomitant medication review
- Randomisation
- Administration of IMP

### 2.1.3 Visits 2, 3 and 5

These were intermediary visits used to check safety and tolerability of IMP/placebo. At these visits, study procedures were completed as follows:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Urine pregnancy test
- Serious Adverse Events and Adverse Events review
- Blood draw for Factor XIII level

### 2.1.4 Visit 4

Visit 4 was performed at week 4 and information was gathered regarding tolerability, safety and trial endpoints. The following study procedures were completed:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Physician assessments (Physician global visual analogue score (VAS), digital ulcer count)
- Patient reported outcomes (PROs) (Scleroderma Health Assessment Questionnaire (SHAQ), Cochin Hand Function Score, Raynaud's Condition Score and 36 item Short Form Health Survey (SF 36))
- Serious Adverse Events and Adverse Events review
- Modified Rodnan Skin Score
- Physical examination
- Concomitant medication review
- Urine pregnancy test
- Blood draw for Factor XIII level

### 2.1.5 Follow up Visit

This visit was to review patient wellbeing following trial completion. Study procedures were completed as listed:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- 12 lead ECG
- Urine pregnancy test
- Physical examination
- Urinalysis
- Serious Adverse Events and Adverse Events review
- Concomitant medication review

***Table 2.1: PK Phase Schedule of Assessments***

| | **Screening** | **Baseline (Visit 1)** | **Visit 2** | **Visit 3** | **Visit 4** | **Visit 5** | **Follow up** |
|---|---|---|---|---|---|---|---|
| Assessments | Week 0 | Week1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 7 |
| Visit windows (day) | -7 | 0 ± 7days | 7 ± 3d | 14 ± 3d | 21 ± 3d | 28 ± 3d | 42 ± 3d |
| Written Informed Consent | x | | | | | | |
| Subject Demography | x | | | | | | |
| Medical History | x | | | | | | |
| Inclusion and Exclusion Criteria | x | x | | | | | |
| Therapy-specific Assessment(s) | | | | | | | |
| Capillaroscopy[9] | | x | | | | | |
| Physician VAS | | x | | | x | | |
| Raynaud condition score | | x | | | x | | |
| mRSS | x | x | | | x | | |
| SHAQ | | x | | | x | | |
| Digital ulcer count | | x | | | x | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SF 36 | | x | | | x | | |
| Cochin hand function | | x | | | x | | |
| Safety Assessments | | | | | | | |
| Concomitant Medication | x | x | | | x | | x |
| Physical Examination | x | x | | | x | | x |
| Vital Signs [1] | x | x | x | x | x | X | x |
| PFT[8] | | x | | | | | |
| 12-lead ECG | | x | | | | | x |
| AE/SAE | | x | x | x | x | x | x |
| Laboratory Assessments | | | | | | | |
| Haematology[5] | | x | | | | | |
| Chemistry[4] | | x | | | | | |
| Urinalysis[6] | | x | | | | | |
| Pregnancy Test [2] | x | x | x | x | x | x | x |
| Factor XIII activity (Berichrom assay)[3] | x | x | x | x | x | x | |
| Thrombospondin (plasma sample collection) | | x | | | | | |
| Investigational product | | | | | | | |
| IMP administration [7] | | x | | | | | |

[1.] Vital signs will include heart rate, blood pressure, pulse, body weight and height. Height will be collected only at baseline

[2.] Urine pregnancy tests at all visits, except baseline when a serum pregnancy test will be performed

[3.] Factor XIII blood sampling for PK at: Pre-infusion, 1 hour after infusion and 7, 14, 21 and 28 days post infusion

[4.] Serum ALT, AST, alkaline phosphatase, GGT, total bilirubin, creatinine, eGFR, amylase, blood urea nitrogen (BUN), sodium, potassium, chloride, bicarbonate, calcium, total cholesterol, uric acid, glucose, total protein and albumin.

[5.] Haemoglobin, haematocrit, red cell count, red cell indices, white blood cell count (total and differential) and platelet count

[6.] Including a microscopic examination of the urine sediment if indicated

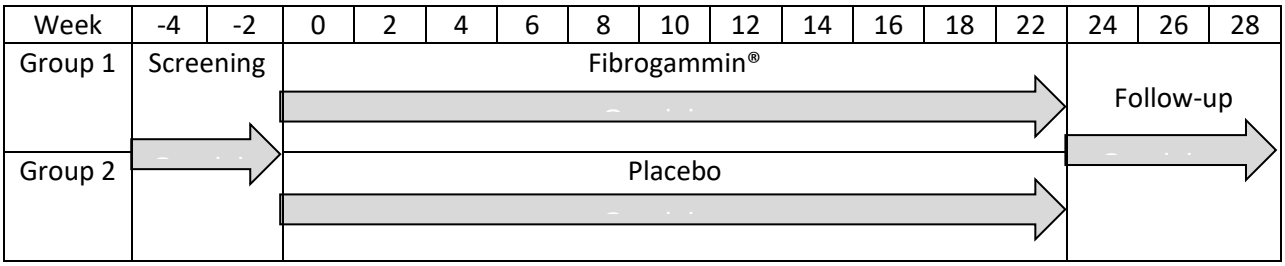
[7.] Patients to be observed for a minimum of 1-hour post dose

[8.] If not performed in the12 months preceding baseline visit

[9.] If never performed previously

### 2.2 Sclero XII Treatment Phase

***Table 2.2: Treatment Phase Participant Flow***



The Treatment phase was a multiple dose, double-blinded, randomised, controlled trial in 18 subjects. Subjects were randomised to the Factor XII or Placebo groups using a 2:1 allocation ratio. Randomisation was stratified by disease subset (limited or diffuse SSc). Factor XIII or Placebo was administered intravenously every 2 weeks for 24 weeks with all infusions taking place at the trial unit under supervision of trial investigators.

In the Treatment phase of the study the intervention was IMP (Fibrogammin®) or placebo (0.9% sodium chloride in solution). Both interventions took the form of an IV infusion.

The Baseline Visit took place within 28 days of the Screening Visit. Other visits occurred at the specified timepoint +/- 3 days.
Blood samples were taken by the investigator and processed by the Haemophilia laboratory at the Royal Free Hospital.
A tabulated view of the assessments performed during the Treatment Phase can be viewed in the Schedule of Assessments (table 2.8). An example of the prescription is shown in table 2.9.

### 2.2.1 Dosing

As before, dose of IMP/Placebo was calculated based on patient weight and endogenous Factor XIII level, assessed at Screening Visit. Patients whose Factor XIII level was ≤150 IU at the Screening Visit received 40 IU/kg as their first dose at Baseline (Visit 1). Patients whose Screening Visit Factor XIII level was 151-170 IU received 30 IU/kg at Baseline Visit and patients whose Screening Visit level was >170 IU received 20 IU/kg at Baseline Visit.

For the second dose (Visit 2), patients whose Screening Visit Factor XIII level was ≤120 IU received 40 IU/kg, and patients whose Screening Visit Factor XIII level was >120 IU received 20 IU/kg.

For the third dose (Visit 3) and subsequent doses, the dose was determined by the pre-dose Factor XIII level from 14 days prior to the visit. If the pre-dose level 14 days prior to the visit was ≤150 IU patients received 40 IU/kg. Patients whose pre-dose level from 14 days previously was 151-170 IU received 30 IU/kg. Patients whose pre-dose level from 14 days previously was >170 IU received 20 IU/kg. In this way the pre-dose level from Visit 2 was used to determine the dose for Visit 3, and the pre-dose level from Visit 3 was used to determine the dose for Visit 4, and so on.

If the level from 14 days prior to the visit was unavailable for any reason, the following structure was followed: patient's whose Screening Factor XIII level was <100 IU received 30 IU/kg, and all others received 20 IU/kg. To ensure patient safety, dosing was postponed for one week if the pre-dose Factor XIII level from the previous visit was ≥200 IU.

***Table 2.3: Treatment Phase Dosing Algorithm***

| **Randomisation (Visit 1) and subsequent doses except Visit 2** | **Dose** |
|---|---|
| Factor XIII level 14 days prior to visit ≤150 % | 40 IU/kg |
| Factor XIII level 14 days prior to visit 151-170 % | 30 IU/kg |
| Factor XIII level 14 days prior to visit 171-199 % | 20 IU/kg |
| Factor XIII level 14 days prior to visit ≥200 % | No dose |
| **Visit 2** | |
| Factor XIII level at screening ≤120 % | 40 IU/kg |

| | |
|---|---|
| Factor XIII level at screening 121-199 % | 20 IU/kg |
| Factor XIII level at screening ≥200 % | No dose |
| **If the Factor XIII level from 14 days prior to visit was unavailable** | |
| Factor XIII level at screening ≤100 % | 30 IU/kg |
| Factor XIII level at screening 101-199 % | 20 IU/kg |
| Factor XIII level at screening ≥200 % | No dose |

### 2.2.2 Maintaining blinding

Patients and trial investigators were not aware of treatment group allocation. Pharmacy staff and study nurses were unblinded.

Factor XIII levels were reviewed only by the unblinded study nurse and were not placed in the patient's records, to maintain the investigator blind. The unblinded team members then communicated to the blinded investigator which range of the dosing algorithm included the current Factor XIII level (table 2.3). The prescription was filled and signed by the Investigator using the dosing algorithm. By communicating to the investigator only a broad range which encompassed the patient's factor XIII level, rather than the true level, the investigator was not able to deduce which treatment arm each patient had been allocated. The intervention was IMP (Fibrogammin®) or placebo (0.9% sodium chloride in solution). Both interventions took the form of an IV infusion. The IMP in solution was clear or slightly opalescent, and the placebo was clear. Fibrogammin® was supplied as white powder in vials and this was reconstituted in normal saline (0.9% sodium chloride solution) for intravenous administration. Vials contained 1250 IU of Factor XIII.

The IMP/placebo was dispensed by the trial pharmacy to the unblinded study nurse. Only unblinded nurses were permitted to collect dispensed IMP/placebo. An opaque box was used to collect the IMP/placebo and move it around the department. IMP/placebo was prepared for administration by the unblinded nurse in a windowless preparation room which the investigators did not have access to. The correct volume of IMP/placebo was reconstituted and drawn into a syringe. The syringe was then covered in opaque material and passed to the study investigator.
Once intravenous access had been gained by the investigator via peripheral cannula, the opacified syringe was connected to the cannula and IMP/placebo administered at the prescribed rate. The opacification process allowed both investigator and patient to remain blinded.

### 2.2.3 Screening Visit and Baseline Visit

These visits were identical to the corresponding visits performed during the PK phase (see sections 2.1.1 and 2.1.2 above).

### 2.2.4 Visit 2, 4, 6, 8, 10, and 12

The main purpose of these visits was to check patient safety and tolerability, and to administer IMP/placebo doses. At these visits, study procedures were completed as follows:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Urine pregnancy test
- Serious Adverse Events and Adverse Events review

- Blood draw for Factor XIII level pre-dose and 1-hour post dose
- IMP/Placebo administration

#### 2.2.5 Visit 3, 7, 9 and 11

Safety and tolerability were checked and IMP/placebo administered as before. These visits also involved data collection for study end points. At these visits, study procedures were completed as follows:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Physician assessments (Physician global visual analogue score (VAS), digital ulcer count)
- Patient reported outcomes (PROs) (Scleroderma Health Assessment Questionnaire (SHAQ), Cochin Hand Function Score, Raynaud's Condition Score and 36 item Short Form Health Survey (SF 36))
- Modified Rodnan Skin Score
- Physical examination
- Serious Adverse Events and Adverse Events review
- Concomitant medication review
- Urine pregnancy test
- Blood draw for Factor XIII level pre-dose and 1-hour post dose
- IMP/Placebo administration

#### 2.2.6 Visit 5

Visit 5 was similar to visit 3, in that IMP/placebo was administered, safety and tolerability check and data were collected for endpoints. At this visit routine blood sampling and Thrombospondin levels were also performed. The study procedures were completed as follows:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Physician assessments (Physician global visual analogue score (VAS), digital ulcer count)
- Patient reported outcomes (PROs) (Scleroderma Health Assessment Questionnaire (SHAQ), Cochin Hand Function Score, Raynaud's Condition Score and 36 item Short Form Health Survey (SF 36))
- Modified Rodnan Skin Score
- Physical examination
- Serious Adverse Events and Adverse Events review
- Concomitant medication review
- Urine pregnancy test
- Blood draw for Factor XIII level pre-dose and 1-hour post dose, Thrombospondin I level, haematology and biochemistry
- IMP/Placebo administration

#### 2.2.7 Visit 13

Visit 13 was completed at week 24 and required data collection for endpoint purposes. No IMP/placebo was administered at this visit. Routine blood monitoring and Thrombospondin levels were collected. Study procedures were completed as follows:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Physician assessments (Physician global visual analogue score (VAS), digital ulcer count)
- Patient reported outcomes (PROs) (Scleroderma Health Assessment Questionnaire (SHAQ), Cochin Hand Function Score, Raynaud's Condition Score and 36 item Short Form Health Survey (SF 36))
- Modified Rodnan Skin Score
- Physical examination
- Serious Adverse Events and Adverse Events review
- Concomitant medication review
- Capillaroscopy (if never completed previously)
- Urine pregnancy test
- Urinalysis
- 12 lead ECG
- Pulmonary function test
- Blood draw for Factor XIII level, Thrombospondin I level, haematology and biochemistry

#### 2.2.8 Follow up Visit

The Follow-up Visit was completed 4 weeks after visit 13. Data were gathered for safety monitoring including routine blood tests, and for study endpoints. Study procedures were completed as listed:

- Vital signs (temperature, body weight, blood pressure, heart rate and respiratory rate)
- Physician assessments (Physician global visual analogue score (VAS), digital ulcer count)
- Patient reported outcomes (PROs) (Scleroderma Health Assessment Questionnaire (SHAQ), Cochin Hand Function Score, Raynaud's Condition Score and 36 item Short Form Health Survey (SF 36))
- Modified Rodnan Skin Score
- Physical examination
- Urine pregnancy test
- Serious Adverse Events and Adverse Events review
- Concomitant medication review
- Blood draw for haematology and biochemistry

#### 2.2.9 Unscheduled Visits

At any unscheduled visit the patient completed study procedures as listed for Visits 3, 7, 9 and 11; plus a blood draw for haematology and biochemistry, and a urinalysis.

#### 2.2.10 Early Withdrawal

Patient undergoing early withdrawal completed study procedures as for the Unscheduled Visit, in addition to a capillaroscopy if one had not been performed previously.

***Table 2.4: Treatment Phase Schedule of Assessments***

| | **Screening** | **Baseline (Visit 1)** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** | **V10** | **V11** | **V12** | | | **End of Therapy (visit 13)** | **V14** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Assessments** | Week 0 | Week 1 | Wk 2 | Wk 4 | Wk 6 | Wk 8 | Wk 10 | Wk 12 | Wk14 | Wk 16 | Wk 18 | Wk 20 | Wk 22 | | Unschld visit | Early Withdrawal | Week 24 | F/up wk28 |
| **Visit windows (day)** | -7 | 0 ± 1d | 14 ± 3d | 28 ± 3d | 42 ± 3d | 56 ± 3d | 70± 3d | 84 ± 3d | 98 ± 3d | 112 ± 3d | 126 ± 3d | 140 ± 3d | 154 ± 3d | | | | 168 ± 3d | 196 ± 3d |
| Written Informed Consent | x | | | | | | | | | | | | | | | | | |
| Subject Demography | x | | | | | | | | | | | | | | | | | |
| Medical History | x | | | | | | | | | | | | | | | | | |
| Inclusion/Exclusion Criteria | x | x | | | | | | | | | | | | | | | | |
| Therapy-specific Assessment(s) | | | | | | | | | | | | | | | | | | |
| Capillaroscopy | | x | | | | | | | | | | | | | | x | x | |
| VAS | | x | | x | | x | | x | | x | | x | | | x | x | x | x |
| Raynaud condition score | | x | | x | | x | | x | | x | | x | | | x | x | x | x |
| mRSS | x | x | | x | | x | | x | | x | | x | | | x | x | x | x |
| SHAQ | | x | | x | | x | | x | | x | | | | | x | x | x | x |
| Digital ulcer count | | x | | x | | x | | x | | x | | x | | | x | x | x | x |
| SF 36 | | x | | x | | x | | x | | x | | x | | | x | x | x | |
| Cochin hand function | | x | | x | | x | | x | | x | | x | | | x | x | x | x |
| Safety Assessments | | | | | | | | | | | | | | | | | | |
| Concomitant Medication | x | x | | x | | x | | x | | x | | x | | | x | x | x | |
| Physical Examination | x | x | | x | | x | | x | | x | | x | | | x | x | x | x |
| Vital Signs [1] | x | x | x | x | x | x | x | x | x | x | x | x | x | | x | x | x | x |
| PFT[5] | x | | | | | | | | | | | | | | | x | x | |
| 12-lead ECG | | x | | | | | | | | | | | | | | x | x | |
| AE/SAE | | x | x | x | X | x | x | x | x | x | x | x | x | | x | x | x | x |
| Laboratory Assessments | | | | | | | | | | | | | | | | | | |
| Haematology[6] | | x | | | | x | | | | | | | | | x | x | x | x |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemistry[7] | | x | | | | x | | | | | | | | | x | x | x | x |
| Urinalysis[8] | | x | | | | | | | | | | | | | | | x | |
| Pregnancy Test [2] | x | x | x | x | X | x | x | x | x | x | x | x | x | | x | x | x | x |
| Factor XIII activity (Berichrom assay)[3] | x | x | x | x | x | x | x | x | x | x | x | x | x | | | | | |
| Thrombospondin (plasma sample collection) | | x | | | | x | | | | | | | | | | | x | |
| Investigational product | | | | | | | | | | | | | | | | | | |
| Randomisation | | x | | | | | | | | | | | | | | | | |
| IMP administration .[4] | | x | x | x | X | x | x | x | x | x | x | x | x | | | | | |

***Table 2.5: Treatment Phase Prescription***

| The Royal Free London NHS Foundation Trust | | | | | RHEUMATOLOGY Unit |
|---|---|---|---|---|---|
| **Patient Name:** | | **Trial Title:** | SCLERO XIII<br>***DOUBLE-BLIND TREATMENT PHASE PRESCRIPTION*** | **Patient Demographics** Height (m) | **FOR CLINICAL TRIAL USE ONLY** |
| **Date of Birth:** | | | | | |
| **Hospital No.:** | | **R&D No.:** | 9337 | Weight (kg) (40 – 100kg) | |
| **Gender:** | | **EudraCT No.:** | 2014-001101-10 | | |
| **Subject No.:** | | **Protocol No.:** | 13/0417 | Date weight measured | |
| **Allergies:** | | **Sponsor:** | UNIVERSITY COLLEGE LONDON (UCL) | **Page 1 of 1** | |

Investigator: Professor Chris Denton  Sub-Investigator: Dr Anna Gill  Research Nurse: Rachel Ochiel  **Version: 2  Date: 16.10.17**

****DOUBLE-BLIND STUDY – PHARMACY DO NOT INFORM RESEARCH TEAM OR PATIENT OF TREATMENT ALLOCATION****
**TREATMENT TO BE COLLECTED BY UNBLINDED RESEARCH NURSE ONLY**

**Dose Information**

| Factor XIII level | FIBROGAMMIN® or PLACEBO | Unblinded nurse (initial) | Pharmacist check (initial) |
|---|---|---|---|
| First dose at Randomisation (Visit 1) and **subsequent doses (apart from second dose)** | | | |
| Level at screening or pre-dose **≤150%** | Dose = 40 IU/kg | | |
| Level at screening or pre-dose **151-170%** | Dose = 30 IU/kg | | |
| Level at screening or pre-dose **>170% to <200%** | Dose = 20 IU/kg | | |
| Level at screening or pre-dose **≥200 %** | **DO NOT DOSE + DELAY 1 WEEK** | | |
| Second dose (Visit 2): | | | |
| Level at screening **≤120%** | Dose = 40 IU/kg | | |
| Level at screening **>120% to < 200%** | Dose = 20 IU/kg | | |
| Level at screening **≥ 200%** | **DO NOT DOSE + DELAY 1 WEEK** | | |

If the level from 14 days prior to the visit is unavailable:

- patients will receive 30 IU/kg if screening Factor XIII level <100%;
- patients will receive 20 IU/kg if screening Factor XIII level ≥ 100% to <200%
- **IF FACTOR XIII LEVEL IS ≥200%: DO NOT DOSE + DELAY 1 WEEK**

**Treatment Duration:** 24 weeks (baseline visit (day 0), weeks 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22)

**Please attach the following documents:**

1) List of concomitant medications including dose & frequency (at Baseline)
2) Blood Results (copy of lab results for FBC, Biochem, **pre-dose Factor XIII levels**)

**Disease Classification (please select):** ☐ **Diffuse SSc** ☐ **Limited SSc**

Changes in concomitant medication since previous visit: ☐ Yes ☐ No; If Yes, specify here:_____

| Week Number & date | Drug | Dose (IU/kg) (Please select) | Dose (IU) (Please complete) | Volume (ml) (Please complete) | Route | Rate of Administration | Admin date and time* | Nurse admin signature* | Pharmacy use: dispense | Pharmacy use: check |
|---|---|---|---|---|---|---|---|---|---|---|
| Date: _____<br>Visit No: _____<br>Week No.: _____ | **PLACEBO (0.9% SODIUM CHLORIDE) OR FIBROGAMMIN® (FACTOR XIII CONCENTRATE)** (Trial Supply) | 0 / 20 IU/kg ☐<br>0 / 30 IU/kg ☐<br>0 / 40 IU/kg ☐ | 0 IUOR<br>_____ IU<br>(dose = dose (IU/kg) x weight (kg)) | _____ (ml)<br>(volume (ml) = dose (IU) / 62.5 IU) | IV | Max. rate = 4ml/min: give over at least _____ minutes<br>(Minimum duration (mins) = Volume (ml) / 4) | | | | |
| | | **MONITOR PATIENT FOR AT LEAST ONE HOUR FOLLOWING DOSE ADMINISTRATION**<br>***DO NOT ENDORSE THE PRESCRIPTION WITH THE TREATMENT RANDOMISATION*** | | | | | | | | |

| Prescribed by: | I confirm that I have read and understood the clinical trial protocol for this study and I have been delegated the responsibility for prescribing by the Principal/Chief Investigator.<br>Prescriber Name _____ Prescriber signature _____ Ext/Blp _____ Date ____/____/____ | Screened by: | **Ensure documents requested attached** ☐<br>Pharmacist name _____ Pharmacist signature _____ Bleep /ext _____ Date ____/____/____ |
|---|---|---|---|

### 2.3 Eligibility

All patients met the eligibility criteria and no changes to the eligibility were made once the trial had commenced. The inclusion and exclusion criteria may be reviewed in Table 2.6 and 2.7 below.

***Table 2.6: Clinical Trial Inclusion Criteria***

| |
|---|
| 1. Subject selection ≥ 18 years |
| 2. Male and female adults |
| 3. Subjects with a diagnosis of SSc according to the 2013 ACR EULAR classification criteria. They will be classified according to LeRoy criteria as limited or diffuse subset. |
| 4. Females of childbearing potential must be willing to use a reliable form of medically acceptable contraception and have a negative pregnancy test |
| 5. Subjects will have serological status for hepatitis A and B assessed at screening. |
| 6. Patients who have given their free and informed consent |
| 7. Patients willing to use an effective method of contraception (hormonal or barrier method of birth control; abstinence) from the time consent is signed until 6 weeks after treatment discontinuation (females of childbearing potential and males). |
| 8. Patients who have a negative pregnancy test within 7 days prior to being registered for trial treatment (females of childbearing potential).<br><br>NOTE: Subjects are considered not of child bearing potential if they are surgically sterile (i.e. they have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are postmenopausal. |

***Table 2.7: Clinical Trial Exclusion Criteria***

| |
|---|
| 1. Females must not be breastfeeding. |
| 2. Have allergies to excipients of IMP and placebo |
| 3. Have uncontrolled systemic hypertension as evidenced by sitting systolic blood pressure > 160 mmHg or sitting diastolic blood pressure > 100 mmHg. |
| 4. Have portal hypertension or chronic liver disease defined as mild to severe hepatic impairment (Child-Pugh Class A-C). Subjects positive for Hepatitis C with evidence of active viral replication on sensitive PCR testing are also excluded. |
| 5. Have hepatic dysfunction, defined as aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) > 3 times the upper limit of the normal range (× ULN) at the Screening Visit. |
| 6. Have chronic renal insufficiency as defined by a serum creatinine > 221 µmol/L or requires dialysis. |
| 7. Have a haemoglobin concentration < 100 g/L at the Screening Visit. |

| |
|---|
| 8. Have a history of left-sided heart disease and/or clinically significant cardiac disease, including but not limited to any of the following:<br>a. Aortic or mitral valve disease (stenosis or regurgitation) defined as more than minimum aortic insufficiency and more than moderate mitral regurgitation; (stenosis or regurgitation>grade 1).<br>b. Pericardial constriction.<br>c. Restrictive or congestive cardiomyopathy.<br>d. Left ventricular ejection fraction < 40 % by multiple gated acquisition scan (MUGA), angiography, or echocardiography.<br>e. Left ventricular shortening fraction < 22 % by echocardiography.<br>f. Symptomatic coronary disease with demonstrable ischaemia. |
| 9. Have a history of malignancies within 5 years of Screening Visit with the exception of localized skin or cervical carcinomas. |
| 10. Have psychiatric, addictive, or other disorder that compromises the ability to give informed consent for participating in this study. This includes subjects with a recent history of abusing alcohol or illicit drugs. |
| 11. Be receiving ongoing treatment with hyperbaric oxygen. |
| 12. Have pulmonary artery hypertension. |
| 13. Have received IV Iloprost within the last 2 months. |
| 14. Have been treated with sympathectomy or toxin botulinum A within the last 3 months. |
| 15. Have had a thrombosis, stroke, pulmonary embolism or myocardial infarction in the last 6 months. |
| 16. Have a diagnosis of diabetes mellitus requiring dietary restriction of carbohydrate. |
| 17. Require a low sodium diet on medical advice. |
| 18. Be participating in another clinical trial involving an investigational medicinal product. |
| 19. Be taking Bosentan or have taken Bosentan within the last 4 weeks. |

### 2.4 Concomitant Medications

Patients were permitted to commence or continue most common medications used in the treatment of systemic sclerosis for the duration of the trial. Of note background immunosuppressive therapy, for example mycophenolate mofetil, was permitted. Additionally, most medications that are commonly used for the treatment of secondary Raynaud's syndrome and digital ulceration in systemic sclerosis were permitted. This included vasodilators, selective serotonin reuptake inhibitors, phosphodiesterase type 5 inhibitors, and IV prostacyclin analogues.

The only medication of note which was prohibited was the endothelin receptor antagonist Bosentan.

### 2.5 Endpoints

No changes were made to trial endpoints after trial commencement.

***Table 2.8: Clinical Trial Primary Endpoints***

| Description of Endpoint | Method of Assessing Endpoint | Timings of Assessments |
|---|---|---|
| Skin involvement | Modified Rodnan skin score (mRSS) | Screening, baseline and week 4 during PK phase<br><br>Screening, baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| Severity of Raynaud's phenomenon | Raynaud's condition score | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |

***Table 2.9: Clinical Trial Secondary Endpoints***

| Description of Endpoint | Method of Assessing Endpoint | Timings of Assessments |
|---|---|---|
| Skin involvement | Modified Rodnan skin score (mRSS) | Screening, baseline and week 4 during PK phase<br><br>Screening, baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| Severity of Raynaud's phenomenon | Raynaud's condition score | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| Pulmonary function | Pulmonary function testing | Baseline during PK phase<br><br>Screening and week 24 during Treatment phase |
| Healing of DU | Digital ulcer count | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| Number of new DU developed during treatment period | Digital ulcer count | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| Hand function | Cochin hand function | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| Prevention of new DU | Digital ulcer count | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| DU Pain assessment | Visual analogue scale for pain (VAS) and Raynaud's condition score | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |
| DU worsening, defined as: | Digital ulcer count | Baseline and week 4 during PK phase<br><br>Baseline, week 4, 8, 12, 16, 20, 24 and 28 during Treatment phase |

| | | |
|---|---|---|
| • Overnight Hospitalization for digital ulcers<br>• Additional surgical treatment for digital ulcer<br>• Gangrene and/or amputation<br>• Need of local sympathectomy<br>• Need of botulinum toxin A<br>• Need for systemic antibiotic<br>• Need of unplanned IV Iloprost | | |
| Safety and tolerability of study medication in scleroderma | Adverse event recording | Baseline, week 1, 2, 3, 4, 5 and week 7<br><br>Baseline, week 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 28 during Treatment phase |

Examples of patient reported outcome measures gathered during the study to provide data for these endpoints can be found in Appendix 2.

### 2.6 Randomisation

There was no randomisation for the PK phase as all patients received Factor XIII.

For the Treatment phase, there was block randomisation of limited or diffuse SSc patients to active treatment or placebo arms, with an allocation ratio of 2:1. For the cohort of 18 patients, we recruited 9 patients with diffuse SSc and 9 with limited. Of these patients, 6 with diffuse and 6 with limited SSc were randomly allocated to receive active treatment; and 3 with diffuse SSc and 3 with limited SSc were randomly allocated to receive placebo. The randomisation was performed using STATA (version 14).

The randomisation list was held by the trial pharmacist who had no contact with the patients and was not reviewed by investigators or study staff. An emergency copy was held in the trial office for the purposes of emergency unblinding. This was not opened. When patients provided informed consent to enter the Treatment phase, they were allocated a trial number in sequence. Each trial number corresponded with the numbered randomisation list held by pharmacy. Once a prescription was provided to pharmacy with the clinical trial number assigned, pharmacy were then able to dispense the allocated treatment.

Patient enrolment was performed by the sub-investigator Dr Anna Leslie with oversight from PI Professor Denton.

### 2.7 Safety and Follow-Up

Where patients were felt to require additional investigations and follow up due to clinical need, this was arranged and performed by the sub-investigator and discussed with the principal investigator.

Following the trial, the patients returned to the routine clinic and their survival status was monitored until trial completion.

## 2.8 Statistical Methods

Clinical and demographic data was used to assess baseline comparability of the randomised groups, including all of the variables that were measured as primary or secondary outcomes.

### 2.8.1 Primary outcome analysis

This was an early stage exploratory study not looking for statistical significance. It included a small number of subjects to assess the safety and feasibility of the study intervention in this patient population. The outcome analysis was descriptive with summary statistics and confidence intervals being determined. For the primary outcomes we assessed the possible effect size by comparing baseline with 24- week values, and these changes were compared with the recognised minimal clinically important difference (MCID) for SSc. The MCID is generally taken as 20% change and at least 4 integer units of skin score (Khanna et al, 2006). This provided insight into potential treatment effect, and values for active treatment were compared with values for those receiving placebo.

### 2.8.2 Secondary outcome analysis

Hypothesis tests were not felt to be appropriate. Secondary analyses should be considered as hypothesis generating rather than providing firm conclusions.

## 2.9 Dates and Timeframe

Recruitment for the PK phase of the trial commenced in January 2016. The first patient was randomised on 1st February 2016. The PK phase was fully recruited by 17th March 2016. The PK phase last patient last visit was 19th May 2016.

Recruitment for the Treatment phase commenced in August 2016 and continued until January 2018. The first patient was randomised into the Treatment Phase on 5th September 2016. The last patient was randomised on 19th February 2018. The last patient last visit was performed on 23rd August 2018.

The trial was completed as planned.

## 2.10 Changes to the Study or Planned Analyses

Following completion of the PK phase of the study, the PK data were analysed and these data were used to generate the dosing algorithm used in the Treatment phase. The protocol underwent an amendment to incorporate this dosing algorithm and information regarding the blinding procedure was also clarified in the protocol at this time.

## 2.11 Clinical Assessments

### 2.11.1 Demographics

The demographic data were recorded at the Screening and Baseline visits. This included, age, ethnicity, gender, height, and weight.

### 2.11.2 History and Examination

Comprehensive clinical history was taken at the Screening visit. This included demographic data, review of previous and current symptoms of SSc, medication history, allergies, social history and allergies. This was followed by more tailored clinical history taking for the duration of the trial, as required based on symptoms and adverse events.

A full clinical examination was performed including the cardiovascular, respiratory, abdominal and neurological systems, ears, nose and throat, and extremities. Skin was assessed for digital ulcers and SSc-related skin thickening. Skin thickening was assessed during clinical examination using the modified Rodnan Skin Score (mRSS) (see section 2.2.5).

### 2.11.3 Adverse Event Reporting

Patients were monitored by study investigators for the duration of the trial and follow up period. Following each intravenous injection, patients were monitored in hospital for one hour, and their vital signs were repeated during this period.

At each study visit the patients were questioned regarding adverse events that had taken place since the preceding study visit, and ongoing adverse events were followed up. Patients were also provided with telephone contact information and encouraged to telephone with any adverse events that they wished to report during the study period, if they did not want to wait until the next scheduled visit.

Adverse events were defined according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines (https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4_2016_1109.pdf). All adverse events occurring following informed consent were recorded. Adverse events occurring before the informed consent were recorded in the patient's medical history. Changes to events recorded in the patient's medical history during the trial period were recorded as adverse events.

Serious adverse events (SAEs) were defined as per the ICH GCP guideline and were reported to the Sponsor in an expedited fashion, within 24 hours of the investigator being made aware of the SAE. SAEs were defined as those adverse events leading to:

- Death
- A life-threatening adverse event
- Inpatient hospitalisation or prolongation of existing hospitalisation
- Persistent or significant disability or incapacity
- A congenital anomaly or birth defect

Other adverse events were considered serious at the discretion of the investigators if they led to action which might reasonably have prevented an SAE from occurring, as defined above.

### 2.11.4 Patient Reported Outcomes

The patient reported outcomes (PROs) used in this study were: the Raynaud's Condition Score, the Scleroderma Health Assessment Questionnaire (SHAQ), the 36 item Short Form Questionnaire (SF-36), and the Cochin Hand Function Score.

The Raynaud's Condition Score is a validated outcome measure for Raynaud's phenomenon (Khanna and Merkel, 2007, Merkel et al 2002).

It assesses the level of difficulty caused to the patient by Raynaud's phenomenon on each day that the test is taken. By taking the test regularly throughout a clinical trial we can gather data regarding changes to the patient's experience of their Raynaud's phenomenon. Patient's self-assess the number and duration of their Raynaud's attacks, as well as rating the degree of difficulty their Raynaud's phenomenon caused them on that day using a 10-point Likert scale.

The Scleroderma Health Assessment Questionnaire (SHAQ) is a patient questionnaire used to assess quality of life in SSc. It is adapted from the Stanford Health Assessment Questionnaire (HAQ) which is used to assess quality of life in rheumatological conditions.

The original HAQ was developed by James Fries in 1980 and was modified by the author in 1982 and 2003 (Bruce and Fries, 2003). The modern HAQ assesses function over 8 domains of daily activities such as eating and washing. The patient selects a response based on the amount of difficult they experience attempting to complete each task. There are also 4 domains in which the patient selects any devices or other people they use for assistance in their activities of daily living, and one visual analogue score relating to pain symptoms.

The SHAQ uses the same initial 13 sections as the HAQ, but also incorporates 5 further visual analogue scores referring to symptoms specific to SSc: respiratory symptoms, gastrointestinal symptoms, Raynaud's phenomenon and digital ulcers. Use of the SHAQ has been validated across several trials, and its language has been shown to correlate with patient's own descriptions of the disease, thereby making the SHAQ applicable and personal to the patient (Johnson et al., 2005, Steen and Medsger 1997)

The Short form-36 Questionnaire (SF-36) is a quality of life assessment tool applicable to a variety of diseases. The SF-36 was developed by Ware et al as part of the Medical Outcomes Study (Ware and Sherbourne 1992). It was later modified to the SF-36v2 which has been used previously in clinical trials and provides useful data on patient wellbeing but is not specific to systemic sclerosis therefore was used in conjunction with the SHAQ (Stewart et al, 1988, Del et al, 2004).

The Cochin Hand Function Score is an eighteen question self-assessment which asks patients to score the level of difficulty they have performing various common tasks on a six-point scale from "impossible to do" to "no difficulty". It was initially called the Duruöz Hand Index and was developed to evaluate hand function in French patients with rheumatoid arthritis (Duruöz et al, 1996). It has been validated in rheumatoid and osteo arthritis (Poiraudeau et al, 2000, Poiraudeau et al, 2001) as well as in systemic sclerosis (Brower and Poole 2004, Rannou et al, 2007).

The PROs used in this trial can be reviewed in Appendix 2.

### 2.11.5 Physician Assessments

The physician assessments for this study were the modified Rodnan Skin Score (mRSS), the physician global visual analogue scale (VAS), and the digital ulcer count.

The mRSS is an internationally recognized and well validated clinical tool for the assessment of skin thickness in SSc. The body is divided into 17 sites which are commonly affected by SSc. Each of these sites is clinically assessed and the skin is given a score of 0-3. A score of 0 represents normal skin, 1 means that skin is mildly thickened, 2 moderately thickened and 3 severely thickened. The 17 sites for assessment are: face, chest, abdomen (each assessed as one area), the upper and lower arms bilaterally, the upper and lower legs bilaterally, and the dorsal surfaces of the hands, fingers and feet bilaterally. The scores from the 17 areas are added together, giving a total score out of 51. When the test is performed by an experienced observer, the clinical score correlates with dermal weight on biopsy. (Perera et al, 2007).

The test was developed by Dr Gerald Rodnan in the 1980s and was first used in a controlled trial in 1982 (Clements et al, 2000).

Over time the test has been modified and in its current form it is the gold standard for the assessment of skin thickness in SSc (Domsic et al, 2011). The validity of the mRSS has been widely proven (Steen et al, 1982, Clements et al, 2000) and it has been used to determine the minimal clinically important difference (MCID) in systemic sclerosis in various clinical trials (Rodnan et al, 1979).

The physician global VAS is a 10cm analogue scale on which the physician marks their opinion on the degree of difficulty caused to the patient by their disease. It is no specific and in this case was used only to judge the degree of suffering caused by symptoms related to the patients' systemic sclerosis. The mark on the analogue scale is then translated into a percentage which is the physician VAS score entered into the trial database.

The digital ulcer count is a simple scoring tool allowing the physician to document how many digital ulcers are present on the patients' hands as well as recording any interventions that might have taken place for the ulcers. The physician assessments used in this study can be found in Appendix 2.

### 2.11.6 Physiological Assessments

Physiological assessments performed during this study were: vital signs (blood pressure, respiratory rate, heart rate and temperature), electrocardiogram (ECG), pulmonary function testing, and capillaroscopy.

## 2.12 Laboratory Assessments

### 2.12.1 Routine Laboratory Assessments

Routine blood analyses were performed at Baseline during the PK phase and at Baseline, week 8, end of therapy (week 24) and follow-up (week 28) during the Treatment phase, as well as during any unscheduled visits. Blood samples were taken for full blood count and biochemistry. Blood samples were analysed by the Haematology and Biochemistry laboratories at the Royal Free Hospital.

Urine samples were taken at Baseline and week 24. Samples were tested in the clinical trials department using urine dipstick. Urine samples with a dipstick abnormality were sent to the Royal Free Hospital biochemistry laboratory for further assessment.

Pregnancy tests were performed at all visits for women of childbearing potential. A serum pregnancy test was performed at Baseline and a urine pregnancy test at all other visits.

### 2.12.2 Factor XIII Analyses

Patient participating in the clinical trial had samples for Factor XIII level taken at every visit during the trial. Where a Factor XIII/placebo infusion was given, the Factor XIII level was drawn pre-infusion and one-hour post-infusion.

Two 4ml BD Vacutainer tubes with 3.2% buffered sodium citrate sodium citrate were obtained. Samples were transferred at ambient temperature to the Haemophilia laboratory at the Royal Free Hospital, where they were processed within 4 hours of being drawn.

The Berichrom FXIII chromogenic ammonia release assay (Siemens Healthcare Diagnostics, Marburg, Germany) was used on the CS-2000i coagulation analyser.

This assay converts the Factor XIII in the sample to Factor XIIIa using thrombin. The fibrin generated by thrombin amplifies the process. A polymerase inhibitor is present to prevent the free fibrin produced in this reaction forming a fibrin clot. However, the cross-linking action of FXIIIa on fibrin continues to occur. FXIIIa cross-links glycine ethyl ester to its peptide substrate, a reaction catalysed by glutamate dehydrogenase and dependent on nicotinamide adenine dinucleotide (NADH). NADH is consumed during the reaction and this can be measured as decreased absorbance spectrophotometrically at 340nm. The concentration change in NADH is considered to be directly proportional to the Factor XIII activity in the sample. There have been some concerns that the consumption of NADH in other reactions may lead to an overestimation of Factor XIII activity, but this does not affect readings in the normal range, only in severe deficiencies (Karimi et al, 2009)

The Berichrom FXIII chromogenic ammonia release assay was developed in the early 2000s as a quantitative alternative to the clot solubility test, and it has since been shown to be more sensitive than the clot solubility test in detecting mild to moderate Factor XIII deficiency (Kárpáti et al, 2000, Fickenscher et al, 1991).

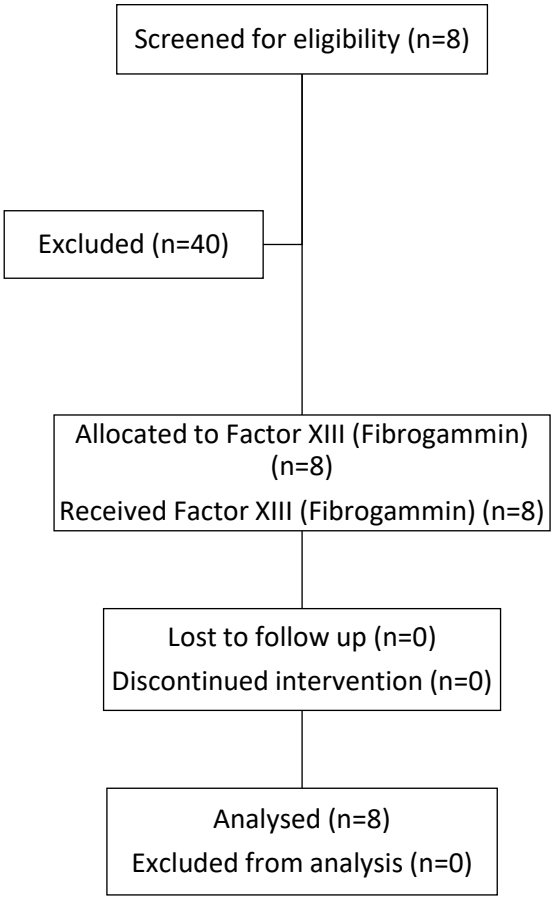
In the clot solubility test the patient's plasma is incubated with thrombin to trigger the final stages of the coagulation cascade (see figure 1) and generate clot formation. The clot is then transferred to an acidic or urea solution for 24 hours. If the clot has dissolved at 24 hours this is indicative of Factor XIII deficiency. (Jennings et al, 2003).

## 3. Results

### 3.1 Pharmacokinetic phase patient flow

Eight patients were screened and randomised into the pharmacokinetic phase. No patients were excluded after being assessed for eligibility. All patients received the full dose of study medication. All patients completed the trial, and none were lost to follow up.

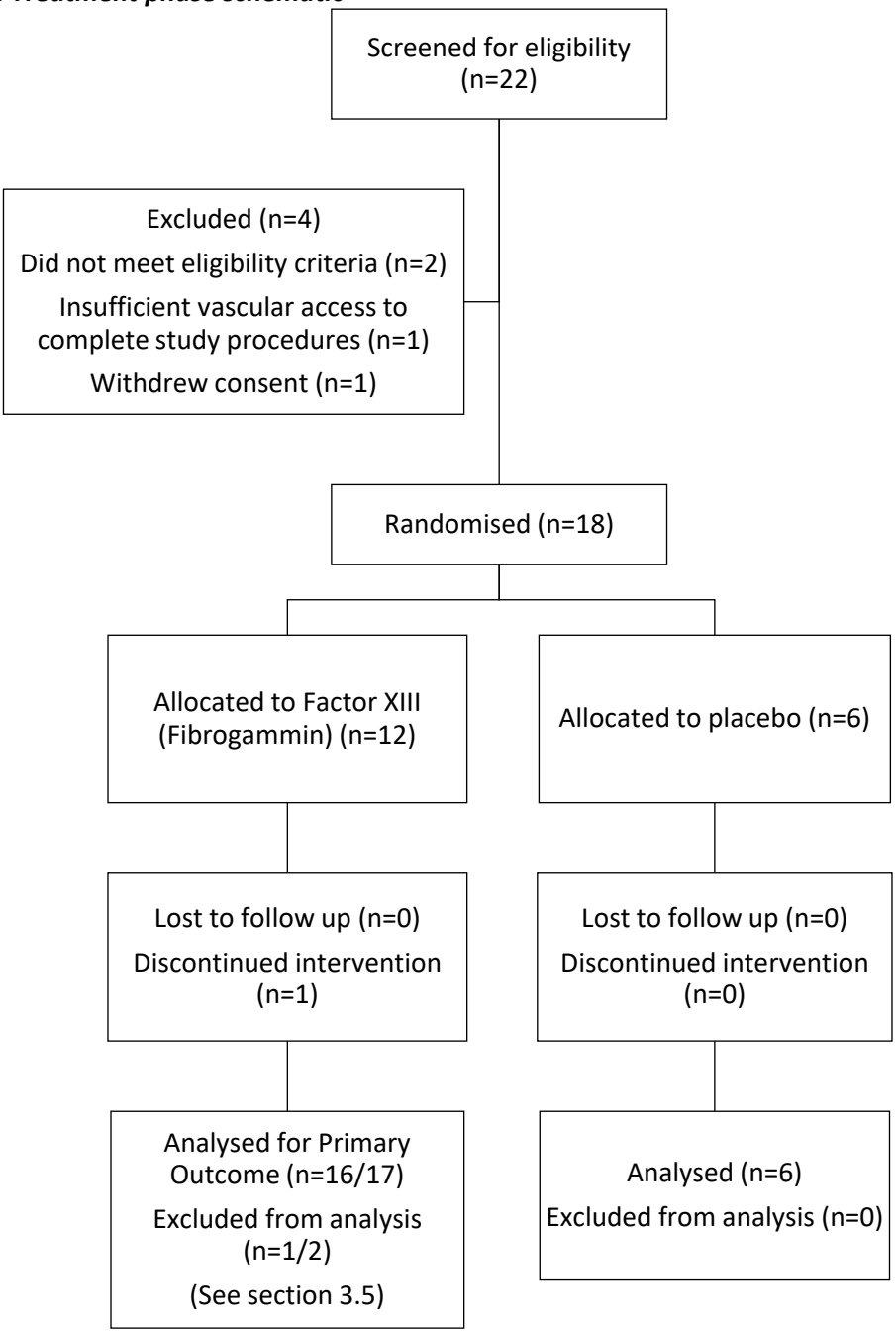
***Figure 3.1: Pharmacokinetic Phase Schematic***




### 3.2 Treatment Phase patient flow

Twenty-two patients were screened for the treatment phase, eighteen were randomised and there were four screen failures. All of the eighteen patients randomised received at least one dose of study medication or placebo. Seventeen patients completed the study. One withdrew consent to receive the study intervention due to poor vascular access which was detected after commencing the IMP/placebo; but participated in the week 24 visit to generate primary endpoint data. No patients were lost to follow up.

***Figure 3.2: Treatment phase schematic***




**3.3 Demographics and disease characteristics**

***Table 3.1: Pharmacokinetic Phase Demographics and Disease Characteristics. The results are presented as mean (SD) or absolute number (percentage).***

| **Baseline criteria** | **Factor XIII (N=8)** |
|---|---|
| Age, years | 59 (8) |
| Gender, No. (%) female | 7 (87.5) |
| Race, No. (%) Caucasian | 6 (75) |
| Weight, kg | 62.75 (12.25) |

***Table 3.2: Treatment Phase Demographics and Disease Characteristics. The results are presented as mean (SD) or absolute number (percentage)***

| **Baseline criteria** | **Factor XIII (N=12)** | **Placebo (N=6)** |
|---|---|---|
| Age, years | 59.8 (11) | 57.5 (11.6) |
| Gender, No. (%) female | 12 (100) | 6 (100) |
| Race, No. (%) Caucasian | 9 (75) | 6 (100) |
| **Vital signs** | | |
| Heart rate, bpm | 69.6 (10) | 66.7 (5.5) |
| Systolic blood pressure, mmHg | 123.5 (14.8) | 129.6 (9) |
| Diastolic blood pressure, mmHg | 70.5 (7.8) | 78.3 (13.9) |
| Temperature, °C | 36.6 (0.1) | 36.6 (0.2) |
| Weight, kg | 68.3 (12.9) | 58.7 (10.2) |
| Height, cm | 167 (7.3) | 164.3 (4.9) |

**3.4 Safety Assessments**

During the PK phase, 13 adverse events were reported. There were no serious adverse events.

During the Treatment phase there were 100 reported adverse events (75 in the FXIII arm and 25 in the placebo arm). There was one events which was recorded as serious based on investigator judgement, although it did not meet the protocol-specified definition of an SAE. The recorded SAE was listed as serious because the patient telephoned an ambulance for her symptoms, though she was not admitted to hospital. The IMP/placebo was temporarily interrupted while she was investigated and then restarted with patient consent. She made a good recovery. No patients withdrew from the trial due to adverse events.

The serious adverse events reported were a traumatic metatarsal fracture which occurred before the randomisation visit, therefore before any study drug had been administered. The second reported serious adverse event was an episode of chest pain for which the patient telephoned an ambulance. Although the patient did not attend hospital, the event was considered serious due to the extent of the pain, and the prolonged period of outpatient investigation and monitoring which followed.

The most common adverse events which were considered likely to be related to the study medication were headaches and diarrhoea. These events were self-resolving and patients reported that they were tolerable.

***Table 3.3: Adverse Events – Pharmacokinetic Phase***

| | **Factor XIII** |
|---|---|
| **Adverse Events** | 13 |
| **Serious adverse events** | 0 |

***Table 3.4: Adverse Events – Treatment Phase***

| | Number | |
|---|---|---|
| | **Factor XIII** | **Placebo** |
| **Adverse events** | 75 | 25 |
| **Adverse events that occurred more than once** | | |
| Chest infection | 4 | 0 |
| Diarrhea | 9 | 2 |
| Digital ulcer | 12 | 0 |
| Dizziness | 3 | 0 |
| Headache | 6 | 0 |
| Leg pain | 0 | 4 |
| Tooth infection | 2 | 0 |
| Upper Respiratory Tract Infection | 5 | 3 |
| **Serious adverse events** | 0 | 1 |

There were no significant chances in vital signs or clinical examination findings between the beginning and the end of the pharmacokinetic or treatment phases of the study.

**3.5 Primary Outcomes**

Sixteen patients were included for the analysis of Raynaud condition score and 17 patients included for the analysis of modified Rodnan skin score (mRSS). Table 3.5 presents mean at baseline and 24 weeks, and mean change from baseline to 24 weeks by treatment group. The mean difference between arms is also computed.

***Table 3.5. Results for primary outcomes***

| | **Baseline Mean (SD)** | | **24 weeks Mean (SD)** | | **Change at 24 weeks Mean (95% CI)** | | **Adjusted difference between arms Mean (95% CI)** |
|---|---|---|---|---|---|---|---|
| | **Factor XIII (N=12)*** | **Placebo (N=6)** | **Factor XIII (N=11)** | **Placebo (N=6)** | **Factor XIII (N=11)** | **Placebo (N=6)** | |
| **Raynaud condition score** | 3.55 (2.02) | 4.33 (2.16) | 2.27 (1.74) | 3.5 (3.56) | -1.2 (-2.26, -0.14) | -0.83 (-3.44, 1.77) | 0.49 (-2.68, 1.69) |
| **Modified Rodnan skin score (mRSS)** | 10.67 (6.46) | 8.5 (4.46) | 9.45 (5.94) | 7 (3.95) | -1.27 (-2.81, 0.26) | -1.5 (-2.95, -0.05) | 0.6 (-1.4, 2.6) |

*For Raynaud condition score, the sample size at baseline for FXIII group is N=11.

The regression analysis for Raynaud condition score suggests that there is a slightly greater reduction (-0.49, 95% CI: -2.68, 1.69) in the score for patients who receive the treatment. However, this difference is not statistically significant (p-value=0.633).

The regression analysis adjusted for baseline score for mRSS suggests that the treatment is not associated with a significant reduction in score (0.6, 95% CI: -1.4, 2.6) with a p-value of 0.528.

The response rate for patients with 20% change and at least 4 integer units of skin score is 16.7% (2/12) and 0% for the FXIII and placebo group, respectively.

Figure 3.3 presents the individual scores at baseline and 24 weeks for the FXIII and placebo groups. Figure 3.4 shows the Raynaud score at the same end points for the two comparison groups.

***Figure 3.3 Modified Rodnan skin score (mRSS) at baseline and 24 weeks for FXIII and placebo group***

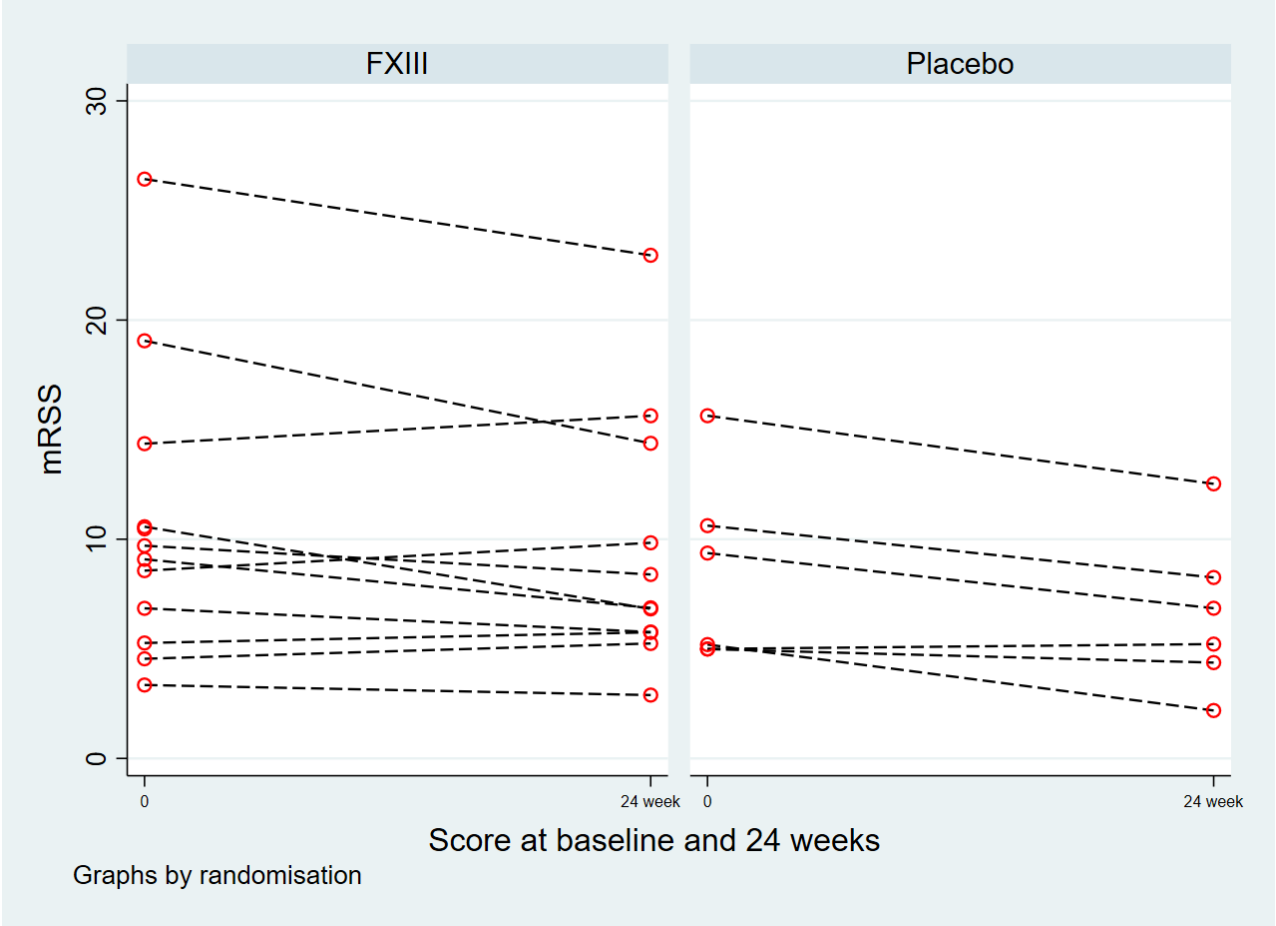
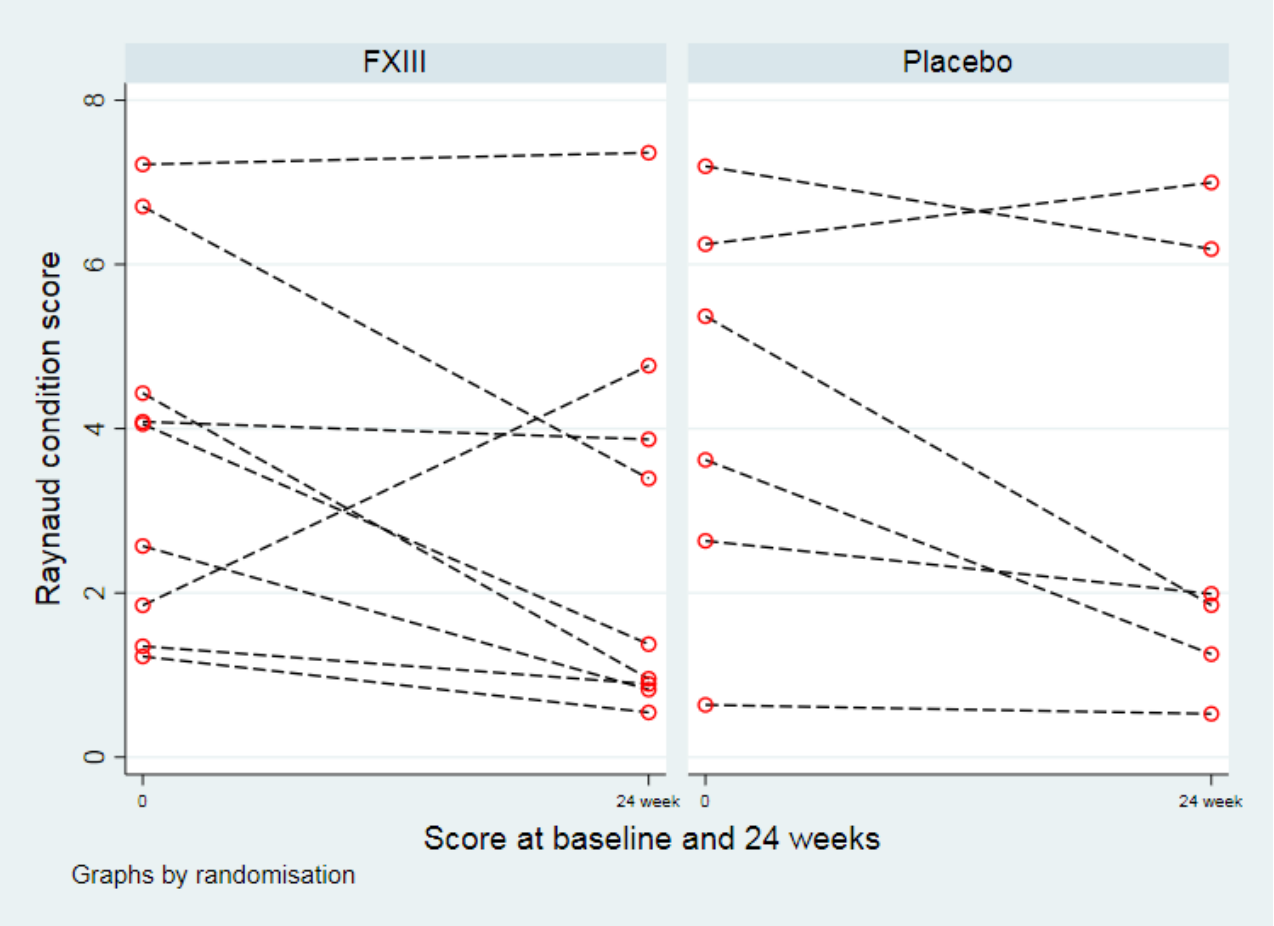


***Figure 3.4 Raynaud condition score at baseline and 24 weeks for FXIII and placebo group.***




### 3.6 Secondary Outcomes

Only descriptive results are reported for the secondary outcomes. Table 3.6 presents the mean at baseline and 24 weeks, and mean change from baseline to 24 weeks in pulmonary function, Cochin hand function, SF36 and digital ulcer counts by treatment group. Figure 3.5 shows the trend for Cochin hand function score over the 24 weeks. It suggests that the Cochin hand function is relatively stable for FXIII group compared with the placebo group where we see a large decrease in both the first 4 weeks and week 8 to 16 in contrast to a rapid increase from week 4 to 8 and week 16 to the end of the trial. Figure 3.6 is profile plot for trend in Cochin hand function by treatment group. One patient in placebo group reported high score (above 40) throughout the study period.

***Table 3.6 Results for secondary outcomes***

| | **Sample size at baseline** | | **Baseline Mean (SD)** | | **Sample size at 24 weeks** | | **24 weeks Mean (SD)** | | **Sample size for change of mean calculation** | | **Change at 24 weeks Mean (95% CI)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Factor XIII | Placebo | Factor XIII | Placebo | Factor XIII | Placebo | Factor XIII | Placebo | Factor XIII | Placebo | Factor XIII | Placebo |
| Pulmonary function | | | | | | | | | | | | |
| Percentage of forced vital capacity (FVC) predicted | 12 | 6 | 99.98 (23.72) | 116.4 (12.47) | 11 | 6 | 100 (26.26) | 113.95 (16.67) | 11 | 6 | -1.25 (-7.34, 4.83) | -2.45 (-10.52, 5.62) |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Percentage of diffusing capacity of the lung for carbon monoxide (DLCO) predicted | 11 | 6 | 63.72 (16.02) | 65.47 (21.36) | 11 | 6 | 64 (21.21) | 59.5 (18.66) | 10 | 6 | -4.05 (-9.17, 1.07) | -1.47 (-9.05, 6.11) |
| Cochin hand function | 12 | 6 | 19.33 (13.34) | 16.83 (22.1) | 11 | 5 | 16.73 (11.87) | 23.2 (23.53) | 11 | 5 | -3.64 (-6.03, -1.25) | 1.26 (-0.51, 6.51) |
| SF36 | | | | | | | | | | | | |
| Physical function | 7 | 1 | 64.3 (24.4) | 50 (-) | 7 | 1 | 50 (0) | 100 (-) | 6 | 1 | -16.7 (-43.8, 10.4) | 50 (-) |
| Physical role function | 12 | 6 | 56.3 (32.2) | 75 (27.4) | 11 | 5 | 50 (35.4) | 75 (17.7) | 11 | 5 | -4.5 (-21, 11.9) | 0 (-21.9, 21.9) |
| Emotional role function | 12 | 6 | 56.3 (32.2) | 75 (27.4) | 11 | 5 | 50 (35.4) | 75 (17.7) | 11 | 5 | -4.5 (-21, 11.9) | 0 (-21.9, 21.9) |
| Energy/fatigue | 12 | 6 | 50 (0) | 50 (0) | 11 | 5 | 50 (0) | 50 (0) | 11 | 5 | 0 | 0 |
| Social function | 12 | 6 | 50 (0) | 50 (0) | 11 | 5 | 50 (0) | 50 (0) | 11 | 5 | 0 | 0 |
| Emotional well-being | | | | | | | | | | | | |
| Pain | 12 | 6 | 49.4 (29) | 32.5 (24.6) | 11 | 5 | 55 (31.8) | 32.5 (15.9) | 11 | 5 | 4.1 (-10.7, 18.9) | 0 (-19.8, 19.8) |
| General health | 12 | 6 | 48.8 (6.4) | 45 (5.5) | 11 | 5 | 50 (7.1) | 45 (3.5) | 11 | 5 | 0.9 (-2.4, 4.2) | 0 (-4.4, 4.4) |
| Digital ulcer count | 11 | 6 | 0.55 (1.04) | 0 | 11 | 6 | 0.55 (1.21) | 0 | 10 | 6 | -0.1 (-0.51, 0.31) | 0 |

***Figure 3.5 Mean values for Cochin hand function score over 24 weeks***

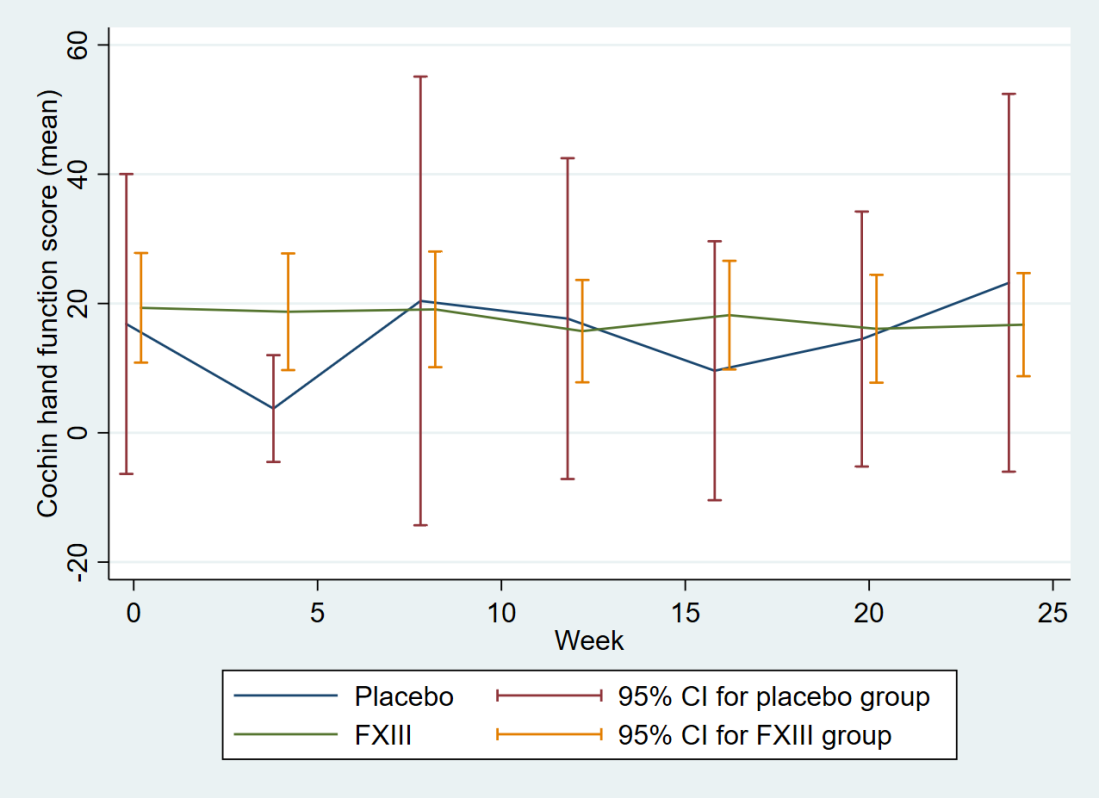



***Figure 3.6 Cochin Hand Function for individual patients over 24 weeks in FXIII and placebo***

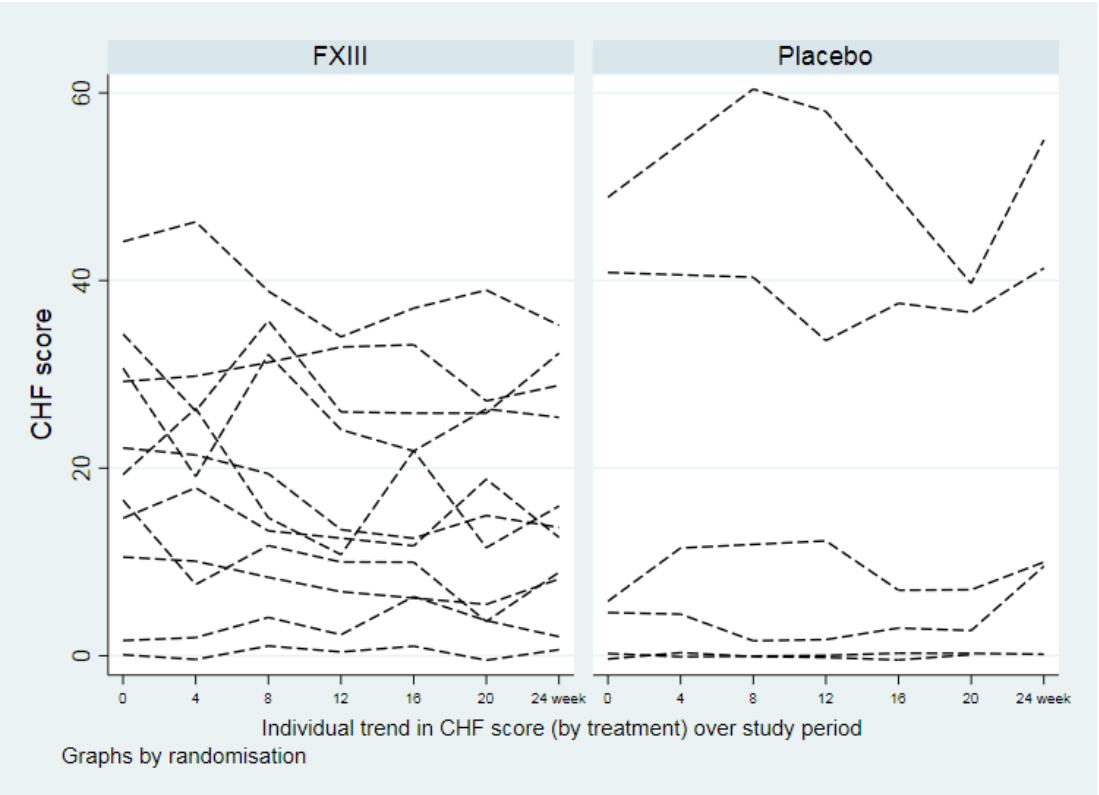


Table 3.7 presents results (mean and SD) for secondary outcomes (i.e. VAS, SHAQ, digital ulcer counts, SF36, Cochin hand function) over the study period from baseline to 24 weeks. Figure 3.7 shows the trend in VAS for individual patients over the study period. In general, patients in FXIII group reported higher VAS compared with the placebo group. The change in digital ulcer counts from baseline to 24 weeks for individual patients by treatment group is displayed in Figure 6. Note that most patients in FXIII group and all patients in placebo group have zero counts at both baseline and 24 weeks.

***Figure 3.7 VAS for individual patients over 24 weeks in FXIII and placebo groups***

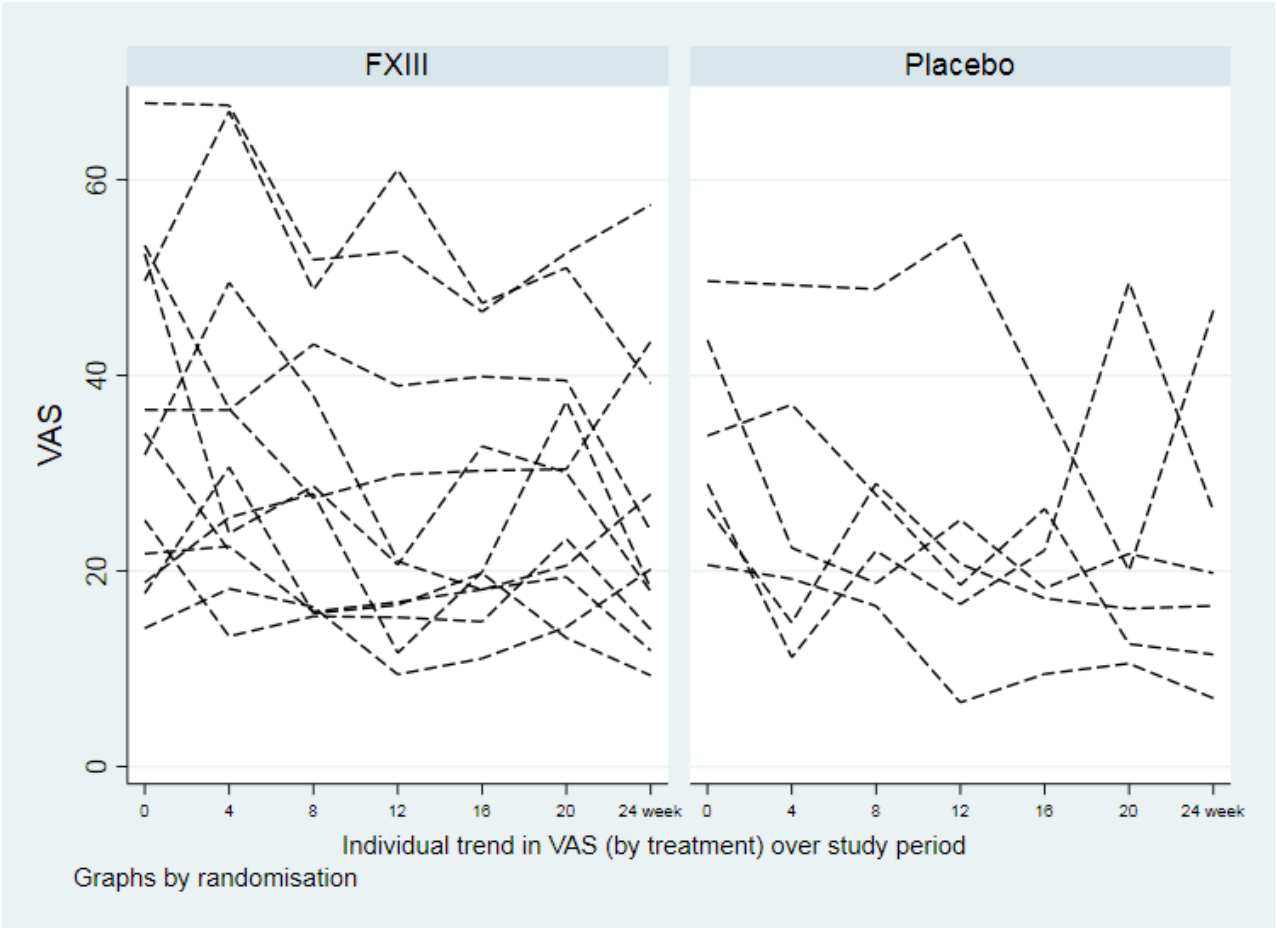


***Figure 3.8 Digital ulcer count for individual patients over 24 weeks in FXIII and placebo groups***

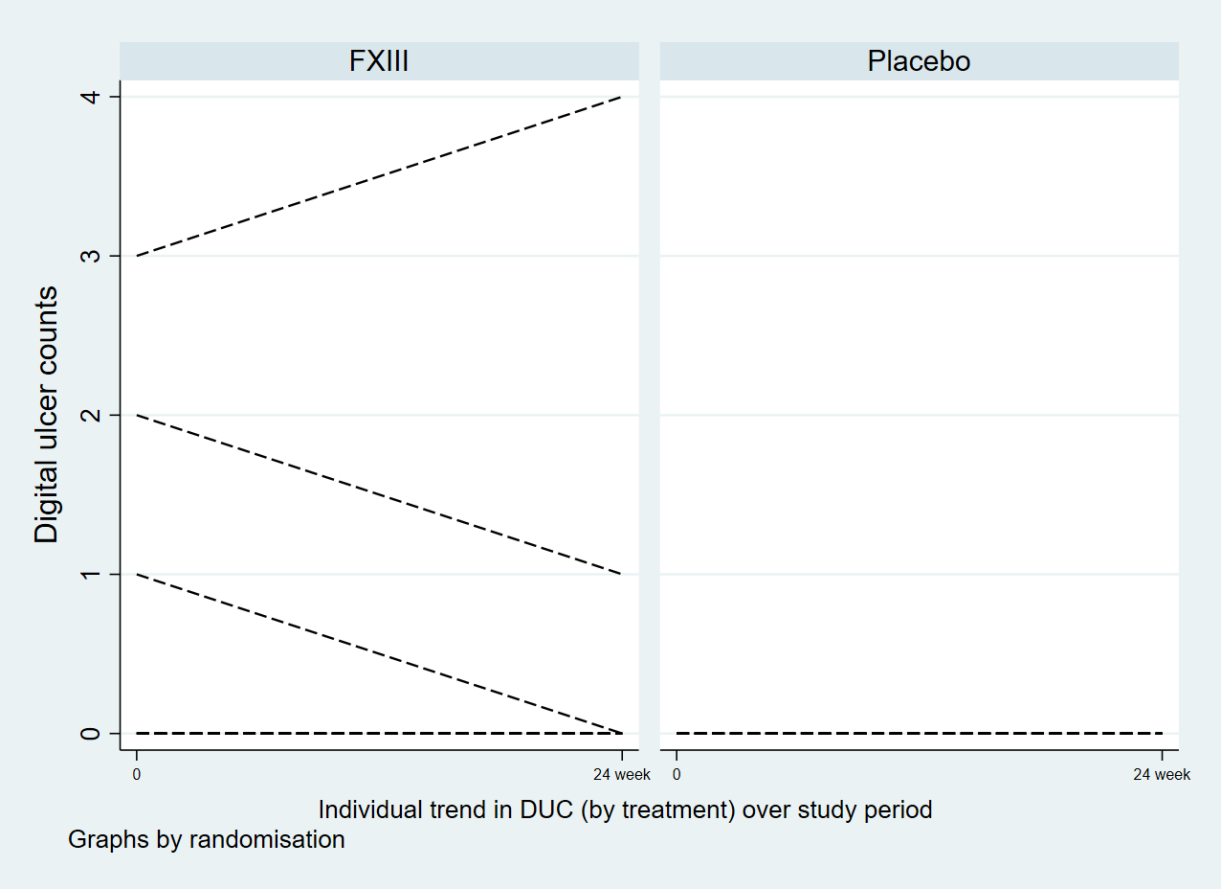



***Table 3.7 Secondary Outcomes over Time***

| Outcome | Baseline | | Week4 | | Week8 | | Week12 | | Week16 | | Week20 | | Week24 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FXIII | Placebo | FXIII | Placebo | FXIII | Placebo | FXIII | Placebo | FXIII | Placebo | FXIII | Placebo | FXIII | Placebo |
| VAS | 35.2 (17.1) | 34.1 (11) | 34.5 (18.3) | 20.7 (10.1) | 29.9 (13.8) | 26.9 (13.2) | 26.7 (17.3) | 23.8 (16) | 28 (13.3) | 18.5 (6.3) | 30 (13.8)) | 22 (13.9) | 25.8 (14.9) | 21.2 (14.3) |
| SHAQ | 1.06 (0.69) | 0.83 (0.93) | 1.07 (0.78) | 0.58 (0.86) | 0.92 (0.84) | 0.83 (1.07) | 0.95 (0.76) | 0.77 (0.97) | 1.01 (0.81) | 0.55 (0.84) | 0.95 (0.8) | 0.77 (0.96) | 0.97 (0.8) | 1.03 (0.93) |
| Digital ulcer count | 0.55 (1.04) | 0 | 0.75 (1.36) | 0 | 0.73 (1.01) | 0 | 0.55 (0.82) | 0 | 0.2 (0.42) | 0 | 0.55 (1.51) | 0 | 0.55 (1.21) | 0 |
| SF36 | | | | | | | | | | | | | | |
| Physical function | 64.3 (24.4) | 50 (-) | 58.3 (20.4) | 50 (-) | 57.1 (18.9) | 50 (-) | - | - | - | - | - | - | 50 (0) | 100 (-) |
| Physical role function | 56.3 (32.2) | 75 (27.4) | 56.8 (33.7) | 80 (32.6) | 52.3 (34.4) | 75 (30.6) | - | - | - | - | - | - | 50 (35.4) | 75 (17.7) |
| Emotional role function | 56.3 (32.2) | 75 (27.4) | 56.8 (33.7) | 80 (32.6) | 52.3 (34.4) | 75 (30.6) | - | - | - | - | - | - | 50 (35.4) | 75 (17.7) |
| Energy/fatigue | 50 (0) | 50 (0) | 50 (0) | 50 (0) | 50 (0) | 50 (0) | - | - | - | - | - | - | 50 (0) | 50 (0) |
| Social function | 50 (0) | 50 (0) | 50 (0) | 50 (0) | 50 (0) | 50 (0) | - | - | - | - | - | - | 50 (0) | 50 (0) |
| Emotional well-being | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pain | 49.4 (29) | 32.5 (24.6) | 48.8 (30.3) | 28 (29.3) | 53 (30.9) | 32.5 (27.6) | - | - | - | - | - | - | 55 (31.8) | 32.5 (15.9) |
| General health | 48.8 (6.4) | 45 (5.5) | 48.6 (6.7) | 44 (6.5) | 49.5 (6.9) | 45 (6.1) | - | - | - | - | - | - | 50 (7.1) | 45 (3.5) |
| **Cochin hand function** | 19.3 (13.3) | 16.8 (22.1) | 18.7 (13.4) | 3.8 (5.2) | 19.1 (13.3) | 20.4 (27.9) | 15.7 (11.8) | 17.7 (23.6) | 18.2 (11.7) | 9.6 (16.1) | 16.1 (12.4) | 14.5 (18.8) | 16.7 (11.9) | 23.2 (23.5) |

Note: All outcomes are summarised as Mean (SD)

### 3.7 Laboratory Studies

#### 3.7.1 Routine laboratory studies

The baseline results of the routine laboratory studies (blood and urine) are presented below in table 3.8.

***Table 3.8 Baseline results for routine laboratory studies***

| | **FXIII** | **Placebo** |
|---|---|---|
| **Haematology** | | |
| White Blood Cells, $\times 10^9$/L | 7 (1.8) | 6.4 (1.3) |
| Red Blood Count, $\times 10^{12}$/L | 4.4 (0.4) | 4.3 (0.5) |
| Haemoglobin, g/L | 117.8 (9.6) | 124.7 (12.8) |
| Haematocrit, L/L | 0.4 (0.02) | 0.4 (0.04) |
| MCV, fL | 87 (7.2) | 90.6 (5.3) |
| MCH, pg | 27.3 (3.3) | 29.3 (2.3) |
| MCHC, g/L | 312.3 (15.6) | 322.8 (7.5) |
| Platelets, $\times 10^9$/L | 298.5 (76.1) | 245 (44.7) |
| Neutrophils, $\times 10^9$/L | 4.8 (1.5) | 4.2 (1.2) |
| Lymphocytes, $\times 10^9$/L | 1.5 (0.5) | 1.5 (0.3) |
| Eosinophils, $\times 10^9$/L | 0.5 (0.2) | 0.5 (0.2) |
| Basophils, $\times 10^9$/L | 0.2 (0.2) | 0.2 (0.04) |
| Reticulocytes, $\times 10^9$/L | 0.05 (0.04) | 0.04 (0.01) |
| **Biochemistry** | | |
| Sodium, mmol/L | 140.8 (1.9) | 140.7 (2.1) |
| Potassium, mmol/L | 4.6 (0.4) | 4.5 (0.4) |

| | | |
|---|---|---|
| Chloride, mmol/L | 101.7 (2.8) | 101.8 (2.3) |
| Bicarbonate, mmol/L | 23.6 (4.1) | 22.5 (2.9) |
| Urea, mmol/L | 5.7 (2.3) | 4.9 (1.9) |
| Creatinine, umol/L | 73 (17.3) | 75 (27.5) |
| Total protein, g/L | 68.9 (3.8) | 66.3 (4) |
| Albumin, g/L | 44.3 (14.4) | 43.5 (3.2) |
| Alkaline phosphatase, U/L | 66.4 (14.4) | 65.8 (13.5) |
| ALT, U/L | 17.8 (6) | 19 (3.9) |
| AST, U/L | 19.1 (5.8) | 20.7 (3.2) |
| Calcium, mmol/L | 2.4 (0.1) | 2.3 (0.1) |
| GGT, U/L | 19.6 (13.6) | 13.3 (4.3) |
| eGFR, mL/min | 75.1 (14.6) | 72.5 (19.8) |
| Amylase, U/L | 77.3 (34.5) | 74.8 (33.2) |
| Total cholesterol, mmol/L | 5.3 (1.1) | 4.7 (1) |
| Glucose, mmol/L | 4.9 (0.6) | 4.6 (0.3) |
| **Urinalysis** | | |
| ***Glucose*** | | |
| Negative, No (%) | 10 (83.3)* | 6 (100) |
| ***Blood*** | | |
| Negative | 7 (70) | 5 (83.3) |
| Trace | 1 (10) | 0 |
| + | 2 (20) | 0 |
| ++ | 0 | 1 (16.7) |
| ***Protein*** | | |
| Negative | 10 (83.3)* | 5 (83.3) |
| + | 0 | 1 (16.7) |

*Note that two patients did not report results for glucose.

### 3.7.2 Factor XIII Analyses

Factor XIII levels taken during the PK phase demonstrated a peak in Factor XIII level within one hour of administration. Factor XIII results taken over the subsequent 28 days showed a gradual drop in Factor XIII level. The endogenous factor XII results were variable, as evidenced by differences in patient levels taken at Screening, and 1 week later at Baseline. The mean factor XIII level at Screening was 131.1 IU (SD 23.3) and the mean at Baseline was 132.3 IU (SD 25.5) units. The mean percentage change in factor XIII level between Screening and baseline was -1.6% however the range of percentage change was wide (-29.0% to 17.8%) with a standard deviation of 13.9%.

The mean time taken for the factor XIII level to return to within 10 IU of the endogenous level (Screening or Baseline) was 16.6 days (SD 8.7).

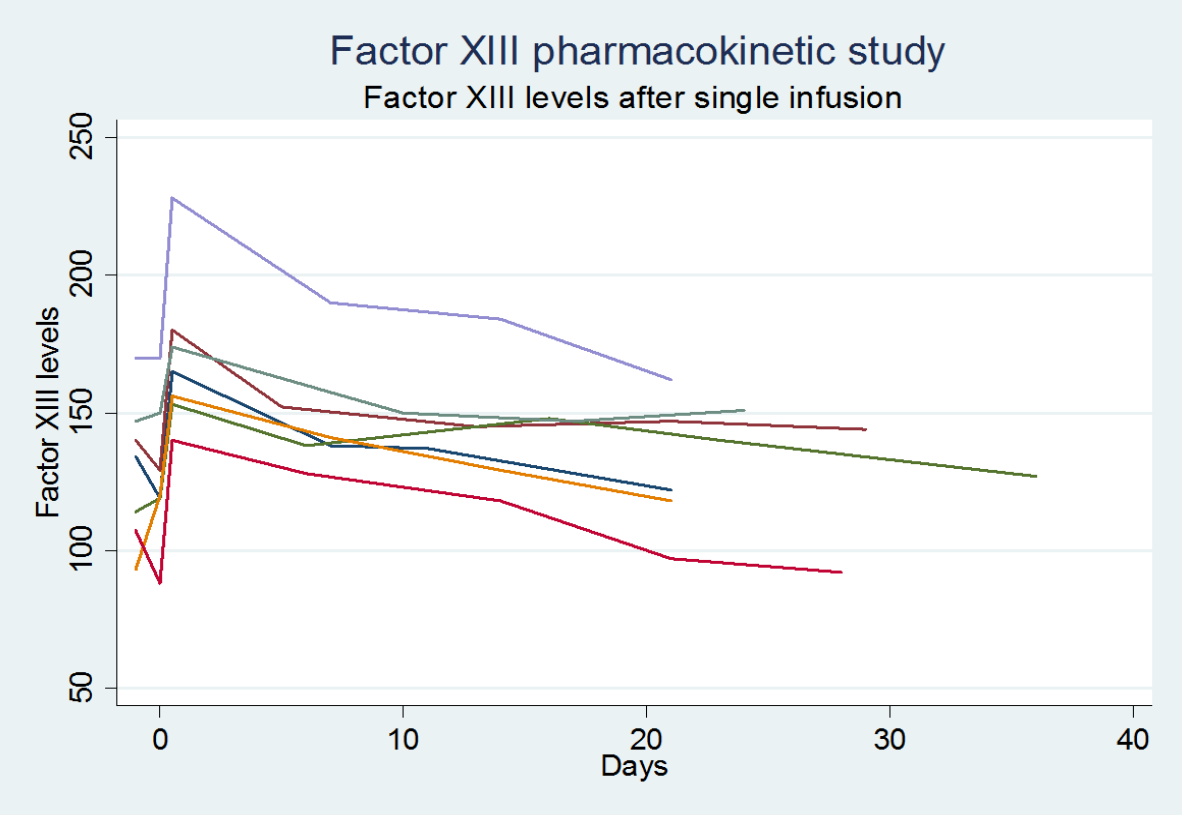
These PK data were analysed by CSL Behring who produce purified Factor XIII and have experience of calculating appropriate doses in non-SSc patient populations. The dosing algorithm for the Treatment phase was then established and incorporated into the protocol (see section 2.2.1).

***Table 3.9: PK phase FXIII levels***

| Outcome | Baseline | Week 2 | Week 4 |
|---|---|---|---|
| Factor XIII activity (IU) | 170.9 (43.7) | 190.6 (60.8) | 195.8 (65.4) |

Note: All outcomes are summarised as Mean (SD)

***Figure 3.9 PK phase Factor XIII levels***



## 4. Discussion

The study was fully recruited. There were no significant safety concerns. As this is a small trial all analysis is descriptive and no robust conclusion about efficacy can be drawn from the data although there was observation of improvement in some variables for both active treatment and placebo treated patients. Therefore, this study confirms feasibility of recruitment to the designed trial and provides a platform for future studies including any further evaluation of Factor XIII in systemic sclerosis. The justification for this is likely to depend upon work outside the present trial including possible mechanistic and preclinical scientific experiments.

## 5. Concluding remarks

This academic trial was completed successfully under Sponsorship from UCL and all necessary reports will be submitted and the data will be presented and published at future academic meetings.

## 6. Appendices

### Appendix 1: Thrombospondin-1 assays

Serum samples were taken to measure Thrombospondin-1 (TSP-1) levels during the Treatment phase of the study at week 1 (pre-treatment), week 8, and week 24.

TSP-1 levels were analysed by CSL-Behring in Australia using the R&D Systems Quantikine ELISA Human Thrombospondin-1 assay (Cat No. DTSP10, Lot no. p176627, expiry 08/04/2019). The assay uses a quantitative sandwich enzyme immunoassay technique. A human Thrombospondin-1 specific enzyme-linked polyclonal antibody is added to the microplate. Any unbound antibody-enzyme reagent is washed away and a substrate solution is added, a colour develops in proportion to the amount of TSP-1 bound. The colour development is stopped and the colour intensity is measured. The results are displayed below.

***Figure A.1: Thrombospondin-1 levels over time for Factor XIII and Placebo groups***

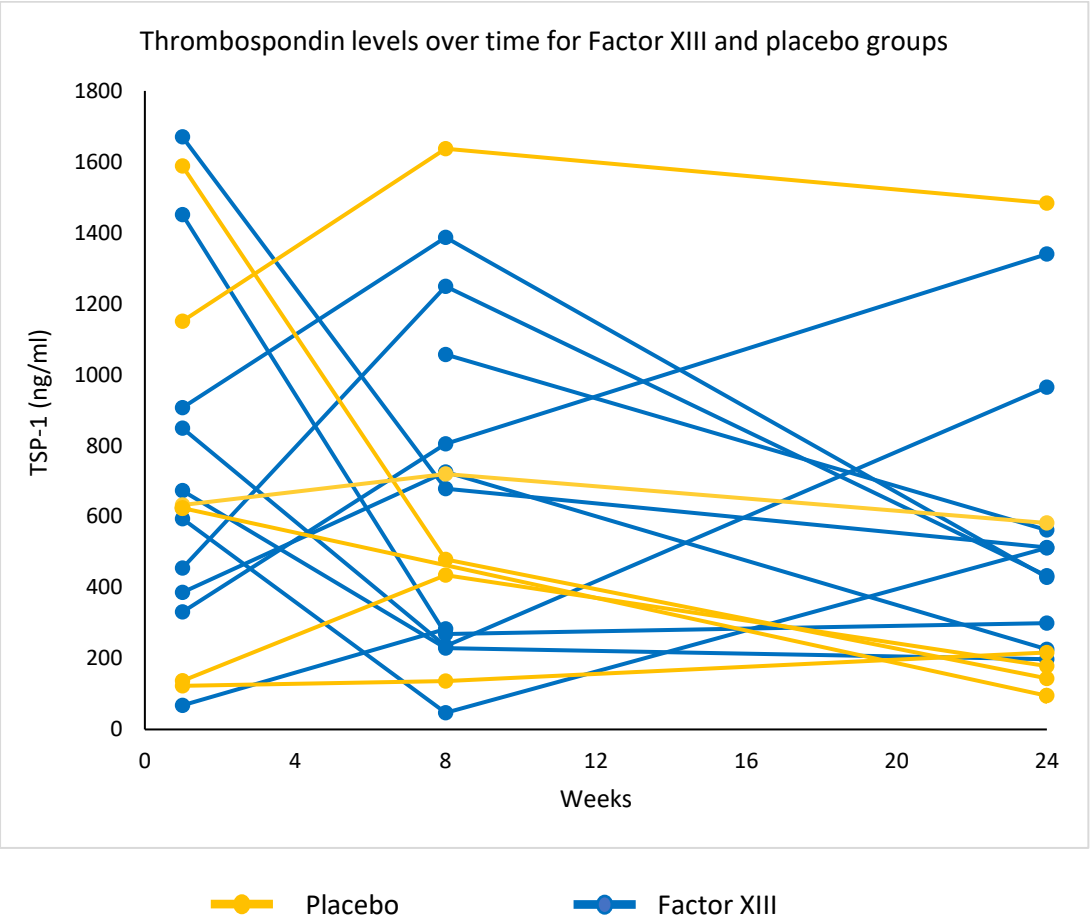
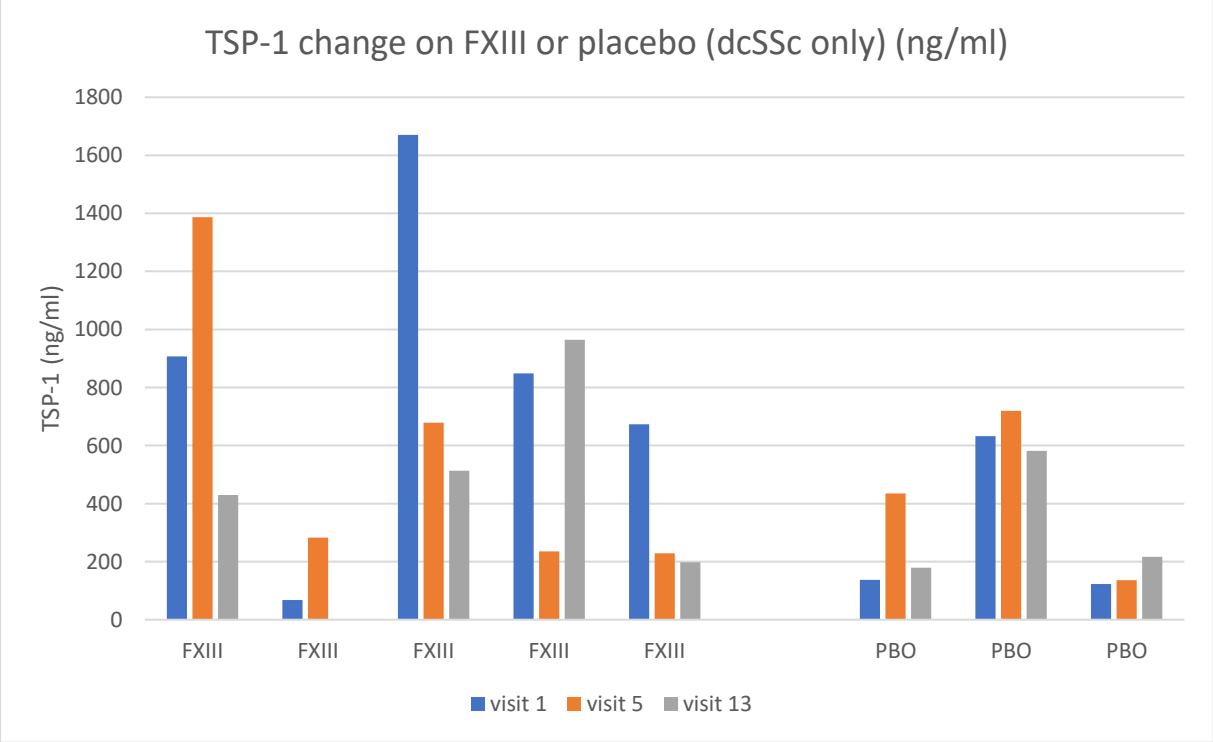



***Figure A.2: Thombospondin-1 level change over time: FXIII or placebo (dcSSc only)***



**Appendix 2: Patient and Physician Reported Outcomes**

# SSc- HEALTH ASSESSMENT QUESTIONNAIRE

**CSL SCLERO XIII (13/0417) – TREATMENT PHASE**

**Subject ID:** _____ **Patient Initials:** ☐☐☐

**Visit Type (circle one):** **Baseline (visit 1)** **Visit 3 / 5 / 7 / 9**

**Unscheduled visit** **Early withdrawal** **End of therapy (visit 13)** **Follow up (visit 14)**

Date of completion: ___ ___ / ___ ___ ___ / 2 0 ___ ___
d d m m m y y y y

Please tick one response in the box which best describes your usual abilities over the past week

| No | Item | Without ANY difficulty | With SOME difficulty | With MUCH difficulty | UNABLE to do |
|---|---|---|---|---|---|
| 1. | **Dressing & Grooming** Are you able to: | | | | |
| | • Dress yourself, including tying shoelaces and doing buttons | ☐ | ☐ | ☐ | ☐ |
| | • Shampoo your hair | ☐ | ☐ | ☐ | ☐ |
| 2. | **Arising** Are you able to: | | | | |
| | • Stand up from an armless straight chair | ☐ | ☐ | ☐ | ☐ |
| | • Get in and out bed? | ☐ | ☐ | ☐ | ☐ |
| 3. | **Eating** Are you able to : | | | | |
| | • Cut your Meat? | ☐ | ☐ | ☐ | ☐ |
| | • Lift a full cup or glass to your mouth? | ☐ | | ☐ | ☐ |
| 4. | **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
☐ Walker
☐ Crutches
☐ Wheelchair
☐ Devices used for dressing (buttonhooks, zipper pull, shoe horn)
☐ Special utensils
☐ Special or built-up chair
☐ Other (specify).....

# SSc- HEALTH ASSESSMENT QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject ID: _____ | Patient Initials: ☐☐☐ |

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

☐ Dressing &Grooming ☐ Eating

☐ Arising ☐ Walking

Please tick one response in the box which best describes your usual abilities over the past week

| No | Item | Without ANY difficulty | With SOME difficulty | With MUCH difficulty | UNABLE to do |
|---|---|---|---|---|---|
| 1. | **Hygiene** Are you able to: | | | | |
| | • Wash and dry your entire body? | ☐ | ☐ | ☐ | ☐ |
| | • Take a bath? | ☐ | ☐ | ☐ | ☐ |
| | • Get on and off the toilet? | ☐ | ☐ | ☐ | ☐ |
| 2. | **Reach** Are you able to: | | | | |
| | • Reach and get down a 2 kilo object (E.g. a bag of sugar) from just above your head? | ☐ | ☐ | ☐ | ☐ |
| | • Bend down to pick up clothing off the floor? | ☐ | ☐ | ☐ | ☐ |
| 3. | **Grip** Are you able to : | | | | |
| | • Open car doors? | ☐ | ☐ | ☐ | ☐ |
| | • Open jars that have been previously opened? | ☐ | ☐ | ☐ | ☐ |
| | • Turn taps on and off? | ☐ | ☐ | ☐ | ☐ |

# SSc- HEALTH ASSESSMENT QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject ID: _____ | Patient Initials: ☐☐☐ |

| No | Item | Without ANY difficulty | With SOME difficulty | With MUCH difficulty | UNABLE to do |
|---|---|---|---|---|---|
| 4. | **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 seat
☐ Bathtub seat
☐ Long handled appliances for reach
☐ Jar opener (for jars previously opened)
☐ Long handled appliances for bathroom
☐ Other (specify)……
☐ Bathtub bar

**Please tick any categories for which you usually need help from another Person:**

☐ Hygiene
☐ Gripping and opening things
☐ Reach
☐ Errands and chores

# SSc- HEALTH ASSESSMENT QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject ID: _____ | Patient Initials: ☐☐☐ |

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 (0) _____ Very severe pain (100)

Measured pain level ☐☐.☐

**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 (0) _____ Very severe Limitation (100)

Measured pain level ☐☐.☐

**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 (0) _____ Very severe Limitation (100)

Measured pain level ☐☐.☐

**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 (RAYNAUDS'S)

Do not limit Activities (0) _____ Very severe Limitation (100)

Measured pain level ☐☐.☐

# SSc- HEALTH ASSESSMENT QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject ID: _____ | Patient Initials: ☐☐☐ |

**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 (0) _____ Very severe Limitation (100)

Measured pain level ☐☐.☐

**Overall, considering how much pain, discomfort, limitation 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 (FINGER ULCERS)

No disease (0) _____ Very severe Limitation (100)

Measured pain level ☐☐.☐

# RAYNAUDS CONDITION SCORE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Initials: ☐☐☐ |

**Visit Type (circle one):** **Baseline (visit 1)** **Visit 3 / 5 / 7 / 9 / 11**

**Unscheduled visit** **Early withdrawal** **End of therapy (visit 13)** **Follow up (visit 14)**

Date of completion: __ __/__ __ __/ 2 0 __ __
d d m m m y y y y

Day (*circle*) Mon Tue Wed Thu Fri Sat Sun

How many times have you been exposed to outdoor temperatures today?

How many Raynaud's attacks have you had today? Please list below

| Raynaud's attack number | Duration (minutes) | Raynaud's attack number | Duration (minutes) | Raynaud's attack number | Duration (minutes) |
|---|---|---|---|---|---|
| 1 | | 6 | | 11 | |
| 2 | | 7 | | 12 | |
| 3 | | 8 | | 13 | |
| 4 | | 9 | | 14 | |
| 5 | | 10 | | 15 | |

**Raynaud's condition score (RSC)**

Circle below the number that best indicates the difficulty you had today with your Raynaud's condition:

1 2 3 4 5 6 7 8 9 **10**

No difficulty — Extreme difficulty

# SF-36 QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Initials: ☐☐☐ |

Visit Type (circle one): Baseline (visit 1) Visit 3 / 5

Unscheduled visit Early withdrawal End of therapy (visit 13) Follow up (visit 14)

**Date of completion:** __ __ / __ __ __ / 2 0 __ __

d d m m m y y y y

**For each of the following questions, please tick the one box that best describes your answer.**

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

| Excellent | Very good | Good | Fair | Poor |
|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

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

| Much better now than one year ago | Somewhat better than one year ago | About the same as one year ago | Somewhat worse now than one year ago | Much worse now than one year ago |
|---|---|---|---|---|
| ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

**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 | Yes, limited A lot | Yes, limited a little | No, not Limited At all |
|---|---|---|---|
| a) **Vigorous activities** such as running, lifting heavy objects, participating in strenuous sports | ☐1 | ☐2 | ☐3 |
| b) **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling or playing golf | ☐1 | ☐2 | ☐3 |
| c) Lifting or carrying groceries | ☐1 | ☐2 | ☐3 |
| d) Climbing **several** flights of stairs | ☐1 | ☐2 | ☐3 |
| e) Climbing **one** flight of stairs | ☐1 | ☐2 | ☐3 |
| f) Bending, kneeling, or stooping | ☐1 | ☐2 | ☐3 |

# SF-36 QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject ID: _____ | Patient Initials: ☐☐☐ |

| Activities | Yes, limited A lot | Yes, limited a little | No, not Limited At all |
|---|---|---|---|
| g) Walking **more than one kilometre** | ☐1 | ☐2 | ☐3 |
| h) Walking **half a kilometre** | ☐1 | ☐2 | ☐3 |
| i) Walking **100 metres** | ☐1 | ☐2 | ☐3 |
| j) **Bathing** or **dressing** yourself | ☐1 | ☐2 | ☐3 |

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

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

**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 any emotional problems (such as feeling depressed or anxious)?**

| | All of the time | Most of the time | Some of time | A little of the time | None of the time |
|---|---|---|---|---|---|
| a. Cut down on the **amount of time** you spent on work or other activities | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| b. **Accomplished less** than you would like | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| c. Did work or other activities **less careful than usual** | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

# SF-36 QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject ID: _____ | Patient Initials: ☐☐☐ |

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

| Not at all | Slightly | Moderate | Quite a bit | Extremely |
|---|---|---|---|---|
| ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

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

| None | Very mild | Mild | Moderate | Severe | Very severe |
|---|---|---|---|---|---|
| ☐1 | ☐2 | ☐3 | ☐4 | ☐5 | ☐6 |

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

| Not at all | Slightly | Moderate | Quite a bit | Extremely |
|---|---|---|---|---|
| ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

8. 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…

| | All of the time | Most of the time | Some of the time | A little the time | None of the time |
|---|---|---|---|---|---|
| a) Did you feel full of life | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| b) Have you been very nervous | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| c) Have you felt calm and peaceful | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| d) Did you have a lot of energy | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| e) Have you felt downhearted and depressed | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| f) Did you feel worn out | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| g) Have you been happy | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| h) Did you feel tired | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

# SF-36 QUESTIONNAIRE

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Initials: ☐☐☐ |

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

| All of the time | Most of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|
| ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

**10. How TRUE or FALSE is <u>each</u> of the following statements for you?**

| | Definitely true | Mostly true | Don't know | Mostly false | Definitely false |
|---|---|---|---|---|---|
| a) I seem to get sick a little easier than other people | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| b) I am as healthy as anybody I know | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| c) I expect my health to get worse | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| d) My health is excellent | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

# Cochin Hand Function Score

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Initials: ☐☐☐ |

Visit Type (circle one): Baseline (visit 1) Visit 3 / 5 / 7 / 9 / 11

Unscheduled visit Early withdrawal End of therapy (visit 13) Follow up (visit 14)

Date of completion: ___ ___ / ___ ___ ___ / 2 0 ___ ___
d d m m m y y y y

**Please indicate to what extent you can perform the following activities.**

| | Yes, without difficulty | Yes, with little difficulty | Yes, with some difficulty | Yes, with much difficulty | Nearly impossible to do | Impossible to do |
|---|---|---|---|---|---|---|
| 1. Can you hold a bowl? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 2. Can you grasp a full bottle and raise it? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 3. Can you hold a plate full of food? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 4. Can you pour liquid from a bottle into a glass? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 5. Can you unscrew the lid from a jar that has been opened before? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 6. Can you cut meat with a knife? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 7. Can you prick things well with a fork? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 8. Can you peel fruit? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 9. Can you button your shirt? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 10. Can you open and close a zipper? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 11. Can you squeeze a new tube of toothpaste? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 12. Can you hold a toothbrush effectively? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 13. Can you write a short sentence with an ordinary pen? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 14. Can you write a letter with an ordinary pen? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

# Cochin Hand Function Score

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Intials: ☐☐☐ |

| | Yes, without difficulty | Yes, with little difficulty | Yes, with some difficulty | Yes, with much difficulty | Nearly impossible to do | Impossible to do |
|---|---|---|---|---|---|---|
| 15. Can you turn a round door knob? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 16. Can you cut a piece of paper with scissors? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 17. Can you pick up coins from a table top? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| 18. Can you turn a key in a lock? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

# MODIFIED RODNAN SKIN SCORE (MRSS)

| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Intials: ☐☐☐ |

| | | | |
|---|---|---|---|
| Visit Type (circle one): | Screening | Baseline (visit 1) | Visit 3 / 5 / 7 / 9 / 11 |
| Unscheduled visit | Early withdrawal | End of therapy (visit 13) | Follow up (visit 14) |

**MODIFIED RODNAN SKIN SCORE(MRSS)**

**Date of assessment** __ __/__ __ __/ 2 0 __ __
d d m m m y y y y

| Body site | Normal skin | Slight thickening | Moderate thickening | Severe thickening |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Face | ☐ | ☐ | ☐ | ☐ |
| Anterior chest | ☐ | ☐ | ☐ | ☐ |
| Abdomen | ☐ | ☐ | ☐ | ☐ |
| Upper arm – left | ☐ | ☐ | ☐ | ☐ |
| Upper arm – right | ☐ | ☐ | ☐ | ☐ |
| Forearm – left | ☐ | ☐ | ☐ | ☐ |
| Forearm – right | ☐ | ☐ | ☐ | ☐ |
| Hand – left | ☐ | ☐ | ☐ | ☐ |
| Hand – right | ☐ | ☐ | ☐ | ☐ |
| Fingers – left | ☐ | ☐ | ☐ | ☐ |
| Fingers – right | ☐ | ☐ | ☐ | ☐ |
| Thigh – left | ☐ | ☐ | ☐ | ☐ |
| Thigh – right | ☐ | ☐ | ☐ | ☐ |
| Leg – left | ☐ | ☐ | ☐ | ☐ |
| Leg – right | ☐ | ☐ | ☐ | ☐ |
| Foot – left | ☐ | ☐ | ☐ | ☐ |
| Foot – right | ☐ | ☐ | ☐ | ☐ |
| | | | | Score ☐☐ /51 |

# SCLERODERMA PHYSICIAN GLOBAL VISUAL ANALOGUE SCALE

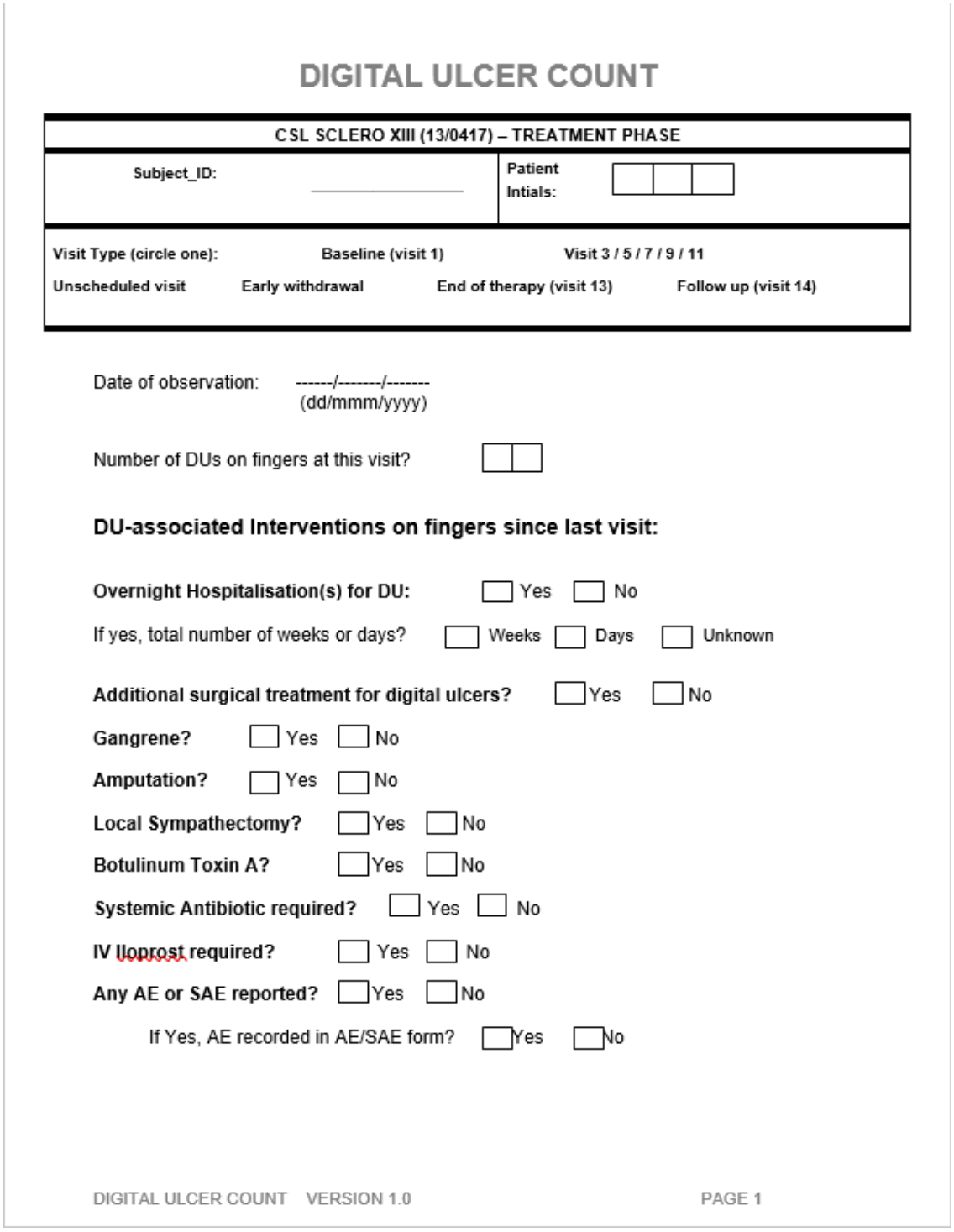
| CSL SCLERO XIII (13/0417) – TREATMENT PHASE | |
|---|---|
| Subject_ID: _____ | Patient Intials: ☐☐☐ |

Visit Type (circle one): Baseline (visit 1) Visit 3 / 5 / 7 / 9 / 11

Unscheduled visit Early withdrawal End of therapy (visit 13) Follow up (visit 14)

Date of completion: ___ ___ / ___ ___ ___ / 2 0 ___ ___
d d m m m y y y y

| SCLERODERMA PHYSICIAN GLOBAL VISUAL ANALOGUE SCALE | |
|---|---|
| ☐ Tick if not Applicable<br>**Overall, considering how much pain, discomfort, limitation in the patient's daily life, how severe would you rate their disease today?**<br>PLACE A MARK ON THE LINE TO INDICATE PHYSICIAN GLOBAL ASSESSMENT<br>Very well (0) — Very poor (100) | |
| Office use only<br>Measured pain level | ☐☐ . ☐ **cm** |



DIGITAL ULCER COUNT

CSL SCLERO XIII (13/0417) – TREATMENT PHASE

Subject_ID: _____ Patient Intials: ☐☐☐

Visit Type (circle one): Baseline (visit 1) Visit 3 / 5 / 7 / 9 / 11

Unscheduled visit Early withdrawal End of therapy (visit 13) Follow up (visit 14)

Date of observation: ------/-------/------- (dd/mmm/yyyy)

Number of DUs on fingers at this visit? ☐☐

**DU-associated Interventions on fingers since last visit:**

**Overnight Hospitalisation(s) for DU:** ☐ Yes ☐ No

If yes, total number of weeks or days? ☐ Weeks ☐ Days ☐ Unknown

**Additional surgical treatment for digital ulcers?** ☐ Yes ☐ No

**Gangrene?** ☐ Yes ☐ No

**Amputation?** ☐ Yes ☐ No

**Local Sympathectomy?** ☐ Yes ☐ No

**Botulinum Toxin A?** ☐ Yes ☐ No

**Systemic Antibiotic required?** ☐ Yes ☐ No

**IV Iloprost required?** ☐ Yes ☐ No

**Any AE or SAE reported?** ☐ Yes ☐ No

If Yes, AE recorded in AE/SAE form? ☐ Yes ☐ No

DIGITAL ULCER COUNT VERSION 1.0 PAGE 1

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