A randomized, parallel-group, double-blind, placebo-controlled phase 3 study to Determine the effectiveness of the type I interferon receptor antibody, Anifrolumab, In SYstemic sclerosis: DAISY study design and rationale

Running title: (42 of 45 characters) Anifrolumab SSc DAISY study design and rationale

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Abstract (240 of 250 words)

Objective

The type I interferon pathway is a promising target for effective treatment of patients with systemic sclerosis (SSc). Here, we describe the design of a multinational, randomized phase 3 study to Determine the effectiveness of the type I interferon receptor antibody, Anifrolumab, In SYstemic sclerosis (DAISY).

Methods

DAISY includes a 52-week double-blind, placebo-controlled treatment period, a 52-week open-label active treatment period, and a 12-week safety follow-up period. The patient population includes a planned 306 adults with limited or diffuse cutaneous (classified by Leroy and Medsger 2001) active SSc (American College of Rheumatology/European Alliance of Associations for Rheumatology 2013 criteria). Use of standard immunosuppressants, including mycophenolate mofetil, at a stable dose prior to randomization is permitted in addition to weekly subcutaneous anifrolumab or placebo. Efficacy will be assessed at Week 52 via Revised-Composite Response Index in SSc (CRISS)-25 response (primary endpoint). Lung function and skin activity will be assessed via change from baseline in forced vital capacity in patients with SSc-associated interstitial lung disease and modified Rodnan Skin Score, respectively (key secondary endpoints).

Conclusion

The DAISY trial will evaluate the efficacy of anifrolumab as a first-in-class treatment option for patients with both limited and diffuse cutaneous SSc and will provide insight into the contributions of type I interferon to SSc pathogenesis. Revised-CRISS-25 can account for improvement and worsening in a broad set of disease activity domains beyond lung function

and skin, including clinician- and patient-reported outcomes, capturing the heterogeneity of SSc.

Introduction

Systemic sclerosis (SSc) is a complex, heterogeneous autoimmune disease in which vasculopathy, inflammation, and fibrosis contribute to damage in multiple organs (1). Dysregulated interferon signalling is a cardinal feature of autoimmune diseases often linked as overlap syndromes including systemic lupus erythematosus (SLE), Sjögren's syndrome, and SSc. There is evidence that type I interferon dysregulation may play an important role in SSc pathogenesis (2, 3). Excess type I interferon signalling has been consistently identified in the blood and tissues of large percentages of patients with SSc (4, 5), and type I IFN pathway activity has been associated with the presence of SSc autoantibodies (6) and the severity level of organ involvement (3, 7). Immunoablation and stem cell rescue in patients with SSc was observed to normalize the levels of two interferon gene expression modules; this decrease in transcript levels was significantly correlated with improvement in forced vital capacity (FVC) (8), an important clinical measure of lung function (9).

Particularly strong support for a pathogenic role of type I interferon in SSc came from a randomized, placebo-controlled trial of patients with early diffuse cutaneous SSc, in which treatment with recombinant type I interferon-α led to SSc disease progression rather than the disease improvement that had been anticipated (10). Conversely, inhibiting the type I interferon pathway in SLE, another disease in which the interferon signature has been associated with disease activity (11), led to the first new biologic drug approval for patients with SLE in many years (12). Anifrolumab is a fully-human, IgG1κ monoclonal antibody that targets the type I interferon receptor α subunit 1 and blocks downstream type I interferon

signalling, which suppresses the interferon gene signature (IFNGS) (13, 14). In phase 3 trials, efficacy of anifrolumab in patients with moderate to severe SLE was demonstrated across organ systems (15) and for the duration of the trial, up to 4 years (16).

The safety/tolerability, pharmacodynamics, and pharmacokinetics of anifrolumab were previously investigated in a phase 1 trial of patients with SSc (17). The phase 1 trial revealed an adequate safety and tolerability profile of anifrolumab in patients with SSc, whereby most treatment-emergent adverse events were mild or moderate, and anifrolumab treatment decreased type I IFNGS expression in whole blood and skin (17). Anifrolumab treatment in the phase 1 trial was also associated with suppression of two T-cell activation markers, a collagen marker, and upregulation of a marker of collagen degradation, which all support the potential of anifrolumab as a SSc treatment (18).

Together, the strong evidence for the role of type I interferon in SSc pathogenesis and the safety and efficacy results from the phase 1 trial in patients with SSc support further investigation of anifrolumab as a treatment for SSc (3, 17, 18). Thus, here we report the study design of a multinational, randomized, placebo-controlled, double-blind phase 3 study (DAISY; NCT05925803). DAISY aims to evaluate the safety and efficacy of subcutaneous (SC) anifrolumab in adult patients with SSc who may be taking one or a combination of protocol-specified standard therapies and to establish a long-term profile of anifrolumab treatment in SSc.

Methods

Study design

DAISY is a multinational, randomized (1:1), parallel-group, double-blind, placebo-controlled, phase 3 study that plans to include 306 patients in at least 22 countries. The study consists of 4 periods with total study duration of ~122 weeks: a ≤6-week screening period

prior to randomization; a 52-week double-blind, placebo-controlled treatment period; a 52-week open-label active treatment period; and a 12-week safety follow-up period (**Figure 1**). The primary and key secondary endpoints will be evaluated at Week 52.

The study visit schedule, self-administered dosing, and visit procedures/activities were discussed with 15 patients from the US and UK living with SSc. Their input contributed to reducing the burden of the trial visits, guiding decisions about visit/assessment frequency and flexibility, and about the digital tools (eg, touch pen/stylus) utilized for collection of patient-reported outcomes.

Ethics

The study is designed to be conducted in accordance with consensus ethical principles derived from international guidelines, including the Declaration of Helsinki as amended in October 2013. The study protocol, any amendments, Investigator's Brochure, and informed consent forms will be reviewed and approved by an Institutional Review Board/Ethic Committee for each study site, and patients will provide informed consent prior to participation.

Patient selection

DAISY will include adults 18–70 years of age (inclusive) with SSc according to 2013 American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) classification criteria (19), and either limited or diffuse cutaneous subset as defined by LeRoy and Medsger (20). Use of standard immunosuppressants, including hydroxychloroquine, methotrexate, azathioprine, mycophenolate mofetil (MMF), mycophenolic acid or mycophenolate sodium (MPA), tacrolimus, or oral glucocorticoids, will be permitted (not required) at a stable dose prior to randomization; the dose should remain stable throughout the study. Key inclusion and exclusion criteria are presented in

Table 1 and a full list, including permitted immunosuppressant treatment dosages and timeframes, is provided in **Supplement S1**.

Treatment

Patients will receive once weekly treatment with 120 mg anifrolumab, administered subcutaneously via an accessorized prefilled syringe (0.8 mL fill volume), or with matching placebo. Study intervention may be administered by study staff, patient, or caregiver, in the clinic or, for weekly doses in between clinic visits, at home.

Efficacy assessments

Efficacy analyses will be performed using the "full analysis dataset" of all patients who were randomized to treatment and received ≥1 dose of study intervention (**Table 2**; endpoint definitions in **Table 3**). The primary efficacy endpoint will be Revised-Composite Response Index in SSc (CRISS)-25 (Revised-CRISS-25) response (yes/no) at Week 52 (21). Revised-CRISS-25 uses a 2-step algorithm, with equal weighting given to each of five validated core measures (modified Rodnan skin score [mRSS], percent predicted forced vital capacity [ppFVC], Health Assessment Questionnaire–Disability Index [HAQ-DI], and patient and clinician global assessments [PtGA and CGA]) (21, 22) and assesses percent improvement and worsening in these measures in patients with SSc. In Step 1, SSc-related events are adjudicated for new onset renal crisis, ppFVC below 80% predicted, pulmonary arterial hypertension, or left ventricular failure (left ventricular ejection fraction ≤45%), for significant worsening of ILD (decline in ppFVC ≥15%), or for significant gastrointestinal dysmotility (requiring enteral or parenteral nutrition) or digital ischemia (ie, with gangrene or requiring hospitalization or amputation). Patients that develop Step 1 events during the study are classified as non-responders and included in Step 2. In patients with no new or worsening major organ involvement, a Revised-CRISS-25 response is determined in Step 2: response is

achieved by showing improvement in ≥2 components and worsening in ≤1 component of the measure. Improvement and worsening thresholds were defined according to published minimal clinically important differences for each measure: a ≥25% change in mRSS, HAQ-DI, CGA, or PtGA, or a ≥5% change in ppFVC (21).

Key secondary endpoints include change from baseline to Week 52 in 1) FVC (mL) in patients with SSc-associated ILD and 2) in mRSS in the full analysis dataset. Other secondary endpoints assessed in the full analysis dataset include improvement in each of the five components of Revised-CRISS-25 at Week 52 and change from baseline to Week 52 in computed tomography (CT) measures of ILD (Quantitative ILD [QILD] and quantitative light-induced fluorescence [QLF]), FVC (mL), and ppFVC. Patient-reported outcome (PRO) secondary endpoints include change from baseline to Week 52 in HAQ-DI, PtGA, and Scleroderma Skin Patient Reported Outcome (SSPRO). Change from baseline to Week 52 in ppFVC in patients with SSc-associated ILD is also a secondary endpoint.

Safety assessments

Safety and tolerability will be evaluated in terms of adverse events (AEs; for each treatment group by system organ class and number and percentage of patients reporting that event, and number of events where appropriate), AEs of special interest (serious non-opportunistic infection, opportunistic infection, herpes zoster, tuberculosis [including latent tuberculosis], malignancy, injection site reaction, and major adverse cardiac events), vital signs, clinical laboratory tests, 12-lead electrocardiograms, physical examination, Columbia Suicide Severity Rating Scale (C-SSRS), and Patient Health Questionnaire Depression Scale (PHQ-8). Patients will be assessed for coronavirus disease-2019 at each clinic visit.

Pharmacodynamics and pharmacokinetics

Whole blood will be collected at screening and pre-dose for pharmacodynamic, pharmacokinetic, and/or immunogenicity tests throughout the study. The suppression of the type I interferon 21-gene signature in peripheral blood as a percent of baseline will be explored as a secondary endpoint to follow the biologic effect of anifrolumab on its target throughout the study (23). A dichotomous 4-gene IFNGS test in peripheral blood will be used to measure the overexpression of mRNA relative to certain types of type I interferon-inducible genes at baseline to evaluate if the participants had high/low IFNGS at study entry. This information will be used as part of an exploratory analysis to understand if interferon activity at baseline would impact clinical outcomes (24). In addition to whole blood samples, optional skin biopsy samples may be collected for biomarker measurement alongside plasma and serum samples to explore the wider mechanism of action of anifrolumab, downstream of IFN blockade, in blood and skin.

Statistical methods

Approximately 460 patients will be screened to achieve 306 eligible patients for randomization. A sample size of 153 participants per arm yields 90% power to detect a treatment difference of 18% in the proportions of patients who achieve Revised-CRISS-25 response at Week 52 between anifrolumab and placebo groups (two-sided α=0.05). The assumed proportion of responders (participants achieving Revised-CRISS-25 at Week 52) is 30% in the placebo group (21).

To control for study-wise Type I error, the primary endpoint will be tested at a 2-sided α=0.05 and, if statistically significant, then key secondary endpoints will be tested using a hierarchical sequential testing strategy in pre-defined order.

The proportions of patients achieving Revised-CRISS-25 response at Week 52 with anifrolumab vs placebo (the primary endpoint) will be compared using a Cochran-Mantel-

Haenszel (CMH) approach, controlling for randomization stratification factors (ILD [yes/no] at Week 0/Day 1; MMF or MPA use [yes/no] at Week 0/Day 1; and disease duration from first non-Raynaud's symptom attributable to SSc [</≥18 months] at Week 0/Day 1).

Models for repeated measures including baseline value, treatment group, visit, treatment x visit interaction, and randomization stratification factors will be used to compare treatment groups and assess key secondary endpoints: change from baseline to Week 52 in 1) FVC and 2) mRSS.

A "safety dataset" consisting of all patients who received ≥1 dose of study intervention will be used to assess safety and tolerability. Patients' results will be reported according to the treatment actually received.

Discussion

There is strong evidence for a role of type I interferon in the pathogenesis of SLE, SSc, and related connective tissue diseases (3, 7, 8, 10). Multiple lines of evidence support that type I interferon receptor inhibition with anifrolumab has the potential to be a first-in-class treatment option for patients with SSc (17, 18). Here, we describe the design of a multinational phase 3 study to evaluate the safety and efficacy and the long-term treatment profile of anifrolumab in adult patients with diffuse or limited cutaneous SSc.

The study partnered with patients to capture and amplify the SSc patient voice throughout the clinical trial, including the study design process. Patients provided ideas to reduce the burden of the trial and to make it fit-for-purpose for an SSc patient population. Discussions with patients also allowed us to better understand how to capture diaries and instruments and how to mitigate SSc-related issues with using touch-screen digital devices. Patients will also be

completing several PRO instruments throughout the trial (including the HAQ-DI and PtGA that are components of the primary endpoint), providing further opportunities to capture the patient voice throughout the drug development process. Furthermore, inclusion of an open-label extension period of 1 year will allow patients to be involved in their treatment decisions.

A unique feature in the study design is the selection of Revised-CRISS-25 the primary endpoint in this study. Revised-CRISS was recently developed and is being utilized in current phase 2 (NCT03844061, NCT05559580) and phase 3 studies. This measure incorporates multiple sources of assessments (PROs, clinician-reported outcomes, and surrogate biomarkers of disease activity [ie, FVC]) (21) to determine patient response according to the validated core set variables selected in ACR-CRISS (22, 25). Use of a composite response measure can provide insights into changes in multiple organ systems, not just skin involvement (26), and therefore provide insights into overall patient health for a population of patients with a complex, heterogenous disease. Use of a composite response measure also enables inclusion of a broad population of patients with SSc, a strength of this study.

Whereas randomized trials for SSc therapies intended to treat overall SSc disease and skin fibrosis tend to include only patients with diffuse cutaneous SSc (26), this study includes patients with limited or diffuse cutaneous SSc, which reflects the real-world target population of the therapy. Given that type I interferon might contribute to SSc pathogenesis across multiple different organs (27, 28), it is important to include a broad population of patients in this trial of anifrolumab. Type I interferon, measured as an elevated IFNGS, has been identified at the earliest phases of SSc, and in patients with both limited and diffuse cutaneous SSc (29). Interferon-regulated gene expression has been implicated as a pathogenic pathway in patients with SSc-related ILD (27), and progression of ILD can be similar in patients from both cutaneous subsets (30). In this trial, we will quantify the degree of total lung involvement and fibrosis at baseline and at the end of the trial, as done in the Phase 3

tocilizumab trial (31). Importantly, there is currently no disease-modifying treatment option available for patients with limited SSc; as such, new clinical trials are needed in this patient population (32).

Another important feature of the DAISY study design is the option for patients to receive background treatment with standard immunosuppressants alongside the trial intervention. MMF is the most common first-line standard therapy for patients with diffuse SSc (26). This is based largely on its benefit for patients who also have ILD (26): >20% of patients can be anticipated to respond on ppFVC with MMF alone (33). Thus, including background therapy may reduce the ability to observe a treatment effect in a clinical trial, as was seen in a separate phase 3 trial of lenabasum in patients with SSc (25), but not in a phase 2 trial of romilkimab (34). Additionally, the option for background immunosuppressive treatment may impact the frequency of Step 1 events (ie, new or worsening major organ involvement) in the determination of Revised/ACR-CRISS response; previous studies without background immunosuppression (eg, (31)) have higher contribution from Step 1 vs Step 2. These potential issues could be mitigated by including MMF use as a stratification factor for randomization and requiring MMF treatment to have been initiated ≥6 months prior to randomization and at a stable dosage ≤3 g/day for ≥3 months prior to randomization. Including MMF as a background therapy in our phase 3 trial will allow this study to provide meaningful information to treating clinicians managing SSc (rheumatologists, dermatologists, and pulmonologists) who might wish to understand the role of anifrolumab alongside standard therapies that are frequently prescribed in the clinic.

As aforementioned, the potential for MMF background therapy to mask treatment response, and the lack of published data with Revised-CRISS-25 as a primary endpoint, can be considered limitations of this study design. Additionally, though the study includes patients

from both limited and diffuse cutaneous subsets, other patient population, such as pediatric or elderly patients, are not represented.

To conclude, DAISY is the first phase 3 trial to investigate a first-in-class type I interferon receptor α subunit 1 antibody in patients with both diffuse and limited cutaneous SSc. The study design was informed by patients and utilizes a composite endpoint that incorporates assessments of how patients feel and function along with clinical disease activity assessments. As it includes a broad patient population, permits the use of standard therapy, and spans a 2-year on-drug period, DAISY will be able to provide important insights into the potential of anifrolumab for SSc treatment.

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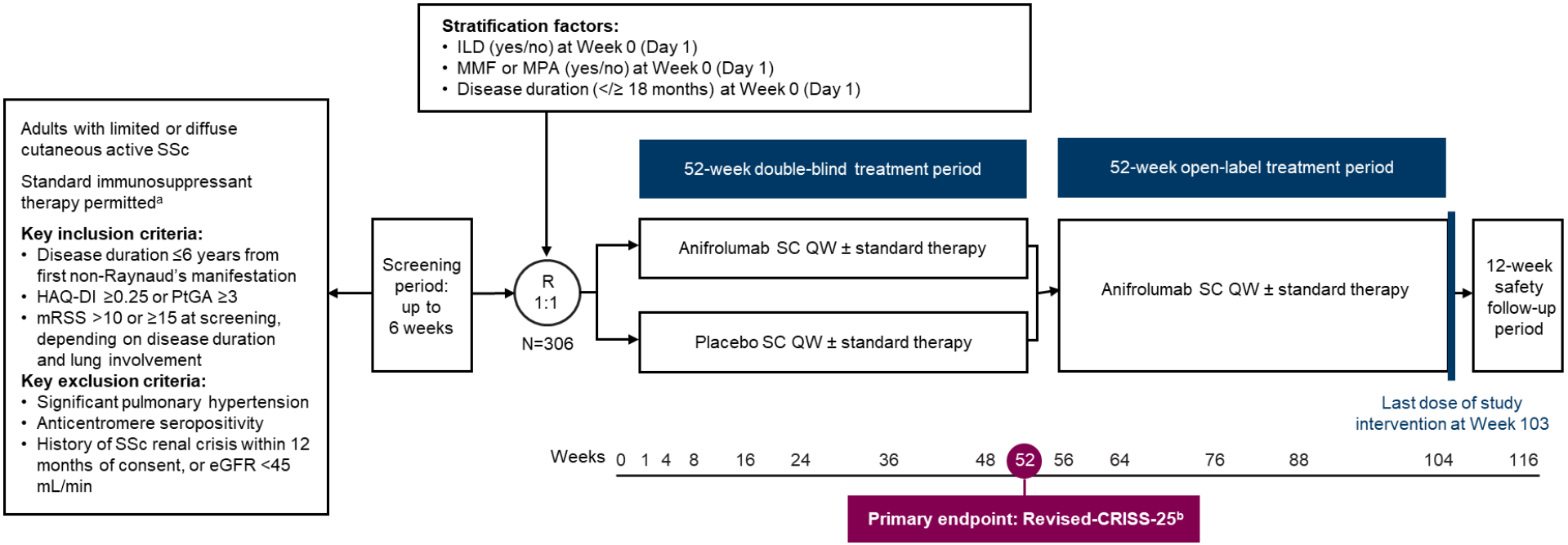
Figures

Figure 1. Study design



[a]Hydroxychloroquine, MMF, MPA, methotrexate, azathioprine, tacrolimus, or oral glucocorticoids; [b]Revised version of the 2-step ACR-CRISS algorithm: Step 1) No significant SSc-related event (new SSc renal crisis; new decline in ppFVC ≥15% in established ILD, or new ppFVC <80% predicted; new onset of left ventricular failure [defined as LVEF ≤45%] requiring treatment; new onset of PAH on right heart catheterization requiring treatment; GI dysmotility requiring enteral or parenteral nutrition; digital ischemia with gangrene, amputation, or hospitalization

requiring treatment); Step 2) Improvement in ≥2 components (mRSS, HAQ-DI, CGA, PtGA: decrease ≥25%; ppFVC: increase ≥5%) and worsening in ≤1 component (mRSS, HAQ-DI, CGA, PtGA: increase ≥25%; ppFVC: decrease ≥5%).

ACR, American College of Rheumatology; CGA, Clinician's Global Assessment; CRISS, Composite Response Index in Systemic Sclerosis; eGFR, estimated glomerular filtration rate; GI, gastrointestinal; HAQ-DI, Health Assessment Questionnaire–Disability Index; ILD, interstitial lung disease; LVEF, left ventricular ejection fraction; MMF, mycophenolate mofetil; MPA, mycophenolic acid or mycophenolate sodium; mRSS, modified Rodnan Skin Score; N, number of patients; PAH, pulmonary arterial hypertension; ppFVC, percent predicted forced vital capacity; PtGA, Patient’s Global Assessment; QW, once weekly; R, randomization; SC, subcutaneous; SSc, systemic sclerosis.

Tables

Table 1. Key inclusion and exclusion criteria

Inclusion criteria
Demographic characteristics
Adult patients from 18 to 70 years of age inclusive
Disease characteristics
SSc according to 2013 ACR/EULAR classification criteria
Limited or diffuse cutaneous subsets according to LeRoy and Medgser
SSc disease duration within 6 years from first non-Raynaud's phenomenon manifestation
Either HAQ-DI score ≥0.25 points or PtGA score ≥3 points
mRSS >10 with early disease or lung involvement as defined by the protocol
mRSS ≥15 with disease duration ≥18 months and active disease as defined by the protocol[*]
Background therapies
Stable background therapies can be used including hydroxychloroquine, methotrexate, azathioprine, MMF, mycophenolic sodium, mycophenolic acid, oral glucocorticoids, or tacrolimus[†]
Sex and contraceptive/barrier requirements
Women of childbearing potential with a negative urine pregnancy test
Other criteria
Uninvolved skin at injection sites
Informed consent
Exclusion criteria
Anticentromere antibody seropositivity on central laboratory
History of SSc renal crisis within past 12 months (eGFR<45 mL/min)
Overlap syndromes, systemic lupus erythematosus with anti-dsDNA antibody seropositivity or anti-citrullinated protein antibodies-positive rheumatoid arthritis, or SSc mimics (*e.g.* scleromyxedema, eosinophilic fasciitis)
History of, or current, other inflammatory diseases (*e.g.* inflammatory bowel disease, skin disease) that, in the opinion of the investigator, could interfere with efficacy and safety assessments or require immunomodulatory therapy
Evidence of moderately severe concurrent nervous system, renal, endocrine, hepatic, or gastrointestinal disease (*e.g.* clinical signs of malabsorption or needing parenteral nutrition) not related to SSc, as determined by the investigator
Hematopoietic stem cell transplantation or solid organ/limb transplantation
Any severe case of herpes zoster infection as defined by the protocol
Known malignancy or a history of malignancy within 5 years, with exception of excised/cured local basal or squamous cell carcinoma of the skin or carcinoma in situ of the uterine cervix
Major surgery within 8 weeks prior to and/or during study enrollment
Known active current or history of recurrent infections
Severe cardiopulmonary disease or significant pulmonary hypertension
Any condition that, in the opinion of the investigator or AstraZeneca, would interfere with the efficacy or safety evaluation of the study intervention or put participant at safety risk

[*]Active disease includes at least one of the following: C-reactive protein ≥0.6 mg/dL that is unrelated to other conditions, erythrocyte sedimentation rate ≥28mm/hr, platelet count $\geq 330 \times 10^9$/L, new skin involvement or skin progression by mRSS ≥3 units, at least one tendon friction rub documented in medical records at or within the previous 3 months of screening;

[†]Patients are permitted to have used MMF or, azathioprine, methotrexate, or tacrolimus in combination with hydroxychloroquine and/or low-dose oral glucocorticoids, if their doses remained stable for ≥3 months prior to randomization. Combinations of MMF or mycophenolic acid or mycophenolate sodium, azathioprine, methotrexate, or tacrolimus with other conventional immunosuppressants are not permitted. Patients receiving low-dose glucocorticoids must have maintained a stable dose for ≥2 weeks prior to randomization.

ACR, American College of Rheumatology; anti-dsDNA, anti-double-stranded DNA; eGFR, estimated glomerular filtration rate; EULAR, European Alliance of Associations for Rheumatology; HAQ-DI, Health Assessment Questionnaire–Disability Index; MMF, mycophenolate mofetil; mRSS, modified Rodnan Skin Score; PtGA, Patient's Global Assessment.

Table 2. Study objectives and endpoints

Objective	Endpoints
Primary	
To demonstrate the superiority of anifrolumab compared with placebo with or without standard therapy on measures of signs, symptoms, and impacts associated with SSc	Revised-CRISS-25 response at Week 52 Where a responder is defined as a patient who shows improvement in at least 2 components, and worsening in no more than one component, with no significant SSc-related event* over 52 weeks • Improvement: ≥5% increase in ppFVC, ≥25% decrease for mRSS, HAQ-DI, PtGA, CGA • Worsening: ≥5% decrease in ppFVC, ≥25% increase for mRSS, HAQ-DI, PtGA, CGA
Key Secondary	
To demonstrate the superiority of anifrolumab compared with placebo with or without standard therapy on lung function in patients with SSc-associated ILD	Change from baseline in FVC (mL) at Week 52
To demonstrate the superiority of anifrolumab compared with placebo with or without standard therapy on skin thickness in patients with SSc	Change from baseline in mRSS at Week 52
Secondary	
To assess the effect of anifrolumab compared with placebo with or without standard therapy on each component of the measures of signs, symptoms, and impacts associated with SSc included in the Revised-CRISS-25 response	Improvement in each component (evaluated separately) of the Revised-CRISS-25 at Week 52, where a responder is defined as follows: • ppFVC ≥ 5% increase • mRSS ≥ 25% decrease • HAQ-DI ≥ 25% decrease • PtGA ≥ 25% decrease • CGA ≥ 25% decrease Otherwise, a participant is a non-responder for the component

To assess the effect of anifrolumab compared with placebo with or without standard therapy on ILD progression as quantified by CT in patients with SSc	Change from baseline at Week 52 in CT measures of interstitial lung disease (Quantitative ILD, Quantitative light-induced fluorescence)
To assess the effect of anifrolumab compared with placebo with or without standard therapy on skin-specific HRQoL assessment in patients with SSc	Change from baseline in SSPRO at Week 52
To assess the effect of anifrolumab compared with placebo with or without standard therapy on lung function in patients with SSc	Change from baseline in ppFVC at Week 52
	Change from baseline in FVC (mL) at Week 52
To assess the effect of anifrolumab compared with placebo with or without standard therapy on lung function in patients with SSc-associated ILD	Change from baseline in ppFVC at Week 52
To evaluate the pharmacokinetics, pharmacodynamics, and immunogenicity of SC anifrolumab	Anifrolumab concentration and pharmacokinetic parameters, anti-drug antibodies, and type I interferon 21-gene signature from blood
	Anti-drug antibody presence and titer during treatment and follow-up
	Relationships between anti-drug antibodies and efficacy, safety, or pharmacokinetic outcome measures

*A significant SSc-related event can be any one of the following: (1) Renal: new scleroderma renal crisis; (2) ILD: new decline in ppFVC ≥ 15% (relative) in established ILD and new ppFVC below 80% predicted; (3) Heart: new onset of left ventricular failure (defined as LVEF ≤ 45%) requiring treatment; (4) Cardiopulmonary: new onset of pulmonary arterial hypertension on right heart catheterization requiring treatment; (5) Gastrointestinal: dysmotility requiring enteral or parenteral nutrition; (6) Digital ischemia: gangrene, amputation, or hospitalization requiring treatment.

CGA, Clinician's Global Assessment; FVC, Forced Vital Capacity; HAQ-DI, Health Assessment Questionnaire–Disability Index; ILD, interstitial lung disease; mRSS, modified Rodnan skin score; ppFVC, percent predicted FVC; PtGA, Patient's Global Assessment; Revised-CRISS-25, Revised-Composite Response Index in SSc Score; SSc, systemic sclerosis; SSPRO, Scleroderma Skin Patient Reported Outcome.

Table 3. Definitions of assessments used in the DAISY study

Assessment	Abbreviation	Definition
Revised-Composite Response Index in SSc Score*	Revised-CRISS-25	A composite endpoint where a responder is defined as a patient who shows improvement in at least 2 components (≥5% increase for ppFVC and/or ≥25% decrease for mRSS, HAQ-DI, PtGA, CGA) and worsening in no more than one component (≥5% decrease for ppFVC and/or ≥25% increase for mRSS, HAQ-DI, PtGA, CGA) with no significant SSc-related event† over the duration evaluated; otherwise, a patient is a non-responder
Forced Vital Capacity‡	FVC	Assessed with a spirometer according to ATS/ERS 2019 guidelines (35)
Modified Rodnan Skin Score	mRSS	Patient's skin thickness is rated by clinical palpation using a 0–3 scale (0=normal skin; 1=mild thickness; 2=moderate thickness; 3=severe thickness with inability to pinch the skin into a fold) for each of 17 surface anatomic areas of the body: face, anterior chest, abdomen, and fingers, forearms, upper arms, thighs, lower legs, dorsum of hands and feet with right and left considered separately. Individual values are added and the sum is defined as the total skin score (36)
Health Assessment Questionnaire–Disability Index	HAQ-DI	20-item instrument with which patient scores their difficulties in performing daily activities over the last week in each of 8 sections (dressing, arising, eating, walking, hygiene, reach, grip, and activities) (37) from 0 ("Without Any Difficulty") to 3 ("Unable to Do"). The overall score is determined by summing the highest item score in each section and dividing by 8. The Disability Index will be used to adjust each section based on the use of an aid, device, or assistance
Patient's Global Assessment	PtGA	11-point numerical rating scale used for patient to report their overall health in the last week from 0 ("Excellent") to 10 ("Extremely Poor")
Clinician's Global Assessment	CGA	Physician assesses patient's overall health in the last week from 0 ("Excellent") to 10 ("Extremely Poor"), with an option to choose "Not Known" if the patient is new to the clinic
Scleroderma Skin Patient Reported Outcome	SSPRO	18-item instrument with 4 domains (Physical Effects, Physical Limitations, Emotional Effects, and Social Effects). Patient reports skin-related HRQoL over the last 4 weeks using

		a 7-point numerical rating scale from 0 to 6, with lower score indicating higher HRQoL (38)

*The primary objective, to demonstrate the superiority of anifrolumab to placebo with or without standard therapy in SSc, was evaluated using Revised CRISS-25 response at Week 52. Improvements in each component of Revised-CRISS-25 at Week 52 were evaluated separately as secondary objectives;

†A significant SSc-related event can be any one of the following: (1) Renal: new scleroderma renal crisis; (2) ILD: new decline in ppFVC ≥15% (relative) in established ILD and new ppFVC below 80% predicted; (3) Heart: new onset of left ventricular failure (defined as LVEF ≤45%) requiring treatment; (4) Cardiopulmonary: new onset of pulmonary arterial hypertension on right heart catheterization requiring treatment; (5) Gastrointestinal: dysmotility requiring enteral or parenteral nutrition; (6) Digital ischemia: gangrene, amputation, or hospitalization requiring treatment;

‡One key secondary objective, to demonstrate the superiority of anifrolumab to placebo with or without standard therapy on lung function in patients with SSc-associated ILD, was evaluated using change from baseline in FVC (mL) at Week 52. The other key secondary objective, to demonstrate the superiority of anifrolumab to placebo with or without standard therapy on skin thickness in patients with SSc, was evaluated using change from baseline in mRSS at Week 52.

ATS/ERS, American Thoracic Society/European Respiratory Society; HRQoL, health related quality of life; ILD, interstitial lung disease; LVEF, left ventricular ejection fraction; ppFVC, percent predicted forced vital capacity; SSc, systemic sclerosis.

Supplementary materials

S1. Full inclusion and exclusion criteria for participants in the DAISY study

INCLUSION CRITERIA

Demographic Characteristics

1 Participant must be 18 to 70 years of age inclusive, at the time of signing the ICF.

2 Body weight ≤ 145.0 kg (≤ 319.7 lb).

Disease Characteristics

3 Participants with a confirmed classification of SSc according to 2013 ACR/EULAR classification criteria.

4 Limited or diffuse cutaneous subsets, as classified by Leroy and Medsger (Leroy and Medsger 2001).

5 SSc disease duration ≤ 6 years from first non-Raynaud's phenomenon manifestation.

6 Either HAQ-DI score ≥ 0.25 points or PtGA score ≥ 3 points at Screening.

7 mRSS > 10 at Screening with at least one of the following:

- Disease duration < 18 months
- Diagnosis of lung involvement (ILD) by chest CT confirmed by central reading assessment (no minimum) either via historical CT or CT performed during Screening

8 If disease duration ≥ 18 months and no confirmed ILD, mRSS must be ≥ 15 AND active disease at Screening with at least one of the following:

- CRP ≥ 6 mg/L (0.6 mg/dL) that is unrelated to other conditions (e.g., infection) or
- ESR ≥ 28mm/hr or platelet count ≥ 330×10^9/L (330000/μL)
- New skin involvement or skin progression by mRSS ≥ 3 units compared with the
- most recent assessment performed within the previous 6 months of signing the ICF
- Presence of at least one tendon friction rub documented in medical records within the
- previous 3 months of Screening and at Screening

9 Participants may use one of the following standard immunosuppressant therapies at a stable dose prior to randomization (dose should be expected to remain stable throughout the course of the study)[a,b]

- Hydroxychloroquine (≤ 400 mg/day), methotrexate (≤ 25 mg/week), azathioprine (≤ 200 mg/day) at a stable dose for ≥ 3 months prior to randomization
- MMF (≤ 3 g/day) or MPA (≤ 2.16 g/day) administered for ≥ 6 months and at a stable dose for ≥ 3 months prior to randomization
- Oral glucocorticoids (≤ 10 mg/day of prednisone or equivalent) at a stable dose for ≥ 2 weeks prior to randomization
- Tacrolimus (≤ 0.2 mg/kg/day) administered for ≥ 3 months prior to randomization; where local practice guidelines mandate or in case of safety concerns, monitoring of serum tacrolimus concentration may be performed at the discretion of investigator, with an aim of keeping a stable tacrolimus concentration targeting 5–10 ng/mL

[a]MMF or MPA, azathioprine, methotrexate, and tacrolimus may be used in combination with hydroxychloroquine and/or low-dose oral glucocorticoids (≤ 10 mg/day); [b]Combinations of MMF or MPA, azathioprine, methotrexate, or tacrolimus with each other or with other conventional immunosuppressants are not permitted

10 Uninvolved or mildly thickened skin (i.e., mRSS score of < 1) at one of the following possible injection-site locations:

- Anterior thigh
- Upper arm
- Abdomen

Sex and Contraceptive/Barrier Requirements

11 Contraceptive use by females or males should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

(a) Female participants:

- Negative serum β-hCG test at Screening (females of childbearing potential only).
- Women of childbearing potential must have a negative urine pregnancy test at randomization (Day 1), prior to administration of study intervention.
- Females of childbearing potential must use 2 effective methods of avoiding pregnancy, only one of which is a barrier method, from Screening until 16 weeks after the final dose of study intervention, unless the participant is surgically sterile (e.g., bilateral oophorectomy, tubal ligation, or complete hysterectomy), has a sterile male partner, is at least 1 year postmenopausal, or practices sustained abstinence consistent with the participant's customary lifestyle. Postmenopausal is defined as at least 1 year since last menses and the participant has an elevated FSH level greater than the central laboratory value of postmenopausal at Screening.

Ineffective methods that should not be used are as follows:

- Periodic abstinence (calendar, sympto-thermal, post-ovulation methods)
- Coitus interruptus (withdrawal method)
- Spermicides
- Lactational amenorrhea
- Non-copper containing intrauterine devices (except Mirena, Liletta)
- Triphasic combined oral contraceptives
- Progesterone only oral contraceptives (except Cerazette)
- Sponge, diaphragm, male or female condoms without secondary methods
- Tubal occlusion without a secondary method.

Male participants:

- All males (sterilized or non-sterilized) who are sexually active must use condom (with spermicide where commercially available for contraception) from Day 1 until at least 16 weeks after receipt of the final dose of study intervention. It is strongly recommended that the female partner of a male participant also use an effective method of contraception from Table 7 (other than a barrier method) throughout this period.
- Male participants must not donate sperm during the course of the study and for 16 weeks after the last dose of the study intervention.

One Barrier Method PLUS One Intrauterine Device or Hormonal Method Required[a]
Barrier Methods (choose one only)
Male condom (with spermicide[b]) Cap (with spermicide cream or jelly[b]) Diaphragm (with spermicide cream or jelly[b])
Intrauterine Device/Hormonal Methods (choose one only)
Intrauterine Device • Levonorgestrel Intra • Uterine System (e.g., Mirena, Liletta) • Intrauterine device (copper)
Hormonal[c] • Contraceptive Implants: Levonorgestrel and Etonogestrel implants (e.g., Norplant, Implanon/Nexplanon) • Hormone shot or Injection: Medroxyprogesterone injections (e.g., Depo-Provera) • Normal and low dose combined (estrogen and progesterone) oral contraceptive pills (except triphasic) • Patch: Norelgestromin/ethinyl estradiol transdermal system (e.g., Evra Patch) • Progestogen-only pill: desogestrel (only Cerazette is acceptable) • Intravaginal device: ethinyl estradiol and etonogestrel (e.g., Nuvaring)

[a]Tubal occlusion PLUS a barrier method is an acceptable effective method of birth control; [b]Where commercially available; [c]Hormonal methods are prone to drug-drug interactions that may occur with co-prescribed medications.

Informed Consent

12 Capable of giving signed informed consent as described in Appendix A which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

13 Provision of signed and dated written Optional Genetic Research Information informed consent prior to collection of samples for optional genetic research that supports the Genomic Initiative (see Appendix E).

EXCLUSION CRITERIA

Participants are excluded from the study if any of the following criteria apply:

SSc-related Exclusions

1 Significant pulmonary hypertension defined by any of the following:

(a) Previous clinical or echocardiographic evidence of right sided heart failure

(b) History of right heart catheterization showing a cardiac index ≤ 2 L/min/m²

(c) PAH requiring parenteral therapy with epoprostenol/treprostinil or combination of oral and inhaled therapy

(d) Class III or higher PAH, as defined by the World Health Organization (Galiè et al 2009).

2 Anticentromere antibody seropositivity on central laboratory.

3 History of SSc renal crisis within 12 months prior to signing the ICF or eGFR < 45 mL/min.

Medical History and Concurrent Diseases

Immunology and Other Immune-mediated Disorders

4 Overlap syndromes, SLE with anti-dsDNA antibody seropositivity or ACPA-positive rheumatoid arthritis, or SSc mimics (e.g., scleromyxedema, eosinophilic fasciitis).

5 History of, or current, any other inflammatory diseases, e.g., inflammatory bowel disease, skin disease, that, in the opinion of the investigator, could interfere with efficacy and safety assessments or require immunomodulatory therapy.

6 History of any non-SSc disease that has required treatment with oral or parenteral corticosteroids for more than a total of 2 weeks within the last 24 weeks prior to signing the ICF.

Cardiopulmonary Disorders

7 Pulmonary disease with any of the following at Screening:
- FVC ≤ 50% of predicted; or
- DLCO hemoglobin corrected ≤ 45% of predicted (historical DLCO from up to 3 months prior to signing the ICF may be used); or
- Airway obstruction (pre-bronchodilator FEV1/FVC < 0.7)

8 Evidence of other severe pulmonary disease as determined by the investigator (e.g., asthma requiring biologic therapy, COPD requiring long-term, at-home oxygen).

9 Cardiovascular disease with significant arrhythmia, congestive heart failure (NYHA Class II-IV), unstable angina, uncontrolled hypertension, cor pulmonale, symptomatic pericardial effusion, or cardiac abnormality such as left ventricular failure with ejection fraction < 50% at Screening.

10 History of thrombotic event (including MI, stroke, and transient ischemic attack) within 12 months prior to signing the ICF.

11 Chest CT scan obtained during Screening which shows any of the following:

(a) Evidence of current active infection (e.g., pneumonia, TB) or previous TB; or

(b) Evidence of malignancy; or

(c) Clinically significant non-SSc-related abnormalities.

Note: If a CT cannot be performed during Screening, a chest x-ray or CT scan performed within 12 weeks prior to signing of the ICF, or a chest x-ray performed during Screening may be acceptable (only for safety).

Other Disorders

12 History or evidence of suicidal ideation (severity of 4 [active: method and intent, but no plan] or 5 [active: method, intent, and plan]) within the past 6 months; or any suicidal behavior within the past 12 months or recurrent suicidal behavior in the lifetime of the participant based on an assessment with the C-SSRS at Screening or at baseline.

13 Evidence of moderately severe concurrent nervous system, renal, endocrine, hepatic (e.g., underlying chronic liver disease [Child Pugh A, B, C hepatic impairment]), or gastrointestinal disease (e.g., clinical signs of malabsorption or needing parenteral nutrition) not related to SSc, as determined by the investigator.

14 Other disease or conditions that may interfere with testing procedures or in the judgment of the investigator may interfere with trial participation or may put the participant at risk when participating in this trial.

Surgery and Transplantation

15 Major surgery within 8 weeks prior to signing the ICF or elective major surgery planned during the study period.

16 Hematopoietic stem cell transplantation or solid organ/limb transplantation.

17 Blood transfusion within 4 weeks prior to signing the ICF.

Infection Risk Factors

18 History of recurrent infection requiring hospitalization and IV antibiotics (e.g., 3 or more of the same type of infection over the previous 52 weeks).

19 Any live or attenuated vaccine within 8 weeks prior to signing the ICF (administration of killed or subunit vaccines is acceptable, AstraZeneca recommends investigators ensure all participants are up to date on required vaccinations, including COVID-19, pneumonia, varicella zoster, and influenza [inactivated/recombinant] vaccine prior to study entry).

20 Known history of a primary immunodeficiency, splenectomy, or any underlying condition that predisposes the participant to infection, or a positive result for HIV infection confirmed by central laboratory at Screening.

(a) Results from the Screening HIV test should be available prior to randomization. A participant is ineligible for enrollment if positive HIV nucleic acid test; participants refusing HIV testing are not eligible.

Active HBV or HCV infection, as confirmed by central laboratory, for:

(a) HBsAg; or

(b) HBcAb and HBV DNA detected above the LLOQ by reflex testing; or

(c) HCV RNA as confirmed by central laboratory.

Note: Participants who are HBcAb or HCV-Ab positive at Screening will be tested at Week 16, Week 52/EDV-DB, Week 76, and Week 104/EDV-OL for HBV or HCV activity. To

remain eligible for the study, the participant's HBV DNA or HCV RNA levels must remain below the LLOQ as per the central laboratory. See Appendix L 1 for Japan.

22 Any severe case, as defined by study guidelines, of HZ infection at any time prior to Week 0 (Day 1), including, but not limited to:

(a) Systemic HZ such as herpes encephalitis or ophthalmic herpes involving the retina at any time prior to signing the ICF;

(b) Recurrent cutaneous HZ defined as 2 or more episodes within 2 years prior to signing the ICF;

(c) Any HZ infection that has not completely resolved within 12 weeks prior to signing the ICF.

23 Any active CMV or Epstein-Barr virus infection that has not completely resolved within 12 weeks prior to signing the ICF.

24 Opportunistic infection (see Section 8.4.8.3) requiring hospitalization or IV antimicrobial treatment within 3 years prior to signing the ICF.

25 Any of the following:

(a) Clinically significant chronic infection (i.e., osteomyelitis, bronchiectasis, etc) within 8 weeks prior to signing the ICF (chronic nail infections are allowed)

(b) Any infection requiring hospitalization or treatment with IV anti-infectives not completed at least 4 weeks prior to signing the ICF.

26 Any infection requiring oral anti-infectives (including antivirals) within 2 weeks prior to signing the ICF.

COVID-19 Requirements

27 Any positive PCR or antigen test result (central or local labs as appropriate) as per local policies at Screening in addition to any known or suspected COVID-19 exposure within 2 weeks prior to Screening based on the COVID-19 assessment.

- If there is a known or suspected exposure, a participant must be negative upon retest obtained after 2 weeks and must remain asymptomatic for inclusion in the study.

Note: Participants positive at Screening may be re-screened after 6 weeks of mild/asymptomatic infections or at the discretion of the investigator, provided there has been no development of severe COVID-19 disease or sequelae. Participants may be re-screened a second time if the primary reason for screen failure was due to positive COVID-19 test.

28 Any history of severe COVID-19 infection (e.g., prolonged hospitalization [hospitalization for observational purposes is not exclusionary]) or any prior COVID-19 infection with documented long COVID and/or clinically significant unresolved sequelae.

29 Any mild/asymptomatic COVID-19 infection (lab confirmed or suspected based on clinical symptoms) within the last 6 weeks prior to first dosing.

TB Requirements

30 At any Screening visit, meets any of the following TB criteria:

(a) Signs or symptoms of active TB;

(b) Medical history or past physical examinations suggestive of active TB;

(c) Recent contact with a person with active TB without well documented evaluation and possible treatment for latent TB by a TB specialist.

31 TB test by QFT-GIT test at Screening with:

(a) Positive result for which active TB has been ruled out, and appropriate treatment for latent TB has been initiated prior to administration of study intervention;

(b) Indeterminate result which has been confirmed indeterminate upon immediate retesting using the same assay.

Malignancy Risk Factors

32 Any known malignancy or a history of malignancy within the past 5 years, apart from:

(a) Squamous or basal cell carcinoma of the skin treated with documented success of curative therapy ≥ 3 months prior to signing the ICF;

(b) Cervical cancer in situ treated with apparent success with curative therapy ≥ 1 year prior to signing the ICF.

33 Females who have been or are sexually active with an intact cervix must have documentation of a cervical cancer screening (Pap smear or HPV tests as per local guidelines) with a normal test result within 2 years prior to randomization. Any abnormal cervical cancer screening result documented within 2 years prior to randomization must be repeated to confirm patient eligibility.

Note: Females aged < 25 years, who have never been sexually active or have well documented HPV vaccination records may not require a cervical cancer screening.

34 Failure to comply with all required Screening procedures due to circumstances related to pandemic or public health emergency.

Prior/Concomitant Therapy

35 Prior receipt of anifrolumab.

36 Any immunosuppressive combinations other than adding hydroxychloroquine and/or oral glucocorticoids ≤ 10 mg/day are not permitted.

37 Treatment with investigational agents, cell therapy, cell-depleting therapies, immunomodulating agents (e.g., tumor necrosis factor antagonists), tyrosine kinase inhibitors, or alkylating agents is prohibited during the study.

38 Receipt of any commercially available biologic agent within 5 half-lives (see Appendix K for a complete list of prohibited medications) prior to signing of the ICF.

39 Receipt of any of B cell-depleting therapies (e.g., rituximab) ≤ 26 weeks prior to signing the ICF or, if therapy was administered > 26 weeks prior to signing the ICF, an absolute B cell count below the lower limit of normal or below baseline value prior to receipt of B cell-depleting therapy (whichever is lower).

40 Any new medicinal cannabinoid should not be started during the course of the study. Participants who are already on a stable regimen 8 weeks prior to signing the ICF may continue their regimen at the same stable dose through the course of study. Increases in strength or frequency are not allowed. Decrease or discontinuation is allowed for safety reasons.

Prior/Concurrent Clinical Study Experience

41 Receipt of any study intervention (small molecule or biologic agent) within 4 weeks or 5 half-lives prior to signing of the ICF, whichever is greater.

42 Concurrent enrollment in another clinical study with a study intervention.

43 Individuals involved with the conduct of the study, their employees, or immediate family members of such individuals.

Diagnostic Assessments

44 At Screening (within 4 weeks prior to signing the ICF), any of the following (note: retesting of laboratory test results during Screening may be repeated once):

(a) AST > 2.0 × ULN

(b) ALT > 2.0 × ULN

(c) TBL > ULN (unless due to Gilbert's syndrome)

(d) eGFR < 45 mL/min

(e) Urine protein/creatinine ratio > 2.0 mg/mg (or > 226.30 mg/mmol)

(f) Neutrophil count < 1000/μL (or < 1.0×10^9/L)

(g) Platelet count 25000/μL (or < 25×10^9/L)

(h) Hemoglobin < 8 g/dL (or < 80 g/L), or < 7 g/dL (or < 70 g/L) if related to participant's SSc such as in active hemolytic anemia

(i) Glycosylated hemoglobin (HbA1c) > 8% (or > 0.08) at Screening (diabetic participants only)

Other Exclusions

45 Any condition that, in the opinion of the investigator or AstraZeneca, would interfere with efficacy or safety evaluation of the study intervention or put participant at safety risk.

46 A known history of allergy or reaction to any component of the study intervention formulation or history of anaphylaxis to any human gamma globulin therapy.

47 Any history of an anaphylactic reaction to human proteins, or monoclonal antibodies.

48 Lactating or pregnant females or females who intend to become pregnant or begin breastfeeding anytime from initiation of Screening until 16 weeks following last dose of study intervention.

49 Spontaneous or induced abortion, still or live birth, or pregnancy ≤ 4 weeks prior to signing the ICF.

50 Current alcohol, drug or chemical abuse, or a history of such abuse within 1 year prior to signing the ICF.

51 Judgment by the investigator that the participant is unlikely to comply with study procedures, restrictions, and requirements (e.g., self-injection or at-home study intervention administration by a caregiver).

Lifestyle Considerations

Participants should avoid high-risk situations for infectious diseases (including but not limited to SARS-CoV-2) due to the immunosuppressive properties of the disease and associated SSc treatments. There are no other specific lifestyle restrictions identified for this study.

Blood Donation

Participants must not donate blood from date of randomization and within 16 weeks after the last study intervention dose.

Sperm Donation

Male participants must not donate sperm during the course of the study and for 16 weeks after the last dose of the study intervention (see Inclusion Criterion 11).

Perioperative Management of Study Intervention

Participants who have had major surgery within 8 weeks of signing the ICF or who are planning elective surgery during the study period are not eligible to participate in the study.

<u>Major surgery</u>

Pre-operative management of study intervention: if a non-urgent major surgical procedure becomes necessary during the study, it should be scheduled at least 4 weeks after the last administration of study intervention, if clinically feasible. The determination of whether or not a surgery is “urgent” will be at the discretion of the investigator, preferably in consultation with the AstraZeneca Global Study Physician.

<u>Non-major surgery</u>

The decision to withhold study intervention administration is at the investigator's discretion.

Post-operative management of study intervention: study intervention administration can be resumed at the investigator's discretion after all of the following criteria are met:

- External wound healing is complete, AND
- Any post-operative antibiotic course is completed, AND
- All acute surgical complications have resolved.