

Supplementary TABLE 1A. Gene Expression by Treatment Group						
THBS1 Expression						
Group 1						
Variable	N	Mean	Median	Std Dev	Minimum	Maximum
baseline	7	14.49	11.63	8.01	7.86	29.59
week 3	6	13.67	12.35	6.55	6.89	26.21
week 7	6	8.28	7.81	3.66	4.33	13.24
week 24	3	6.30	4.32	4.95	2.65	11.93
chg wk 3	6	-1.90	0.32	8.91	-18.60	6.78
chg wk 7	6	-5.90	-5.32	6.01	-16.35	-0.28
chg wk 24	3	-5.70	-5.33	1.65	-7.51	-4.26
Group 2						
Variable	N	Mean	Median	Std Dev	Minimum	Maximum
baseline	8	6.55	5.97	2.28	4.44	10.29
week 3	8	4.44	4.17	1.44	2.38	6.76
week 7	8	5.38	4.47	3.08	2.86	12.70
week 24	7	7.03	5.59	4.63	2.97	14.90
chg wk 3	8	-2.11	-2.20	1.49	-4.33	0.05
chg wk 7	8	-1.17	-0.72	2.62	-6.01	2.94
chg wk 24	7	0.94	1.09	5.74	-7.32	10.03

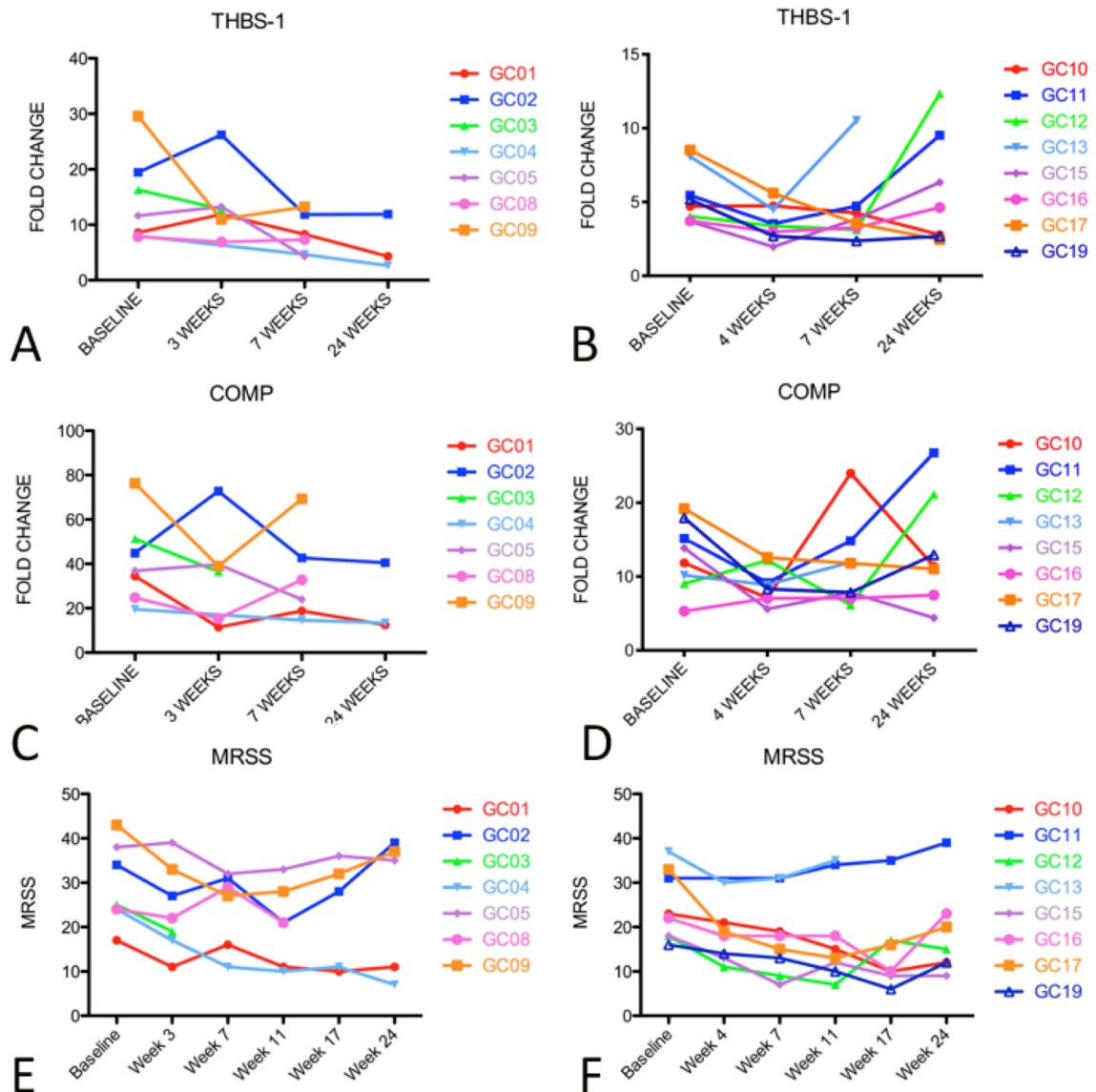
COMP Expression						
Group 1						
Variable	N	Mean	Median	Std Dev	Minimum	Maximum
baseline	7	41.17	36.84	18.94	19.54	76.33
week 3	6	35.80	37.75	21.92	11.45	72.75
week 7	6	33.68	28.38	20.15	14.62	69.31
week 24	3	22.12	13.34	15.95	12.49	40.53
chg wk 3	6	-8.98	-12.13	22.52	-37.35	27.86
chg wk 7	6	-5.80	-5.97	8.40	-15.73	7.95
chg wk 24	3	-10.85	-6.21	9.69	-21.99	-4.36
Group 2						
Variable	N	Mean	Median	Std Dev	Minimum	Maximum
baseline	8	16.16	16.20	5.88	6.68	24.22
week 3	8	11.17	10.83	3.10	7.06	15.87
week 7	8	14.41	12.40	7.41	7.76	30.22
week 24	7	17.14	14.39	9.81	5.56	33.71
chg wk 3	8	-4.99	-6.76	5.89	-12.16	3.96
chg wk 7	8	-1.75	-2.03	8.78	-12.72	15.28
chg wk 24	7	0.50	-0.54	11.10	-11.92	15.24

Supplementary TABLE 1B. Modified Rodnan Skin Score by Treatment Group

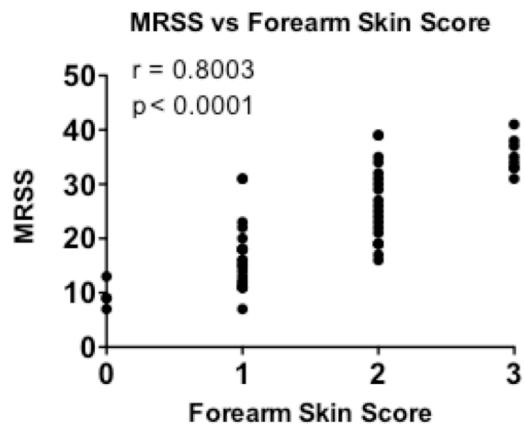
Modified Rodnan Skin Score						
Group 1						
Variable	N	Mean	Median	Std Dev	Minimum	Maximum
baseline	7	29.29	25.00	9.23	17.00	43.00
week 3	7	24.00	22.00	9.68	11.00	39.00
week 7	6	24.33	28.00	8.71	11.00	32.00
week 11	6	20.67	21.00	9.09	10.00	33.00
week 17	5	23.40	28.00	12.12	10.00	36.00
week 24	5	25.80	35.00	15.47	7.00	39.00
chg wk 3	7	-5.29	-6.00	3.64	-10.00	1.00
chg wk 7	6	-5.67	-4.50	7.79	-16.00	5.00
chg wk 11	6	-9.33	-9.50	5.24	-15.00	-3.00
chg wk 17	5	-7.80	-7.00	4.32	-13.00	-2.00
chg wk 24	5	-5.40	-6.00	7.89	-17.00	5.00
Group 2						
Variable	N	Mean	Median	Std Dev	Minimum	Maximum
baseline	8	24.75	22.50	7.89	16.00	37.00
week 3	7	18.00	18.00	6.38	11.00	30.00
week 7	8	17.88	16.50	9.06	7.00	31.00
week 11	8	18.00	14.00	10.69	7.00	35.00
week 17	7	14.71	10.00	9.76	6.00	35.00
week 24	7	18.57	15.00	10.24	9.00	39.00
chg wk 3	7	-5.86	-5.00	4.14	-14.00	-2.00
chg wk 7	8	-6.88	-5.00	5.67	-18.00	0.00
chg wk 11	8	-6.75	-6.00	6.78	-20.00	3.00
chg wk 17	7	-8.29	-10.00	7.30	-17.00	4.00
chg wk 24	7	-4.43	-4.00	7.35	-13.00	8.00

Supplementary Table 2. Comparison of Clinical Trial Demographics

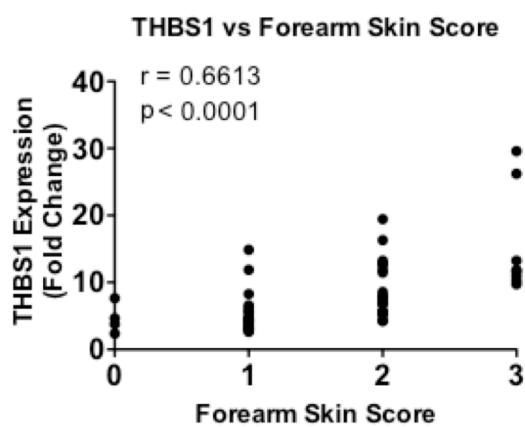
Characteristic	Fresolimumab Trial (n=15)	Nilotinib Trial (n=10)
Age (Year)		
Mean (SD)	52 (11)	44 (15)
Median (Range)	51 (35-64)	47 (18-70)
Sex		
Female (n)	73% (11)	80% (8)
Male (n)	27% (4)	20% (2)
Modified Rodnan Skin		
Mean (SD)	27 (8)	26 (10)
Median (Range)	24 (16-43)	24 (10-46)
Disease duration		
Mean (SD)	19 (20)	12 (8)
Median	15.5	8



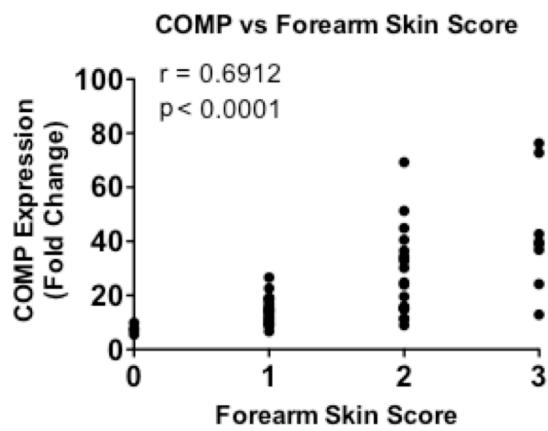
Supplementary Figure 1. Fresolimumab treated patients show decreased biomarkers and skin scores. Graphs show thrombopondin-1 (THBS1, panels a and b) and cartilage oligomeric protein (COMP, panels c and d) gene expression, and MRSS (panels e and f) from study patients in group 1, receiving two doses of 1 mg/kg fresolimumab, (a, c, and e) and group 2, receiving one dose of 5 mg/kg fresolimumab (b, d and f). Line graphs show changes in individual patients over time.



A

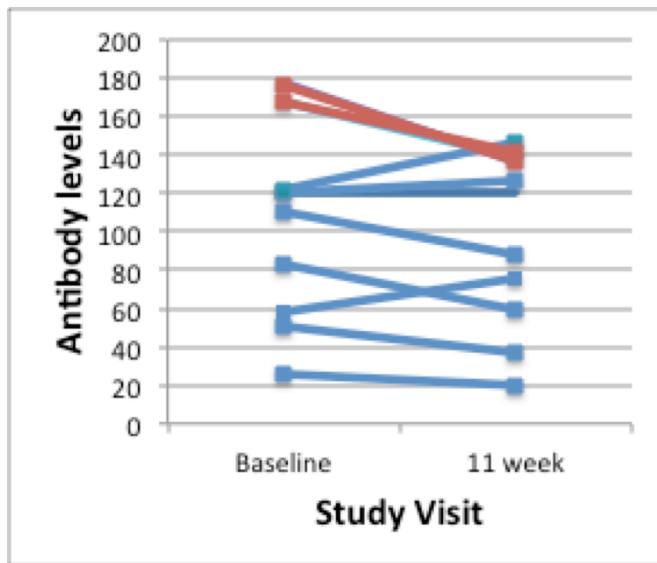


B

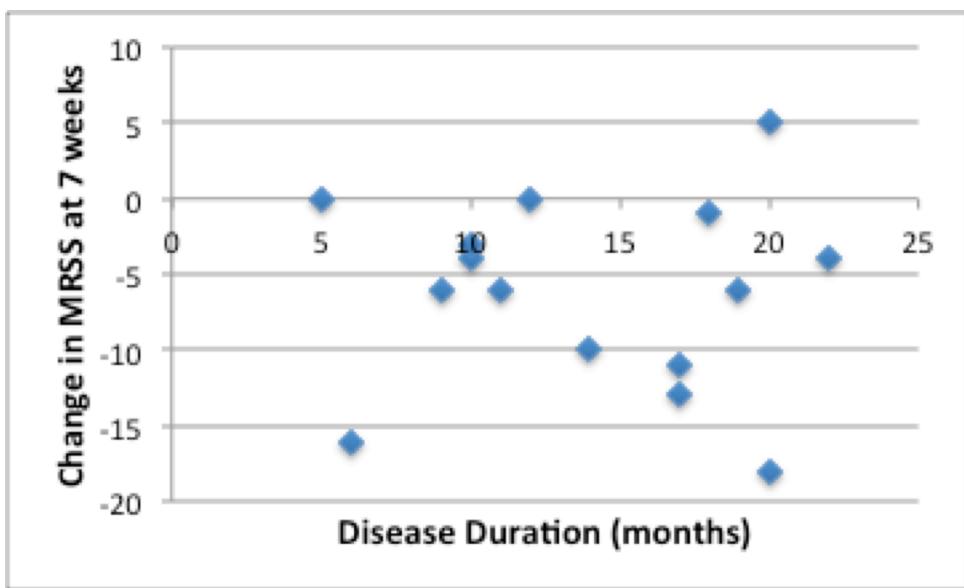


C

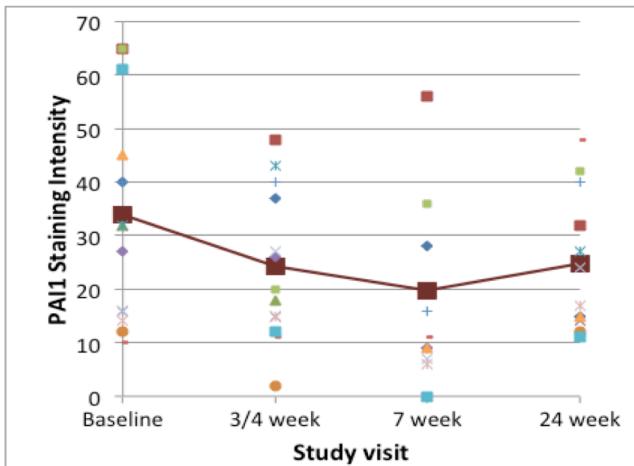
Supplementary Figure 2: Biomarker gene expression correlates with the local skin score. The local forearm skin score correlates highly with the MRSS (panel a), as well as THBS1 (panel b) and COMP (panel c) gene expression.



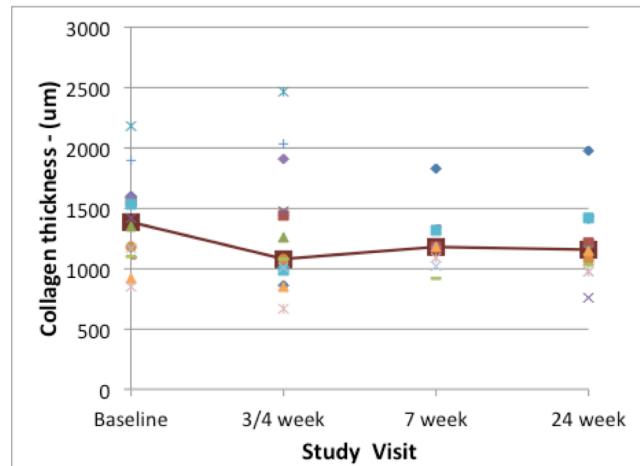
Supplementary Figure 3. Autoantibody levels measured at baseline and 11 weeks after fresolimumab treatment. Anti-RNA polymerase III levels for anti-RNA polymerase III-positive patients are shown in blue. Anti-Scl70 levels for anti-Scl70-positive patients are shown in red. Autoantibody levels in two of the four anti-Scl70-positive patients lie on top of the other two.



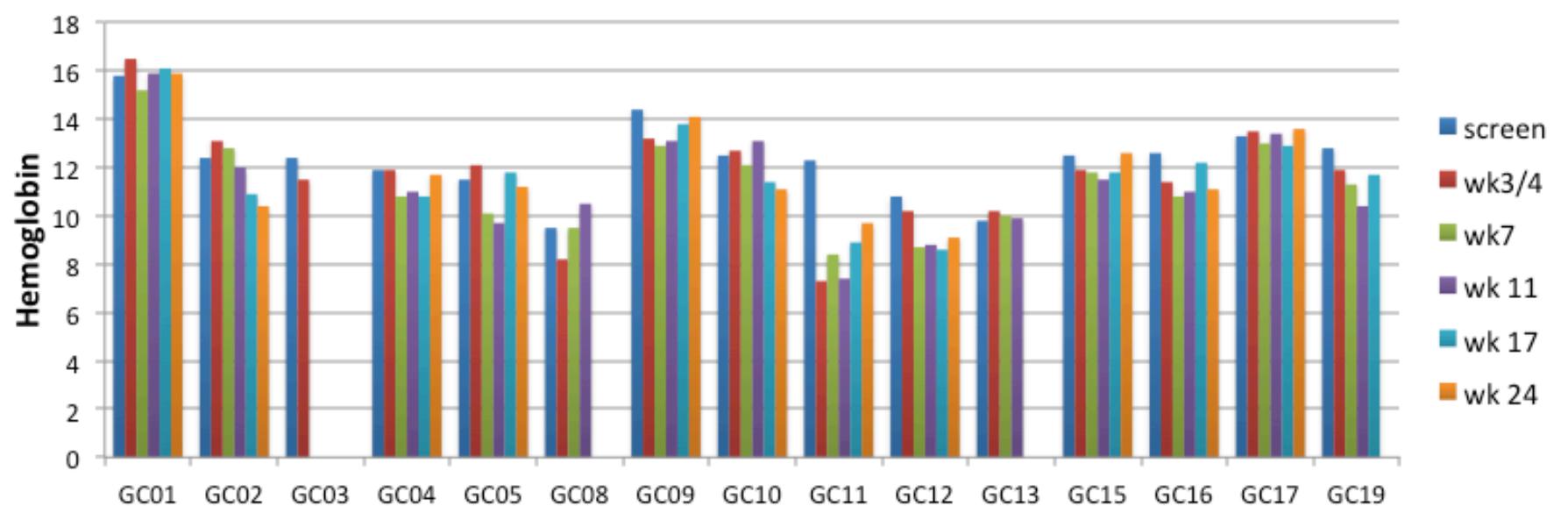
Supplementary Figure 4. Disease duration compared to the change in MRSS 7 weeks after fresolimumab treatment. The change in MRSS after fresolimumab treatment showed no correlation with the disease duration ($r^2 = 0.00063$)



Supplementary Figure 5A. Immunohistochemical staining of plasminogen activator protein-1 (PAI-1/serpine1). Each patient is represented by a different color/style of marker. The median of the values is represented by dark red boxes connected by a line. At 3/4 weeks compared to baseline, $p=0.136$; at 7 weeks compared to baseline, $p \leq 0.05$; and 24 week compared to baseline, $p=0.205$ (Wilcoxon signed-rank test).



Supplementary Figure 5B. Skin collagen thickness. Based on trichrome staining, dermal thickness was measured from the dermal-epidermal junction to subcutaneous fat. Each patient is shown with a different symbol, with the median shown by the line connecting large red boxes



	GC02	GC04	GC05	GC08	GC09	GC10	GC 11	GC12	GC 16	GC19
Iron	NT	L	NT	NT	NT	L	L	L	L	L
TIBC	NT	N	NT	NT	NT	L	L	L	L	L
Retic %	NT	N	NT	NT	NT	N	H	N	N	N
History of gastrointestinal bleeding	Hx GAVE	Hx bleeding ulcer	Hx GAVE	Hx GAVE						
Gastrointestinal bleeding (during trial)				GAVE			GAVE			
Anemia-classification			ACD	Bleeding from GAVE W3		ACD	Bleeding from GAVE W6, ACD, Fe def	ACD	ACD	ACD
Comment	Started on methotrexate W11, then cyclophosphamide W17			Hgb nadir = 6.0 Pt did not receive second dose fresolimumab						

Supplementary Figure 6. Hemoglobin changes and evaluation of anemia in study patients. Hemoglobin level at each study visit is graphed by patient (upper panel). Anemia evaluation, previous history of gastrointestinal bleeding, and GAVE discovered during the study is shown for patients showing a significant decrease in hemoglobin during the study (lower panel).

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**Title: OPEN LABEL TRIAL OF anti-TGF β MAB, FRESOLIMUMAB, IN SYSTEMIC
SCLEROSIS –A PHASE ONE BIOMARKER TRIAL**

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Table 3.6 Clinical Experience with Fresolimumab.....

1. OVERVIEW OF STUDY

Title of Study: Open Label Trial of Fresolimumab-anti TGF β mAB in Systemic Sclerosis – A Phase one Biomarker Trial

Study Phase: Phase I

Research Hypothesis: This study will test safety of TGF β inhibition by fresolimumab in patients with systemic sclerosis (SSc). This study will also test the hypothesis that TGF β drives expression of TGF β -responsive genes upregulated in skin in SSc, and that inhibition of TGF β by fresolimumab will downregulate expression of these genes, providing a proof-of-concept for a larger clinical trial of this agent in SSc.

Primary Objectives:

1. To evaluate safety of fresolimumab in patients with Systemic Sclerosis
2. To investigate the effect of fresolimumab on TGF β -responsive gene expression in skin after treatment with fresolimumab compared to pre-treatment TGF β -responsive gene expression

Study Drug: Fresolimumab is a high affinity humanized antibody that neutralizes activity of TGF β 1, TGF β 2 and TGF β 3. Fresolimumab has a half-life in the circulation of approximately 3 weeks.

Study Design: This will be as a dose escalation open-label trial with no placebo group. The study will evaluate the effect of short-term treatment with fresolimumab/anti-TGF β on skin TGF β -responsive gene expression making up part of a 4-gene biomarker of skin disease. The first 8 patients entered will receive fresolimumab (1 mg/kg), the next eight patients entered will receive fresolimumab (5 mg/kg). Each treated patient will receive two doses with a 4-week intervening period. Skin biopsies will be taken to test for TGF β -responsive gene expression within two weeks before treatment and 3-weeks after each of the study drug doses. Safety assessments will extend to 17 weeks after the second dose of study drug.

Number of Subjects per Group: Eight subjects will be studied in each of two dosing groups for a total of 16 subjects

Study Population: Men and women at least 18 years of age. Subjects with diffuse cutaneous SSc as defined by the American College of Rheumatology (ACR) with a modified Rodnan skin score of greater than or equal to 15.

Study Duration: The duration of this study will be 24 weeks. Treatment efficacy will be assessed during the first 7 weeks of the study and safety assessed for additional 17 weeks, thus encompassing more than 5 drug half-lives.

Statistical Methods: Once all subjects complete the study or discontinue prematurely, the final analyses will be completed. All available data from all subjects who receive at least one infusion of fresolimumab will be included in the safety and efficacy analyses. TGF β -responsive gene expression at 3 and 7 weeks will be compared to baseline using a paired t-test. If changes in TGF β -responsive gene expression do not follow a normal distribution then these comparisons will be made using a Wilcoxon matched pairs test. Secondary outcome measures, including skin myofibroblast score, modified Rodnan skin score (MRSS) and scleroderma modified health assessment questionnaire (SHAQ) at 7 and 24 weeks, will be compared to baseline. Only descriptive statistics will be reported for these secondary measures.

2. STUDY OBJECTIVES

2.1 Primary Objectives: Safety and Efficacy

Primary Safety endpoint:

- Adverse and serious adverse events during the study period, including metabolic or hematologic adverse events, will be assessed at each study visit up to 24 weeks.

Primary Efficacy endpoint:

- TGF β -responsive gene components (cartilage oligomeric protein, COMP and thrombospondin-1, THS1) of the 4-gene SSc skin biomarker skin score will be compared at 3 and 7 weeks (and optional 24 week biopsy) to week 0 values.

2.2 Secondary Objectives

- 4-gene SSc skin biomarker skin score will be compared at 3 and 7 weeks (and optional 24 week biopsy) to baseline score.
- MRSS at 3, 7 and 24 weeks will be compared to week 0 MRSS.
- The scleroderma health assessment questionnaire (SHAQ) the six component scores of the SSc VAS at 7 weeks and 24 weeks will be compared to week 0 values.
- Myofibroblast score in skin biopsies at 3 and 7 weeks (and optional 24 week biopsy) after treatment will be compared to week 0 myofibroblast score.

2.3 Exploratory Objectives

- Microarray gene expression in skin at 3 and 7 weeks (and optional 24 week biopsy) will be compared to week 0 gene expression.

3. BACKGROUND AND STUDY RATIONALE

3.1 Overview of Systemic Sclerosis

Scleroderma, also known as systemic sclerosis (SSc), is a multisystem disease affecting skin and, more variably, other tissues, commonly including joints, muscles, lungs, the gastrointestinal tract and kidneys. It is one of a group of diseases in which fibrosis is associated with organ dysfunction. Fibrosis can involve the liver (Lefton et al., 2009; Pinzani et al., 2005), lung (Frankel and Schwarz, 2009), kidneys (Schnaper, 2005), and less commonly other organs, representing a final common pathway to organ dysfunction. In SSc tissue fibrosis is widespread, variably involving skin, lungs and the gastrointestinal tract.

Although SSc can affect almost any part of the body, skin disease is the most consistent clinical manifestation. Skin disease typically starts in the hands with an edematous phase of hand swelling lasting one to several months. The skin then progressively thickens and tethers to underlying tissues. In diffuse cutaneous SSc (dcSSc), skin thickening, induration and tethering typically extend proximally up the arm and can involve the torso, abdomen, face and legs. Patients with limited cutaneous SSc (lcSSc) have skin disease limited to below the elbow and face and neck as well as other characteristic clinical features. SSc skin pathology (diffuse and limited cutaneous SSc) shows fibrosis and variable perivascular lymphocyte infiltration in the deep reticular dermis.

SSc affects multiple other body systems. Most severe complications are seen more frequently in dcSSc with considerable morbidity and mortality (Steen and Medsger, 2000). Lung disease manifests as interstitial fibrosis or pulmonary arterial hypertension (PAH, more common in lcSSc). Lung disease remains the leading cause of death among SSc patients. Gastrointestinal disease primarily results from dysmotility. In the esophagus and stomach this most commonly leads to esophagitis. In the small and large bowel this most commonly leads to constipation, bowel obstruction and/or malnutrition. Renal disease is primarily manifest as accelerated hypertension and renal insufficiency. Angiotension converting enzyme inhibitors

are generally though not uniformly effective for treating this manifestation, which previously led to significant mortality.

Other important clinical manifestations include cold-induced vasospastic disease in extremities (Raynaud's phenomenon) and digital ulcers. SSc can also have cardiac manifestations. Pericarditis is the most frequent cardiac manifestation. Subclinical pericarditis is common with large effusions developing occasionally. Myocardial involvement with low-grade myocardial fibrosis is relatively common, but not frequently of clinical importance (Follansbee et al., 1985). Fibrosis most commonly manifests as the appearance of a septal infarction pattern on EKG in patients with normal coronary arteries, or as ventricular conduction delays. Occasionally myocardial fibrosis leads to heart failure. Cardiac arrhythmias are seen in ~5% of patients with SSc. Most common are atrial or ventricular ectopy, generally not associated with more serious rhythm disturbances. However, thallium perfusion defects are associated with sudden cardiac death (Steen et al., 1996).

Current treatment for SSc is limited (Steen, 2001). For most disease manifestations treatment is primarily symptomatic and generally inadequate. The exception is renal disease, scleroderma renal crisis, once a major cause of mortality in SSc patients, can often be treated successfully with angiotensin converting enzyme inhibitors. Pulmonary complications now represent the major cause of mortality. Cyclophosphamide provides some benefit in patients with interstitial lung disease (ILD), the most lethal complication of SSc. However, the effect of this agent on SSc-associated ILD is modest and transient (Tashkin et al., 2006; Tashkin et al., 2007). Pulmonary arterial hypertension (PAH) also leads to considerable mortality in SSc patients. PAH may respond to vasodilators such as epoprostanol and bosentan, but frequently responses are incomplete and mortality still high (Badesch et al., 2009). Bowel hypomotility also leads to considerable morbidity and sometimes mortality. Esophageal hypomotility is treated, frequently without success, with pro-motility and acid-blocking agents. Dysmotility of the lower bowel and its complications are even more difficult to treat with pro-motility agents providing modest relief in some patients and antibiotics helping in cases of small

bowel overgrowth. Thus there are limited therapeutic alternatives for SSc patients faced with progressive lung or bowel disease.

Skin fibrosis, the hallmark feature of SSc remains without effective treatment. Although skin changes in SSc are not a cause of mortality, they cause considerable morbidity, may reflect similar pathological processes to those that occur in the bowel and lungs, correlate highly with prognosis and disease progression in other organ systems and can be reproducibly assessed by skin score testing. Skin disease is of particular interest for evaluation in clinical trials since it is easily biopsied and can thus be repeatedly assessed for pathological changes during clinical trials (Lafyatis et al., 2009).

Part of the difficulty in finding effective treatments for SSc has been a continuing uncertainty regarding what initiates pathogenesis. The cause of disease manifestations in SSc remains obscure, although three major pathophysiologic explanations have been advanced. Prominent pathologic changes in dermal and pulmonary tissues show fibrosis, suggesting abnormalities in matrix deposition. Vascular disease, resulting in scleroderma renal crisis, digital ischemia and pulmonary hypertension suggests dysfunction of the vascular endothelium. Autoantibodies in SSc patient sera suggest that immune dysfunction and autoimmunity may contribute to or cause disease. The different pathological features in different organs have provided support for each of these mechanisms, but not clarified which is most important in overall pathogenesis.

3.2 Background: Transforming Growth Factor

Transforming growth factor-beta (TGF β) is a pleiotropic cytokine which belongs to a superfamily of ligands, including bone morphogenetic proteins and activins (Blobe et al. 2000; Dumont, Arteaga. 2000; Akhurst, 2002; Wakefield, Roberts, 2002; Dumont, Arteaga, 2003). Under normal conditions, members of the TGF β family maintain homeostasis in many organ systems. In normal and non-cancerous cells, TGF β limits the growth of epithelial, endothelial, neuronal, and hematopoietic cell lineages through anti-proliferative and apoptotic responses. In addition, TGF β exerts potent effects that influence immune

function, cell proliferation/ functional differentiation, cell adhesion, extracellular matrix production, cell motility, angiogenesis, and cytokine production.

TGF β exists in 3 isoforms: TGF β 1, β 2, and β 3. Each isoform is encoded by distinct, highly conserved genes and is a 25-kilodalton homodimeric, disulphide-bonded protein. TGF β members are expressed in a tissue-specific and developmentally regulated fashion. TGF β 1 is expressed most commonly and is found in endothelial, hematopoietic, and connective tissues. TGF β 2 is found primarily in epithelial and neuronal tissues, and TGF β 3 resides in mesenchymal tissues. In vitro, each has similar activities. However, data from knockout mice suggest that each may be associated with distinct phenotypes.

TGF β is secreted by cells in a biologically inactive “latent form” by virtue of its association with latency-associated proteins (LAPs). Much of the TGF β /LAP “pro-drug” is stored in the extracellular matrix as a complex. However, other notable sites exist, including platelet granules and the surface of certain cells such as regulatory T cells. The mechanism of release of active TGF β may allow for local control. Activation can occur either under acidic conditions or through the action of proteases such as thrombospondin-1, plasmin, and prostate-specific antigen (PSA).

TGF β binds to cells via 3 major receptors: TGF β RI, TGF β RII, and TGF β RIII (transforming growth factor-beta receptor type I, type II, and type III). The binding of TGF β to receptors occurs in a specific sequence and results in a cascade of events leading to the formation of a receptor complex and the phosphorylation and activation of T β RI. The receptor complex has serine/threonine kinase activity and can activate the Smad (Sma- [small body size] and Mad-related protein) pathway by phosphorylation of Smad2 or 3. A key event is the formation of an activated Smad2(3)/4 complex, which is then transported to the nucleus where it induces gene transcription. This leads to a variety of effects on cell differentiation and growth.

Although TGF β /TGF β R/Smad is an essential pathway, TGF β 's influence on cellular activities appears to be much more complex. TGF β also binds to other receptors, such as endoglin, a cell-surface glycoprotein associated with proliferation of human endothelial cells and angiogenesis. Other signaling pathways, such as ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal protein kinase), MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase), Rho-kinase, Akt (agammaglobulinemia tyrosine kinase), and GTPases (guanosine triphosphatases) may also be involved. In addition, other receptor/signal pathways may intersect with the Smad pathway – including estrogen receptor (ER), androgen receptor (AR), steroid, epidermal growth factor receptor (EGFR), and other TGF β family members such as the activins. Because of these interactions, the overall effects of TGF β cannot always be predicted based on examination of any single pathway such as Smad.

3.3 TGF β in Systemic Sclerosis: Rationale for Anti-TGF β Antibody Therapy

TGF β is the most potent known profibrotic cytokine and the most consistently implicated cytokine in SSc pathogenesis (Varga and Abraham, 2007). A large body of in vitro data point to its role in fibrosis: it stimulates collagen and collagen processing to mature collagen, and it stimulates the conversion of fibroblasts into profibrotic myofibroblasts (Mauviel, 2005). Several observations in SSc skin suggest that altered TGF β activity might cause the fibrotic manifestations of SSc. Skin biopsy specimens from patients with SSc are characterized by increased new collagen synthesis in the reticular dermis as well as by increased numbers of myofibroblasts, activated fibroblasts that express the smooth muscle marker, α -smooth muscle actin (SMA) and actively form type I collagen, and other matrix components up-regulated in SSc skin (Sappino et al., 1990). In addition, SSc fibroblasts show increased p300 (Bhattacharyya et al., 2005), phosphorylated smad1 and smad2/3 (Ihn et al., 2006; Pannu et al., 2008), and $\alpha v \beta 5$ -mediated autocrine TGF β activation (Asano et al., 2006).

Although the etiology of vascular disease is uncertain, TGF β might also contribute to pathological features of intimal hyperplasia and obliteration, and perivascular fibrosis that characterize vascular disease in SSc (Trojanowska). Endothelial cells in SSc tissues show

reduced expression of typical endothelial cell markers such as vascular endothelial cadherin (Fleming et al., 2008). Data support a role for TGF β in the process of endothelial-mesenchymal transformation, a possible explanation for the loss of endothelial cell markers and smooth muscle hyperplasia in SSc vasculature (Arciniegas et al., 2007; Liebner et al., 2004; Morrell et al., 2009).

Clinical-pathological correlations have further implicated TGF β in SSc fibrosis. Although the origin of myofibroblasts in SSc remains uncertain, myofibroblasts can be induced in vitro by TGF β stimulation of normal dermal fibroblasts, suggesting that their presence in SSc skin reflects TGF β stimulation in vivo (Desmouliere et al., 1993). Supporting the notion that TGF β -induced myofibroblasts are key in skin fibrosis, “myofibroblast score”, a semi-quantitative assessment of myofibroblast infiltration of skin, in biopsies of lesional skin from the forearm of dcSSc patients correlates highly with the modified Rodnan skin score (MRSS), the current standard for evaluating the clinical extent of skin disease based on scoring the degree of skin involvement (0-3+) at 17 sites (Kissin et al., 2006; Lafyatis et al., 2009). Recent studies have extended these observations, showing that expression of four genes in a biopsy of mid-forearm skin correlates highly with the MRSS (Farina et al., 2010). Notably two of the genes in the 4-gene skin biomarker (COMP and THS1) are known transforming growth factor-beta (TGF β)-responsive genes. Biomarkers of disease activity might supplement or, in early phase trials, replace clinical outcome measures, such as the MRSS, potentially permitting short (open label) trials where the skin score would not normally be expected to change significantly. Further validating the 4-gene biomarker, in a small patient subset over 6-12 months the 4-gene biomarker changed with the MRSS (Farina et al., 2010).

3.4 Description of Fresolimumab

Genzyme and Cambridge Antibody Technology (CAT) have collaborated to produce human antibodies to TGF β . Fresolimumab is a human IgG4 kappa monoclonal antibody capable of neutralizing all mammalian isoforms of TGF β (i.e., β 1, β 2, and β 3). Fresolimumab is a high-affinity antibody with dissociation constants (Kds) of 1.8 nM, 2.8 nM, and 1.4 nM for

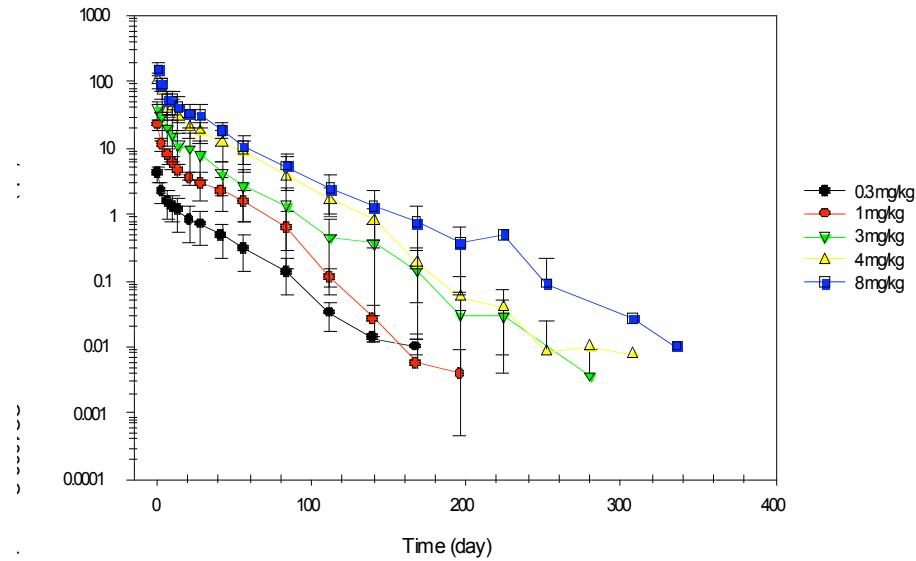
TGF β 1, 2, and 3, respectively. Three clinical studies with fresolimumab have been completed in patients with advanced Melanoma/Renal cell Carcinoma, Idiopathic Pulmonary Fibrosis (IPF), and Focal Segmental Glomerulosclerosis (FSGS).

3.4.1 Pharmacokinetics and Pharmacology

The pharmacokinetics of fresolimumab have been evaluated following a single 30 minute infusion administered to patients with IPF at doses of 0.3, 1, 2, 4 and 8 mg/kg. Single and multiple-dose pharmacokinetics of fresolimumab have also been assessed in patients with renal cell carcinoma and melanoma following administration of 0.1, 0.3, 1, 3, 10 and 15 mg/kg infused over 0.5 to 3 hours.

The results of the single-dose pharmacokinetic study in IPF patients showed that fresolimumab is eliminated in a biphasic manner with an elimination half-life of approximately 3 weeks, consistent with IgG monoclonal antibodies. The distribution volume (6.5-15.8 L) suggests that fresolimumab is confined mostly within serum. An analysis of the relationship of C_{max} and AUC with dose showed that the pharmacokinetics of fresolimumab are dose proportional over the dose range of 0.3 to 8 mg/kg. The clearance of fresolimumab appeared to be dose independent, suggesting that the target antigen has not been saturated in the studied dose range. Mean fresolimumab serum concentrations by dose group is presented on a semi-log scale in Figure 3.4.1-1.

Figure 3.4.1-1: Mean Fresolimumab Serum Concentrations As a Function of Time By Dose Group on a Log-Linear Scale



A preliminary analysis of fresolimumab serum concentrations in patients with renal carcinoma and myeloma showed similar pharmacokinetic properties to the IPF population. The elimination half-life ranged from 21-30 days, and clearance and distribution volume were dose independent. Additionally, systemic exposure increased in proportion to the total dose administered. The dosing regimen in patients with renal carcinoma and myeloma (first and second doses separated by four weeks, with administration every two weeks for the three remaining doses), did not appear to result in substantial accumulation of maximal serum fresolimumab concentrations over the duration of the study.

3.5 Preclinical Studies

3.5.1 TGF- β Antagonism in Murine Models of Disease

A more complete summary of studies, evaluating the effects of a murine analog TGF- β monoclonal antibody (1D11) and fresolimumab in animal models of fibrosis and cancer can be found in the Investigator Brochure. In general, TGF- β antagonism results in inhibition of fibrosis as well as reversal of preexisting fibrosis in models of liver and kidney fibrosis.

3.5.2 Toxicology Studies

Genzyme has performed toxicology studies in rodents and non-human primates. Details of each of these studies can be found in the Investigator Brochure.

3.5.2.1 Non-Human Primate Toxicity Studies

Genzyme has performed both single- and multiple-dose toxicology studies of fresolimumab in Cynomolgus monkeys. Highlights of these studies are discussed briefly below (for details refer to the Investigator Brochure).

In the single-dose study, groups of 4 female animals received 0.5, 5, or 50 mg/kg of fresolimumab and 2 female animals received vehicle control alone. Under the conditions of this study, the agent was well tolerated and the no-observed-adverse-effect level (NOAEL) for a single dose of fresolimumab in female Cynomolgus monkeys was 50 mg/kg.

In the 3 separate repeat- dose toxicity studies in cynomolgus monkeys, the effects of bi-weekly administration of fresolimumab were assessed at doses ranging from 0.1-50 mg/kg, over periods of 3 to 6 months. In all 3 studies, repeat administration of fresolimumab resulted in dose-dependent and time-dependent epithelial cell hyperplasia. Depending on the study, the epithelial hyperplasia was observed in different tissues, including in the gingival and/or nasal epithelium, or the urinary bladder. Disruption of the gingival and nasal epithelium resulted in bleeding and was associated with reductions in erythrocyte parameters (red blood cell, hematocrit, and hemoglobin) at doses of 10 or 50 mg/kg. Clinically meaningful anemia was observed in some animals receiving 10 or 50 mg/kg and could, on occasion, be correlated with clinical observations of gingival bleeding. At 50 mg/kg, the severity of the anemia observed required cessation of antibody administration following the 5th dose. At 10 mg/kg, transient reticulocytosis enabled animals to respond to anemia so that there was no need to discontinue antibody administration throughout the course of the study. In all 3 studies, cessation of fresolimumab administration resulted in time-dependent reversal of epithelial hyperplasia, reduction in bleeding and normalization of hematocrit. The only other toxicological effect of chronic neutralization of TGF β that was noted in these studies was the dose-dependent induction of subcutaneous masses consistent with organizing hematomas in

some animals that were histologically described as foci of fibrovascular proliferation into central areas of abundant hemorrhage and organizing fibrin. Given time, the hematoma organized and eventually regressed, leaving fibrosis and vascular spaces behind. All other clinical and histological assessments of these animals were observed to be within normal ranges.

3.6 Clinical Experience with Fresolimumab

Three clinical studies have been completed with fresolimumab: a single-dose Phase 1 study in patients with idiopathic pulmonary fibrosis (IPF), a single dose Phase 1 study in focal segmental glomerulosclerosis (FSGS), and a multiple dose Phase 1 study in melanoma and renal cell carcinoma patients. Thus far, the number of patients receiving fresolimumab is small, and only limited information regarding human experience is available at this time (see Table 3.6).

Study Title	Route of Administration/ Dose Range/ Frequency	Number of Patients
A Phase 1, Multicentre, Open-label, Dose-Escalating Study of Single Doses of GC1008 in Patients with Treatment Resistant Idiopathic Focal Segmental Glomerulosclerosis	IV administration Cohort A: 1 mg/kg Cohort B: 2 mg/kg Cohort C: 4 mg/kg Cohort D: 0.3 mg/kg (mg/kg body weight) Single Dose	16 (4 per cohort)
A Phase 1, Open-Label, Multi-Center, Single Dose, Dose-Escalating, Safety, Tolerability, and Pharmacokinetic Study of GC1008 in Patients with Idiopathic Pulmonary Fibrosis	IV administration Cohort 1: 0.3 mg/kg Cohort 2: 1 mg/kg Cohort 3: 2 mg/kg Cohort 4: 4 mg/kg Cohort 5: 8 mg/kg (mg/kg body weight) Single Dose	25 (5 per cohort)
A Phase 1 Study of the Safety and Efficacy of GC1008: A Human Anti Transforming Growth Factor- β Monoclonal Antibody in Patients with Advanced Renal Cell Carcinoma or Malignant Melanoma*	IV administration Cohort 1: 0.1 mg/kg Cohort 2: 0.3 mg/kg Cohort 3: 1 mg/kg Cohort 4: 3 mg/kg Cohort 5: 10 mg/kg Cohort 6: 15 mg/kg (mg/kg body weight) Single dose, followed 28 days later by 3 additional doses given every 14 days	29 Cohort 1: 3 Cohort 2: 3 Cohort 3: 3 Cohort 4: 4 Cohort 5: 3 Cohort 6 Part 1: 6 Cohort 6 Part 2: 7

Note: GC1008 is the research number for fresolimumab

* This study was comprised of 2 parts: Part 1, Dose Escalation and Part 2, Patient Expansion. The highest dose level examined (15 mg/kg) in Part 1 was found to be the maximal safe dose tested and thus was selected for evaluation in Part 2.

Table 3.6. Clinical Experience with Fresolimumab
PROPRIETARY AND CONFIDENTIAL

The safety, tolerability, pharmacokinetics, and exploratory clinical efficacy of fresolimumab were assessed in an open-label, single-dose, dose-escalating study in patients with idiopathic FSGS. A total of 16 patients were enrolled in the study, 4 patients in each dose cohort. Patients in each cohort received a single dose of fresolimumab infusion at 0.3, 1, 2 or 4 mg/kg body weight. Patients returned regularly over the following 112 days for safety evaluations. Exploratory clinical outcomes were evaluated; however, the results are limited due to the small sample size, lack of placebo controlled comparison group and single-dose treatment design. Potential improved outcome was shown in 3 patients (2 from the fresolimumab 1 mg/kg cohort and 1 from the 2 mg/kg cohort) who demonstrated substantial decreases in urine protein:creatinine ratio levels at the final study visit compared to baseline. Fresolimumab was well tolerated at single doses up to 4 mg/kg in patients with FSGS. Reporting of treatment-emergent adverse events (TEAEs) was similar across the dose cohorts. Fifteen (93.8%) of 16 patients reported a total of 73 TEAEs. The most frequent TEAEs reported were peripheral edema peripheral (4 patients) and nasopharyngitis (3 patients); all other TEAEs were reported by \leq 2 patients. All TEAEs except 1 (Grade 3 urticaria in a patient with a history of urticaria) were of Grade 1 or 2 intensity (mild or moderate).

The safety, tolerability, pharmacokinetics, potential clinical outcomes and bioactivity of fresolimumab were assessed in an open-label, single-dose, dose-escalating study of IPF patients. Twenty-five patients were enrolled and allocated to 5 dosing cohorts, with 5 patients in each cohort. Patients in each cohort received a single infusion of fresolimumab at 0.3, 1, 2, 4, or 8 mg/kg, respectively. The cohorts were enrolled and started treatment in a dose-escalating manner. Patients returned periodically for follow-up visits for safety evaluations and clinical outcome assessments. Efficacy was not assessed in this study; however, exploratory clinical outcomes were assessed. In terms of the pulmonary function test evaluation there were no clinically significant changes from screening in forced vital capacity and carbon monoxide diffusing capacity at Days 28 and 84. Fresolimumab was well tolerated at single doses up to 8 mg/kg in patients with IPF. Overall, there was no evidence of different adverse event (AE) patterns by cohort. Of the 25 patients treated with

fresolimumab in this study, 23 patients reported a total of 116 TEAEs. The most frequently reported TEAEs, regardless of relationship to study drug, were fatigue reported by 6 (24%) patients, headache reported by 4 (16%) patients, and bronchitis, diarrhea and dyspnea, each reported by 3 (12%) patients. In addition, 2 patients reported a total of 7 events of epistaxis during the study. All nonserious treatment emergent adverse events were Grade 1 or Grade 2 in intensity.

Interim results of the fresolimumab oncology study were presented at ASCO 2008 (Morris et al, 2008). The study was a Phase I multi-center, open-label, dose-escalation study designed to characterize the safety, tolerability, pharmacokinetics and potential anti-tumor activity of fresolimumab in patients with renal cell carcinoma (RCC) and melanoma. Dose escalation occurred at doses of 0.1, 0.3, 1, 3, 10 and 15 mg/kg fresolimumab in a 3+3 design. If no dose limiting toxicity (DLT) occurred within 28 days of the first dose, 3 additional doses were administered 2 weeks apart. Extended treatment (fresolimumab every 2 weeks x 4 doses) was offered to patients achieving stable disease, partial remission or complete remission. Of the 22 patients enrolled and treated in Part 1 of the study, no dose limiting toxicities were reported, and the highest dose of fresolimumab intended for administration, 15 mg/kg, was determined to be a safe dose (Morris et al, 2008). Fresolimumab was generally well tolerated and the majority of adverse events deemed (possibly, probably or definitely) related to study drug were classified as National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) grade 1 or 2. The most frequently reported related events included skin lesions (2 patients at grade 1, 1 patient at grade 2, and 2 patients at grade 3), fatigue (3 patients at grade 1), headache (2 patients at grade 1 or 2) and gingival bleeding (2 patients at grade 1).

Based on the clinical experience to date, the main epithelial adverse reaction to fresolimumab appears to be adverse events involving the skin, most notably keratoacanthomas (KA) and squamous cell carcinoma (SCC). Keratoacanthoma-like epithelial lesions developed in 4 of 29 patients who received multiple doses of fresolimumab in the Phase 1 oncology study of patients with advanced melanoma or RCC. Biopsies of these lesions were read as KA in

some patients and as SCC in others. Since it may be difficult to distinguish KA from well-differentiated SCC, both clinically and histologically, it is possible that all patients had the same process. One of the patients with KA and a history of basal cell carcinoma had a skin lesion biopsied that revealed basal cell carcinoma. The clinical course of the fresolimumab-associated skin lesions observed to date has been more consistent with KA since the lesions either spontaneously improved or resolved off therapy. The development of these lesions appears to be related to both dose and duration of exposure. Keratoacanthomas or SCC was not observed in the Phase 1 IPF or FSGS studies. Important differences between the studies could have impacted the occurrence of KA/SCC in the oncology, IPF, and FSGS studies include the following:

1. Underlying disease: All patients in the oncology study diagnosed with KA/SCC had metastatic melanoma and some had a history of SCC (2 patients) and basal cell carcinoma (1 patient). These patients may be expected to have a higher rate of KA/skin cancer relative to patients without history of melanoma and potential sun damaged skin.
2. Dose: Patients in the oncology study received multiple doses of up to 15 mg/kg administered every 2 weeks. In contrast, the highest dose in IPF and FSGS studies were 8 mg/kg and 4 mg/kg, respectively, each administered as a single dose.

Three melanoma patients also developed more diffuse drug reaction rashes. Self-limited, mild rashes and/or erythema considered by the treating physician to be drug-related occurred in the oncology and FSGS Phase 1 clinical studies. Skin exams, evaluating for treatment emergent lesions such as keratoacanthomas and drug reactions should be performed in all patients receiving fresolimumab. Patients who develop concerning treatment-emergent skin lesions should be referred to a dermatologist for evaluation.

3.7 Rationale for Anti-TGF β Antibody Therapy In This Study

SSc presents special problems for developing therapies due to the heterogeneous clinical presentation, the variability of disease progression and the difficulty quantifying the extent of disease. The variability of disease progression presents particular challenges for deciding

whom to treat, leading to overtreatment of patients, as well as misinterpretation of open label trials. This heterogeneity of disease progression has also required recruitment of relatively large patient numbers into clinical trials, many with skin disease that is going to stabilize or improve spontaneously (Amjadi et al., 2009).

This study will utilize skin TGF β -responsive gene biomarkers as the primary efficacy outcome in a short duration, open-label clinical trial of anti-TGF β , designed to provide preliminary data for a larger trial. These gene biomarkers should provide a strong surrogate for such trials in the future and, if TGF β is indeed the cytokine leading to fibrosis in this disease, provide a highly significant start to finding a therapeutic for SSc that for the first time might dramatically affect fibrosis. A central hypothesis of this study is that TGF β inhibition will downregulate the two TGF β -responsive genes (cartilage oligomeric protein, COMP and thrombospondin-1, THS1) components of the 4-gene biomarker over a relatively short period of time, much shorter than is historically thought necessary to see changes in the MRSS. This is consistent with in vitro studies showing that TGF β quickly and transiently activates a profibrotic phenotype in fibroblasts.

TGF β presents the most widely studied target in SSc, yet for a variety of reasons it has not been adequately studied as a therapeutic target. An earlier trial seeking to block TGF β did not show a change in skin score (Denton et al., 2007). However, this study had several significant limitations, most significantly the antibody studied, CAT192. CAT192 blocks only TGF β 1, where both TGF β 1 and TGF β 2 have been implicated in SSc. In addition, the antibody affinity of CAT192 for TGF β is much lower than the currently available anti-TGF β therapeutic, fresolimumab, and most animal data showing effects of anti-TGF β uses 1D11, an antibody with similar characteristics to fresolimumab (high affinity blocker of all three TGF β isoforms).

Thus, in this trial patients will be treated in a short-term open label trial with the high affinity pan-anti-TGF β antibody, fresolimumab, under development by Genzyme. We hypothesize that this antibody will rapidly inhibit TGF β signature mRNA expression in the 4-gene

biomarker, providing preliminary proof-of-concept data for a larger clinical trial using this agent. This outcome will be tested before treatment is started and 3 weeks after each of two doses of fresolimumab, the two doses separated by 4 weeks. The study will include a dose escalation from 1 mg/kg to 5 mg/kg after the first 8 patients have been enrolled.

In addition to testing for changes in biomarkers after fresolimumab treatment, the other primary outcome for this phase I study will be to determine safety in this patient population. Entry criteria will include the recent onset of dcSSc as this is the population most likely to show progressive skin disease and also the population examined in previous studies showing correlations between MRSS and the 4-gene biomarker. Patient selection will be designed to maximize safety by excluding patients with significant pulmonary, renal or bowel disease. As all KA/SSC adverse events observed in the oncology trial were in patients treated with multiple doses (4 doses) of fresolimumab, safety will also be increased by limiting exposure to the study medication to 2 doses. In addition patients with a history of KA or SCC will be excluded from this trial.

Secondary outcomes will include other validated measures of SSc disease activity. MRSS (Appendix C), SSc health assessment questionnaire (SHAQ, Appendix D), which includes the health assessment questionnaire (HAQ) and a SSc specific, patient visual analogue scale (VAS) for organ specific involvement will be followed during the trial. Several studies suggest that the SHAQ accurately measures disease activity and may detect smaller changes in health status. With these outcome measures we will test whether short-term therapy with fresolimumab affects skin and/or internal organ system involvement. In addition we will test the effect of fresolimumab on global skin gene expression using microarray analyses of skin biopsies.

4. INVESTIGATIONAL PLAN

4.1 Study Design

Patients will be recruited into the study from the Boston University Medical Center, Doctors Office Building, Rheumatology Clinic. Informed consent will be obtained at this first face-to-face contact with the study coordinator. Procedures performed are summarized in Appendix A: Study Flow Sheet. After informed consent to participate in the study has been obtained, demographic information will be taken and the patient will be scheduled for all pre-screening tests and a complete history and physical examination, including MRSS, careful skin cancer screening exam and complete examination of the tongue, oral and nasal mucosa (ENT exam). Laboratory testing will include an electrocardiogram, complete blood count (CBC), comprehensive metabolic panel, INR PT/PTT, urinalysis and HIV/HCV/HBV serologies. Blood will be banked for sera and peripheral blood mononuclear cell (PBMC) RNA. Based on these results patients meeting eligibility requirements will be scheduled for the first study visit.

Subjects with any skin lesions suspicious for possible malignant or pre-malignant disease identified during the screening visit will be referred for dermatology evaluation to include biopsy of any suspicious lesions. Subjects with KA or SCC will be excluded from treatment with fresolimumab. Subjects with hematuria will have repeat urinalysis. Patients with persistent hematuria will be referred for evaluation by a urologist and patients with any suspicious lesions on ENT exam will be referred to an otolaryngologist. Any patients with cancerous or precancerous bladder or ENT lesions will be excluded from treatment with fresolimumab.

Study Visit 1 (week 0): After all screening evaluations have been completed (within 4 weeks of laboratory tests), the subjects will be scheduled for the first study visit in the Boston University Medical Center, General Clinical Research Center (GCRC). On this visit, inclusion and exclusion criteria will be reviewed, and eligibility for study entry confirmed and documented. Eligible subjects will then have skin biopsies performed, SHAQ administered, MRSS assessed, and interim history (in this and future visits to include review

of adverse events and concomitant medications) and physical exam evaluated, including complete skin and ENT exams. Women of child-bearing potential (WOCP) will have a urine pregnancy test performed prior to study drug administration. Fresolimumab treatment will then be administered.

Study Visit 2 (week 3): At approximately week 3 subjects will return to the GCRC. An interim medical history, safety monitoring, and physical examination, including complete skin and ENT exam will be performed. Skin biopsies will be carried out and the MRSS will be assessed. Laboratory evaluations will include a CBC with differential, comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

Subjects with any skin lesions suspicious for possible malignant or pre-malignant disease identified during this or any subsequent visit will be referred for dermatology evaluation to include biopsy of any suspicious lesions. Subjects identified with new KA or SCC lesions will be excluded from further treatment with fresolimumab. Subjects with hematuria persistent on retesting during this or any subsequent visit will be referred to a urologist for further evaluation. Subjects showing any suspicious lesions on ENT exam on this or any subsequent visit will be referred to an otolaryngologist for further evaluation. Any patients with cancerous or precancerous bladder or ENT lesions will be excluded from further treatment with fresolimumab.

Study Visit 3 (week 4): The patient will return at approximately week 4 to the GCRC for the second of two doses of fresolimumab. Laboratories and interim history will be reviewed. A urine pregnancy test will be tested prior to administration of study medication, which must be negative for administration of the second dose. Fresolimumab will then be given.

Study Visit 4 (week 7): At approximately week 7 patients will return to the GCRC. An interim medical history, safety monitoring, and PE, including complete skin and ENT exam, and skin biopsies will be performed. The MRSS will be assessed, and the SHAQ

administered. Laboratory evaluation will include a CBC, comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

Study Visits 5 and 6 (weeks 11 and 17): At approximately weeks 11 and 17 patients will return to the GCRC. An interim medical history, safety monitoring, and PE, including complete skin and ENT exam will be performed. The MRSS will be assessed. Laboratory evaluation will include CBC, comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

Study Visit 7 (week 24): At approximately week 24, patients will return to the GCRC for the final study visit. An interim medical history, safety monitoring, and PE, including complete skin and ENT exam will be performed. Subjects who agree to will have optional skin biopsies carried out. The MRSS will be assessed and the SHAQ administered. Laboratory evaluation will include a CBC with differential a comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

5. ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

Patients must meet the following inclusion criteria to be eligible for study entry:

- Must meet the American College of Rheumatology criteria for systemic sclerosis with diffuse cutaneous involvement and <24 months since the onset of the first SSc manifestation other than Raynaud's phenomenon.
- Must have a MRSS of ≥ 15
- Male or female patients ≥ 18 years of age.
- Able and willing to give written informed consent and comply with the requirements of the study protocol

5.2 Exclusion Criteria

Patients will be excluded from the study based on the following criteria:

- Treatment with any investigational agent within 4 weeks of screening or 5 half-lives of the investigational drug (whichever is longer).
- Ongoing use of high dose steroids (>10 mg/day) or unstable steroid dose in the past 4 weeks.
- Treatment with immunosuppressive (other than low dose steroids), cytotoxic or anti-fibrotic drug within 4 weeks of screening.
 - The patient reactive or known reactive for HIV
 - The patient has positive viral hepatitis B or hepatitis C serologies on screening laboratories. (Patients with a positive hepatitis B surface antibody (HBsAb) test with a history of prior hepatitis B immunization are eligible as long as other criteria are met (i.e., negative tests for: hepatitis B surface antigen [HBsAg], hepatitis B core antibody [HBcAb], and hepatitis C virus antibody [HCVAb]).)
- Known active bacterial, viral fungal mycobacterial, or other infection (including tuberculosis or atypical mycobacterial disease, but excluding fungal infections of nail beds) or any major episode of infection requiring hospitalization or treatment with i.v. antibiotics within 4 weeks of screening.
- Patients with a history of malignancy or lesion considered premalignant

- Patients with a prior history of keratoacanthoma or squamous cell carcinoma
- Moderate to severe hepatic impairment, i.e., Child-Pugh Class B or C.
- Scleroderma renal crisis within 6 months or creatinine greater than 2.0
- Lack of intravenous access for medication administration
- Pregnancy (a negative pregnancy test will be performed for all women of childbearing potential on the day of treatment).
- Male and female patients of child-producing potential must agree to use effective contraception while enrolled on study and receiving the experimental drug, and for at least 3 months after the last treatment.
- Nursing mothers
- Gastrointestinal involvement requiring total parenteral nutrition or hospitalization within the past 3 months for pseudo-obstruction
- Moderately severe pulmonary disease with FVC <80%, or DLCO <70% predicted, or ground glass and fibrosis involving greater than 20% of the lung fields by HRCT.
- Moderately severe cardiac disease with either a history of significant arrhythmia (not to include conduction delays other than trifascicular block, or PVCs or PACs <5/min), clinically significant heart failure, or unstable angina.
- Hemoglobin: < 8.5 gm/dL
- Platelets: < 100,000/mm
- AST or ALT >2.5 x Upper Limit of Normal.
 - total bilirubin > 1.5 x upper limit of normal (ULN). Patients with Gilbert's Disease may be included if their total bilirubin is \leq 3.0 mg/dL.
- PT, PTT, INR > ULN
- History of ascites or pleural effusion, unless successfully treated, completely resolved, and the patient has not been treated for these conditions for >4 months.

- Active thrombophlebitis, thromboembolism, hypercoagulability states, bleeding, or use of anti-coagulation therapy (including anti-platelet agents such as aspirin, clopidogrel, ticlopidine, dipyridamole, and other agents used to induce long-acting platelet dysfunction). Patients with a history of deep venous thrombosis may participate if successfully treated, completely resolved, and no treatment has been given for >4 months.
- Patients with an organ transplant, including those that have received an allogeneic bone marrow transplant.
- Patients who, in the opinion of the Investigator, have significant medical or psychosocial problems that warrant exclusion. Examples of significant problems include, but are not limited to:
 - Other serious non-malignancy-associated medical conditions that may be expected to limit life expectancy or significantly increase the risk of SAEs.
 - Any condition, psychiatric, substance abuse, or otherwise, that, in the opinion of the Investigator, would preclude informed consent, consistent follow-up, or compliance with any aspect of the study

6. STUDY TREATMENT

6.1 Guidelines for Fresolimumab Administration

- Patients 1-8 will receive on Days 1 and 28 Fresolimumab 1 mg/kg administered by IV infusion for each of the two doses
- Patients 9-16 will receive on Days 1 and 28 Fresolimumab 5 mg/kg administered by IV infusion for each of the two doses

6.2 Treatments Administered

Fresolimumab will be administered as IV infusions administered over 30-60 minutes as deemed appropriate by the PI. (Dose of GC1008 should be diluted in Dextrose 5% in Water (D5W) prior to infusion. GC1008 is physically stable in D5W at a concentration of **0.3 to 7 mg/ml**. For GC1008 dose of 1mg/kg, the total dose should be further diluted with 50ml of D5W, and for a dose of 5mg/kg, the total dose should be further diluted with 100ml of D5W. Withdraw from the D5W infusion bag a volume equal to the amount of volume of GC1008 that will be required to prepare the dose.). A 0.22- μ m low-protein-binding inline filter must be used during each infusion. Infusions may be administered through either a peripheral IV or a central line. Further details are provided in the Investigational Product Handling Guidelines.

Subjects will be monitored for 30 minutes after each infusion for any infusion reaction. Patients treated with fresolimumab who develop acute infusion adverse reactions will be monitored as deemed medically appropriate and the reaction reviewed by the Investigator to determine if administration of fresolimumab may continue in this patient.

Prior to each subsequent dose of fresolimumab, each patient will be evaluated for the development of AEs. All AEs should be considered related to the study drug unless there is clear and identifiable reason or explanation to reject causal relation. Any patients

experiencing an AE of grade 2 related to study drug (as defined above) may receive the second dose if AE grade has resolved to grade 1 or less, if the Investigator determines and DSMB agrees by unanimous vote that continuing fresolimumab is not likely to pose a significant safety risk. Any patients experiencing an AE of grade 2 related to study drug (as defined above) that does not improve to grade 1 or less, or experiencing an AE of grade 3 or higher (thought related or unrelated to study drug) will not be given a second dose. Any patient developing KA or SCC during the study will not receive further doses of medication. Any patient experiencing an AE in which there is a clear and identifiable reason or explanation to reject causal relation to the study drug may continue fresolimumab, if the Investigator determines that continuing study drug is not likely to pose a significant safety risk.

6.3 Dose Modifications

The dose of fresolimumab administered will be based on the patient's actual body weight. Subsequent doses will be recalculated if the patient's weight changes by $\geq 10\%$.

6.4 Concomitant Therapy

No concomitant immunosuppressive agents or anti-coagulation therapies are permitted during the study unless specified below. Heparin flush of a central line is allowed. Transient use of ibuprofen is permitted. Inhaled or topical corticosteroids are allowed, and in patients who develop skin lesions, emollients and topical corticosteroids may be used as needed. Use of alternative medications (herbals, botanicals, etc.) is strongly discouraged during the entire study period. In addition, starting new medications may be associated with side effects; therefore, medications should not be started for the first time during or around the time of fresolimumab administration.

6.5 Duration of Therapy

Patients will receive a maximum of 2 cycles of study treatment unless criteria are met for patient withdrawal.

6.6 Patient Withdrawal

Patients are free to withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment. A patient's participation in the study may also be discontinued at any time at the discretion of the Investigator.

The following are reasons why the Investigator may remove a patient from study treatment and further follow-up:

- The patient withdraws consent;
- The patient is found to be not eligible after enrollment;
- The patient is non-compliant with study requirements;
- The Study is terminated

The following are reasons why the Investigator or Sponsor may remove a patient from study treatment but continue follow-up:

- General or specific changes in the patient's condition render the patient unacceptable for further treatment per the investigator's judgement;
- The patient becomes pregnant during the study;
- Any patient experiencing any grade 2 AE resolving to grade 1, thought related to the study drug, i.e. in which there is not a clear and identifiable reason or explanation to reject causal relation to the study drug, may receive further study medication only if the principal investigator determines and the DSMB agrees by unanimous vote that continuing study drug is not likely to pose a significant safety risk;
- Any patient experiencing any grade 3 or higher AE should be discontinued from further treatment;
- Any patient developing KA or SCC or other malignant or premalignant condition should be discontinued from further treatment;

- The patient develops progressive disease
- The patient requires a prohibited medication;
- There is new information to suggest a significant change in risk/benefit for fresolimumab;

7. INVESTIGATIONAL PRODUCT

Fresolimumab is an engineered human monoclonal antibody against human TGF β 1, β 2, and β 3. It is supplied as a sterile, non-pyrogenic, white to off-white lyophilized powder.

7.1 Packaging and Labeling

The study drug will be packaged in single-use USP Type I borosilicate, 5-mL glass vials with a siliconized butyl rubber stopper. Each vial contains approximately 50 mg of fresolimumab.

The label text for the study drug will include the contents of the vial (i.e., fresolimumab 50 mg/5 mL), lot number, appropriate caution statement, storage conditions, and name and address of Genzyme.

Kits and vials will be packaged in compliance with Good Manufacturing Practice and labeled according to United States Code of Federal Regulations. Each Kit will contain 10 vials and labeled with a single panel label.

7.2 Study Treatment Preparation

The study drug will be prepared according to the patient's treatment assignment. The study drug will be reconstituted with 5.1 mL of sterile Water for Injection to yield a concentration of 10 mg/mL. Further dilution guidelines are included in the Investigational Product Handling Manual.

7.3 Drug Shipment and Storage

Genzyme will arrange the shipment of the study drug to the clinical site.

- The study drug will be shipped overnight for next day delivery.
- Shipments will be made on Monday through Thursday only.
No shipments will be made on Friday, Saturday, or Sunday.

Upon receipt by the Investigator or designee, the study drug must be stored at 2 to 8°C, in a limited-access area until preparation for infusion.

7.4 Study Drug Accountability

Fresolimumab will be provided by Genzyme. Full records must be maintained to account for the study drug supplied to the Investigators, the disposition of the study drug, and the return or destruction of unused supplies.

7.5 Expected Toxicities

As reported at ASCO 2008 (Morris et al, 2008), in 22 melanoma and renal cell carcinoma patients treated with fresolimumab, the most frequently reported related events included skin lesions (2 patients at grade 1, 1 patient at grade 2, and 2 patients at grade 3), fatigue (3 patients at grade 1), headache (2 patients at grade 1 or 2) and gingival bleeding (2 patients at grade 1). Skin lesions including KA/SCC are an expected toxicity of fresolimumab.

There may be an increased risk of herpes zoster associated with fresolimumab. To date, 3/29 melanoma patients and 1/12 FSGS patients who have received fresolimumab developed non-disseminated self-limited herpes zoster. The FSGS patient had a prior history of recurrent herpes zoster.

8. STUDY PROCEDURES (See Appendix A)

8.1 Pre-Treatment Evaluations (Screening Visit)

Unless otherwise specified, the following evaluations will be performed within four weeks prior to initial fresolimumab treatment date:

- Medical history and documentation of the rationale for treatment of the patient's disease with fresolimumab.
- Physical examination, including vital signs, and MRSS.
- Thorough dermatologic evaluation. Any significant dermatologic findings (aside from SSc related) will be recorded on the case report form (CRF). Suspicious lesions will be evaluated by dermatologist
- Hematology: complete blood count (CBC) with differential and platelet count,
- INR/PTT.
- Serum Chemistries (metabolic panel): glucose, BUN, creatinine, electrolytes, total bilirubin, alkaline phosphatase, total protein, albumin, SGOT (AST), SGPT (ALT), and calcium.
- Urinalysis
- Two CPT tubes (10 ml) for PBMC mRNA extraction
- Two red top tubes (10 ml) to be banked for future studies.
- Hep B, C and HIV serologies

8.2 Treatment visits- week 0 and 4

- Urine pregnancy test for women of childbearing potential.
- Scleroderma Modified Health Assessment Questionnaire (SHAQ, week 0, which will also be repeated on weeks 7 and 24).
- Skin biopsies. Two, 3 mm punch skin biopsies will be carried out at the first treatment visit (week 0, which will also be repeated on weeks 3 and 7, and optionally for patient at week 24 study visits. Biopsies will be carried out at adjacent sites over the same mid-forearm. One biopsy will be placed in formalin and the other in RNAlater. Formalin fixed samples will be processed for hematoxylin and eosin staining and smooth muscle actin

(SMA) staining, and myofibroblast score assessed as described (Kissin et al., 2006). Samples in RNAlater will be stored in the freezer until RNA preparation for biomarker skin score.

8.3 Follow-up Evaluations (week 3, 7, 11, 17 and 24)

Safety assessments

- Interim medical history and physical examination, including complete skin exam.
- CBC with differential, comprehensive metabolic panel
- U/A will be assessed (study visits on weeks 3 and 7 only).
- Adverse events (AEs) will be recorded and evaluated as detailed in section 11.

Efficacy Assessments

- Skin biopsies (described section 8.2; weeks 0, 3, 7 and optionally week 24).
- MRSS will be assessed by two of the same, trained physician scorers at screen, 3, 7, 11, 17 and 24 weeks. Drs. Simms, Merkel, Kissin and Lafyatis have taken classes on MRSS. The same two physicians will perform these measures at all treatment time points for each patient.
- SHAQ will be assessed at 0, 7 and 24 weeks (as described in section 8.2).
- Skin RNA (100 mg) prepared from microarray gene expression will be compared at 0, 3, 7 and 24 weeks by microarray.
- Peripheral blood mononuclear cells will be prepared from one 5 ml CPT tubes, lysed in RLT buffer (Qiagen) and stored at -80. RNA will be collected and banked for potential utilization in the context of ongoing biomarker studies. 5 ml serum will be aliquoted and banked at each study visit for future studies (screen, 3, 7, 11, 17 and 24 week visits only).

Study Calendar

See Appendix A

9. CRITERIA FOR RESPONSE

9.1 Primary evaluation of response.

Safety evaluation:

Study safety will be evaluated by a tabulation and review of all AEs and SAEs. The data from all patients who received fresolimumab during the study will be included for safety analysis. Safety data will include laboratory, history, physical exam, and adverse event reports on systemic signs or symptoms of study patients. These descriptive summaries will be provided for all patients for each safety parameter (and body system) by cycle, grade, and relationship to treatment. Safety data will also be summarized as cumulative incidence of specified safety events of interest.

Efficacy evaluation:

Skin expression of the two TGF β -responsive genes (COMP, THS1) in the 4-gene biomarker at 3 and 7 weeks will be compared to week 0 expression. Gene expression will be measured by quantitative real-time PCR and quantified using plasmid standards for reproducibly measuring gene expression between samples (Farina et al., 2010).

9.2 Secondary evaluations of response.

4-Gene Biomarker:

Skin expression of the 4-gene biomarker score (derived from COMP, THS1, SIG1 and IFI44 mRNA expression) at 3 and 7 weeks will be compared to baseline (week 0) 4-gene biomarker score.

Modified Rodnan skin score:

The MRSS at 3, 7, 11, 17 and 24 weeks will be compared to week 0. Improvement of skin score in diffuse SSc correlates with improvement in joint function, overall functional status and physician's global assessment (Clements et al., 2000). At six months compared to baseline, no statistically significant change in Rodnan skin scores were found in either the low-dose or high-dose penicillamine treatment groups (Clements et al., 1999). Based on this

historical control population an improvement in MRSS at 24 weeks or any other earlier time point compared to baseline in our study population will suggest treatment efficacy.

SSc Health Assessment Questionnaire (SHAQ): The SHAQ will be evaluated at study entry compared to 7 and 24 weeks. The HAQ-DI component of the SHAQ (pages 1-3, Appendix D) has been validated as accurately measuring changes in disease status in SSc (Clements et al., 2001; Steen and Medsger, 1997). It correlates directly with skin involvement, SSc heart and kidney disease, tendon friction rubs, hand contractures and proximal muscle strength. It also is a good predictor of outcome and survival. The SSc VAS component of the SHAQ provides additional organ specific information (Steen and Medsger, 1997). Organ specific components correlate well with vascular, GI and pulmonary involvement. The HAQ-DI and the VAS will be analyzed separately. The total HAQ-DI score at 7 and 24-week will be compared to the entry scores. Since these values were not significantly changed in the high-dose versus low-dose penicillamine trial from baseline to 6 months (Clements et al., 1999), any improvement at 24 weeks or at any earlier time point in our study patients would suggest treatment efficacy within the limitations of an open-label study. Each of the 6 components of the VAS will also be analyzed separately, comparing 7 and 24-week to study entry scores. These analyses may detect organ specific efficacy that might otherwise be missed.

Skin biopsy: Skin biopsies will be immunostained for smooth muscle actin+ (myofibroblasts) and scored by semiquantitative evaluation using a 0-10 grading scale in a blinded fashion as described previously (Kissin et al., 2006; Lafyatis et al., 2009).

Exploratory endpoints:

3 and 7-week (and optional-24 week) skin microarray RNA gene expression will be compared to baseline (week 0) skin gene expression to define whether a subset of dcSSc patients, as defined previously (Milano et al., 2008), appear to selectively respond to therapy, and to explore whether a gene signature can be defined that indicates a fresolimumab

response.

Baseline, 3, 7, 11, 17 and 24 week PBMC RNA will be prepared and stored at -80° C for possible future gene array analyses for prognostic correlations, and 5 ml serum will be aliquoted and stored at -80° C for possible future analyses of serum prognostic markers.

10. FORMS TO BE MAINTAINED

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

11. REGULATORY AND REPORTING REQUIREMENTS

11.1 Adverse Event Monitoring and Reporting

The principal investigator is responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of the study drug will be followed until resolution. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be used for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (<http://ctep.cancer.gov/reporting/ctc.html>; see also Appendix B).

Definition of Adverse Event

An AE is any untoward medical occurrence in a clinical investigation patient, which does not necessarily have a causal relationship with the investigational product (active or placebo drug, biologic, or device). An AE can, therefore, be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality whether or not considered related to the investigational product.

Adverse events include:

- Symptoms described by the patient or signs observed by the Investigator or medical staff.
- Test abnormalities (laboratory tests, ECG, X-rays, etc.) that result in an alteration in medical care (diagnostic or therapeutic).

Abnormalities present at baseline are considered AEs only if they reoccur after resolution or they worsen during the study.

Definition of a Serious Adverse Event

An SAE is any AE that results in any of the following:

Death: The patient died as the result of the event.

Life-threatening event: Any AE that places the patient, in the view of the Investigator, at immediate risk of death from the AE as it occurred, i.e., does not include an AE that had it occurred in a more severe form, might have caused death.

Required or prolonged inpatient hospitalization: The AE resulted in an initial inpatient hospitalization or prolonged an existing hospitalization of the patient. If a patient is

hospitalized as part of the clinical use of the product, a period of normal hospitalization will be outlined in the protocol or by the judgment of the Investigator. Hospitalizations longer than this period will be prolonged hospitalizations.

Persistent or significant disability/incapacity: An AE that results in a substantial disruption of a person's ability to conduct normal life functions.

Congenital anomaly/birth defect: A congenital anomaly/birth defect that occurs in the offspring of a patient exposed to the investigational product.

Important medical events: An AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Definition of Medical Events of Interest

Medical events of interest are specific events not meeting SAE criteria, but important enough to require expedited or special reporting. Events to be reported in this manner for this protocol include: herpes zoster, treatment-emergent skin lesions, cancers, bleeding events, and other events deemed of interest per the Sponsor.

Evaluation of Adverse Events/Serious Adverse Events

Relationship to Study Treatment

Assessment of the association between the AE and study exposure is important for regulatory reporting. This assessment is to be made in blinded studies and also for known comparators. For each AE/SAE the Investigator determines whether there is a reasonable possibility that the AE may have been caused by the study treatment according to the categories below:

- Not Related: There is no suspicion of a causal relationship between exposure and the AE.
- Unlikely Related: There is no evidence for a causal relationship between exposure and the AE; however, such a relationship cannot be ruled out.Possibly Related: There is some evidence supporting the possibility of a causal relationship between exposure and the AE.
- Related: There is strong evidence that there is a causal relationship between exposure and the AE.

A relationship to the investigational product must be given for each AE/SAE recorded, even if there is only limited information at the time.

The Investigator may change his/her opinion of causality in light of follow-up information, amending the AE/SAE report accordingly.

Severity Grading of Adverse Event Scoring

Note that this is not the same as “seriousness,” which is defined above. Seriousness serves as a guide for defining regulatory reporting obligations.

Severity Grading

The Investigator will assess the severity of all AEs/SAEs as Mild, Moderate, or Severe, based on the following definitions (developed from CDISC SDTM standard terminology v3.1.1).

Definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the research participant.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Outcome

Outcome describes the status of the AE. The Investigator will provide information regarding the patient outcome of each AE.

Definitions for possible results of an AE outcome:

- Fatal: The termination of life as a result of an AE.
- Not recovered/not resolved: The patient has not recuperated or the AE has not improved.
- Recovering/resolving: The patient is recuperating or the AE is improving.

- Recovered/resolved: The patient has recuperated or the AE has resolved.
- Recovered with sequelae/resolved with sequelae: The AE has resolved, but the patient has been left with symptoms or pathology.
- Unknown: Not known, not observed, not recorded, or refused.

Action Taken Regarding the Investigational Product

The Investigator will be required to provide the action taken regarding investigational product in response to the AE.

Options include:

- Dose not changed: No change in administration of the investigational product.
- Drug (investigational product) interrupted: Temporary interruption (termination) in administration of the investigational product.
- Drug (investigational product) withdrawn: Administration of the investigational product terminated (no further dosing).
- Not applicable: Determination of a value is not relevant in the current context.
- Unknown: Not known, not observed, not recorded, or refused.

Timeframe for Collection of Adverse Events/Serious Adverse Events

Adverse Events Occurring Prior to Study Treatment

Adverse events, including MEOIs and SAEs, will be collected from the time the patient signs the informed consent form.

Adverse Events Occurring After Study Treatment

Adverse events will be collected from the time of the patient's first receipt of investigational product until week 24.

Serious AEs will be collected from the time of the patient's first receipt of investigational product until week 24.

Medical Events of Interest will be collected from the time of the patient's first receipt of investigational product until week 24. **Adverse Events Occurring Following Patient Discontinuation of Treatment**

Patients who prematurely discontinue study treatment and are withdrawn from the study will be followed for new AEs, MEOIs, and SAEs for 45 days after their last dose of fresolimumab.

For patients who prematurely discontinue study treatment but who are not withdrawn from the study, AEs will continue to be recorded until the patient completes the study.

Serious Adverse Events Occurring Following Patient Completion of the Study

If, at any time after the patient has completed participation in the study, the Investigator becomes aware of an SAE that they believe is possibly related or related to the investigational product, then the event and any known details should be reported promptly to Genzyme.

11.2 Reporting Serious Adverse Events

All SAEs occurring during the study or within 45 days of the last administration of fresolimumab must be reported to the principal investigator and to Genzyme within 24 hours of occurrence. The principal investigators are responsible for reporting SAEs to the IRB and the FDA (21 CFR §312.32] or other applicable regulatory authority. The principal investigator is responsible for submitting follow-up reports for all SAEs regarding the patient's subsequent course until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment), or the patient dies.

Institution and Investigator understand and agree that Investigator and Institution are obligated under applicable law and regulations to report any serious and related adverse event, if any, that occurs during treatment with the Product to the Institution's IRB/Ethics Committee and to the governing regulatory authority in accordance with applicable filing timelines promptly after any such event occurs. Investigator, within 24 hours (US) or one business day (EU) of first knowledge of such serious adverse event, will notify Genzyme via fax, attention Genzyme Global Patient Safety and Risk Management (GPS-RM), 617-761-8506 (US) or +1-617-761-8506 or by e-mail to pharmacovigilancesafety@genzyme.com. Prior to or at the time of filing any such report with the governing regulatory authority, the Investigator will also transmit an information copy of the report as sent to the authorities to Genzyme GPS-RM as listed above. . The Investigator shall make available to Genzyme promptly such

records as may be necessary and pertinent to investigate any such expedited adverse event, if specifically requested by Genzyme.

Furthermore, the Investigator will inform Genzyme of the following:

- any events that result in protocol amendments for safety reasons, as well as any safety related regulatory action such as a clinical hold of the Research
- any pregnancies occurring in patients who are exposed to the Product in connection with the Research.
- In addition, the Investigator shall notify Genzyme within 24 hours (US) or one business day (EU) of first knowledge of any Product complaints (communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, effectiveness, safety, labeling, purity, stability, and appearance) by email to GEMG@genzyme.com or via fax to 508-661-8771 (US) or Genzyme Customer Services Europe, +31 (0)35 699 1222.
- The Investigator will also inform Genzyme within 1 business day of becoming aware of any actions from any authority that may affect the performance of the Research

11.3 Medical Events of Interest

Sponsors-Investigators are requested to report to Genzyme serious and non-serious events of treatment-emergent skin lesions of unknown etiology and varicella zoster infections within 15 days of becoming aware of the event using medical events of interest forms that will be provided by Genzyme. Medical event of interest forms should be faxed to Genzyme GPS-RM at 617-761-8506. In addition, Sponsors-Investigators will provide updates to Genzyme regarding safety issues arising in this study via regular conference calls.

11.4 Data Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be formed to act in an advisory capacity to the study investigators to monitor participant safety in the study.

The DSMB responsibilities are to:

- protect the safety of the study participants;
- report to IRB on the safety issues or concerns of the trial;
- make recommendations to the Principal Investigator, and, if required, to the Food and Drug Administration (FDA) concerning continuation, termination or other modifications of the trial based on the observed adverse effects of the treatment under study.

Membership

The DSMB will be composed of two members, Dr. Arthur Theodore (Pulmonologist) and Dr. Deborah Cummins (Dermatologist), who are independent of the investigators, and who have no financial, scientific, or other conflict of interest with the trial.

Board Process

The DSMB will meet in two settings. In the first setting the DSMB will meet in a planned meeting after all of the first 8 patients have completed study treatment and been evaluated at the week 7 study visit. Further recruitment into the study will pause until this review is completed. All adverse events will be reviewed and afterwards a meeting held with the study principal investigator and other study investigators as available to discuss any safety concerns before proceeding to the second dose escalation phase of the trial.

DSMB members will be informed of all SAEs within 7 days after the investigator becomes aware. The principal investigator will also inform the DSMB of the study investigators' assessment of causality to study medication. This event may trigger the second setting for

DSMB meeting, an emergency meeting of the DSMB. An emergency meeting of the DSMB may be called at any time by either DSMB member or any study investigator to address any safety questions or other unanticipated problems. Meetings may be convened as conference calls as well as in-person. A recommendation to terminate the study may be made by the DSMB at any time.

Meeting Format

DSMB meetings will consist of open and closed sessions. The interim report and any SAE reports for the DSMB will be prepared by the study staff, typically the Clinical Coordinator. The Principal Investigator and key members of the study team attend the **open sessions**. The **closed session** will only be attended by DSMB members. Typically a DSMB meeting may include both an open session to gather information from study investigators and a closed session to consider and vote regarding study continuation versus termination. DSMB vote and a brief description of rationale for recommendations will be provided to the principal investigator as a written report.

A split or consensus vote by the DSMB for study termination will not be binding on the principal investigator but will be reported to the Institutional review Board and Sponsor. Upon a consensus DSMB vote for study termination no further subjects will be recruited or given study medication until full review and discussion with the IRB and FDA regarding any identified safety questions or issues, and a final decision made whether to terminate the study.

Reports from the DSMB

A formal report containing the recommendations for continuation or modifications of the study will be prepared by the DSMB and forwarded to the Principal Investigator. As previously stated, the formal DSMB report must include a recommendation to continue or to terminate the study. This recommendation should be made by formal vote.

Confidentiality

All materials, discussions and proceedings of the DSMB are completely confidential.
Members and other participants in DSMB meetings are expected to maintain confidentiality.

12. STATISTICAL CONSIDERATIONS

Once all subjects complete the study or discontinue prematurely, the final analyses will be completed. All available data from all subjects who receive at least one infusion of fresolimumab will be included in the safety and efficacy analyses. TGF β -responsive gene expression at 3 and 7 weeks will be compared to baseline using a paired t-test. If changes in TGF β -responsive gene expression do not follow a normal distribution then these comparisons will be made using a Wilcoxon matched pairs test.

Secondary outcome measures skin myofibroblast score, modified Rodnan skin score (MRSS) and scleroderma modified health assessment questionnaire (SHAQ) at 3 and 7 weeks will be compared to baseline. Only descriptive statistics, summarizing changes at 3, 7 and 24 weeks for secondary efficacy endpoints will be utilized for these comparisons.

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APPENDIX A: STUDY CALENDAR

APPENDIX B: Common Terminology Criteria for Adverse Events (CTCAE) version 4.0

APPENDIX C: Modified Rodnan Skin Score

APPENDIX D: Scleroderma Health Assessment Questionnaire

IRB Protocol #: H-30142

Protocol Version: 3.0

Protocol Version Date: March 15, 2012

**Title: An Open Label Trial of Anti-TGF β MAB, FRESOLIMUMAB, IN SYSTEMIC
SCLEROSIS—A PHASE ONE BIOMARKER TRIAL**

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Table 3.6 Clinical Experience with Fresolimumab.....

1. OVERVIEW OF STUDY

Title of Study: Anti-TGF β MAB, FRESOLIMUMAB, IN SYSTEMIC SCLEROSIS—A PHASE ONE BIOMARKER TRIAL

Study Phase: Phase I

Research Hypothesis: This study will test safety of TGF β inhibition by fresolimumab in patients with systemic sclerosis (SSc). This study will also test the hypothesis that TGF β drives expression of TGF β -responsive genes upregulated in skin in SSc, and that inhibition of TGF β by fresolimumab will downregulate expression of these genes, providing a proof-of-concept for a larger clinical trial of this agent in SSc.

Primary Objectives:

1. To evaluate safety of fresolimumab in patients with Systemic Sclerosis
2. To investigate the effect of fresolimumab on TGF β -responsive gene expression in skin after treatment with fresolimumab compared to pre-treatment TGF β -responsive gene expression

Study Drug: Fresolimumab is a high affinity humanized antibody that neutralizes activity of TGF β 1, TGF β 2 and TGF β 3. Fresolimumab has a half-life in the circulation of approximately 3 weeks.

Study Design: This will be as an open-label dose escalation trial. The study will evaluate the effect of short-term treatment with fresolimumab/anti-TGF β on skin, TGF β -responsive gene expression making up part of a 4-gene biomarker of skin disease. The first eight subjects entered will receive a single dose of fresolimumab at 1 mg/kg (addendum: seven subjects have already received two doses under the previously approved protocol).. The Data Safety Monitoring Board will review all adverse events prior to initiation of the second part of the study after all of the first 8 patients have completed study treatment and been evaluated at the week 7 study visit.

In the second part of the study, eight subjects will be entered to receive a single dose of fresolimumab at 5 mg/kg. In both the first and second parts of the study skin biopsies will be taken to test for TGF β -responsive gene expression within two weeks before treatment and 3 and 7-weeks after the single dose of study drug. An optional biopsy will also be taken during at Week 24. Safety assessments will extend to 24 weeks after the single dose of study drug.

Number of Subjects per Group: Eight subjects will be studied in the first and eight subjects in the second dosing group for a total of 16 subjects.

Study Population: Men and women at least 18 years of age. Subjects with diffuse cutaneous SSc as defined by the American College of Rheumatology (ACR) with a modified Rodnan skin score of greater than or equal to 15.

Study Duration: The duration of this study for each subject will continue for 24 weeks after the single dose of study drug is administered, thus encompassing more than 5 drug half-lives.

Statistical Methods: Once all subjects complete the study or discontinue prematurely, the final analyses will be completed. All available data from all subjects who receive at one infusion of fresolimumab will be included in the safety and efficacy analyses. TGF β -responsive gene expression at 7 weeks will be compared to baseline using a paired t-test for the first part of the study. If changes in TGF β -responsive gene expression do not follow a normal distribution then these comparisons will be made using a Wilcoxon matched pairs test. Secondary outcome measures, including skin myofibroblast score, modified Rodnan skin score (MRSS) and scleroderma modified health assessment questionnaire (SHAQ) at 7 and 24 weeks, will be compared to baseline. Only descriptive statistics will be reported for these secondary measures.

2. STUDY OBJECTIVES

2.1 Primary Objectives: Safety and Efficacy

Primary Safety endpoint:

- Adverse and serious adverse events during the study period, including metabolic or hematologic adverse events, will be assessed at each study visit up to 24 weeks after the single dose of study drug is administered.

Primary Efficacy endpoint:

- For the first part of the study, TGF β -responsive gene expression at 7 weeks will be compared to baseline using a paired t-test. TGF β -responsive gene components (cartilage oligomeric protein, COMP and thrombospondin-1, THS1) of the 4-gene SSc biomarker skin score will be analyzed for these comparisons.

2.2 Secondary Objectives

- The 4-gene SSc biomarker skin score will be compared at 3 and 7 weeks (and optional 24 week biopsy) to baseline score in all fresolimumab-treated subjects.
- MRSS at 3, 7 and 24 weeks will be compared to week 0 MRSS.
- The scleroderma health assessment questionnaire (SHAQ), at 7 weeks and 24 weeks will be compared to week 0 values. The myofibroblast score in skin biopsies at 3 and 7 weeks (and optional 24 week biopsy) after treatment will be compared to week 0 myofibroblast score.

2.3 Exploratory Objectives

- Microarray gene expression in skin at 3 and 7 weeks (and optional 24 week biopsy) will be compared to week 0 gene expression in both subject groups.

3. BACKGROUND AND STUDY RATIONALE

3.1 Overview of Systemic Sclerosis

Scleroderma, also known as systemic sclerosis (SSc), is a multisystem disease affecting skin and, more variably, other tissues, commonly including joints, muscles, lungs, the gastrointestinal tract and kidneys. It is one of a group of diseases in which fibrosis is associated with organ dysfunction. Fibrosis can involve the liver (Lefton et al., 2009; Pinzani et al., 2005), lung (Frankel and Schwarz, 2009), kidneys (Schnaper, 2005), and less commonly other organs, representing a final common pathway to organ dysfunction. In SSc tissue fibrosis is widespread, variably involving skin, lungs and the gastrointestinal tract.

Although SSc can affect almost any part of the body, skin disease is the most consistent clinical manifestation. Skin disease typically starts in the hands with an edematous phase of hand swelling lasting one to several months. The skin then progressively thickens and tethers to underlying tissues. In diffuse cutaneous SSc (dcSSc), skin thickening, induration and tethering typically extend proximally up the arm and can involve the torso, abdomen, face and legs. Patients with limited cutaneous SSc (lcSSc) have skin disease limited to below the elbow and face and neck as well as other characteristic clinical features. SSc skin pathology (diffuse and limited cutaneous SSc) shows fibrosis and variable perivascular lymphocyte infiltration in the deep reticular dermis.

SSc affects multiple other body systems. Most severe complications are seen more frequently in dcSSc with considerable morbidity and mortality (Steen and Medsger, 2000). Lung disease manifests as interstitial fibrosis or pulmonary arterial hypertension (PAH, more common in lcSSc). Lung disease remains the leading cause of death among SSc patients. Gastrointestinal disease primarily results from dysmotility. In the esophagus and stomach this most commonly leads to esophagitis. In the small and large bowel this most commonly leads to constipation, bowel obstruction and/or malnutrition. Renal disease is primarily manifest as accelerated hypertension and renal insufficiency. Angiotension converting enzyme inhibitors

are generally though not uniformly effective for treating this manifestation, which previously led to significant mortality.

Other important clinical manifestations include cold-induced vasospastic disease in extremities (Raynaud's phenomenon) and digital ulcers. SSc can also have cardiac manifestations. Pericarditis is the most frequent cardiac manifestation. Subclinical pericarditis is common with large effusions developing occasionally. Myocardial involvement with low-grade myocardial fibrosis is relatively common, but not frequently of clinical importance (Follansbee et al., 1985). Fibrosis most commonly manifests as the appearance of a septal infarction pattern on EKG in patients with normal coronary arteries, or as ventricular conduction delays. Occasionally myocardial fibrosis leads to heart failure. Cardiac arrhythmias are seen in ~5% of patients with SSc. Most common are atrial or ventricular ectopy, generally not associated with more serious rhythm disturbances. However, thallium perfusion defects are associated with sudden cardiac death (Steen et al., 1996).

Current treatment for SSc is limited (Steen, 2001). For most disease manifestations treatment is primarily symptomatic and generally inadequate. The exception is renal disease, scleroderma renal crisis, once a major cause of mortality in SSc patients, can often be treated successfully with angiotensin converting enzyme inhibitors. Pulmonary complications now represent the major cause of mortality. Cyclophosphamide provides some benefit in patients with interstitial lung disease (ILD), the most lethal complication of SSc. However, the effect of this agent on SSc-associated ILD is modest and transient (Tashkin et al., 2006; Tashkin et al., 2007). Pulmonary arterial hypertension (PAH) also leads to considerable mortality in SSc patients. PAH may respond to vasodilators such as epoprostanol and bosentan, but frequently responses are incomplete and mortality still high (Badesch et al., 2009). Bowel hypomotility also leads to considerable morbidity and sometimes mortality. Esophageal hypomotility is treated, frequently without success, with pro-motility and acid-blocking agents. Dysmotility of the lower bowel and its complications are even more difficult to treat with pro-motility agents providing modest relief in some patients and antibiotics helping in cases of small

bowel overgrowth. Thus there are limited therapeutic alternatives for SSc patients faced with progressive lung or bowel disease.

Skin fibrosis, the hallmark feature of SSc, remains without effective treatment. Although skin changes in SSc are not a cause of mortality, they cause considerable morbidity, may reflect similar pathological processes to those that occur in the bowel and lungs, correlate highly with prognosis and disease progression in other organ systems and can be reproducibly assessed by skin score testing. Skin disease is of particular interest for evaluation in clinical trials since it is easily biopsied and can thus be repeatedly assessed for pathological changes during clinical trials (Lafyatis et al., 2009).

Part of the difficulty in finding effective treatments for SSc has been a continuing uncertainty regarding what initiates pathogenesis. The cause of disease manifestations in SSc remains obscure, although three major pathophysiologic explanations have been advanced. Prominent pathologic changes in dermal and pulmonary tissues show fibrosis, suggesting abnormalities in matrix deposition. Vascular disease, resulting in scleroderma renal crisis, digital ischemia and pulmonary hypertension suggests dysfunction of the vascular endothelium. Autoantibodies in SSc patient sera suggest that immune dysfunction and autoimmunity may contribute to or cause disease. The different pathological features in different organs have provided support for each of these mechanisms, but not clarified which is most important in overall pathogenesis.

3.2 Background: Transforming Growth Factor

Transforming growth factor-beta (TGF β) is a pleiotropic cytokine which belongs to a superfamily of ligands, including bone morphogenetic proteins and activins (Blobe et al. 2000; Dumont, Arteaga. 2000; Akhurst, 2002; Wakefield, Roberts, 2002; Dumont, Arteaga, 2003). Under normal conditions, members of the TGF β family maintain homeostasis in many organ systems. In normal and non-cancerous cells, TGF β limits the growth of epithelial, endothelial, neuronal, and hematopoietic cell lineages through anti-proliferative and apoptotic responses. In addition, TGF β exerts potent effects that influence immune

function, cell proliferation/ functional differentiation, cell adhesion, extracellular matrix production, cell motility, angiogenesis, and cytokine production.

TGF β exists in 3 isoforms: TGF β 1, β 2, and β 3. Each isoform is encoded by distinct, highly conserved genes and is a 25-kilodalton homodimeric, disulphide-bonded protein. TGF β members are expressed in a tissue-specific and developmentally regulated fashion. TGF β 1 is expressed most commonly and is found in endothelial, hematopoietic, and connective tissues. TGF β 2 is found primarily in epithelial and neuronal tissues, and TGF β 3 resides in mesenchymal tissues. In vitro, each has similar activities. However, data from knockout mice suggest that each may be associated with distinct phenotypes.

TGF β is secreted by cells in a biologically inactive “latent form” by virtue of its association with latency-associated proteins (LAPs). Much of the TGF β /LAP “pro-drug” is stored in the extracellular matrix as a complex. However, other notable sites exist, including platelet granules and the surface of certain cells such as regulatory T cells. The mechanism of release of active TGF β may allow for local control. Activation can occur either under acidic conditions or through the action of proteases such as thrombospondin-1, plasmin, and prostate-specific antigen (PSA).

TGF β binds to cells via 3 major receptors: TGF β RI, TGF β RII, and TGF β RIII (transforming growth factor-beta receptor type I, type II, and type III). The binding of TGF β to receptors occurs in a specific sequence and results in a cascade of events leading to the formation of a receptor complex and the phosphorylation and activation of T β RI. The receptor complex has serine/threonine kinase activity and can activate the Smad (Sma- [small body size] and Mad-related protein) pathway by phosphorylation of Smad2 or 3. A key event is the formation of an activated Smad2(3)/4 complex, which is then transported to the nucleus where it induces gene transcription. This leads to a variety of effects on cell differentiation and growth.

Although TGF β /TGF β R/Smad is an essential pathway, TGF β 's influence on cellular activities appears to be much more complex. TGF β also binds to other receptors, such as endoglin, a cell-surface glycoprotein associated with proliferation of human endothelial cells and angiogenesis. Other signaling pathways, such as ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal protein kinase), MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase), Rho-kinase, Akt (agammaglobulinemia tyrosine kinase), and GTPases (guanosine triphosphatases) may also be involved. In addition, other receptor/signal pathways may intersect with the Smad pathway – including estrogen receptor (ER), androgen receptor (AR), steroid, epidermal growth factor receptor (EGFR), and other TGF β family members such as the activins. Because of these interactions, the overall effects of TGF β cannot always be predicted based on examination of any single pathway such as Smad.

3.3 TGF β in Systemic Sclerosis: Rationale for Anti-TGF β Antibody Therapy

TGF β is the most potent known profibrotic cytokine and the most consistently implicated cytokine in SSc pathogenesis (Varga and Abraham, 2007). A large body of in vitro data point to its role in fibrosis: it stimulates collagen and collagen processing to mature collagen, and it stimulates the conversion of fibroblasts into profibrotic myofibroblasts (Mauviel, 2005). Several observations in SSc skin suggest that altered TGF β activity might cause the fibrotic manifestations of SSc. Skin biopsy specimens from patients with SSc are characterized by increased new collagen synthesis in the reticular dermis as well as by increased numbers of myofibroblasts, activated fibroblasts that express the smooth muscle marker, α -smooth muscle actin (SMA) and actively form type I collagen, and other matrix components up-regulated in SSc skin (Sappino et al., 1990). In addition, SSc fibroblasts show increased p300 (Bhattacharyya et al., 2005), phosphorylated smad1 and smad2/3 (Ihn et al., 2006; Pannu et al., 2008), and α v β 5-mediated autocrine TGF β activation (Asano et al., 2006).

Although the etiology of vascular disease is uncertain, TGF β might also contribute to pathological features of intimal hyperplasia and obliteration, and perivascular fibrosis that characterize vascular disease in SSc (Trojanowska). Endothelial cells in SSc tissues show

reduced expression of typical endothelial cell markers such as vascular endothelial cadherin (Fleming et al., 2008). Data support a role for TGF β in the process of endothelial-mesenchymal transformation, a possible explanation for the loss of endothelial cell markers and smooth muscle hyperplasia in SSc vasculature (Arciniegas et al., 2007; Liebner et al., 2004; Morrell et al., 2009).

Clinical-pathological correlations have further implicated TGF β in SSc fibrosis. Although the origin of myofibroblasts in SSc remains uncertain, myofibroblasts can be induced in vitro by TGF β stimulation of normal dermal fibroblasts, suggesting that their presence in SSc skin reflects TGF β stimulation in vivo (Desmouliere et al., 1993). Supporting the notion that TGF β -induced myofibroblasts are key in skin fibrosis, “myofibroblast score”, a semi-quantitative assessment of myofibroblast infiltration of skin, in biopsies of lesional skin from the forearm of dcSSc patients correlates highly with the modified Rodnan skin score (MRSS), the current standard for evaluating the clinical extent of skin disease based on scoring the degree of skin involvement (0-3+) at 17 sites (Kissin et al., 2006; Lafyatis et al., 2009). Recent studies have extended these observations, showing that expression of four genes in a biopsy of mid-forearm skin correlates highly with the MRSS (Farina et al., 2010). Notably two of the genes in the 4-gene skin biomarker (COMP and THS1) are known transforming growth factor-beta (TGF β)-responsive genes. Biomarkers of disease activity might supplement or, in early phase trials, replace clinical outcome measures, such as the MRSS, potentially permitting short (open label) trials where the skin score would not normally be expected to change significantly. Further validating the 4-gene biomarker, in a small patient subset over 6-12 months the 4-gene biomarker changed with the MRSS (Farina et al., 2010).

3.4 Description of Fresolimumab

Genzyme and Cambridge Antibody Technology (CAT) have collaborated to produce human antibodies to TGF β . Fresolimumab is a human IgG4 kappa monoclonal antibody capable of neutralizing all mammalian isoforms of TGF β (i.e., β 1, β 2, and β 3). Fresolimumab is a high-affinity antibody with dissociation constants (Kds) of 1.8 nM, 2.8 nM, and 1.4 nM for

TGF β 1, 2, and 3, respectively. Three clinical studies with fresolimumab have been completed in patients with advanced Melanoma/Renal cell Carcinoma, Idiopathic Pulmonary Fibrosis (IPF), and Focal Segmental Glomerulosclerosis (FSGS).

3.4.1 Pharmacokinetics and Pharmacology

The pharmacokinetics of fresolimumab have been evaluated following a single 30 minute infusion administered to patients with IPF at doses of 0.3, 1, 2, 4 and 8 mg/kg. Single and multiple-dose pharmacokinetics of fresolimumab have also been assessed in patients with renal cell carcinoma and melanoma following administration of 0.1, 0.3, 1, 3, 10 and 15 mg/kg infused over 0.5 to 3 hours.

The results of the single-dose pharmacokinetic study in IPF patients showed that fresolimumab is eliminated in a biphasic manner with an elimination half-life of approximately 3 weeks, consistent with IgG monoclonal antibodies. The distribution volume (6.5-15.8 L) suggests that fresolimumab is confined mostly within serum. An analysis of the relationship of C_{max} and AUC with dose showed that the pharmacokinetics of fresolimumab are dose proportional over the dose range of 0.3 to 8 mg/kg. The clearance of fresolimumab appeared to be dose independent, suggesting that the target antigen has not been saturated in the studied dose range.

A preliminary analysis of fresolimumab serum concentrations in patients with renal carcinoma and myeloma showed similar pharmacokinetic properties to the IPF population. The elimination half-life ranged from 21-30 days, and clearance and distribution volume were dose independent. Additionally, systemic exposure increased in proportion to the total dose administered. The dosing regimen in patients with renal carcinoma and myeloma (first and second doses separated by four weeks, with administration every two weeks for the three remaining doses), did not appear to result in substantial accumulation of maximal serum fresolimumab concentrations over the duration of the study.

3.5 Preclinical Studies

3.5.1 TGF- β Antagonism in Murine Models of Disease

A more complete summary of studies, evaluating the effects of a murine analog TGF- β monoclonal antibody (1D11) and fresolimumab in animal models of fibrosis and cancer can be found in the Investigator Brochure. In general, TGF- β antagonism results in inhibition of fibrosis as well as reversal of preexisting fibrosis in models of liver and kidney fibrosis.

3.5.2 Toxicology Studies

Genzyme has performed toxicology studies in rodents and non-human primates. Details of each of these studies can be found in the Investigator Brochure.

3.5.2.1 Non-Human Primate Toxicity Studies

Genzyme has performed both single- and multiple-dose toxicology studies of fresolimumab in Cynomolgus monkeys. Highlights of these studies are discussed briefly below (for details refer to the Investigator Brochure).

In the single-dose study, groups of 4 female animals received 0.5, 5, or 50 mg/kg of fresolimumab and 2 female animals received vehicle control alone. Under the conditions of this study, the agent was well tolerated and the no-observed-adverse-effect level (NOAEL) for a single dose of fresolimumab in female Cynomolgus monkeys was 50 mg/kg.

In the 3 separate repeat- dose toxicity studies in cynomolgus monkeys, the effects of bi-weekly administration of fresolimumab were assessed at doses ranging from 0.1-50 mg/kg, over periods of 3 to 6 months. In all 3 studies, repeat administration of fresolimumab resulted in dose-dependent and time-dependent epithelial cell hyperplasia. Depending on the study, the epithelial hyperplasia was observed in different tissues, including in the gingival and/or nasal epithelium, or the urinary bladder. Disruption of the gingival and nasal epithelium resulted in bleeding and was associated with reductions in erythrocyte parameters (red blood cell, hematocrit, and hemoglobin) at doses of 10 or 50 mg/kg. Clinically meaningful anemia was observed in some animals receiving 10 or 50 mg/kg and could, on occasion, be correlated with clinical observations of gingival bleeding. At 50 mg/kg, the severity of the anemia observed required cessation of antibody administration following the 5th dose. At 10

mg/kg, transient reticulocytosis enabled animals to respond to anemia so that there was no need to discontinue antibody administration throughout the course of the study. In all 3 studies, cessation of fresolimumab administration resulted in time-dependent reversal of epithelial hyperplasia, reduction in bleeding and normalization of hematocrit. The only other toxicological effect of chronic neutralization of TGF β that was noted in these studies was the dose-dependent induction of subcutaneous masses consistent with organizing hematomas in some animals that were histologically described as foci of fibrovascular proliferation into central areas of abundant hemorrhage and organizing fibrin. Given time, the hematoma organized and eventually regressed, leaving fibrosis and vascular spaces behind. All other clinical and histological assessments of these animals were observed to be within normal ranges.

3.6 Clinical Experience with Fresolimumab

Three clinical studies have been completed with fresolimumab: a single Phase 1 study

Study Title	Route of Administration/ Dose Range/ Frequency	Number of Patients
A Phase 1, Multicentre, Open-label, Dose-Escalating Study of Single Doses of GC1008 in Patients with Treatment Resistant Idiopathic Focal Segmental Glomerulosclerosis	IV administration Cohort A: 1 mg/kg Cohort B: 2 mg/kg Cohort C: 4 mg/kg Cohort D: 0.3 mg/kg (mg/kg body weight) Single Dose	16 (4 per cohort)
A Phase 1, Open-Label, Multi-Center, Single Dose, Dose-Escalating, Safety, Tolerability, and Pharmacokinetic Study of GC1008 in Patients with Idiopathic Pulmonary Fibrosis	IV administration Cohort 1: 0.3 mg/kg Cohort 2: 1 mg/kg Cohort 3: 2 mg/kg Cohort 4: 4 mg/kg Cohort 5: 8 mg/kg (mg/kg body weight) Single Dose	25 (5 per cohort)
A Phase 1 Study of the Safety and Efficacy of GC1008: A Human Anti Transforming Growth Factor- β Monoclonal Antibody in Patients with Advanced Renal Cell Carcinoma or Malignant Melanoma*	IV administration Cohort 1: 0.1 mg/kg Cohort 2: 0.3 mg/kg Cohort 3: 1 mg/kg Cohort 4: 3 mg/kg Cohort 5: 10 mg/kg Cohort 6: 15 mg/kg (mg/kg body weight) Single dose, followed 28 days later by 3 additional doses given every 14 days	29 Cohort 1: 3 Cohort 2: 3 Cohort 3: 3 Cohort 4: 4 Cohort 5: 3 Cohort 6 Part 1: 6 Cohort 6 Part 2: 7

Note: GC1008 is the research number for fresolimumab

* This study was comprised of 2 parts: Part 1, Dose Escalation and Part 2, Patient Expansion. The highest dose level examined (15 mg/kg) in Part 1 was found to be the maximal safe dose tested and thus was selected for evaluation in Part 2.

in patients with idiopathic pulmonary fibrosis (IPF), a single dose Phase 1 study in focal segmental glomerulosclerosis (FSGS) , and a multiple dose Phase 1 study in melanoma and renal cell carcinoma patients. Thus far, the number of patients receiving fresolimumab is small, and only limited information regarding human experience is available at this time (see Table 3.6).

The safety, tolerability, pharmacokinetics, and exploratory clinical efficacy of fresolimumab were assessed in an open-label, single-dose, dose-escalating study in patients with idiopathic FSGS. A total of 16 patients were enrolled in the study, 4 patients in each dose cohort. Patients in each cohort received a single dose of fresolimumab infusion at 0.3, 1, 2 or 4 mg/kg body weight. Patients returned regularly over the following 112 days for safety evaluations. Exploratory clinical outcomes were evaluated; however, the results are limited due to the small sample size, lack of placebo controlled comparison group and single-dose treatment design. Potential improved outcome was shown in 3 patients (2 from the fresolimumab 1 mg/kg cohort and 1 from the 2 mg/kg cohort) who demonstrated substantial decreases in urine protein: creatinine ratio levels at the final study visit compared to baseline. Fresolimumab was well tolerated at single doses up to 4 mg/kg in patients with FSGS. Reporting of treatment-emergent adverse events (TEAEs) was similar across the dose cohorts. Fifteen (93.8%) of 16 patients reported a total of 73 TEAEs. The most frequent TEAEs reported were peripheral edema peripheral (4 patients) and nasopharyngitis (3 patients); all other TEAEs were reported by \leq 2 patients. All TEAEs except 1 (Grade 3 urticaria in a patient with a history of urticaria) were of Grade 1 or 2 intensity (mild or moderate).

The safety, tolerability, pharmacokinetics, potential clinical outcomes and bioactivity of fresolimumab were assessed in an open-label, single-dose, dose-escalating study of IPF patients. Twenty-five patients were enrolled and allocated to 5 dosing cohorts, with 5 patients in each cohort. Patients in each cohort received a single infusion of fresolimumab at 0.3, 1, 2, 4, or 8 mg/kg, respectively. The cohorts were enrolled and started treatment in a dose-escalating manner. Patients returned periodically for follow-up visits for safety evaluations

and clinical outcome assessments. Efficacy was not assessed in this study; however, exploratory clinical outcomes were assessed. In terms of the pulmonary function test evaluation there were no clinically significant changes from screening in forced vital capacity and carbon monoxide diffusing capacity at Days 28 and 84. Fresolimumab was well tolerated at single doses up to 8 mg/kg in patients with IPF. Overall, there was no evidence of different adverse event (AE) patterns by cohort. Of the 25 patients treated with fresolimumab in this study, 23 patients reported a total of 116 TEAEs. The most frequently reported TEAEs, regardless of relationship to study drug, were fatigue reported by 6 (24%) patients, headache reported by 4 (16%) patients, and bronchitis, diarrhea and dyspnea, each reported by 3 (12%) patients. In addition, 2 patients reported a total of 7 events of epistaxis during the study. All nonserious treatment emergent adverse events were Grade 1 or Grade 2 in intensity.

Interim results of the fresolimumab oncology study were presented at ASCO 2008 (Morris et al, 2008). The study was a Phase I multi-center, open-label, dose-escalation study designed to characterize the safety, tolerability, pharmacokinetics and potential anti-tumor activity of fresolimumab in patients with renal cell carcinoma (RCC) and melanoma. Dose escalation occurred at doses of 0.1, 0.3, 1, 3, 10 and 15 mg/kg fresolimumab in a 3+3 design. If no dose limiting toxicity (DLT) occurred within 28 days of the first dose, 3 additional doses were administered 2 weeks apart. Extended treatment (fresolimumab every 2 weeks x 4 doses) was offered to patients achieving stable disease, partial remission or complete remission. Of the 22 patients enrolled and treated in Part 1 of the study, no dose limiting toxicities were reported, and the highest dose of fresolimumab intended for administration, 15 mg/kg, was determined to be a safe dose (Morris et al, 2008). Fresolimumab was generally well tolerated and the majority of adverse events deemed (possibly, probably or definitely) related to study drug were classified as National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) grade 1 or 2. The most frequently reported related events included skin lesions (2 patients at grade 1, 1 patient at grade 2, and 2 patients at grade 3), fatigue (3 patients at grade 1), headache (2 patients at grade 1 or 2) and gingival bleeding (2 patients at grade 1).

Based on the clinical experience to date, the main epithelial adverse reaction to fresolimumab appears to be adverse events involving the skin, most notably keratoacanthomas (KA) and squamous cell carcinoma (SCC). Keratoacanthoma-like epithelial lesions developed in 4 of 29 patients who received multiple doses of fresolimumab in the Phase 1 oncology study of patients with advanced melanoma or RCC. Biopsies of these lesions were read as KA in some patients and as SCC in others. Since it may be difficult to distinguish KA from well-differentiated SCC, both clinically and histologically, it is possible that all patients had the same process. One of the patients with KA and a history of basal cell carcinoma had a skin lesion biopsied that revealed basal cell carcinoma. The clinical course of the fresolimumab-associated skin lesions observed to date has been more consistent with KA since the lesions either spontaneously improved or resolved off therapy. The development of these lesions appears to be related to both dose and duration of exposure. Keratoacanthomas or SCC was not observed in the Phase 1 IPF or FSGS studies. Important differences between the studies could have impacted the occurrence of KA/SCC in the oncology, IPF, and FSGS studies include the following:

1. Underlying disease: All patients in the oncology study diagnosed with KA/SCC had metastatic melanoma and some had a history of SCC (2 patients) and basal cell carcinoma (1 patient). These patients may be expected to have a higher rate of KA/skin cancer relative to patients without history of melanoma and potential sun damaged skin.
2. Dose: Patients in the oncology study received multiple doses of up to 15 mg/kg administered every 2 weeks. In contrast, the highest dose in IPF and FSGS studies were 8 mg/kg and 4 mg/kg, respectively, each administered as a single dose.

Three melanoma patients also developed more diffuse drug reaction rashes. Self-limited, mild rashes and/or erythema considered by the treating physician to be drug-related occurred in the oncology and FSGS Phase 1 clinical studies. Skin exams, evaluating for treatment emergent lesions such as keratoacanthomas and drug reactions should be performed in all patients receiving fresolimumab. Patients who develop concerning treatment-emergent skin

lesions will be referred to a dermatologist for evaluation. Patients who develop treatment emergent oropharyngeal lesions or bladder lesions will be referred to an otolaryngologist or urologist, respectively.

3.7 Rationale for Anti-TGF β Antibody Therapy In This Study

SSc presents special problems for developing therapies due to the heterogeneous clinical presentation, the variability of disease progression and the difficulty quantifying the extent of disease. The variability of disease progression presents particular challenges for deciding whom to treat, leading to overtreatment of patients, as well as misinterpretation of open label trials. This heterogeneity of disease progression has also required recruitment of relatively large patient numbers into clinical trials, many with skin disease that is going to stabilize or improve spontaneously (Amjadi et al., 2009).

This study will utilize skin TGF β -responsive gene biomarkers as the primary efficacy outcome in a short duration clinical trial of anti-TGF β , designed to provide preliminary data for a larger trial. These gene biomarkers should provide a strong surrogate for such trials in the future and, if TGF β is indeed the cytokine leading to fibrosis in this disease, provide a highly significant start to finding a therapeutic for SSc that for the first time might dramatically affect fibrosis. A central hypothesis of this study is that TGF β inhibition will downregulate the two TGF β -responsive genes (cartilage oligomeric protein, COMP and thrombospondin-1, THS1) components of the 4-gene biomarker over a relatively short period of time, much shorter than is historically thought necessary to see changes in the MRSS. This is consistent with in vitro studies showing that TGF β quickly and transiently activates a profibrotic phenotype in fibroblasts.

TGF β presents the most widely studied target in SSc, yet for a variety of reasons it has not been adequately studied as a therapeutic target. An earlier trial seeking to block TGF β did not show a change in skin score (Denton et al., 2007). However, this study had several significant limitations, most significantly the antibody studied, CAT192. CAT192 blocks only TGF β 1, where both TGF β 1 and TGF β 2 have been implicated in SSc. In addition, the

antibody affinity of CAT192 for TGF β is much lower than the currently available anti-TGF β therapeutic, fresolimumab, and most animal data showing effects of anti-TGF β have used 1D11, an antibody with similar characteristics to fresolimumab (high affinity blocker of all three TGF β isoforms).

Thus, in this trial subjects will be treated in a short-term open label trial with the high affinity pan-anti-TGF β antibody, fresolimumab, under development by Genzyme. We hypothesize that this antibody will rapidly inhibit TGF β signature mRNA expression in the 4-gene biomarker, providing preliminary proof-of-concept data for a larger clinical trial using this agent. This outcome will be tested before treatment is started and 3 weeks after the single dose of fresolimumab. The study will include a dose escalation from 1 mg/kg to 5 mg/kg.

In addition to testing for changes in biomarkers after fresolimumab treatment, the other primary outcome for this phase I study will be to determine safety in this patient population. Entry criteria will include the recent onset of dcSSc as this is the population most likely to show progressive skin disease and also the population examined in previous studies showing correlations between MRSS and the 4-gene biomarker. Subject selection will be designed to maximize safety by excluding patients with significant pulmonary, renal or bowel disease. As all KA/SSC adverse events observed in the oncology trial were in patients treated with multiple doses (4 doses) of fresolimumab, safety will also be increased by limiting exposure to the study medication to a single dose. In addition patients with a history of KA, SSC, or bladder lesionwill be excluded from this trial.

Secondary outcomes will include other validated measures of SSc disease activity. MRSS (Appendix C), SSc health assessment questionnaire (SHAQ, Appendix D), which includes the health assessment questionnaire (HAQ) and a SSc specific, patient visual analogue scale (VAS) for organ specific involvement will be followed during the trial. Several studies suggest that the SHAQ accurately measures disease activity and may detect smaller changes in health status. With these outcome measures we will test whether short-term therapy with fresolimumab affects skin and/or internal organ system involvement. In addition we will test

the effect of fresolimumab on global skin gene expression using microarray analyses of skin biopsies.

4. INVESTIGATIONAL PLAN

4.1 Study Design

Patients will be recruited into the study from the Boston University Medical Center, Shapiro Ambulatory Care, Rheumatology Clinic. Informed consent will be obtained at this face-to-face contact with the study coordinator. Procedures performed are summarized in Appendix A: Study Flow Sheet. After informed consent to participate in the study has been obtained, demographic information will be taken and the patient will be scheduled for all screening tests and a complete history and physical examination, including MRSS, careful skin cancer screening exam and complete examination of the tongue, oral and nasal mucosa (ENT exam). Laboratory testing will include an electrocardiogram (EKG), complete blood count (CBC), comprehensive metabolic panel, INR PT/PTT, urinalysis and HIV/HCV/HBV serologies. The urinalysis will screen for hematuria, a potential indicator of bladder cancer. This will be tested at least two times during the screening phase prior to fresolimumab dosing and at all subsequent safety visits. Blood will be banked for sera and peripheral blood mononuclear cell (PBMC) RNA. Based on these results patients meeting eligibility requirements will be scheduled for the first study visit.

Subjects with any skin lesions suspicious for possible malignant or pre-malignant disease identified during the screening visit will be referred for dermatology evaluation to include biopsy of any suspicious lesions. Subjects with KA or SCC will be excluded from treatment with fresolimumab. Subjects with hematuria will have repeat urinalysis. Patients with persistent hematuria will be referred for evaluation by an urologist and patients with any suspicious lesions on ENT exam will be referred to an otolaryngologist. Any patients with cancerous or precancerous bladder or ENT lesions will be excluded from treatment with fresolimumab.

Study Visit 1 (week 0): After all screening evaluations have been completed (within 4 weeks of laboratory tests), the subjects will be scheduled for the first study visit in the Boston University Medical Center, General Clinical Research Unit (GCRU). On this visit, inclusion and exclusion criteria will be reviewed, and eligibility for study entry confirmed and

documented. Eligible subjects will then have skin biopsies performed, SHAQ administered, MRSS assessed, and interim history (in this and future visits to include review of adverse events and concomitant medications) and physical exam evaluated, including complete skin and ENT exams. Women of child-bearing potential (WOCP) will have a urine pregnancy test performed prior to fresolimumab administration (either 1 mg/kg or 5 mg/kg). A single dose of fresolimumab will be administered.

Subjects that develop a suspicious skin, or ear, nose or throat lesion after receiving the study drug will be referred to a dermatologist or otolaryngologist, respectively. Subjects showing hematuria after drug treatment on follow-up visits will be retested 2 times within 2 weeks. Subjects who present with persistent hematuria will be referred to an urologist for cystoscopic evaluation.

Study Visits 2 and 3 (week 3 and 7): At approximately week 3 and week 7, subjects will return to the GCRU. An interim medical history, safety monitoring, and physical examination, including complete skin and ENT exam will be performed. Skin biopsies will be carried out at both these visits and the MRSS will be assessed. Durometry will be performed. Subjects will fill out a questionnaire (SHAQ). Laboratory evaluations will include a CBC with differential, comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

Subjects with any skin lesions suspicious for possible malignant or pre-malignant disease identified during this or any subsequent visit will be referred for dermatology evaluation to include biopsy of any suspicious lesions. Subjects identified with new KA or SCC lesions will be excluded from further treatment with fresolimumab. Subjects with hematuria persistent on retesting during this or any subsequent visit will be referred to a urologist for further evaluation. Subjects showing any suspicious lesions on ENT exam on this or any subsequent visit will be referred to an otolaryngologist for further evaluation.

Study Visits 4 and 5 (weeks 11 and 17): At approximately weeks 11 and 17 subjects will return to the GCRU. An interim medical history, safety monitoring, and PE, including complete skin and ENT exam will be performed. The MRSS will be assessed. Laboratory evaluation will include CBC, comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

Study Visit 6 (week 24): At approximately week 24, subjects will return to the GCRU for the final study visit. An interim medical history, safety monitoring, and PE, including complete skin and ENT exam will be performed. Subjects who agree to will have optional skin biopsies carried out. The MRSS will be assessed and the SHAQ administered. Durometry will be performed. Laboratory evaluation will include a CBC with differential a comprehensive metabolic panel and urinalysis. Blood will be banked for sera and PBMC RNA.

5. ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

Patients must meet the following inclusion criteria to be eligible for study entry:

- Must meet the American College of Rheumatology criteria for systemic sclerosis with diffuse cutaneous involvement and <24 months since the onset of the first SSc manifestation other than Raynaud's phenomenon.
- Must have a MRSS of ≥ 15
- Male or female patients ≥ 18 years of age.
- Able and willing to give written informed consent and comply with the requirements of the study protocol

5.2 Exclusion Criteria

Patients will be excluded from the study based on the following criteria:

- Treatment with any investigational agent within 4 weeks of screening or 5 half-lives of the investigational drug (whichever is longer).
- Ongoing use of high dose steroids (>10 mg/day) or unstable steroid dose in the past 4 weeks.
- Treatment with immunosuppressive (other than low dose steroids), cytotoxic or anti-fibrotic drug within 4 weeks of screening.
 - The patient reactive or known reactive for HIV
 - The patient has positive viral hepatitis B or hepatitis C serologies on screening laboratories. (Patients with a positive hepatitis B surface antibody (HBsAb) test with a history of prior hepatitis B immunization are eligible as long as other criteria are met (i.e., negative tests for: hepatitis B surface antigen [HBsAg], hepatitis B core antibody [HBcAb], and hepatitis C virus antibody [HCVAb]).)
- Known active bacterial, viral fungal mycobacterial, or other infection (including tuberculosis or atypical mycobacterial disease, but excluding fungal infections of nail beds) or any major episode of infection requiring hospitalization or treatment with i.v. antibiotics within 4 weeks of screening.
- Patients with a history of malignancy or lesion considered premalignant

- Patients with a prior history of keratoacanthoma or squamous cell carcinoma
- Moderate to severe hepatic impairment, i.e., Child-Pugh Class B or C.
- Scleroderma renal crisis within 6 months or creatinine greater than 2.0
- Lack of intravenous access for medication administration
- Pregnancy (a negative pregnancy test will be performed for all women of childbearing potential on the day of treatment).
- Male and female patients of child-producing potential must agree to use effective contraception while enrolled on study and receiving the experimental drug, and for at least 3 months after the last treatment.
- Nursing mothers
- Gastrointestinal involvement requiring total parenteral nutrition or hospitalization within the past 3 months for pseudo-obstruction
- Moderately severe pulmonary disease with FVC <70%, or DLCO <60% predicted, or ground glass and fibrosis involving greater than 20% of the lung fields by HRCT.
- Moderately severe cardiac disease with either a history of significant arrhythmia (not to include conduction delays other than trifascicular block, or PVCs or PACs <5/mintue), clinically significant heart failure, or unstable angina.
- Hemoglobin: < 8.5 gm/dL
 - Patients with anemia will have reticulocyte count and iron binding laboratory tests performed at screen and subsequent study visits
- Platelets: < 100,000/mm³
- AST or ALT >2.5 x Upper Limit of Normal.
 - total bilirubin > 1.5 x upper limit of normal (ULN). Patients with Gilbert's Disease may be included if their total bilirubin is \leq 3.0 mg/dL.
- PT, PTT, INR > ULN
- History of ascites or pleural effusion, unless successfully treated, completely resolved, and the patient has not been treated for these conditions for >4 months.

- Coagulation disorders including, but not limited to: active thrombophlebitis, thromboembolism, hypercoagulability states, bleeding, or use of anti-coagulation therapy (including anti-platelet agents such as aspirin, all non-steroidal anti-inflammatory drugs (NSAIDS), clopidogrel, ticlopidine, dipyridamole, and other agents used to induce long-acting platelet dysfunction). Patients with a history of deep venous thrombosis may participate if successfully treated, completely resolved, and no treatment has been given for >4 months.
- Patients with an organ transplant, including those that have received an allogeneic bone marrow transplant.
- Patients who, in the opinion of the Investigator, have significant medical or psychosocial problems that warrant exclusion. Examples of significant problems include, but are not limited to:
 - Other serious non-malignancy-associated medical conditions that may be expected to limit life expectancy or significantly increase the risk of SAEs.
 - Any condition, psychiatric, substance abuse, or otherwise, that, in the opinion of the Investigator, would preclude informed consent, consistent follow-up, or compliance with any aspect of the study

6. STUDY TREATMENT

6.1 Guidelines for Fresolimumab Administration

- Subjects 1-8 will receive on Visit 1/Week 0 GC1008 1 mg/kg administered by IV infusion (7 subjects already received 2 doses, 4 weeks apart under the previously approved protocol).
- Patients 9-18 will receive on Visit1/Week 0 GC1008 5 mg/kg administered by IV infusion

The dose of fresolimumab administered will be based on the patient's actual body weight. Subsequent doses will be recalculated if the patient's weight changes by $\geq 10\%$.

6.2 Treatments Administered

Fresolimumab will be administered as IV infusions administered over 30-60 minutes as deemed appropriate by the PI. (Dose of GC1008 should be diluted in Dextrose 5% in Water (D5W) prior to infusion. GC1008 is physically stable in D5W at a concentration of **0.3 to 7 mg/ml**. For GC1008 dose of 1mg/kg, the total dose should be further diluted with 50ml of D5W, and for a dose of 5mg/kg, the total dose should be further diluted with 100ml of D5W. Withdraw from the D5W infusion bag a volume equal to the amount of volume of GC1008 that will be required to prepare the dose.). A 0.22- μ m low-protein-binding inline filter must be used during each infusion. Infusions may be administered through either a peripheral IV or a central line. Placebo will consist of D5W in an equal volume to that administered to patients receiving study medication. In order to maintain blinding, during the placebo-controlled part of the trial, the same 0.22- μ m low-protein-binding inline filter will be used as for GC1008, infusion bags will be covered with a paper bag and labeled GC1008/placebo. Further details are provided in the Investigational Product Handling Guidelines.

Subjects will be monitored for 30 minutes after each infusion for any infusion reaction. Patients treated with fresolimumab who develop acute infusion adverse reactions will be

monitored as deemed medically appropriate and the reaction reviewed by the Investigator to determine if administration of fresolimumab may continue in this patient.

Each patient will be evaluated for the development of adverse events (AEs). All AEs should be considered related to the study drug unless there is clear and identifiable reason or explanation to reject causal relation.

6.3 Concomitant Therapy

No concomitant immunosuppressive agents or anti-coagulation therapies are permitted during the study unless specified below. Heparin flush of a central line is allowed. Transient use of ibuprofen is permitted. Inhaled or topical corticosteroids are allowed, and in patients who develop skin lesions, emollients and topical corticosteroids may be used as needed. Use of alternative medications (herbals, botanicals, etc.) is strongly discouraged during the entire study period. In addition, starting new medications may be associated with side effects; therefore, medications should not be started for the first time during or around the time of fresolimumab administration.

6.4 Duration of Therapy

Patients will receive a maximum of one dose of study drug unless criteria are met for patient ineligibility (7 subjects already received two doses of fresolimumab at 1 mg/kg, 4 weeks apart under the previously approved protocol).

6.5 Subject Withdrawal

Subjects are free to withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment. A subject's participation in the study may also be discontinued at any time at the discretion of the Investigator.

The following are reasons why the Investigator may remove a patient from study treatment

and further follow-up:

- The subject withdraws consent;
- The subject is found to be not eligible after enrollment;
- The subject is non-compliant with study requirements;
- The Study is terminated

7. INVESTIGATIONAL PRODUCT

Fresolimumab is an engineered human monoclonal antibody against human TGF β 1, β 2, and β 3. It is supplied as a sterile, non-pyrogenic, white to off-white lyophilized powder.

7.1 Packaging and Labeling

The study drug will be packaged in single-use USP Type I borosilicate, 5-mL glass vials with a siliconized butyl rubber stopper. Each vial contains approximately 50 mg of fresolimumab.

The label text for the study drug will include the contents of the vial (i.e., fresolimumab 50 mg/5 mL), lot number, appropriate caution statement, storage conditions, and name and address of Genzyme.

Kits and vials will be packaged in compliance with Good Manufacturing Practice and labeled according to United States Code of Federal Regulations. Each Kit will contain 10 vials and labeled with a single panel label.

7.2 Study Treatment Preparation

The study drug will be prepared according to the patient's treatment assignment. The study drug will be reconstituted with 5.1 mL of sterile Water for Injection to yield a concentration of 10 mg/mL. Further dilution guidelines are included in the Investigational Product Handling Manual.

7.3 Drug Shipment and Storage

Genzyme will arrange the shipment of the study drug to the clinical site.

- The study drug will be shipped overnight for next day delivery.
- Shipments will be made on Monday through Thursday only.
No shipments will be made on Friday, Saturday, or Sunday.

Upon receipt by the Investigator or designee, the study drug must be stored at 2 to 8°C, in a limited-access area until preparation for infusion.

7.4 Study Drug Accountability

Fresolimumab will be provided by Genzyme. Full records must be maintained to account for the study drug supplied to the Investigators, the disposition of the study drug, and the return or destruction of unused supplies.

7.5 Expected Toxicities

As reported at ASCO 2008 (Morris et al, 2008), in 22 melanoma and renal cell carcinoma patients treated with fresolimumab, the most frequently reported related events included skin lesions (2 patients at grade 1, 1 patient at grade 2, and 2 patients at grade 3), fatigue (3 patients at grade 1), headache (2 patients at grade 1 or 2) and gingival bleeding (2 patients at grade 1). Skin lesions including KA/SCC are an expected toxicity of fresolimumab.

There may be an increased risk of herpes zoster associated with fresolimumab. To date, 3/29 melanoma patients and 1/12 FSGS patients who have received fresolimumab developed non-disseminated self-limited herpes zoster. The FSGS patient had a prior history of recurrent herpes zoster.

8. STUDY PROCEDURES (See Appendix A)

8.1 Pre-Treatment Evaluations (Screening Visit)

Unless otherwise specified, the following evaluations will be performed within four weeks prior to initial fresolimumab treatment date:

- Medical history and documentation of the rationale for treatment of the patient's disease with fresolimumab.
- Physical examination, including vital signs, and MRSS.
- A thorough skin exam will be performed. Any significant dermatologic findings (aside from SSc related) will be recorded on the case report form (CRF). Suspicious lesions will be evaluated by dermatologist
- A through ears, nose, and throat exam will be performed. Any significant ENT findings will be recorded in the case report form. Suspicious lesions will be evaluated by an otolaryngologist.
- Hematology: complete blood count (CBC) with differential and platelet count,
- INR/PTT.
- Serum Chemistries (metabolic panel): glucose, BUN, creatinine, electrolytes, total bilirubin, alkaline phosphatase, total protein, albumin, SGOT (AST), SGPT (ALT), and calcium.
- Urinalysis (U/A): screen for hematuria as a potential indicator of bladder cancer. Will be performed at least two times during the screening phase. Any subject showing hematuria will be excluded from the study and referred for urology evaluation.
- Two CPT tubes (10 ml) for PBMC mRNA extraction
- Two red top tubes (10 ml) to be banked for future studies.
- Hep B, C and HIV serologies

8.2 Treatment visit- Week 0

- Urine pregnancy test for women of childbearing potential.

- Skin biopsies. Two, 3 mm punch skin biopsies will be carried out at the treatment visit (week 0), and repeated on subsequent follow-up visits as described below. Biopsies will be carried out at adjacent sites over the same mid-forearm. One biopsy will be placed in formalin and the other in RNAlater. Formalin fixed samples will be processed for hematoxylin and eosin staining and smooth muscle actin (SMA) staining, and myofibroblast score assessed as described (Kissin et al., 2006). Samples in RNAlater will be stored in the freezer until RNA preparation for biomarker skin score.

8.3 Follow-up Evaluations- Week 3, 7, 11, 17, and 24

Safety assessments

- Interim medical history and physical examination, including complete skin exam and ENT exam.
- CBC with differential, comprehensive metabolic panel
- U/A will be assessed for hematuria
- Adverse events (AEs) will be recorded and evaluated as detailed in section 11.

Efficacy Assessments

- Skin biopsies (described section 8.2) at weeks 0, 3, 7 and optionally week 24.MRSS will be assessed by two, trained physician scorers at each visit. Drs. Simms, Kissin and Lafyatis have taken classes on MRSS. The same physician will perform these measures at treatment time points as described in section 4.1 for each patient (see also Study Calendar, Appendix A).
- SHAQ will be assessed at visits as described in section 4.1(see also Study Calendar, Appendix A).
- Peripheral blood mononuclear cells will be prepared from one 5 ml CPT tubes, lysed in RLT buffer (Qiagen) and stored at -80. RNA will be collected and banked for potential utilization in the context of ongoing biomarker studies. 5 ml serum will be aliquoted and banked at study visits as described in section 4.1(see also Study Calendar, Appendix A).

Study Calendar

See Appendix A

9. CRITERIA FOR RESPONSE

9.1 Primary evaluation of response.

Safety evaluation:

Study safety will be evaluated by a tabulation and review of all AEs and SAEs. The data from all patients who received fresolimumab during the study will be included for safety analysis. Safety data will include laboratory, history, physical exam, and adverse event reports on systemic signs or symptoms of study patients. These descriptive summaries will be provided for all patients for each safety parameter (and body system) by cycle, grade, and relationship to treatment. Safety data will also be summarized as cumulative incidence of specified safety events of interest.

Efficacy evaluation:

Skin expression of the two TGF β -responsive genes (COMP, THS1) in the 4-gene biomarker at 3 and 7 weeks will be compared to week 0 expression. Gene expression will be measured by quantitative real-time PCR and quantified using plasmid standards for reproducibly measuring gene expression between samples (Farina et al., 2010).

9.2 Secondary evaluations of response.

Biomarker gene expression:

The 4-gene SSc skin biomarker skin score will be compared at 3 and 7 weeks (and optional 24 week biopsy) to baseline score in all fresolimumab-treated patients.

Modified Rodnan skin score:

The MRSS at follow-up study visits will be compared to week 0. Improvement of skin score in diffuse SSc correlates with improvement in joint function, overall functional status and physician's global assessment (Clements et al., 2000). At six months compared to baseline, no statistically significant change in Rodnan skin scores were found in either the low-dose or high-dose penicillamine treatment groups (Clements et al., 1999). Based on this historical control population an improvement in MRSS at 24 weeks or any other earlier time point compared to baseline in our study population will suggest treatment efficacy.

SSc Health Assessment Questionnaire (SHAQ): The SHAQ will be evaluated at follow-up visits will be compared to study entry.. The HAQ-DI component of the SHAQ (pages 1-3, Appendix D) has been validated as accurately measuring changes in disease status in SSc (Clements et al., 2001; Steen and Medsger, 1997). It correlates directly with skin involvement, SSc heart and kidney disease, tendon friction rubs, hand contractures and proximal muscle strength. It also is a good predictor of outcome and survival. The SSc VAS component of the SHAQ provides additional organ specific information (Steen and Medsger, 1997). Organ specific components correlate well with vascular, GI and pulmonary involvement. The HAQ-DI and the VAS will be analyzed separately. The total HAQ-DI score at 7 and 24-week will be compared to the entry scores. Since these values were not significantly changed in the high-dose versus low-dose penicillamine trial from baseline to 6 months (Clements et al., 1999), any improvement at 24 weeks or at any earlier time point in our study patients would suggest treatment efficacy within the limitations of an open-label study. Each of the 6 components of the VAS will also be analyzed separately, comparing 7 and 24-week to study entry scores. These analyses may detect organ specific efficacy that might otherwise be missed.

Skin biopsy: Skin biopsies will be immunostained for smooth muscle actin+ (myofibroblasts) and scored by semiquantitative evaluation using a 0-10 grading scale in a blinded fashion as described previously (Kissin et al., 2006; Lafyatis et al., 2009).

Exploratory endpoints:

Skin microarray RNA gene expression at follow-up visits will be compared to baseline (week 0) skin gene expression to define whether a subset of dcSSc patients, as defined previously (Milano et al., 2008), appear to selectively respond to therapy, and to explore whether a gene signature can be defined that indicates a fresolimumab response.

Baseline and follow-up visit PBMC RNA will be prepared and stored at -80°C for possible

future gene array analyses for prognostic correlations, and 5 ml serum will be aliquoted and stored at -80° C for possible future analyses of serum prognostic markers.

10. FORMS TO BE MAINTAINED

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

11. REGULATORY AND REPORTING REQUIREMENTS

11.1 Adverse Event Monitoring and Reporting

The principal investigator is responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of the study drug will be followed until resolution. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be used for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (<http://ctep.cancer.gov/reporting/ctc.html>; see also Appendix B).

Definition of Adverse Event

An AE is any untoward medical occurrence in a clinical investigation patient, which does not necessarily have a causal relationship with the investigational product (active or placebo drug, biologic, or device). An AE can, therefore, be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality whether or not considered related to the investigational product.

Adverse events include:

- Symptoms described by the patient or signs observed by the Investigator or medical staff.
- Test abnormalities (laboratory tests, ECG, X-rays, etc.) that result in an alteration in medical care (diagnostic or therapeutic).

Abnormalities present at baseline are considered AEs only if they reoccur after resolution or they worsen during the study.

Definition of a Serious Adverse Event

An SAE is any AE that results in any of the following:

Death: The patient died as the result of the event.

Life-threatening event: Any AE that places the patient, in the view of the Investigator, at immediate risk of death from the AE as it occurred, i.e., does not include an AE that had it occurred in a more severe form, might have caused death.

Required or prolonged inpatient hospitalization: The AE resulted in an initial inpatient hospitalization or prolonged an existing hospitalization of the patient. If a patient is

hospitalized as part of the clinical use of the product, a period of normal hospitalization will be outlined in the protocol or by the judgment of the Investigator. Hospitalizations longer than this period will be prolonged hospitalizations.

Persistent or significant disability/incapacity: An AE that results in a substantial disruption of a person's ability to conduct normal life functions.

Congenital anomaly/birth defect: A congenital anomaly/birth defect that occurs in the offspring of a patient exposed to the investigational product.

Important medical events: An AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Definition of Medical Events of Interest

Medical events of interest are specific events not meeting SAE criteria, but important enough to require expedited or special reporting. Events to be reported in this manner for this protocol include: herpes zoster, treatment-emergent skin lesions, cancers, bleeding events, and other events deemed of interest per the Sponsor.

Evaluation of Adverse Events/Serious Adverse Events

Relationship to Study Treatment

Assessment of the association between the AE and study exposure is important for regulatory reporting. This assessment is to be made in blinded studies and also for known comparators. For each AE/SAE the Investigator determines whether there is a reasonable possibility that the AE may have been caused by the study treatment according to the categories below:

- Not Related: There is no suspicion of a causal relationship between exposure and the AE.
- Unlikely Related: There is no evidence for a causal relationship between exposure and the AE; however, such a relationship cannot be ruled out.
- Possibly Related: There is some evidence supporting the possibility of a causal relationship between exposure and the AE.
- Related: There is strong evidence that there is a causal relationship between exposure and the AE.

Any AE in which there is not a clear and identifiable reason or explanation to reject causal relational to the study drug will be considered possibly related or probably related.

A relationship to the investigational product must be given for each AE/SAE recorded, even if there is only limited information at the time.

The Investigator may change his/her opinion of causality in light of follow-up information, amending the AE/SAE report accordingly.

Severity Grading of Adverse Event Scoring

Note that this is not the same as “seriousness,” which is define above. Seriousness serves as a guide for defining regulatory reporting obligations.

Severity Grading

The Investigator will assess the severity of all AEs/SAEs as Mild, Moderate, or Severe, based on the following definitions (developed from CDISC SDTM standard terminology v3.1.1).

Definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the research participant.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Outcome

Outcome describes the status of the AE. The Investigator will provide information regarding the patient outcome of each AE.

Definitions for possible results of an AE outcome:

- Fatal: The termination of life as a result of an AE.
- Not recovered/not resolved: The patient has not recuperated or the AE has not improved.

- Recovering/resolving: The patient is recuperating or the AE is improving.
- Recovered/resolved: The patient has recuperated or the AE has resolved.
- Recovered with sequelae/resolved with sequelae: The AE has resolved, but the patient has been left with symptoms or pathology.
- Unknown: Not known, not observed, not recorded, or refused.

Action Taken Regarding the Investigational Product

The Investigator will be required to provide the action taken regarding investigational product in response to the AE.

Options include:

- Dose not changed: No change in administration of the investigational product.
- Drug (investigational product) interrupted: Temporary interruption (termination) in administration of the investigational product.
- Drug (investigational product) withdrawn: Administration of the investigational product terminated (no further dosing).
- Not applicable: Determination of a value is not relevant in the current context.
- Unknown: Not known, not observed, not recorded, or refused.

Timeframe for Collection of Adverse Events/Serious Adverse Events

Adverse Events Occurring Prior to Study Treatment

Adverse events, including MEOIs and SAEs, will be collected from the time the patient signs the informed consent form.

Adverse Events Occurring After Study Treatment

Adverse events will be collected from the time of the patient's first receipt of investigational product until week 24.

Serious AEs will be collected from the time of the patient's first receipt of investigational product until week 24.

Medical Events of Interest will be collected from the time of the patient's first receipt of investigational product until week 24.

Adverse Events Occurring Following Patient Discontinuation of Treatment

Patients who prematurely discontinue study treatment and are withdrawn from the study will be followed for new AEs, MEOIs, and SAEs for 45 days after their last dose of fresolimumab.

For patients who prematurely discontinue study treatment but who are not withdrawn from the study, AEs will continue to be recorded until the patient completes the study.

Serious Adverse Events Occurring Following Patient Completion of the Study

If, at any time after the patient has completed participation in the study, the Investigator becomes aware of an SAE that they believe is possibly related or related to the investigational product, then the event and any known details should be reported promptly to Genzyme.

11.2 Reporting Serious Adverse Events

All SAEs occurring during the study or within 45 days of the last administration of fresolimumab must be reported to the principal investigator and to Genzyme within 24 hours of first knowledge and provide follow-up reports until the SAE has resolved. The principal investigators are responsible for reporting SAEs to the IRB and the FDA (21 CFR §312.32] or other applicable regulatory authority. The principal investigator is responsible for submitting follow-up reports for all SAEs regarding the patient's subsequent course until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment), or the patient dies.

Institution and Investigator understand and agree that Investigator and Institution are obligated under applicable law and regulations to report any serious and related adverse event, if any, that occurs during treatment with the Product to the Institution's IRB/Ethics Committee and to the governing regulatory authority in accordance with applicable filing timelines promptly after any such event occurs. Investigator, within 24 hours (US) or one business day (EU) of first knowledge of such serious adverse event, will notify Genzyme via fax, attention Genzyme Global Patient Safety and Risk Management (GPS-RM), 617-761-8506 (US) or +1-617-761-8506 or by e-mail to pharmacovigilancesafety@genzyme.com. Prior to or at the time of filing any such report with the governing regulatory authority, the

Investigator will also transmit an information copy of the report as sent to the authorities to Genzyme GPS-RM as listed above. . The Investigator shall make available to Genzyme promptly such records as may be necessary and pertinent to investigate any such expedited adverse event, if specifically requested by Genzyme. In addition, all SAEs will be reported and reviewed by the DSMB (detailed in Section 11.4).

The Principal Investigator and study coordinator will convene monthly safety meetings with Genzyme. All adverse events, both serious and non-serious will be discussed to track any safety issues or concerns, in addition to required review of SAEs and expedited reporting of serious, unexpected, related SAEs (per 21 CFR 312.32[c]).

Furthermore, the Investigator will inform Genzyme of the following:

- any events that result in protocol amendments for safety reasons, as well as any safety related regulatory action such as a clinical hold of the Research
- any pregnancies occurring in patients who are exposed to the Product in connection with the Research.
- In addition, the Investigator shall notify Genzyme within 24 hours (US) or one business day (EU) of first knowledge of any Product complaints (communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, effectiveness, safety, labeling, purity, stability, and appearance) by email to GEMG@genzyme.com or via fax to 508-661-8771 (US) or Genzyme Customer Services Europe, +31 (0)35 699 1222.
- The Investigator will also inform Genzyme within 1 business day of becoming aware of any actions from any authority that may affect the performance of the Research

11.3 Medical Events of Interest

Sponsors-Investigators are requested to report to Genzyme serious and non-serious events of treatment-emergent skin lesions of unknown etiology and varicella zoster infections within

15 days of becoming aware of the event using medical events of interest forms that will be provided by Genzyme. Medical event of interest forms should be faxed to Genzyme GPS-RM at 617-761-8506. In addition, Sponsors-Investigators will provide updates to Genzyme regarding safety issues arising in this study via regular conference calls.

11.4 Data Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be formed to act in an advisory capacity to the study investigators to monitor participant safety in the study.

The DSMB responsibilities are to:

- protect the safety of the study participants;
- report to IRB on the safety issues or concerns of the trial;
- make recommendations to the Principal Investigator, and, if required, to the Food and Drug Administration (FDA) concerning continuation, termination or other modifications of the trial based on the observed adverse effects of the treatment under study.

Membership

The DSMB will be composed of two members, Dr. Arthur Theodore (Pulmonologist) and Dr. Deborah Cummins (Dermatologist), who are independent of the investigators, and who have no financial, scientific, or other conflict of interest with the trial.

Board Process

The DSMB will meet in two settings. In the first setting the DSMB will meet in a planned meeting after all of the first 8 patients have completed study treatment and been evaluated at the week 7 study visit. Further recruitment into the study will pause until this review is

completed. All adverse events will be reviewed and afterwards a meeting held with the study principal investigator and other study investigators as available to discuss any safety concerns before proceeding to the second dose escalation phase of the trial.

DSMB members will be informed of all SAEs within 7 days after the investigator becomes aware. The principal investigator will also inform the DSMB of the study investigators' assessment of causality to study medication. This event may trigger the second setting for DSMB meeting, an emergency meeting of the DSMB. An emergency meeting of the DSMB may be called at any time by either DSMB member or any study investigator to address any safety questions or other unanticipated problems. Meetings may be convened as conference calls as well as in-person. A recommendation to terminate the study may be made by the DSMB at any time.

Meeting Format

DSMB meetings will consist of open and closed sessions. The interim report and any SAE reports for the DSMB will be prepared by the study staff, typically the Clinical Coordinator. The Principal Investigator and key members of the study team attend the **open sessions**. The **closed session** will only be attended by DSMB members. Typically a DSMB meeting may include both an open session to gather information from study investigators and a closed session to consider and vote regarding study continuation versus termination. DSMB vote and a brief description of rationale for recommendations will be provided to the principal investigator as a written report.

A split or consensus vote by the DSMB for study termination will not be binding on the principal investigator but will be reported to the Institutional review Board and Sponsor. Upon a consensus DSMB vote for study termination no further subjects will be recruited or given study medication until full review and discussion with the IRB and FDA regarding any identified safety questions or issues, and a final decision made whether to terminate the study.

Reports from the DSMB

A formal report containing the recommendations for continuation or modifications of the study will be prepared by the DSMB and forwarded to the Principal Investigator. As previously stated, the formal DSMB report must include a recommendation to continue or to terminate the study. This recommendation should be made by formal vote.

Confidentiality

All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

12. STATISTICAL CONSIDERATIONS

Once all subjects complete the study or discontinue prematurely, the final analyses will be completed. All available data from all subjects who receive at least one infusion of fresolimumab will be included in the safety and efficacy analyses. TGF β -responsive gene expression at 3 and 7 weeks will be compared to baseline using a paired t-test. If changes in TGF β -responsive gene expression do not follow a normal distribution then these comparisons will be made using a Wilcoxon matched pairs test.

Secondary outcome measures skin myofibroblast score, modified Rodnan skin score (MRSS) and scleroderma modified health assessment questionnaire (SHAQ) at 3 and 7 weeks will be compared to baseline. Only descriptive statistics, summarizing changes at 3, 7 and 24 weeks for secondary efficacy endpoints will be utilized for these comparisons.

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APPENDIX A: STUDY CALENDAR

Open-label Fresolimumab Study Visit Calendar

	Screening (BL)	*Visit 1 (Week 0)	Visit 2 (Week 3)	Visit 3 (Week 7)	Visit 4 (Week 11)	Visit 5 (Week 17)	Visit 6 (Week 24)
Inclusion/exclusion criteria	X						
Informed Consent	X						
Medical History	X						
Demographics	X						
Efficacy Assessment							
Skin biopsy		X	X	X			**X
Modified Rodnan Skin Score	X	X	X	X	X	X	X
4-gene Biomarker Score		X	X	X			**X
Durometry (FTR + TFR)		X	X	X			X
Scleroderma Modified Health Assessment (SHAQ)		X	X	X			X
Safety Assessment							
Physical exam	X						
Interim physical exam		X	X	X	X	X	X
Vital signs		X	X	X	X	X	X
EKG	X						
ENT exam	X	X	X	X	X	X	X
Interim medical history		X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X
AE/safety monitoring		X	X	X	X	X	X
Skin examination	X	X	X	X	X	X	X
Labs							
Urine/serum pregnancy (WOCBP only)		X					
CBC with differential	X		X	X	X	X	X
Comprehensive Metabolic Panel	X		X	X	X	X	X
CRP	X			X			X
Urinalysis (U/A)	X ^a	X	X	X	X	X	X
INR/PT/PTT	X						
HIV, HBV, HCV serologies	X						
PBMC-RNA sampling	X		X	X	X	X	X
Serum sampling (stored)	X		X	X	X	X	X
Clinical Supplies							
Skin biopsy Kit		X	X	X			**X
Fresolimumab infusion		X					

*Visit 1 must take place within 4 weeks of screening labs

**Optional

^a at least 2 U/A during screening phase

APPENDIX B: Common Terminology Criteria for Adverse Events (CTCAE) version 4.0

APPENDIX C: Modified Rodnan Skin Score

Modified Rodnan skin score

Patient Study#:

Name: _____

Date: _____

Examiner: _____

Visit#: _____

The same trained tester throughout the study, administers the Modified Rodnan skin score. Skin thickening will be assessed by palpation and scored as follows.

- 0 UNINVOLVED
 - 1 MILD THICKENING
 - 2 MODERATE THICKENING
 - 3 SEVERE THICKENING

The score will be reported below for 17 body areas

FACE 0 1 2 3

ANTERIOR CHEST 0 1 2 3

ABDOMEN 0 1 2 3

	RIGHT				LEFT			
FINGERS	0	1	2	3	0	1	2	3
HANDS	0	1	2	3	0	1	2	3
FOREARMS	0	1	2	3	0	1	2	3
UPPER ARMS	0	1	2	3	0	1	2	3
THIGHS	0	1	2	3	0	1	2	3
LEGS	0	1	2	3	0	1	2	3
FEET	0	1	2	3	0	1	2	3

TOTAL SCORE

Total score is the sum of the 17 body area scores

APPENDIX D: Scleroderma Health Assessment Questionnaire

Subject ID	Visit #	Patient Initials	Date
[] [] [] []	- [] [] []	[] [] []	[] [] / [] [] / [] []
			Month Day Year

Scleroderma Health Assessment Questionnaire

(1 of 3)

Please check the response which best describes your usual abilities (**over the past week**)

At this moment are you able to:		Without any difficulty	With some difficulty	With much difficulty	Unable to do
Dressing/ Grooming	<u>Are you able to:</u> 1. Dress yourself, including tying shoelaces and doing buttons? 2. Shampoo your hair?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Arising	<u>Are you able to:</u> 1. Stand up from an armless straight chair? 2. Get in and out of bed?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eating	<u>Are you able to:</u> 1. Cut your meat? 2. Lift a full cup or glass to your mouth? 3. Open a new carton of milk?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking	<u>Are you able to:</u> 1. Walk outdoors on flat ground? 2. Climb up 5 steps?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hygiene	<u>Are you able to:</u> 1. Wash and dry your entire body? 2. Take a tub bath? 3. Get on and off the toilet?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Subject ID	Visit #	Patient Initials	Date
[] [] [] []	- [] [] []	[] [] []	[] [] / [] [] / [] [] []
			Month Day Year

Scleroderma Health Assessment Questionnaire

(3 of 3)

Instruction: For questions 1-5, if you did not have the problem during the past week, place a mark at the far left hand side of the line.

1. In the past week how much have your intestinal problems interfered with your daily activities? Place a mark on the line to indicate limitations of activity.

Intestinal problems
did not limit activities

Very severe
limitation mm

2. In the past week how much have your breathing problems interfered with your daily activities? Place a mark on the line to indicate limitations of activity.

Breathing problems
did not limit activities

Very severe
limitation mm

3. In the past week how much have Raynaud's attacks interfered with your daily activities?
Place a mark on the line to indicate limitations of activity.

Raynaud's did
not limit activities

Very severe
limitation mm

4. In the past week how much have finger ulcers interfered with your daily activities? Place a mark on the line to indicate limitations of activity.

Finger ulcers did
not limit activities

Very severe
limitation mm

5. Overall, considering pain, discomfort, limitations in your daily life and other changes in your body and life, how severe would you rate your disease today? Place a mark on the line to indicate limitations of activity.

No disease

Very severe
limitation mm

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported?	
			✓	Pg #
Title and Abstract				
Title and Abstract	1	• Information on how units were allocated to interventions	✓	1,2
		• Structured abstract recommended	✓	1,2
		• Information on target population or study sample	✓	1,2
Introduction				
Background	2	• Scientific background and explanation of rationale	✓	4,5
		• Theories used in designing behavioral interventions	NA	—
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	✓	18
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	✓	18
		• Recruitment setting	✓	18
		• Settings and locations where the data were collected	✓	18
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:	✓	18
		○ Content: what was given?	✓	18
		○ Delivery method: how was the content given?	✓	18
		○ Unit of delivery: how were the subjects grouped during delivery?	✓	18
		○ Deliverer: who delivered the intervention?	✓	18
		○ Setting: where was the intervention delivered?	✓	18
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	✓	18
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	✓	18
		○ Activities to increase compliance or adherence (e.g., incentives)	✓	18
Objectives	5	• Specific objectives and hypotheses	✓	18,19
Outcomes	6	• Clearly defined primary and secondary outcome measures	✓	18-21
		• Methods used to collect data and any methods used to enhance the quality of measurements	✓	19,20
		• Information on validated instruments such as psychometric and biometric properties	NA	—
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	NA	—
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	✓	19
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	✓	19
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	NA	—

TREND Statement Checklist

Blinding (masking)	9	<ul style="list-style-type: none"> Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed. 		✓ 6, ✓ 19
Unit of Analysis	10	<ul style="list-style-type: none"> Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community) 	✓	6
		<ul style="list-style-type: none"> If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis) 	✓	6
Statistical Methods	11	<ul style="list-style-type: none"> Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data 	✓	20,21
		<ul style="list-style-type: none"> Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis 	✓	20,21
		<ul style="list-style-type: none"> Methods for imputing missing data, if used 	✓	20,21
		<ul style="list-style-type: none"> Statistical software or programs used 	✓	20,21

Results

Participant flow	12	<ul style="list-style-type: none"> Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended) <ul style="list-style-type: none"> Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study Assignment: the numbers of participants assigned to a study condition Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition Analysis: the number of participants included in or excluded from the main analysis, by study condition Description of protocol deviations from study as planned, along with reasons 	✓ Fig 1	✓ Fig 1
Recruitment	13	<ul style="list-style-type: none"> Dates defining the periods of recruitment and follow-up 	✓	18-19
Baseline Data	14	<ul style="list-style-type: none"> Baseline demographic and clinical characteristics of participants in each study condition 	✓	Table 1
		<ul style="list-style-type: none"> Baseline characteristics for each study condition relevant to specific disease prevention research 	✓	
		<ul style="list-style-type: none"> Baseline comparisons of those lost to follow-up and those retained, overall and by study condition 	✓	
		<ul style="list-style-type: none"> Comparison between study population at baseline and target population of interest 	✓	↓
Baseline equivalence	15	<ul style="list-style-type: none"> Data on study group equivalence at baseline and statistical methods used to control for baseline differences 	NA	—

TREND Statement Checklist

Numbers analyzed	16	<ul style="list-style-type: none"> Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible 	/	6
		<ul style="list-style-type: none"> Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses 	/	7-8
Outcomes and estimation	17	<ul style="list-style-type: none"> For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision 	/	6-11
		<ul style="list-style-type: none"> Inclusion of null and negative findings 	/	6-11
		<ul style="list-style-type: none"> Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any 	/	6-11
Ancillary analyses	18	<ul style="list-style-type: none"> Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory 	/	6-11
Adverse events	19	<ul style="list-style-type: none"> Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals) 	/	11-12

DISCUSSION

Interpretation	20	<ul style="list-style-type: none"> Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study 	/	13-17
		<ul style="list-style-type: none"> Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations 	/	13-17
		<ul style="list-style-type: none"> Discussion of the success of and barriers to implementing the intervention, fidelity of implementation 	/	13-17
		<ul style="list-style-type: none"> Discussion of research, programmatic, or policy implications 	/	13-17
Generalizability	21	<ul style="list-style-type: none"> Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues 	/	13-17
Overall Evidence	22	<ul style="list-style-type: none"> General interpretation of the results in the context of current evidence and current theory 	/	13-17

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health, 94*, 361-366. For more information, visit: <http://www.cdc.gov/trendstatement/>