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Recombinant Human Anti–Transforming Growth Factor β1 Antibody Therapy in Systemic Sclerosis

A Multicenter, Randomized, Placebo-Controlled Phase I/II Trial of CAT-192

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Objective. **To evaluate CAT-192, a recombinant human antibody that neutralizes transforming growth factor β1 (TGFβ1), in the treatment of early-stage diffuse cutaneous systemic sclerosis (dcSSc).**

Methods. **Patients with SSc duration of <18 months were randomly assigned to the placebo group or to 1 of 3 CAT-192 treatment groups: 10 mg/kg, 5 mg/kg, 0.5 mg/kg. Infusions were given on day 0 and weeks 6, 12, and 18. The primary objective of this study was to evaluate the safety, tolerability, and pharmacokinetics of CAT-192. Secondary outcomes included the modified Rodnan skin thickness score (MRSS), the Scleroderma Health Assessment Questionnaire, assessment of organ-based disease, serum levels of soluble interleukin-2 receptor, collagen propeptides (N propeptide of type I [PINP] and type III collagen), and tissue levels of messenger RNA for procollagens I and III and for TGFβ1 and TGFβ2.**

Results. **Forty-five patients were enrolled. There was significant morbidity and mortality, including 1 death in the group receiving 0.5 mg/kg of CAT-192 and 3 deaths in the group receiving 5 mg/kg of CAT-192. There were more adverse events and more serious adverse events in patients receiving CAT-192 than in those receiving placebo, although these events were not more frequent in the high-dose treatment group. The MRSS improved in all groups during the study, but there was no evidence of a treatment effect for CAT-192. Improvement in the MRSS correlated with the disease duration ($r = -0.54$, $P = 0.0008$). Changes in the PINP level from baseline correlated with changes in the MRSS ($r = 0.37$, $P = 0.027$).**

Conclusion. **We report the first evaluation of a systemically administered and repeatedly dosed anti-TGFβ1 drug. In this pilot study, CAT-192, in doses up to 10 mg/kg, showed no evidence of efficacy. The utility of**

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clinical and biochemical outcome measures and the feasibility of multicenter trials of early dcSSc were confirmed.

A growing body of evidence indicates a role for sustained activity of the transforming growth factor β (TGFβ) ligand–receptor axis in the pathogenesis of systemic sclerosis (SSc; scleroderma). Numerous studies have demonstrated increased expression of TGFβ1 or TGFβ2 in early skin lesions and in lung tissue from patients with SSc-associated interstitial lung disease, including immunostaining and in situ hybridization studies (1,2). Investigators have demonstrated increased levels of the high-affinity receptors for TGFβ on explanted SSc fibroblasts and have suggested that, ex vivo, some of the hallmark biochemical properties of lesional SSc fibroblasts, such as overproduction of type I collagen, may be reversed or abrogated by neutralizing antibodies against TGFβ (3). Indirect evidence supporting a role of TGFβ in the pathogenesis of SSc comes from a growing body of evidence demonstrating that the altered biochemical properties of fibroblasts from patients with SSc are similar to those induced by recombinant TGFβ in control fibroblasts. For example, upregulation of a large number of key TGFβ-regulated genes and gene products has been observed (4). Furthermore, in a variety of animal models, TGFβ-blocking strategies have been shown to prevent or diminish fibrosis, including some models of SSc, such as a murine model of graft-versus-host disease (5).

Current therapies for SSc focus upon treatment of organ-based manifestations, with successful use of prostacyclin analogs and endothelin receptor antagonists in the treatment of SSc-associated pulmonary arterial hypertension, angiotensin-converting enzyme inhibitors in the treatment of scleroderma renal crisis, and proton-pump inhibitors in the treatment of gastrointestinal reflux (6). In contrast trials of potential overall disease-modifying strategies in SSc have been disappointing. Immunomodulatory agents that have been evaluated include chlorambucil, methotrexate, and extracorporeal photopheresis (7–9). Putative antifibrotic or matrix-altering agents tested in SSc have included D-penicillamine, interferon-α, ketanserin, and recombinant human relaxin (10–13).

Confounding aspects of the design of SSc clinical trials include disease heterogeneity and insufficient, validated tools for disease assessment in this complex multisystem disorder (14). Furthermore, several agents have only been evaluated in relatively late-stage disease, when spontaneous improvement in the skin score or relatively stable disease in patients who are not improving may make it difficult to demonstrate treatment effects. One alternative strategy is to focus on very early-stage disease, when deterioration is more likely to occur without intervention and when prevention of worsening would be clinically meaningful.

The potentially pivotal role of TGFβ in the pathogenesis of SSc, combined with the availability of fully humanized anti-TGFβ antibodies of defined specificity generated by phage-display technology, led us to consider anti-TGFβ therapy as a potentially valuable novel treatment of fibrosis in SSc. We performed a phase I/II trial of repeated doses of CAT-192, a recombinant human antibody against active TGFβ1, in a cohort of patients with early-stage diffuse cutaneous SSc (dcSSc).

PATIENTS AND METHODS

Study design. The primary objective of this study was to evaluate the safety, tolerability, and pharmacokinetics of repeated intravenous infusions of CAT-192 (Cambridge Antibody Technology, Cambridge, UK) in patients with early-stage dcSSc. There was an independent Drug Safety Monitoring Board for the study, and all serious adverse events (SAEs) were reviewed in real time, with an interim safety analysis. The secondary objective was to evaluate the potential utility of various clinical outcomes and biomarkers for future use in a larger, similarly designed trial. This was an international, multicenter, randomized, double-blind, placebo-controlled, dose-ranging, and repeated-dosing study. Blood samples were taken to evaluate the pharmacokinetics of repeat administrations of CAT-192. The protocol and patient informed consent forms were approved by Institutional Review Board or Ethics Committee at each participating site.

Study drug. CAT-192 is a recombinant human monoclonal antibody with specific neutralizing activity against active human TGFβ1. Subjects were randomized to receive treatment with either placebo or CAT-192 at 1 of 3 doses (0.5 mg/kg, 5 mg/kg, or 10 mg/kg), administered on day 0, week 6, week 12, and week 18. Each received the same treatment assignment for all 4 doses and received a test dose of CAT-192 or comparably prepared placebo on day 0 and subsequent visits according to the study protocol. The study drug was administered intravenously over ~30 minutes. Subjects were followed up for evaluation of drug safety and for exploration of clinical outcomes at 6 months, and a complete safety analysis was performed at 9 months.

Study subjects. Subjects were recruited from 11 scleroderma centers in the US and Europe. Demographic characteristics of the patients are summarized in Table 1. All patients fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) classification criteria for SSc (15); however, positive findings on tests for anticentromere antibodies (ACAs) was an exclusion criterion, given the strong association of ACAs with limited cutaneous SSc.

To enrich the study cohort for patients in the progressive phase of dcSSc, the following inclusion criteria were

Table 1. Demographics of the patient cohort with diffuse cutaneous systemic sclerosis, by treatment group

Parameter	Placebo treatment (n = 11)	CAT-192 treatment			All patients (n = 43)
		0.5 mg/kg (n = 11)	5 mg/kg (n = 11)	10 mg/kg (n = 10)	
Age, years					
Mean ± SD	50.2 ± 9.8	48.1 ± 10.1	52.3 ± 12.8	42.8 ± 11.0	48.5 ± 11.1
Median	52.0	49.0	51.0	41.5	49.0
Minimum, maximum	36.0, 65.0	31.0, 64.0	31.0, 76.0	24.0, 63.0	24.0, 76.0
Sex, no. (%)					
Male	1 (9)	2 (18)	3 (27)	4 (40)	10 (23)
Female	10 (91)	9 (82)	8 (73)	6 (60)	33 (77)
Race, no. (%)					
White	11 (100)	9 (82)	10 (91)	7 (70)	37 (86)
Black	–	–	–	1 (10)	1 (2)
Hispanic	–	1 (9)	–	–	1 (2)
Asian	–	–	1 (9)	1 (10)	2 (5)
Other	–	1 (9)	–	1 (10)	2 (5)

required for any study subject: enrollment within 18 months of the onset of their disease (defined as the time from the first non–Raynaud's phenomenon manifestation); a modified Rodnan skin thickness score (MRSS) between 10 and 28 (inclusive); and evidence of worsening disease by any of the following 3 criteria: progression of skin disease to a new skin score site within the previous 3 months; an increase in the skin score from 1 to 3 at any skin score location; or the presence of tendon friction rubs at 3 or more joints (16). The clinical features of the study cohort are given in Table 2.

Exclusion criteria included the following: a decrease in the total MRSS of >3 points between the screening and baseline visits; clinical evidence of another definable connective tissue or autoimmune disease; exposure to environmental agents associated with scleroderma-like disease; severe renal involvement (serum creatinine >2 mg/dl [180 μmoles/liter]); actual creatinine clearance of <40 ml/minute; severe proteinuria (4+ by dipstick or >500 mg/24 hours if so measured); history of scleroderma renal crisis; severe lung involvement, defined by a forced vital capacity (FVC) of <50% predicted or a diffusing capacity for carbon monoxide (DLCO) of <40% predicted or severe dyspnea on exertion; significant heart disease, defined by the presence of congestive heart failure; left ventricular ejection fraction <40%, as determined by echocardiography, or dysrhythmia needing therapy; history of neoplasia; evidence of retinal detachment in view of the recognized role of TGFβ in vitreoretinal repair. Additional exclusion criteria consisted of any previous treatment with cyclophosphamide or treatment with any of the following drugs within 4 weeks of enrollment: cyclosporine, methotrexate, chlorambucil, azathioprine, mycophenolate mofetil, colchicine, or D-penicillamine.

Dosages of angiotensin-converting enzyme inhibitors and/or vasodilators were required to be stable for at least 4 weeks prior to enrollment. Treatment with systemic glucocorticoids at a dosage >10 mg/day of prednisolone or equivalent was not permitted.

Serum from 100 healthy subjects who were matched with the SSc patients for age and sex was obtained. Skin biopsies were performed on 10 healthy volunteers with a similar sex distribution as the SSc cohort (data not shown). These samples served as controls.

Clinical outcomes. Clinical assessment of study subjects followed routine clinical practice and standardized clinical trial design by focusing on determining the extent of skin involvement and the presence of major visceral involvement. The 17-site MRSS was used, with each site assessed by global average integer scoring (0–3 scale). Consistent with clinical trial guidelines (16,17), and to minimize interobserver variation, all investigators attended skin scoring standardization sessions, since earlier studies suggest that this reduces interindividual variability in skin scoring (18). Renal function was determined by serum creatinine and creatinine clearance values. Pulmonary function testing was performed by standard techniques and included assessments of FVC, total lung capacity, forced expiratory volume in 1 second, and DLCO.

All subjects completed the Scleroderma Health Assessment Questionnaire (HAQ), which is comprised of the Disability Index (DI) of the HAQ and visual analog scales for severity assessments of overall SSc, Raynaud's phenomenon, lung disease, digital ulcers, and gastrointestinal disease (19,20).

Biomarker evaluation. Biomarkers of collagen turnover were evaluated in serum samples and skin biopsy tissues. Values at baseline and after 6 months were compared. Serum samples were assayed, and baseline values were compared with the values in healthy controls (n = 100). Skin biopsy samples (4-mm^2) were taken from clinically affected and clinically unaffected skin (usually, the lower back) of patients with SSc. Repeat biopsies were taken from sites within 5 cm of the site of the first procedure. Serum samples and biopsy specimens were shipped on dry ice to a central laboratory for analysis. Total RNA was extracted from the skin biopsy tissues and analyzed by real-time quantitative polymerase chain reaction (PCR). For comparison with baseline values in the SSc patients, RNA was extracted from punch biopsies of skin from healthy volunteers (n = 10). Collagen production was assessed by measuring messenger RNA (mRNA) encoding the α1 chain of type I and type III procollagen. The mRNA encoding TGFβ1 and TGFβ2 was measured, since these are known to be up-regulated by TGFβ1 (21). Serum amino-terminal propep-

Table 2. Disease characteristics at baseline, by treatment group

Parameter*	Placebo treatment	CAT-192 treatment			All patients
		0.5 mg/kg	5 mg/kg	10 mg/kg	
Disease duration, months					
No. of patients	10	11	11	10	42
Mean ± SD	8.8 ± 7.0	6.0 ± 4.7	7.3 ± 4.6	8.3 ± 5.0	7.6 ± 5.3
Median	5.9	4.6	7.2	9.4	6.4
Minimum, maximum	0.3, 22.5	0.7, 13.8	1.2, 16.7	1.6, 16.2	0.3, 22.5
MRSS					
No. of patients	11	11	11	10	43
Mean ± SD	23.9 ± 7.1	20.5 ± 4.0	22.1 ± 5.1	22.1 ± 5.8	22.1 ± 5.5
Median	25.0	21.0	23.0	24.0	22.0
Minimum, maximum	11.0, 38.0	14.0, 27.0	14.0, 30.0	13.0, 30.0	11.0, 38.0
Anti–Scl-70, no (%)	1 (9.1)	5 (45.5)	4 (36.4)	2 (20.0)	12 (27.9)
Serum creatinine, μmoles/liter					
No. of patients	11	11	11	10	43
Mean ± SD	71.2 ± 12.5	71.0 ± 9.9	77.9 ± 23.4	70.1 ± 17.8	72.6 ± 16.4
Median	70.0	70.0	75.0	68.5	70.0
Minimum, maximum	53.0, 97.0	61.0, 91.0	44.0, 122.0	44.0, 99.0	44.0, 122.0
Creatinine clearance, ml/minute					
No. of patients	9	10	8	9	36
Mean ± SD	103.1 ± 34.2	104.8 ± 24.0	94.6 ± 35.7	125.8 ± 47.0	107.4 ± 36.1
Median	102.0	105.0	91.1	127.7	103.1
Minimum, maximum	62.2, 148.0	72.0, 138.0	39.5, 156.0	58.9, 200.0	39.5, 200.0
FVC, % predicted					
No. of patients	11	11	11	10	43
Mean ± SD	92.5 ± 9.7	92.9 ± 17.5	88.8 ± 13.3	88.1 ± 15.6	90.6 ± 13.9
Median	92.0	93.0	92.0	83.5	92.0
Minimum, maximum	75.0, 106.0	60.0, 125.0	69.0, 107.0	69.0, 120.0	60.0, 125.0
DLCO, % predicted					
No. of patients	11	11	11	10	43
Mean ± SD	72.0 ± 16.4	83.1 ± 22.9	79.0 ± 18.3	69.0 ± 17.3	75.9 ± 19.1
Median	74.0	75.0	76.0	70.5	75.0
Minimum, maximum	47.0, 100.0	52.0, 117.0	55.0, 117.0	37.0, 93.0	37.0, 117.0

* MRSS = modified Rodnan skin thickness score; FVC = forced vital capacity; DLCO = diffusing capacity for carbon monoxide.

tides of type III collagen (PIIINP) and of type I collagen (PINP) were measured by commercial radioimmunoassay (Behringwerke, Marburg, Germany, and Orion Diagnostica, Helsinki, Finland, respectively). A quantitative immunoassay was used to determine levels of interleukin-2 receptor (IL-2R; R&D Systems, Minneapolis, MN).

Adverse events. All AEs were summarized by preferred term for the number of times reported and the number of patients who reported that AE in each treatment group and were tabulated by body system, severity, time to event, and relationship to study treatment. In addition, SAEs as well as discontinuations because of SAEs were similarly generated for each treatment group.

Sample size. The study was designed to evaluate the safety of CAT-192 and to measure the pharmacokinetics of the agent, although potential efficacy data were also collected. These data included exploratory end points that would help determine variability and utility in a future controlled trial in early-stage dcSSc. It was initially envisaged that 36 subjects would be enrolled into the study, but a total of 44 subjects were randomized. This increased sample size was used in order to increase the number of evaluable cases at study completion.

Statistical analysis. Analysis of clinical outcomes was performed on the study population that included all patients who completed the baseline assessment, received at least 1 infusion of study medication, and had at least 1 postinfusion assessment. Changes from baseline in the MRSS and biomarker values at weeks 12 and 24 were analyzed using analysis of variance (ANOVA), and the mean differences, *P* values, and 95% confidence intervals (95% CIs) are reported. In addition, the proportion of responders (defined as no change, or any improvement, in the MRSS) was compared between the active treatment groups and control groups at weeks 12 and 24 using the Cochran-Mantel-Haenszel test. These data were analyzed using Wilcoxon's rank sum test. Mean or median differences between each active treatment group and placebo, along with *P* values, 95% CIs, and interquartile ranges (IQRs) are presented. Changes in biomarker values at weeks 12 and 24 were compared between study subjects and healthy controls using ANOVA. All data management and statistical analyses were performed with Oracle (Redwood Shores, CA) and BBN/Clintrial (Cambridge, MA) software.

RESULTS

Characteristics of the study subjects. Forty-five patients were enrolled into this trial. Of these, 43 patients received at least 1 infusion of study drug, 31 completed the study, and none were lost to followup. None had received potential disease-modifying treat-

Table 3. Summary of deaths and serious adverse events occurring during the study, by treatment group

Parameter	Placebo treatment (n = 11)	CAT-192 treatment				No. (%) of all patients (n = 43)
		0.5 mg/kg (n = 11)	5 mg/kg (n = 11)	10 mg/kg (n = 10)	No. (%) of all CAT-192 (n = 32)	
No. of patients experiencing an event*						
Serious adverse event†	2	5	4	2	11 (34)	13 (30)
Death‡	0	1	3	0	4 (12.5)	4 (9)
No. of individual serious adverse events						
Progression of skin involvement	1	2	3	0	5	6
Gastrointestinal manifestations	0	3	1	2	6	6
Weight loss	0	1	0	0	1	1
Gastrointestinal hemorrhage/anemia	1	2	1	1	4	5
Infusion reaction	0	0	1	0	1	1
Autoantibody response	0	0	0	1	1	1
Constitutional symptoms	0	1	1	2	4	4
Musculoskeletal pain	0	3	0	1	4	4
Cardiac manifestations	0	1	3	0	4	4
Infection	1	0	2	0	2	3
Pulmonary manifestations	0	4	3	0	7	7
Impaired mobility	0	2	0	0	2	2
Total no. of serious adverse events	3	19	15	7	41	44

* Patients often experienced multiple serious adverse events: 41 occurred in 11 patients receiving CAT-192 (34%), and 3 occurred in 2 patients receiving placebo (18%).
† Defined as life-threatening, causing serious incapacity, or leading to additional hospitalization; or requiring treatment to prevent any of these outcomes.
‡ See Results for causes of death.

ment prior to study entry. All patients had evidence of proximal skin sclerosis at study entry, and so, fulfilled the ACR preliminary criteria for the classification of SSc. Of the 14 subjects who withdrew (13 in the CAT-192 group; 1 in the placebo group), the majority (n = 9) did so because of adverse events.

Pharmacokinetics of CAT-192. Serum levels of CAT-192 were detectable in all 3 active treatment groups. The half-life of the agent (mean ± SEM 24.0 ± 2.1 days) was comparable across the dosage groups.

Safety and tolerability of CAT-192. Four deaths were reported during the study, 1 in the group receiving 0.5 mg/kg of CAT-192 and 3 in the group receiving 5 mg/kg. All deaths were attributed to complications of the underlying disease, and none was considered to be related to the administration of CAT-192. In the group receiving 0.5 mg/kg, the patient had progressive skin and muscle disease and had received 2 infusions of CAT-192. Treatment was then changed to cyclophosphamide, but the patient died of cardiac failure despite therapy. In the group receiving 5 mg/kg, 1 death resulted from progressive skin and gastrointestinal tract disease in the context of end-stage renal failure following scleroderma renal crisis. This patient received 1 infusion of CAT-192, but the drug was withdrawn after renal crisis occurred; this developed 6 weeks after the study drug infusion and was considered to be unrelated to the study drug. The second death in this group resulted from progression of the SSc, with cardiac and lung involvement. This patient had received 3 infusions of CAT-192. The third death occurred in a patient who developed progressive pulmonary fibrosis. This patient had received 1 infusion of CAT-192 and was subsequently treated with intravenous cyclophosphamide. Despite this treatment, however, the patient developed respiratory failure and died.

AEs and SAEs were frequent in this study and are consistent with the predictably high morbidity of early-stage dcSSc. Overall, there were more AEs and SAEs in patients receiving CAT-192 than in those receiving placebo. A total of 19 SAEs occurred in 5 patients receiving 0.5 mg/kg, 15 occurred in 4 receiving 5 mg/kg, and 7 occurred in 2 receiving 10 mg/kg of CAT-192, and 3 SAEs occurred in 2 patients receiving placebo. Details of the SAEs are provided in Table 3.

There were a total of 275 unique AEs, including 54 AEs in patients receiving placebo and 75 in those receiving 0.5 mg/kg, 87 in those receiving 5 mg/kg, and 59 in those receiving 10 mg/kg of CAT-192. All but 1 of the patients (receiving 0.5 mg/kg of CAT-192) experienced an AE.

The most commonly reported AEs affected the gastrointestinal, musculoskeletal, respiratory, and skin systems. This included skin ulceration in all but the group taking 10 mg/kg of CAT-192. Although more AEs

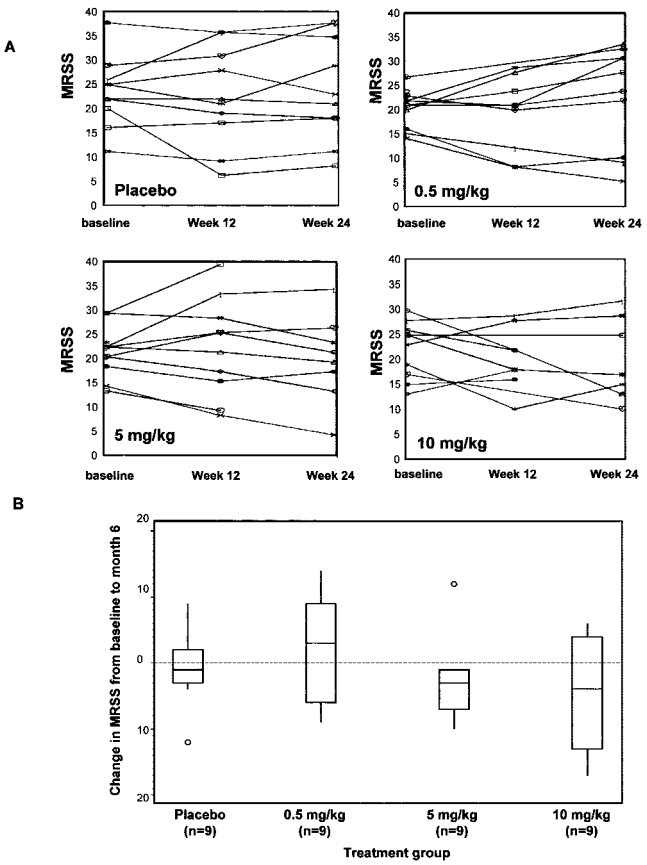

Figure 1. Change in the modified Rodnan skin thickness score (MRSS) from baseline to month 6 in patients with diffuse cutaneous systemic sclerosis treated with placebo or with 3 different doses (0.5, 5, or 10 mg/kg) of CAT-192. **A,** Individual MRSS changes in each treatment group. **B,** Box-and-whisker plot showing overall changes in MRSS during the study as compared with baseline. Boxes show the interquartile range. Lines within the boxes show the median. Vertical lines indicate the range. Circles indicate outliers.

and SAEs occurred in the active treatment groups, there was no relationship to the dosage, in that there were similar numbers of AEs and SAEs in the groups receiving 0.5 mg/kg and 10 mg/kg, and perhaps more importantly, the number of patients experiencing an AE or an SAE was similar for placebo-treated patients and those in the 10 mg/kg groups, despite apparent differences in the total number of events. Thirteen patients experienced 30 unique SAEs, most of which appeared to be related to the underlying dcSSc. None were considered to be related to the study medication. Many of the events occurred in the same patient and reflected disease progression; 2 patients experienced repeated episodes of gastrointestinal hemorrhage.

Overall, intravenous administration of CAT-192 was well tolerated among the 32 patients who received the active study drug, with the majority of reported AEs being mild or moderate in intensity and unrelated to the treatment. The most common AEs leading to discontinuation of therapy were progression of SSc skin disease or worsening breathlessness, which occurred in 9 patients (1 taking placebo, 4 taking 5 mg/kg of CAT-192, and 4 taking 10 mg/kg).

Changes in skin scores. Median values for the MRSS were compared among the 4 treatment groups, from screening to completion of the assessment period. The data are summarized in Figure 1. Our results do not show any evidence of efficacy for CAT-192, since although there was overall improvement in skin sclerosis during the study, the change in the MRSS appeared to be independent of treatment group ($P = 0.49$). The duration of disease at baseline was significantly correlated with the change in skin score between baseline and 6 months ($r = -0.54$, $P = 0.0008$) (Figure 2).

Other outcomes. As expected, there was substantial evidence of functional impairment at baseline in the study cohort, with mean ± SD HAQ DI scores of 1.0 ± 0.70 (range 0.00–2.60). There were no statistically significant differences in HAQ DI scores among any of the 4 treatment subgroups, ranging from 0.80 ± 0.60 in the group receiving 0.5 mg/kg of CAT-192 to 1.40 ± 0.90 in the group receiving 5 mg/kg. The mean ± SD change

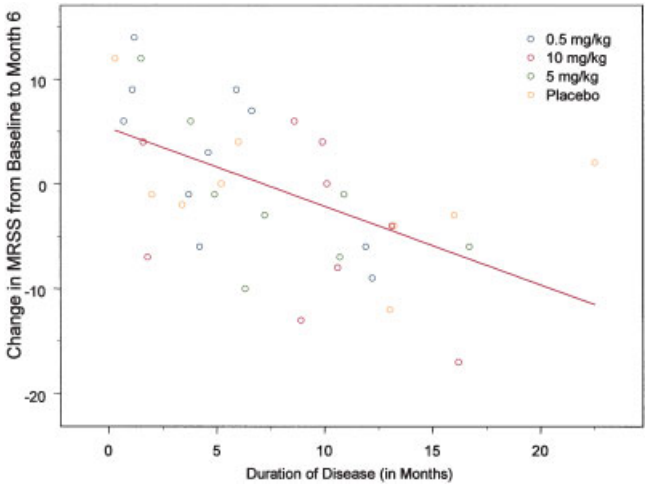

Figure 2. Relationship between change in the modified Rodnan skin thickness score (MRSS) and disease duration in patients with diffuse cutaneous systemic sclerosis (dcSSc) treated with placebo or with 3 different doses (0.5, 5, or 10 mg/kg) of CAT-192. There was a highly statistically significant association between the duration of dcSSc at baseline and the change observed in the MRSS between baseline and week 24 of study ($r = -0.54$, $P = 0.0008$). This suggested that disease duration at baseline was a major determinant of outcome.

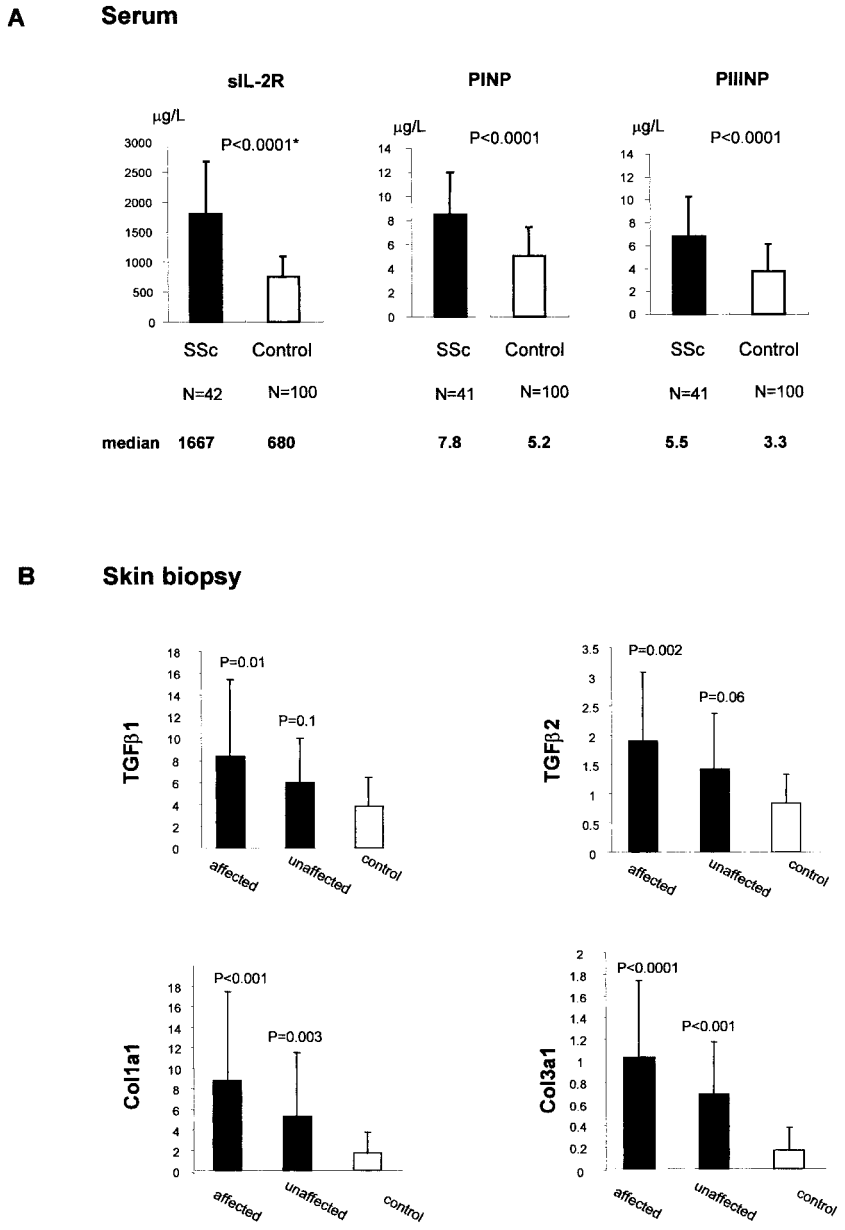

Figure 3. Analysis of biomarkers at baseline in patients with diffuse cutaneous systemic sclerosis (dcSSc) and in healthy control subjects. **A,** Serum biomarkers are elevated in patients with dcSSc as compared with those in age- and sex-matched healthy control subjects, confirming immunologic activation and fibrillar collagen overproduction. sIL-2R = soluble interleukin-2 receptor; PINP = N-propeptide of type I collagen; PIIINP = N-propeptide of type III collagen. **B,** Expression of mRNA for transforming growth factor β1 (TGFβ1) and TGFβ2 is elevated in lesional skin biopsy tissue from patients with dcSSc. There was also a trend toward elevated expression in biopsy tissue from nonlesional skin as compared with that in skin biopsy tissue from sex-matched healthy controls. For the procollagen genes *COL1A3* and *COL1A1*, there were significant elevations in mRNA levels in both lesional and nonlesional skin from dcSSc patients, although the levels tended to be greater in lesional skin. Together, these results suggest that gene expression in uninvolved skin is also altered. This may reflect background genetic susceptibility or subclinical skin involvement. Values are the mean and SD. *P* values are versus controls, as determined by analysis of variance.

from baseline was 0.15 ± 0.50. The magnitude of change overall was below the level suggested to be a minimal clinically significant difference in other rheumatic diseases (20,22).

Analysis of changes in indices of pulmonary function revealed few changes in any patient. Only 2 patients showed >10% worsening in the FVC over 6 months: one in the group receiving placebo and the other in the group receiving 0.5 mg/kg of CAT-192. In contrast, 4 patients in various groups demonstrated at least a 15% decrease in the DLCO.

There were variable changes in renal function from the time of screening between the treatment groups, but these did not appear to reflect the treatment received. There was 1 confirmed hypertensive renal crisis, which occurred in a patient receiving 5 mg/kg of CAT-192 and required hemodialysis. In addition, there were 3 episodes of new-onset systemic hypertension without renal impairment, 1 in the group receiving placebo and 2 in the group receiving 0.5 mg/kg of CAT-192. Treatment with angiotensin-converting enzyme inhibitors was given for all 3 episodes.

Serum levels of biomarkers. The serum biomarkers selected for analysis were those that indicated collagen biosynthesis and immunologic activity. There were clear differences at baseline in the levels of PINP (mean ± SD 8.5 ± 3.5 μg/liter versus 5.1 ± 2.4 μg/liter; $P < 0.0001$) and PIIINP (6.8 ± 6.2 μg/liter versus 3.5 ± 1.1 μg/liter; $P < 0.0001$) in the SSc patients versus the healthy controls. There were changes in the levels of these propeptides during the study period, but these changes did not differ between treatment groups. Association with disease duration was not observed.

Serum levels of IL-2R (ng/liter) were much higher in the patients with SSc (1.8 ± 0.9) than in healthy control subjects (0.8 ± 0.3; $P < 0.0001$). The scleroderma-associated autoantibody anti–topoisomerase I did not change during the treatment period, and no significant difference between the baseline and end of study levels of sIL-2R, PIIINP, or PINP was observed in any treatment group. However, when changes in the MRSS were compared with changes in the various biomarker levels, only PINP levels showed statistically significant changes that correlated with the MRSS ($r = 0.37$, $P = 0.027$).

Findings of mRNA analysis of skin biopsy specimens. Real-time quantitative PCR analysis of markers of fibrosis (*COL1A1*, *COL3A1*, *TGFB1*, and *TGFB2*) demonstrated that levels of mRNA for these markers were increased in affected skin obtained from patients with SSc, but there was no change during treatment. There were changes in transcript levels between the time of screening and completion of the treatment phase of study, but these were not significantly associated with changes in the MRSS. There were no significant differences in transcript levels at the completion of treatment among the treatment groups. Despite changes in skin scores that occurred between baseline and the end of the treatment phase, there was no significant correlation between skin score changes and changes in the levels of mRNA for any of the markers of fibrosis that were examined. Baseline data for the biomarkers examined are summarized in Figure 3.

DISCUSSION

This study represents an important step in the development and exploration of the concept of opposing the action of TGFβ as a therapeutic intervention for SSc. There is a large body of indirect evidence implicating the overexpression of TGFβ ligand or receptor in the pathogenesis of SSc (23), and several recent studies have demonstrated that blocking TGFβ ligand may abrogate fibrosis in a number of mouse models (24). In addition, activation of TGFβ signaling pathways constitutively in fibroblasts replicates many of the key histologic and biochemical features of established SSc (25). In this context, it is disappointing that our study did not show any evidence of efficacy for CAT-192. In animal models of fibrosis, effective prevention of fibrosis has generally required the blockade of multiple isoforms of TGFβ, and this is a potential explanation for the lack of effect on markers of efficacy in this study. Thus, soluble TGFβ receptors that block all active TGFβ ligand and the pan-isoform–specific antibody 1D11 are both effective in blocking fibrosis, as is a polyclonal anti-TGFβ and a recombinant latency-associated peptide (26,27). Other blocking strategies, such as overexpression of Smad7 or decorin, have also been tested in animal models (28).

Concerns about the safety of CAT-192 reflect the well-recognized association between defective TGFβ signaling and immunologic activation, the antiproliferative effect of TGFβ on epithelial cells, and the recognition that several downstream products in the TGFβ signaling cascade may function as tumor-suppressor genes (29). An additional concern at the outset was that no human had ever received multiple doses of CAT-192. With this background, it is appropriate that a mono-isoform–specific antibody was chosen for initial evaluation. Such treatment would be predicted to provide partial blockade of the TGFβ axis and allow the evaluation of safety. Interestingly, results of animal studies suggest that blocking of TGFβ is relatively safe. Recent strategies that induce very significant blockade

in tumor models do not seem to increase the degree of metastasis (30).

This was primarily a safety and tolerability study. There were more AEs, SAEs, and deaths in the active treatment groups than in the placebo treatment group. This may have been a consequence of CAT-192. However, multiple events occurred in the same patients with progressive disease, and when the number of patients in each subgroup that experienced AEs or SAEs is considered, a possible relationship to therapy seems much less likely, particularly since similar numbers of patients in the placebo and high-dose CAT-192 groups were affected by AEs and SAEs. Nevertheless, the high mortality rate observed in this study cohort is noteworthy and is above that seen in other recent trials of dcSSc. This may reflect the selection of cases with early progressive disease and the severe cases referred to the specialist centers that participated in the study. Direct comparison with other trials is complicated by differences in entry criteria; for example, the median disease duration was lower in the present study than in the other trials that have been published. It is quite possible that a more complete blockade of the TGFβ axis might, in turn, be associated with substantial toxicity.

Robust evaluation of novel therapies in SSc remains a major challenge and is complicated by heterogeneity of the disease and the absence of any agents with proven effectiveness. Previous studies yielding negative results have hampered the development of validated assessment tools that are sensitive to change over the time course of a therapeutic clinical trial. There is continued interest in this area, and several groups of investigators have reported guidelines for disease assessment and for the performance of clinical trials (16,17,31). At present, for a short-term study, the MRSS remains the most frequently used tool for the assessment of skin sclerosis. To minimize interobserver variations in the MRSS, standardization of skin scores was performed at baseline to ensure that there was consistency among observers. In addition, wherever possible, the same observer assessed individual subjects during the study. A limitation of the MRSS as a trial end point highlighted by the present study is that even when very early-stage dcSSc patients are recruited, there is an overall tendency for the MRSS to improve. This challenges the feasibility of a "prevention of worsening" study design despite the success of this trial in targeting and enrolling an early disease cohort. It suggests that improvement in skin involvement is not confined to late-stage disease (32).

The HAQ DI data confirm the severe functional impact of dcSSc. The mean levels of disability are similar to those reported for other chronic multisystem diseases that affect hand function, such as rheumatoid arthritis (33). Changes in the HAQ DI scores did not seem to depend upon treatment with CAT-192, but in general, patients with longer disease duration had higher HAQ DI scores. Renal and lung function test results provided additional information concerning the impact of dcSSc in this cohort. However, no changes in these variables that might be attributed to treatment with CAT-192 were observed.

Serial assessment of lesional and nonlesional (clinically unaffected) skin biopsy samples represents the most direct way of assessing changes in scleroderma patients at a biochemical level. The transcripts selected for analysis by real-time quantitative PCR included 2 profibrotic cytokines, TGFβ1 and TGFβ2, and 2 extracellular matrix molecules, PINP and PIIINP. Elevation of these cytokines in lesional skin confirms the validity of these markers. This is one of the few studies to use such a rigorous biochemical evaluation of treatment effects, and our results provide validity for this approach. Clear differences were identified between lesional and nonlesional sites as well as between SSc and healthy control samples. Abnormalities in the samples from nonlesional sites are consistent with earlier reports that unaffected skin from patients with SSc is diseased (34); this has been shown by histologic assessment as well as by assessment of skin biomechanics (35). Most recently, gene expression profiling data support this finding (36) and are perhaps the most relevant comparisons to our study. In the future, groups of genes or protein profiles may be defined as biomarkers of bioactivity in SSc clinical trials, especially early-stage, proof-of-concept studies where skin scores may not change greatly in a short time.

The serum biomarkers examined in this study were selected to demonstrate effects on collagen biosynthesis and immune system activation. The N-terminal propeptides are cleaved from procollagen molecules during posttranslational processing and are an index of newly synthesized collagen. It is interesting that PINP was the only variable to be associated with changes in skin scores, adding validity to this outcome measure in SSc (37). Levels of sIL-2R are increased in conditions associated with immunologic activation (38). Thus, the increased levels observed in the present study provide confirmation of immune system activation in early dcSSc. This is consistent with the presence of T cell infiltrates in lesional tissues and with the body of evidence pointing toward immunopathogenesis, and it justifies the use of caution in treating patients with an antibody to a known immunosuppressive cytokine. The role of other immunosuppressive factors, such as IL-10,

in SSc remains to be determined. There was no increase in sIL-2R levels in any treatment group.

In conclusion, this is the first time that a biologic agent has been used to specifically target one of the key candidate factors in the pathogenesis of SSc. Overall, our findings suggest that early-stage dcSSc can be identified and targeted and that levels of biomarkers are abnormal in this group, but the findings do not show efficacy for CAT-192 in this cohort of patients with SSc.

AUTHOR CONTRIBUTIONS

Dr. Denton had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Drs. Denton, Merkel, Furst, Streisand, Powell, Herrick, Haas, Black, and Seibold.

Acquisition of data. Drs. Denton, Merkel, Furst, Khanna, Emery, Hsu, Åkesson, Coppock, van den Hoogen, Herrick, Mayes, Veale, Haas, Ledbetter, Black, and Seibold.

Analysis and interpretation of data. Drs. Denton, Merkel, Furst, Silliman, Streisand, Powell, van den Hoogen, Mayes, Haas, Ledbetter, and Seibold.

Manuscript preparation. Drs. Denton, Merkel, Furst, Khanna, Emery, Silliman, Streisand, Powell, Herrick, Mayes, Veale, Haas, Ledbetter, Black, and Seibold.

Statistical analysis. Drs. Silliman and Haas.

Patient enrollment. Dr. Hsu.

ROLE OF THE STUDY SPONSORS

Genzyme (Cambridge, MA) and Cambridge Antibody Technology (Cambridge, UK) were equal partners in undertaking the study design, data collection, data analysis, and review of the completed analysis, and have approved the final version of the manuscript for publication.

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