CJASN ePress. Published on June 3, 2010 as doi: 10.2215/CJN.09321209

Phase 1 Study of Anti-CTGF Monoclonal Antibody in Patients with Diabetes and Microalbuminuria

Sharon G. Adler,* Sherwyn Schwartz,† Mark E. Williams,‡ Carlos Arauz-Pacheco,§ Warren K. Bolton,‖ Tyson Lee,¶ Dongxia Li,¶ Thomas B. Neff,¶ Pedro R. Urquilla,¶ and K. Lea Sewell¶

**Division of Nephrology and Hypertension, Los Angeles BioMedical Research Institute, Torrance, California; †Diabetes and Glandular Disease Clinic, San Antonio, Texas; ‡Renal Unit, Joslin Clinic, Boston Massachusetts; §Radiant Research, Dallas Texas; ‖Division of Nephrology, University of Virginia School of Medicine, Charlottesville, Virginia; and ¶FibroGen, Inc., San Francisco, California*

Background and objectives: This report summarizes the first phase 1 trial treating patients with microalbuminuric diabetic kidney disease (DKD) using FG-3019, a human monoclonal antibody to connective tissue growth factor (CTGF). CTGF is critically involved in processes of progressive fibrosis, including DKD. This phase 1, open-label, dose-escalation trial evaluated safety, pharmacokinetics, and possible therapeutic effects of FG-3019 on albuminuria, proteinuria, and tubular proteins.

Design, setting, participants, and measurements: Microalbuminuric subjects (n = 24) with type 2 (79%) or type 1 (21%) diabetes received 3 or 10 mg/kg FG-3019 dosed intravenously every 14 days for four doses. Albuminuria and safety follow-up were to days 62 and 365, respectively.

Results: No infusion was interrupted for symptoms, although 5 of 24 subjects had mild infusion-day adverse events thought to be possibly drug-related. No subject developed anti-FG-3019 antibodies. FG-3019 clearance was lower at 10 mg/kg than at 3 mg/kg, suggesting a saturable elimination pathway. Although this study was not designed for efficacy testing, it was notable that urinary albumin/creatinine ratio (ACR) decreased significantly from mean pretreatment ACR of 48 mg/g to mean post-treatment (day 56) ACR of 20 mg/g (P = 0.027) without evidence for a dose-response relationship.

Conclusions: Treatment of microalbuminuric DKD subjects using FG-3019 was well tolerated and associated with a decrease in albuminuria. The data demonstrate a saturable pathway for drug elimination, minimal infusion adverse events, and no significant drug-attributable adverse effects over the year of follow-up. Changes in albuminuria were promising but require validation in a prospective, randomized, blinded study.

Clin J Am Soc Nephrol 5: ●●●–●●●, 2010. doi: 10.2215/CJN.09321209

Patients with diabetic kidney disease (DKD) are at increased risk for cardiovascular complications and early mortality. Those who survive long enough tend to progress to ESRD requiring dialysis or transplantation. Although advances in therapy with angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor type II blockers (ARBs) have attenuated the incident rate of ESRD (1), disease progression remains common (2–4) and diabetes continues to be the leading cause for initiation of dialysis in the United States (1).

Connective tissue growth factor (CTGF) is a 349-amino-acid secreted pleiotropic protein belonging to the cysteine-rich CCN (CTGF/Cyr61/Cef10/NOVH) family. Numerous glomerular, tubulointerstitial, and vascular cells types can produce CTGF, and many factors associated with the diabetic condition can stimulate CTGF expression, including hypertension, hyperglycemia, and hyperlipidemia (5–24).

CTGF is a critical mediator of extracellular matrix accumulation and coordinates a final common pathway of fibrosis (5,25,26). CTGF has been shown to amplify the fibrogenic activity of TGFβ (27) and IGF-1 (17) and to inhibit the action of antifibrotic and regenerative factors bone morphogenic protein-7 (27,28) and vascular endothelial growth factor (29,30).

In type 1 diabetes, plasma and urine CTGF levels correlate with the level of albuminuria and the stage of progressive renal insufficiency (31–34), and the plasma CTGF level is an independent predictor of vascular disease as assessed by intimal medial thickness (35) and of mortality and progression to ESRD (36). In renal biopsy specimens from patients with diabetes, elevated levels of CTGF mRNA are associated with chronic tubulointerstitial damage, albuminuria, and progression of renal insufficiency (37–39).

FG-3019 is a recombinant human anti-CTGF monoclonal IgG_1 antibody that has shown activity in rodent models of kidney dysfunction associated with type 1 and 2 diabetes (40–

Received December 23, 2009. Accepted April 16, 2010.

Published online ahead of print. Publication date available at www.cjasn.org.

Correspondence: Dr. Sharon G. Adler, Los Angeles BioMed Research Institute, 1124 West Carson Street, Building C1 Annex, Torrance, CA 90502. Phone: 310-222-3891; Fax: 310-782-1837; E-mail: sadler@labiomed.org

ISSN: 1555-9041/508–0001

42). Here, we report results of an open-label dose-escalation trial of FG-3019 infusions administered biweekly over 56 days in patients with DKD, the first study designed to evaluate safety and potential therapeutic effect of FG-3019 in this patient population.

Materials and Methods

Patients

Eligible subjects had type 1 or 2 diabetes mellitus, were at least 21 years old, had a body mass index ≤32 kg/m^2, normal serum creatinine (Cr) ≤1.1 (females), and ≤1.5 mg/dl (males), and microalbuminuria by two first-morning urine albumin/creatinine ratio (ACR) of 30 to 300 mg/g, measured at the investigator's laboratory in two samples collected 2 to 3 days apart and obtained 1 to 15 days before randomization. Subjects were excluded from participation if they had a malignancy within 5 years; aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >1.5 times the upper limit of normal; and history of allergy to previous antibody treatment and for myocardial infarction, angioplasty or bypass surgery, congestive heart failure, angina, or transient ischemic attack or stroke within the past 6 months. Concomitant therapy with insulin, oral hypoglycemic agents, ACEIs, ARBs, other antihypertensive medications, and cholesterol-lowering drugs were required to be stable for 4 weeks before the first study infusion.

The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice guidelines, and local ethics committees. All study participants provided written informed consent.

Study Treatment

FG-3019, a recombinant human IgG_1 kappa monoclonal antibody that binds to domain 2 of CTGF (K_d = 0.1 to 0.2 nM), was diluted in a 250-ml bag of 0.9% sodium chloride for injection, USP, and infused over at least 120 minutes using a 0.22-μm in-line filter. Although not required, some sites premedicated patients with acetaminophen or antihistamines depending on the center's protocol. Drug was administered every other week for a total of four doses. Dose escalation to 10 mg/kg was approved by an independent data safety monitoring committee after all subjects assigned to the 3-mg/kg arm had received all four study infusions. Dose cohorts were independent. Efficacy assessments were performed on day 56 (14 days after the fourth infusion).

Study Purpose

Primary end points were pharmacokinetic parameters and safety. Secondary end points included urinary protein normalized to Cr, estimated GFR (eGFR), and hemoglobin A1c (HbA1c).

Laboratory Testing

Screening electrocardiogram, chest x-ray, and standard laboratory testing were performed at each clinical site. eGFR (ml/min per 1.73 m^2) was calculated using the Modifications of Diet in Renal Disease equation (43), where $eGFR = 186\ (S_{Cr})^{-1.154} \times (Age)^{-0.203} \times (0.742\ if\ female) \times (1.210\ if\ African\ American)$.

Timed urine samples were collected overnight before each infusion and at 2, 4, and 6 weeks after the final infusion. Urinary protein assays were performed in a central laboratory on samples that had been stored at −70°C. Urinary albumin (assayed by nephelometry), Cr, protein, and β-2-microglobulin (β2M) were measured by Esoterix (East Windsor, NJ). Urinary *N*-acetyl-β-D-glucosaminidase (NAG), α-1-microglobulin (α1M), transferrin, and IgG were measured by Alta Analytical Laboratory, Inc. (San Diego, CA). Urinary protein concentrations were expressed relative to Cr concentration.

FG-3019 Pharmacokinetics, CTGF, and Immune Response

Assays for FG-3019, CTGF, and anti-FG-3019 antibodies in heparin plasma were performed at FibroGen, Inc. (San Francisco, CA) and Alta Analytical Laboratory, Inc. (San Diego, CA) by ELISA using frozen samples stored at −70°C.

CTGF has four domains and an interconnecting central protease-susceptible "hinge" region; cleavage of this region yields two 19-kD N- and C-terminal halves (44). In previous studies, full-length CTGF (CTGF-whole) and N-terminal fragments of the CTGF molecule (CTGF N-fragment) have been detected in plasma samples; in urine, mainly CTGF N-fragment has been found. Therefore, all plasma and urine samples were tested with two CTGF assays—one that measures CTGF-whole (W-CTGF), and one that measures the sum of CTGF-whole and CTGF N-fragment (N+W-CTGF).

Development of an antibody response against the drug was evaluated by measuring anti-FG-3019 antibodies at baseline and after FG-3019 drug washout on days 126, 180, and/or 365.

Statistical Analyses

All subjects who received at least one infusion of FG-3019 (n = 24) were included in the safety analyses. Subjects who received at least one infusion of FG-3019 and had no major protocol deviations (n = 20) were included in the efficacy analyses (the four major protocol deviations involved incorrect dosing as described in the *Results* section). No imputation was performed for missing data with the exception of carry-forward to day 56 of day 42 preinfusion laboratory data for one 10-mg/kg subject. Adverse events were summarized by their MedDRA (version 7.1) system organ class and preferred term. Noncompartmental pharmacokinetic parameters for FG-3019 were calculated using WinNonlin, version 4.0.1 (Pharsight Corporation, Mountain View, CA). Data are reported as mean ± SD.

Results

Baseline Characteristics

Tables 1 and 2 summarize baseline characteristics. Ten subjects each received 3 or 10 mg/kg FG-3019 on days 0, 14, 28, and 42; one 3-mg/kg subject withdrew after one dose. Four additional subjects at one site were assigned to receive 10 mg/kg but were dosed incorrectly with 3 mg/kg. These four subjects did not receive a full course of treatment and were therefore only included in the safety evaluation. Subjects had longstanding diabetes (most with type 2), a mean age of 58 years, and a body mass index of 30.3 (±4.0) (Table 1). Serum Cr was normal, and mean eGFR was 84 ± 21 ml/min per 1.73 m^2. Despite normal serum Cr, baseline eGFR for two subjects in the 10-mg/kg cohort classified them as stage 3 chronic kidney disease. Baseline renal function and urinary protein were evenly balanced although the latter tended to be higher for the 10-mg/kg cohort (Table 2). The overall average mean arterial pressure (MAP) was 94.5 (±8.2) mmHg on the basis of the average of three blood pressure measurements before treatment. Over half were receiving insulin, and 88% were receiving an ACEI or an ARB, including 19 of 20 in the efficacy population. Four subjects received both drug types. Three subjects had changes in ACEIs/ARBs during the protocol; one decreased dose on study

Table 1. Patient demographics (safety population)

Mean (±SD) or *N* (%)	FG-3019 3 mg/kg (*n* = 14)	FG-3019 10 mg/kg (*n* = 10)	All (*n* = 24)
Age (years)	54 (±14)	63 (±7.5)	58 (±12)
range	(33 to 78)	(48 to 75)	(33 to 78)
Female (%)	4 (29%)	4 (40%)	8 (33%)
Caucasian (%)	6 (43%)	6 (60%)	12 (50%)
African American (%)	2 (14%)	0	2 (8%)
Hispanic (%)	6 (43%)	3 (30%)	9 (38%)
Other (%)	0	1 (10%)	1 (4%)
Weight (kg)	83.3 (±13)	89.1 (±18)	85.7 (±15)
Body mass index (kg/m^2)	29.5 (±3.4)	31.4 (±4.6)	30.3 (±4.0)
Type 2 diabetes (%)	11 (79%)	8 (80%)	19 (79%)
Years of diabetes	17 (±6)	15 (±8)	16 (±7)
MAP[a] (mmHg)	92.6 (±6.8)	97.0 (±9.7)	94.5 (±8.2)
Insulin (%)	10 (71%)	5 (50%)[b]	15 (63%)
Oral hypoglycemic (%)	8 (57%)	8 (80%)[c]	16 (67%)
ACEIs and/or ARBs (%)	12 (86%)	9 (90%)	21 (88%)

[a]Average of three pretreatment blood pressure measurements.
[b]One receiving pramlintide.
[c]One receiving exenatide.

Table 2. Baseline clinical variables (efficacy population)

Mean (±SD)	FG-3019 3 mg/kg (*n* = 10)	FG-3019 10 mg/kg (*n* = 10)	All (*n* = 20)
Serum Cr (mg/dl)	0.9 (±0.1)	1.0 (±0.3)	0.9 (±0.2)
eGFR (MDRD) (ml/min per 1.73 m^2)	84.8 (±16.2)	79.4 (±22.9)	81.9 (±19.7)
Urine ACR (mg/g)	48.0 (±26.6)	73.0 (±74.8)	61.2 (±57.3)
Urine albumin excretion rate (mg/24 h)	83.2 (±81.5)	140.8 (±145.1)	117.1 (±123.2)
Urine protein/Cr ratio (mg/g)	137.1 (±38.8)	218.2 (±127.3)	170.9 (±91.9)
Urine protein excretion rate (mg/24 h)	233.6 (±160.1)	392.9 (±286.6)	327.3 (±249.7)
Urine NAG (IU/L)	3.9 (±2.2)	5.5 (±2.6)	4.7 (±2.5)
Urine α1M (mg/L)	5.7 (±4.5)	6.7 (±5.3)	6.2 (±4.8)
Urine β2M (μg/L)	34.2 (±28.6)	96.5 (±104.2)	67.4 (±82.5)
Plasma CTGF N+W (ng/ml)	6.5 (±1.1)	11.2 (±3.6)	9.8 (±3.8)

MDRD, Modifications of Diet in Renal Disease study equation.

day 42, and two increased their dose, one on study day 4 and the other on study day 42.

FG-3019 Pharmacokinetics

At the lower dose of 3 mg/kg, there was linear clearance of FG-3019 and a steady-state half-life ($T_{1/2}$) of 4.3 days, with minimal accumulation from dose 1 to dose 4 (Figure 1). The rate of clearance was slower and $T_{1/2}$ was longer at 56 days for the 10-mg/kg dose. Slower clearance at the higher dose of 10 mg/kg suggested a saturable clearance pathway and resulted in a greater than proportional increase in exposure to drug (steady-state area under the curve) than expected from the dose increase alone (Table 3; Figure 1).

Plasma and Urine CTGF Levels

Pretreatment plasma N+W-CTGF was within normal range in both dose groups with mean levels of 7.3 (±2.7) ng/ml and 12.2 (±3.2) ng/ml for the 3- and 10-mg/kg dose groups, respectively (Table 4). After FG-3019 infusions of 3 mg/kg, plasma N+W-CTGF peaked at 72 hours with mean levels of 372.1 (±135.4) ng/ml and 430.3 (±169.7) ng/ml after the first and fourth infusions, respectively. In the 10-mg/kg cohort, N+W-CTGF peaked at 7 days after the first infusion and 3 days after the fourth, with peak levels of 947.8 (±180.9) ng/ml and 1296.2 (±355.7) ng/ml, respectively. Determination of the apparent peak is an estimation based on limited sampling after the 24-hour time point. However, even at the apparent peak of N+W-CTGF, FG-3019 was at a >10-fold molar excess over N+W-CTGF. Plasma N+W-CTGF gradually decreased over the 14-day period until the next infusion in the 3-mg/kg cohort, paralleling the clearance of FG-3019 from plasma, but did not become undetectable in all subjects before the next infusion. In contrast, in the 10-mg/kg cohort, plasma N+W-CTGF re-

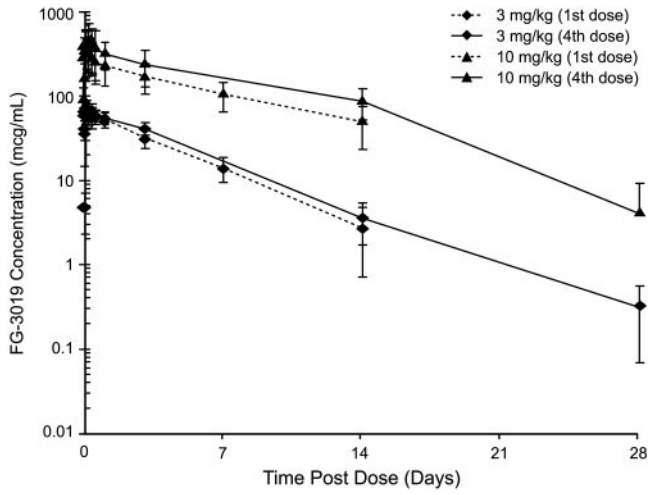

Figure 1. FG-3019 plasma concentration before and after the first infusion, showing no accumulation for 3 mg/kg ($n = 13$, first dose; $n = 9$, fourth dose), but slower clearance for 10 mg/kg ($n = 10$, first dose; $n = 7$ to 10, fourth dose) (mean ± SD; safety population). The slower clearance rate and longer $T_{1/2}$ with the higher dose suggests a saturable clearance pathway and higher drug exposure (steady-state area under the curve).

mained elevated over the 14-day period until the next infusion. Plasma N+W-CTGF had returned to levels within the normal range by 12 weeks after the final infusion in the 3-mg/kg cohort and by the end of the follow-up period (day 365) for the 10-mg/kg cohort. CTGF-whole was detectable in only a small subpopulation of patients in the 3-mg/kg cohort and was below quantitation (<3.8 ng/ml) in the 10-mg/kg cohort. Therefore, the transient increase of N+W-CTGF consisted entirely of increased CTGF N-fragment (Table 4), which is likely due to binding of CTGF N-fragment with FG-3019 in plasma. Urine N+W-CTGF was below quantitation (< 2.8 ng/ml) at all time points with the exception of one subject who had a detectable level of 3 ng/ml on day 14. CTGF-whole was not detected in any of the urine samples (data not shown).

Safety

Infusions were well tolerated with no interruptions for symptoms. Centers reported a total of 11 infusion-day adverse events possibly related to study drug in five subjects, including two headaches and one episode each of fatigue, sore throat, dizziness, flushing, anemia, anxiety, hyperglycemia, hypoglycemia, and increased lactate dehydrogenase. Overall, throughout the treatment period including the 2 weeks after the final infusion, 88% of subjects reported mild or moderate adverse events (Table 5). Adverse events did not increase in frequency with dose escalation, with the possible exception of two subjects with cold sores in the 10-mg/kg cohort. Two patients experienced serious adverse events after day 300 that were considered unrelated to study drug: One patient had severe gastroenteritis and the other had a stroke. One subject in the 3-mg/kg cohort had a transient AST/ALT rise. Another subject in the 3-mg/kg cohort experienced increases in AST and ALT levels from baseline levels of 15 and 18 U/L to 119 and 85 U/L, respectively, at 4 days after the first infusion (2.4- and 1.4-fold upper limit of normal, respectively). The values were improving on day 7, but the subject was then lost to follow-up; because of the temporal association, the liver enzyme elevations were considered possibly related to FG-3019, although recreational drug use was also suspected. No subject developed an antibody response to FG-3019.

Efficacy: Urinary Albumin Excretion Rate, Tubular Dysfunction, and eGFR

Mean urinary ACR tended to be decreased at the efficacy end point on day 56 (2 weeks after the final infusion) in both dose groups (Table 6). In the 3-mg/kg cohort, mean ACRs on days 56 and 84 were 29 (±15) and 45 (±28) mg/g, respectively, compared with 48 (±27) mg/g at baseline. Mean ACRs in the 10-mg/kg cohort on days 56 and 84 were 39 (±25) and 37 (±17) mg/g, respectively, as compared with 73 (±75) mg/g at baseline. Because differences between doses were not apparent, data from both dose groups were combined. Analysis of pooled data showed a significant average decline in mean ACR from baseline to day 56 of 27 (±49) mg/g ($P = 0.027$) (Table 6, Figure 2). The subjects with the most pronounced (>50%) decline in ACR ($n = 6$) tended to have higher baseline ACR than those remaining more stable. Their mean baseline ACR was 100 (±84) mg/g (range = 42 to 268 mg/g), which fell to 30 mg/g (±18) (range = 14 to 63 mg/g) on day 56. In contrast, subjects with <50% ACR decrease had a mean baseline ACR of only 43 (±30) mg/g (range = 13 to 117 mg/g), which had not changed significantly on day 56 (mean = 36 ± 23 mg/g; range = 9 to 84 mg/g). During the washout period from the last infusion on day 42 to day 84, urinary ACR returned to baseline in the 3-mg/kg cohort but remained low in the 10-mg/kg cohort.

Mean overnight urinary NAG concentrations remained consistent with baseline values with no apparent trend over the FG-3019 dosing period. Urine concentrations of β2M and α1M were variable over time with no apparent trend. Overnight urinary β2M/Cr showed a decline at day 56 relative to baseline (25 ± 50% decrease for all subjects; $P = 0.043$). Overnight NAG/Cr and α1M/Cr showed no trend over time.

Serum Cr and eGFR were stable in the efficacy population. Mean percent change in eGFR from a mean baseline value of 84 (±21) ml/min per 1.73 m^2 to day 56 was 3.6% ± 14%. However, the eGFR formula utilized is now recognized not to be highly precise in subjects with eGFRs in this range.

Additional Clinical Assessments

HbA1c was measured in 12 subjects (8 receiving FG-3019 at 10 mg/kg and 4 receiving FG-3019 at 3 mg/kg) and remained stable over time. The overall mean HbA1c values were 8.2% ± 1.57% at baseline and 8.3% ± 1.66% at day 56, reflecting a mean change of 0.1% ± 0.64%. For the safety population, average MAPs at baseline and day 56, respectively, were 92.6 (±6.8) mmHg and 88.2 (±11.2) mmHg for the 3-mg/kg cohort and 97.0 (±9.7) mmHg and 93.5 (±9.2) mmHg for the 10-mg/kg cohort. A meaningful difference in MAP after therapy between responders and nonresponders was not apparent.

Table 3. FG-3019 plasma concentration (safety population)

Mean (±SD)	Day	*n*	3 mg/kg	*n*	10 mg/kg
Cmax (μg/ml)	0	13	72.9 (±20.9)	10	419.8 (±211.4)
	42	9	76.0 (±11.1)	9	511.0 (±309.5)
Cmax (nM)	0	13	486 (±139)	10	2799 (±1409)
	42	9	507 (±74)	9	3407 (±2063)
Median Tmax (hours)	0	13	2.25	10	3.04
	42	9	4.12	9	6.00
$T_{1/2}$ (hours)	0	13	73.3 (±16.8)	10	141 (±25.3)
	42	9	102 (±17.2)	6	135 (±41.3)
CL (ml/h per kg)	0	13	0.43 (±0.11)	10	0.23 (±0.11)
	42	9	0.30 (±0.08)	9	0.17 (±0.07)
AUC_{Inf} (h·μg/ml)	0	13	7446 (±1718)	10	53,894 (±24,565)
AUCss (h·μg/ml)	42	9	11,648 (±4477)	9	79,995 (±33,891)

Cmax, maximum plasma concentration; Tmax, time to Cmax; $T_{1/2}$, half-life; CL, clearance; AUC, area under the curve; AUCss, steady-state AUC; AUC_{Inf}, AUC extrapolated to infinity.

Table 4. Plasma levels of CTGF-whole (W-CTGF assay) and CTGF-whole + CTGF N-fragment (N + W-CTGF assay) (efficacy population)

Levels	3 mg/kg *n* = 10	10 mg/kg *n* = 10
Plasma W-CTGF[a] (ng/ml)		
preinfusion	4.15	3.7
after infusion 1		
24 hours	3.89 (±0.5)	3.7
72 hours	3.7 (±0.3)	3.7
7 days	3.81 (±0.4)	3.7
day 56	4.27 (±1.7)	3.7
Plasma N+W-CTGF[b]		
preinfusion (ng/ml)	7.3 (±2.7)	12.2 (±3.2)
after infusion 1		
Tcmax[c]	3 days	7 days
Cmax (ng/ml)	372.1 (±135.4)	947.8 (±180.9)
Cmax (nM)[d]	19.6 (±7.1)	49.9 (±9.5)
FG-3019: N+W-CTGF molar ratio at Tcmax	11	14
after infusion 4		
Tcmax[c]	3 days	3 days
Cmax (ng/ml)	430.3 (±169.7)	1296.2 (±355.7)
Cmax (nM)[d]	22.6 (±8.9)	68.2 (±18.7)
FG-3019: N+W-CTGF molar ratio at Tcmax	12	23
day 56 (ng/ml)	58.6 (±60.1)	1010.2 (±415.2)

[a]The lower quantitation limit of the W-CTGF assay is 3.8 ng/ml; samples with concentration below the quantitation limit were assigned a value of 3.7 ng/ml.
[b]The lower quantitation limit of the N+W-CTGF assay is 3.8 ng/ml.
[c]Tcmax = the time point of maximum N+W-CTGF concentration after the first or fourth infusion.
[d]For calculation of the molar concentration, it was assumed that all of the measured N+W-CTGF was CTGF N-fragment.

Discussion

This is the first phase 1, open-label, dose-escalation trial of FG-3019, an investigational human monoclonal antibody to CTGF, in subjects with type 1 or 2 diabetes and microalbuminuria. Both doses of FG-3019 (3 and 10 mg/kg) were well tolerated during four infusions over 42 days.

Pharmacokinetic analysis indicated that drug clearance was saturable. Clearance was slower for the 10-mg/kg dose than for the 3-mg/kg dose and slower for the fourth dose than the first dose. These results suggest a high-affinity, low-capacity clearance mechanism such as binding to target CTGF, superimposed on the background low-affinity clearance of antibody. $T_{1/2}$ was approximately 5 to 6 days after four doses of FG-3019, which is shorter than the typical IgG $T_{1/2}$ of 10 to 14 days. This is

Table 5. Adverse events reported by two or more subjects through study day 56 (safety population)

Adverse Event	FG-3019 3 mg/kg (n = 14)	FG-3019 10 mg/kg (n = 10)	All (n = 24)
Overall	14 (100%)	7 (70%)	21 (88%)
Headache	3 (21%)	0	3 (13%)
AST increased	2 (14%)	0	2 (8%)
LDH increased	2 (14%)	0	2 (8%)
Herpes simplex	0	2 (20%)	2 (8%)
Fatigue	1 (7%)	1 (10%)	2 (8%)
Edema	1 (7%)	1 (10%)	2 (8%)
Excoriation	1 (7%)	1 (10%)	2 (8%)
Laceration	2 (14%)	0	2 (8%)
Pain in extremity	1 (7%)	1 (10%)	2 (8%)
Hyperglycemia	2 (14%)	0	2 (8%)
Anemia	1 (7%)	1 (10%)	2 (8%)
Anxiety	1 (7%)	1 (10%)	2 (8%)

Data presented as n (%). LDH, lactate dehydrogenase.

Table 6. Overnight microalbumin (nephelometry)/Cr ratios (mg/g) (mean ±SD) at days 0 (baseline), 56 (2 weeks after final infusion), and 84 (during washout period) (efficacy population)

Day	3 mg/kg (n = 9)			10 mg/kg (n = 10)			All (n = 19)		
	0	56	84	0	56	84[a]	0	56	84[a]
First morning urine ACR (mg/g)	48 (±27)	29 (±15)	45 (±28)	73 (±75)	39 (±25)	37 (±17)	61 (±57)	34 (±21)	41 (±23)
ΔACR to day 56	−66 to 10 (P = 0.052[b])			−205 to 10 (P = 0.124[b])			−205 to 10 (P = 0.027[b])		
Pt with ≥50% ↓ ACR	3 of 9			3 of 10			6 of 19		

[a] n = 1 fewer subject.
[b] P compared with baseline.

unlikely to reflect antigen-mediated clearance because the assay for FG-3019 measures free (not antigen-bound) antibody, and FG-3019 was present in significant molar excess over CTGF N-fragment bound to the antibody. FG-3019 was previously evaluated in an open-label, dose-escalation trial in subjects with moderately severe idiopathic pulmonary fibrosis, and the single-dose pharmacokinetic data were similar (45).

Although there are varying reports regarding association of urinary CTGF with DKD (32,46), the results reported here show that diabetic subjects with microalbuminuria did not have elevated urinary CTGF values. Plasma levels of CTGF-whole were low in these subjects, and changes could not be measured. Plasma levels of CTGF N-fragment significantly increased over a period of 3 to 7 days after each FG-3019 infusion, possibly reflecting slower clearance of the CTGF N-fragment-FG-3019 complex compared with the small (approximately 19 kD) uncomplexed CTGF N-fragment, which may be freely filtered by the kidney.

Our observations are subject to some limitations. This was an exploratory open-label study, underpowered for efficacy and subject to confounding effects that might affect apparent efficacy. Nevertheless, urine albumin excretion and tubular dysfunction as reflected by β2M/Cr decreased at the primary end point (day 56). These declines were not correlated with starting concentrations of CTGF, and there was no clear and consistent correlation between ACR response and starting concentrations of any other biomarker. Both dose groups had apparent responders, and although there was no obvious dose-response relationship, the duration of response trended longer in the 10-mg/kg cohort. Small MAP decrements of approximately 4 mmHg were noted in the 3- and 10-mg/kg dosing groups at day 56, and confounding by blood pressure cannot be completely excluded. However, no meaningful difference between responders and nonresponders in blood pressure or MAP after therapy was found.

Although an antibody to CTGF would be anticipated to modulate extracellular matrix synthesis, it may have additional biologic effects. FG-3019 may block CTGF-mediated effects on glomerular capillary permeability, possibly related to functional and structural changes in podocytes or tubular cells. Studies using rodent models of diabetes have demonstrated early upregulation of CTGF before TGFβ with CTGF expression

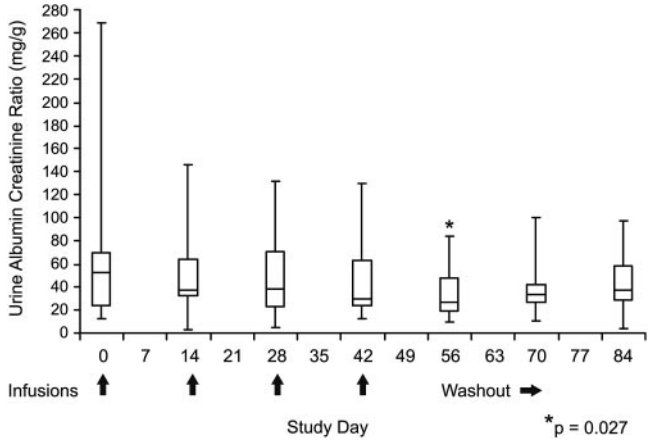

Figure 2. Urine ACR at baseline, at each infusion day, 14 days after the last infusion (day 56, designated primary outcome for efficacy), and during washout. There was a statistically significant decrement in urine ACR at day 56 compared with baseline ($P = 0.027$).

primarily in podocytes, (6) and the development of more severe DKD in mice overexpressing CTGF specifically in podocytes (47). Heterozygous CTGF knockout (±) mice developed less severe albuminuria and glomerular basement membrane thickening with diabetes (48), and a CTGF antisense oligonucleotide decreased albuminuria, mesangial hypertrophy, and the increased serum Cr in murine models (streptozotocin and *db/db*) of type 1 and 2 diabetes (49).

In conclusion, this open-label phase 1 trial showed that FG-3019, a human monoclonal antibody to CTGF, is well tolerated for at least 6 weeks in type 1 and type 2 diabetic subjects with microalbuminuria when administered by sequential intravenous infusion given every other week. Anti-FG-3019 antibodies were not detected in this study, which is consistent with other studies in which FG-3019 was infused. Preliminary efficacy data, to be interpreted with caution because of the uncontrolled open-label design, suggest a rapid and significant decline in urine ACR under treatment, particularly in subjects with higher baseline microalbuminuria, and warrant further clinical trials in subjects with more advanced DKD.

Acknowledgments

The authors thank George Coker, Ph.D., (former employee of FibroGen) for project oversight; Seth Porter, Ph.D., (FibroGen employee) for critical review, data analysis, and editing of manuscript; Ken Lipson, Ph.D., (FibroGen employee) and Roel Goldschmeding, MD, Ph.D., (former fellow at FibroGen) for critical review and editing of manuscript; and Laura Hansen, Ph.D., (FibroGen employee) for editorial assistance. The study results were originally published in abstract form: (a) Adler SG, Schwartz S, Williams ME, 3 Arauz-Pacheco C, Bolton WK, Lee T, Coker G, Sewell KL: Dose-escalation phase I study of FG-3019, anti-CTGF monoclonal antibody, in patients with type I/II diabetes mellitus and microalbuminuria [Abstract]. *J Am Soc Nephrol* 17: 157A, 2006; and (b) Schwartz S, Williams ME, Arauz C, Bolton WK, Lee T, Coker G, Sewell KL, Adler SG: Phase I study of FG-3019, an anti-CTGF monoclonal antibody, in type 1/2 diabetes mellitus with microalbuminuria [Abstract]. *Diabetes* 56: A151, 2007.

Disclosures

The authors' institutional or corporate grant offices received financial support from FibroGen, Inc. for clinical trial execution, and M.W. was a consultant for a FibroGen development meeting during the trial. T.L., D.L., and T.N. are employees of FibroGen, Inc. and therefore have direct financial relationships. P.U. and L.S. are former employees of FibroGen, Inc. This was an industry-sponsored study funded by FibroGen, Inc. Employees at FibroGen performed the analyses. Upon data sharing, the manuscript was written through a process of close collaboration of all of the cited authors.

References

1. U.S. Renal Data System: *USRDS 2006 Annual Data Report: Atlas of End-Stage Renal Disease in the United States*, Bethesda MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2006
2. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 865–869, 2001
3. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345: 851–860, 2001
4. Ruggenenti P, Pagano E, Tammuzzo L, Benini R, Garattini L, Remuzzi G: Ramipril prolongs life and is cost effective in chronic proteinuric nephropathies. *Kidney Int* 59: 286–294, 2001
5. Wahab NA, Schaefer L, Weston BS, Yiannikouris O, Wright A, Babelova A, Schaefer R, Mason RM: Glomerular expression of thrombospondin-1, TGF-beta and CTGF at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli. *Diabetalogica* 48: 2650–2660, 2005
6. Roestenberg P, van Nieuwenhoven FA, Joles JA, Trischberger C, Martens PP, Oliver N, Aten J, Hoppener JW, Goldschmeding R: Temporal expression profile and distribution pattern indicate a role of CTGF/CCN-2 in diabetic nephropathy in mice. *Am J Physiol Renal Physiol* 290: F1344–F1354, 2006
7. Keil A, Blom IE, Goldschmeding R, Rupprecht HD: Nitric oxide down-regulates CTGF in rat mesangial cells. *Kidney Int* 62: 401–411, 2002
8. Hishikawa K, Oemar BS, Nakaki T: Static pressure regulates CTGF expression in human mesangial cells. *J Biol Chem* 276: 16797–16803, 2001
9. Wahab NA, Weston BS, Mason RM: Modulation of the TGFbeta/Smad signaling pathway in mesangial cells by CTGF/CCN2. *Exp Cell Res* 307: 305–314, 2005
10. Han KH, Kang YS, Han SY, Jee YH, Lee MH, Han JY, Kim HK, Kim YS, Cha DR: Spironolactone ameliorates renal injury and CTGF expression in type II diabetic rats. *Kidney Int* 70: 111–120, 2006
11. Sohn M, Tan Y, Wang B, Klein RL, Trojanowska M, Jaffa AA: Mechanisms of LDL-induced expression of CTGF in human aortic endothelial cells. *Am J Physiol Heart Circ Physiol* 290: H1624–H1634, 2006
12. Finckenberg P, Inkinen K, Ahonen J, Merasto S, Louhelainen M, Vapaatalo H, Muller D, Ganten D, Luft F, Mervaala E: Angiotensin II induces CTGF gene expression via cal-

cineurin-dependent pathways. *Am J Pathol* 163: 355–366, 2003
13. Rodriguez-Vita J, Ruiz-Ortega M, Ruperez M, Esteban V, Sanchez-Lopez E, Plaza JJ, Egido J: Endothelin-1, via ETA and independently of TGFbeta, increases the CTGF in vascular smooth muscle cells. *Circ Res* 97: 125–134, 2005
14. Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, Ruperez M, Egido J, Ruiz-Ortega M: Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a TGFbeta-independent mechanism. *Circ Res* 111: 2509–2517, 2005
15. Igarashi A, Okochi H, Bradham DM, Grotendorst GR: Regulation of CTGF gene expression in human skin fibroblasts and during wound repair. *Mol Biol Cell* 4: 637–645, 1993
16. Graness A, Cicha I, Goppelt-Streube M: Contribution of Src-FAK signaling to the induction of connective tissue growth factor in renal fibroblasts. *Kidney Int* 69: 1341–1349, 2006
17. Lam S, van der Geest RN, Verhagen NA, Van Nieuwenhoven FA, Blom IE, Aten J, Goldschmeding R, Daha MR, van Kooten C: CTGF and IGF-I are produced by human renal fibroblasts and cooperate in the induction of collagen production by high glucose. *Diabetes* 52: 2975–2983, 2003
18. Burns WC, Twigg SM, Forbes JM, Pete J, Tikellis C, Thallas-Bonke V, Thomas MC, Cooper ME, Kantharidis P: CTGF plays an important role in advanced glycation end product-induced tubular epithelial-to-mesenchymal transition: Implications for diabetic renal disease. *J Am Soc Nephrol* 17: 2484–2494, 2006
19. Kobayashi T, Inoue T, Okada H, Kikuta T, Kanno Y, Nishida T, Takigawa M, Sugaya T, Suzuki H: CTGF mediates the profibrotic effects of TGF-beta produced by tubular epithelial cells in response to high glucose. *Clin Exp Nephrol* 9: 114–121, 2005
20. Twigg SM, Joly AH, Chen MM, Tsubaki J, Kim HS, Hwa V, Oh Y, Rosenfeld RG: CTGF/IGF-binding protein-related protein-2 is a mediator in the induction of fibronectin by advanced glycosylation end-products in human dermal fibroblasts. *Endocrinol* 143: 1260–1269, 2002
21. Chaqour B, Goppelt-Struebe M: Mechanical regulation of the Cyr61/CCN1 and CTGF/CCN2 proteins. Implications in mechanical stress-associated pathologies. *FEBS J* 273: 3639–3649, 2006
22. Liu BC, Sun J, Chen Q, Ma KL, Ruan XZ, Phillips AO: Role of CTGF in mediating hypertrophy of human proximal tubular cells induced by angiotensin II. *Am J Nephrol* 23: 429–437, 2003
23. Andersen S, van Nieuwenhoven FA, Tarnow L, Rossing P, Rossing K, Wieten L, Goldschmeding R, Parving HH: Reduction of urinary CTGF by Losartan in type 1 patients with diabetic nephropathy. *Kidney Int* 67: 2325–2329, 2005
24. Higgins DF, Biju MP, Akai Y, Wutz A, Johnson RS, Haase VH: Hypoxic induction of CTGF is directly mediated by Hif-1. *Am J Renal Physiol* 287: F1223–F1232, 2004
25. van Nieuwenhoven FA, Jensen LJN, Flyvbjerg A, Goldschmeding R: Imbalance of growth factor signalling in diabetic kidney disease: Is CTGF (CCN2) the perfect intervention point? *Nephrol Dial Transplant* 20: 6–10, 2005
26. Liu Y: Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int* 69: 213–217, 2006
27. Abreu JG, Ketpura NI, Reversade B, De Robertis EM: CTGF modulates cell signaling by BMP and TGF-beta. *Nat Cell Biol* 4: 599–604, 2002
28. Nguyen TQ, Roestenberg P, van Nieuwenhoven FA, Bovenschen N, Li Z, Xu L, Oliver N, Aten J, Joles JA, Vial C, Brandan E, Lyons KM, Goldschmeding R: CTGF inhibits BMP-7 signaling in diabetic nephropathy. *J Am Soc Nephrol* 19: 2098–2107, 2008
29. Hashimoto G, Inoki I, Fujii Y, Aoki T, Ikeda E, Okada Y: Matrix metalloproteinases cleave CTGF and reactivate angiogenic activity of vascular endothelial growth factor 165. *J Biol Chem* 277: 36288–36295, 2002
30. Inoki I, Shiomi T, Hashimoto G, Enomoto H, Nakamura H, Makino K, Ikeda E, Takata S, Kobayashi K, Okada Y: CTGF binds VEGF and inhibits VEGF-induced angiogenesis. *FASEB J* 16: 219–221, 2002
31. Gilbert RE, Akdeniz A, Weitz S, Usinger WR, Molineaux C, Jones SE, Langham RG, Jerums G: Urinary CTGF excretion in patients with type 1 diabetes and nephropathy. *Diabetes Care* 26: 2632–2636, 2003
32. Nguyen TQ, Tarnow L, Andersen S, Hovind P, Parving HH, Goldschmeding R, Van Nieuwenhoven FA: Urinary CTGF excretion correlates with clinical markers of renal disease in a large population of type I diabetic patients with diabetic nephropathy. *Diabetes Care* 29: 83–88, 2006
33. Roestenberg P, van Nieuwenhoven FA, Wieten L, Boer P, Diekman T, Tiller AM, Wiersinga WM, Oliver N, Usinger W, Weitz S, Schlingemann RO, Goldschmeding R: CTGF is increased in plasma of type 1 diabetic patients with nephropathy. *Diabetes Care* 27: 1164–1170, 2004
34. Jaffa AA, Weitz S, Zheng D, Usinger WR, Garvey WT: CTGF N fragment: A maker of progressive diabetic nephropathy [Abstract]. *J Am Soc Nephrol* 13: 249, 2002
35. Jaffa AA, Usinger WR, McHenry B, Jaffa MA, Lipstiz SR, Lackland D, Lopes-Virella M, Luttrell LM, Wilson PW: Connective tissue growth factor and susceptibility to renal and vascular disease risk in type 1 diabetes. *J Clin Endocrinol Metab* 93: 1893–1900, 2008
36. Nguyen TQ, Tarnow L, Jorsal A, Oliver N, Roestenberg P, Ito Y, Parving HH, Rossing P, Van Nieuwenhoven FA, Goldschmeding R: Plasma connective tissue growth factor is an independent predictor of end-stage renal disease and mortality in type 1 diabetic nephropathy. *Diabetes Care* 31: 1177–1182, Jun 2008
37. Adler SG, Kang SW, Feld S, Cha DR, Barba L, Striker L, Striker G, Riser BL, LaPage J, Nast CC: Glomerular mRNAs in human type 1 diabetes: Biochemical evidence for microalbuminuria as a manifestation of diabetic nephropathy. *Kidney Int* 60: 2330–2336, 2001
38. Adler SG, Kang SW, Feld S, Cha DR, Barba L, Striker L, Striker G, Riser BL, LaPage J, Nast CC: Can glomerular mRNAs in human type 1 diabetes be used to predict transition from normoalbuminuria to microalbuminuria? *Am J Kidney Dis* 40: 184–188, 2002
39. Ito Y, Aten J, Bende RJ, Oemar BS, Rabelink TJ, Weening JJ, Goldschmeding R: Expression of CTGF in human renal fibrosis. *Kidney Int* 53: 853–861, 1998
40. Flyvbjerg A, Khatir D, Jensen LJN, Lomongsod E, Liu DY, Rasch R, Usinger WR: Long-term renal effects of a neutralizing CTGF-antibody in obese type 2 diabetic mice [Abstract]. *J Am Soc Nephrol* 15: 261A, 2004
41. Langsetmo I, Jacob CT, Nichols B, Zhang W, Oliver N, Liu DY: Anti-CTGF therapy with FG-3019 prevents diabetes-induced cardiovascular complications in streptozotocin treated rats [Abstract]. *J Am Soc Nephrol* 16: 199A, 2005

42. Langsetmo I, Jacob CT, Zhang W, Oliver N, Lin A, Coker G, Liu DY: Anti-CTGF human antibody therapy with FG-3019 prevents and reverses diabetes-induced cardiovascular complications in streptozotocin treated rats. *Diabetes* 55[Suppl 1]: A122, 2006
43. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 130: 461–470, 1999
44. Rachfal AW, Brigstock DR: Structural and functional properties of CCN proteins. *Vitam Horm* 70: 69–103, 2005
45. Mageto Y, Flaherty K, Brown K, Fong A, Raghu G: Safety and tolerability of human monoclonal antibody FG-3019, anti-connective tissue growth factor, in patients with idiopathic pulmonary fibrosis [Abstract]. *Chest* 126: 7735-a, 2004
46. Tam FW, Riser BL, Meeran K, Rambow J, Pusey CD, Frankel AH: Urinary monocyte chemoattractant protein-1 (MCP-1) and connective tissue growth factor (CCN2) as prognostic markers for progression of diabetic nephropathy. *Cytokine* 47: 37–42, 2009
47. Yokoi H, Mukoyama M, Mori K, Kasahara M, Suganami T, Sawai K, Yoshioka T, Saito Y, Ogawa Y, Kuwabara T, Sugawara A, Nakao K: Overexpression of connective tissue growth factor in podocytes worsens diabetic nephropathy in mice. *Kidney Int* 73: 446–455, 2008
48. Yokoi H, Mukoyama M, Nagae T, Mori K, Suganami T, Sawai K, Yoshioka T, Koshikawa M, Nishida T, Takigawa M, Sugawara A, Nakao K: Reduction in connective tissue growth factor by antisense treatment meliorates renal tubulointerstitial fibrosis. *J Am Soc Nephrol* 15: 1430–1440, 2004
49. Guha M, Xu ZG, Tung D, Lanting L, Natarajan R: Specific down- regulation of CTGF attenuates progression of nephropathy in mouse models of type 1 and type 2 diabetes. *FASEB J* 21: 3355–3368, 2007