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A phase 1, single-dose study of fresolimumab, an anti-TGF-β antibody, in treatment-resistant primary focal segmental glomerulosclerosis

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Primary focal segmental glomerulosclerosis (FSGS) is a disease with poor prognosis and high unmet therapeutic need. Here, we evaluated the safety and pharmacokinetics of single-dose infusions of fresolimumab, a human monoclonal antibody that inactivates all forms of transforming growth factor-β (TGF-β), in a phase I open-label, dose-ranging study. Patients with biopsy-confirmed, treatment-resistant, primary FSGS with a minimum estimated glomerular filtration rate (eGFR) of 25 ml/min per 1.73 m^2, and a urine protein to creatinine ratio over 1.8 mg/mg were eligible. All 16 patients completed the study in which each received one of four single-dose levels of fresolimumab (up to 4 mg/kg) and was followed for 112 days. Fresolimumab was well tolerated with pustular rash the only adverse event in two patients. One patient was diagnosed with a histologically confirmed primitive neuroectodermal tumor 2 years after fresolimumab treatment. Consistent with treatment-resistant FSGS, there was a slight decline in eGFR (median decline baseline to final of 5.85 ml/min per 1.73 m^2). Proteinuria fluctuated during the study with the median decline from baseline to final in urine protein to creatinine ratio of 1.2 mg/mg with all three Black patients having a mean decline of 3.6 mg/mg. The half-life of fresolimumab was ~14 days, and the mean dose-normalized Cmax and area under the curve were independent of dose. Thus, single-dose fresolimumab was well tolerated in patients with primary resistant FSGS. Additional evaluation in a larger dose-ranging study is necessary.

Kidney International (2011) **79,** 1236–1243; doi:10.1038/ki.2011.33; published online 2 March 2011

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Received 8 October 2010; revised 20 December 2010; accepted 4 January 2011; published online 2 March 2011

KEYWORDS: clinical study; fresolimumab; FSGS; TGF-β antagonism

Focal segmental glomerulosclerosis (FSGS) is a clinical entity that impacts renal function through progressive fibrosis of the glomerulus. FSGS is not a single disease; rather, it is a heterogeneous clinicopathological process identified in renal biopsies that are generally performed in patients with proteinuria or nephrotic syndrome. Idiopathic or primary FSGS has a distinctive histopathological appearance, characterized by hyalinization and sclerosis of a portion of the glomerular tuft, minimal deposition of immune complexes, and effacement of visceral epithelial cell (podocyte) foot processes. Idiopathic FSGS can be distinguished from secondary causes of FSGS (for example, genetic mutations, medications, infections, reflux nephropathy, or surgical reduction in renal mass) by more widespread podocyte effacement together with the presence of nephrotic syndrome.[1] The implication for making the distinctions is that primary FSGS may respond to corticosteroids or other immunosuppression therapy, whereas secondary forms are treated by addressing the underlying cause. Regardless of the etiology of FSGS, the majority of cases are characterized by progressive renal fibrosis and steady deterioration in kidney function.[2–4]

In FSGS, the beneficial effects of angiotensin-converting enzyme inhibitor treatment on proteinuria and preservation of renal function have led to their widespread use in this population.[5–12] High-dose and/or prolonged oral corticosteroid therapy has been the primary treatment for FSGS and a treatment response indicates a better long-term outcome in a small percentage of patients.[13,14] Cyclosporine is the only agent that has been documented to be efficacious for reduction of proteinuria in controlled trials in both children and adults with steroid-resistant FSGS.[15–18] There are also uncontrolled studies suggesting that tacrolimus, sirolimus,

mycophenolate mofetil, or rituximab may induce remission in patients with primary FSGS.[19–25] However, in the majority of primary FSGS patients, treatment with steroids and other therapeutic interventions result in only transient improvement in proteinuria and does not alter disease course.[26] This may be especially true in patients with genetic mutations leading to FSGS.[1,27] Because of the poor prognosis of and the lack of a proven treatment for patients with steroid-resistant FSGS, there is a pressing need for new treatments that can reduce proteinuria and slow down or stop the progression of renal damage.

Several lines of evidence point toward an important role of transforming growth factor-β (TGF-β) in disease pathogenesis and progression of primary FSGS. TGF-β is well known to be a modulator of extracellular matrix production and is associated with interstitial fibrosis in renal disease.[28] More recently, TGF-β has been recognized as a key regulator of the glomerular visceral epithelial cell, or podocyte, a pivotal cell in the pathogenesis of FSGS. Overexpression of TGF-β in podocytes leads to podocytopenia and glomerulosclerosis.[29] In cultured podocytes, TGF-β influences cell survival[29–32] and induces changes in the cytoskeleton and cell adhesion that are analogous to *in vivo* foot process effacement.[33] TGF-β also activates several signaling pathways, including the Smad cascade, that have demonstrated roles in glomerular pathogenesis in animal models.[34,35] Both FSGS patients and experimental animal models demonstrate increased expression of TGF-β in the kidney and increased urinary excretion of the growth factor.[36] Biopsies of FSGS patients reveal increased immunostaining for TGF-β in glomerular endothelial cells.[37] Therefore, these findings suggest that modulation of TGF-β activity within the kidney, with consequent effects on key cell components of the glomerulus and signaling molecules, may be renoprotective and have a beneficial effect on the severity or progression of FSGS.

One strategy for altering TGF-β is by antagonism with a monoclonal antibody. Fresolimumab, a member of the G4 immunoglobulin (IgG4) subclass, is an engineered human monoclonal antibody that neutralizes all three isoforms of TGF-β. This IgG subclass does not activate the complement pathway, a potential favorable feature of the antibody. Data from diverse animal models demonstrate that neutralization of TGF-β can inhibit tissue fibrosis.[38] For example, therapeutic administration of a mouse analog of fresolimumab (1D11) to a murine model of chronic cyclosporine nephropathy reduced collagen deposition, epithelial cell apoptosis, and normalized tissue hypoxia.[39] 1D11 has also been shown to preserve glomerular selectivity and prevent ultrastructural changes to the glomerular filtration barrier during hypertension.[40] In a model of diabetic nephropathy, administration of 1D11 combined with enalapril was antihypertensive, antiproteinuric, reduced glomerulosclerosis, and preserved podocyte number.[41] These results provide evidence that TGF-β antagonism is effective in preventing and reducing the structural and functional consequences of chronic renal injury.

The primary objectives of this phase I clinical trial in patients with treatment-resistant primary FSGS and nephrotic-range proteinuria were to determine: (1) the safety and tolerability of single-dose infusions of fresolimumab; and (2) the pharmacokinetics of fresolimumab following single-dose infusions of fresolimumab. The secondary objective was to obtain preliminary data about the effect of single-dose infusions of fresolimumab on proteinuria and kidney function.

RESULTS

Patients

All 16 patients who were enrolled completed the study, 4 at each dose level. Of the 16 patients, 9 (4 patients in the 1 mg/kg group, 2 patients in the 2 mg/kg group, and 3 patients in the 4 mg/kg group) had detectable levels of fresolimumab at day 112. They returned for follow-up visits until fresolimumab was no longer detectable in the blood. The longest duration of additional follow-up after day 112 was 141 days.

The mean age of the patients was 37 ± 12 years, mean FSGS duration was 3.0 ± 2.1 years, half were male, 13 were White, and 3 were Black. Overall, the baseline characteristics of the patients were similar between dose groups (Table 1 and Supplementary Table S1 online).

At the time of enrollment, 15 out of 16 (94%) patients were on a concomitant medication. The most commonly prescribed drugs were agents acting on the renin-angiotensin system in 14 cases. A total of 12 subjects were receiving a lipid-lowering agent, 11 were given a diuretic, and 4 were receiving aspirin. The use of these agents was comparable in the four patient cohorts.

Safety results

Fresolimumab was well tolerated at single doses up to the maximum level of 4 mg/kg in patients with FSGS. No patient withdrew consent or discontinued participation before completing the study. No profound immunologic or systemic inflammatory reactions were seen in any patient. The DMC (Data Monitoring Committee) recommended continued dosing following each dosing cohort.

Infusion-associated reactions, defined as events that occurred within 24 h of infusion and assessed by the investigator as related to fresolimumab, were noted in only one patient who had a cough during infusion of the antibody.

The frequencies of reported treatment-emergent adverse events (TEAEs) were similar across the dose groups. Of the 16 patients, 15 (93.8%) reported a total of 73 TEAEs. The most frequent TEAEs reported were peripheral edema (4 patients) and nasopharyngitis (3 patients); all other TEAEs were reported by $\leqslant 2$ 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). Overall, 7 patients (43.8%) experienced at least 1 TEAE that was considered by the investigator to be related to the study drug (Table 2). Pustular rash, reported by 2 (12.5%) patients, was the most frequently reported related TEAE. All other

Table 1 | Patient demographics in patients receiving fresolimumab by dose

	0.3 mg/kg (*N*=4)	1 mg/kg (*N*=4)	2 mg/kg (*N*=4)	4 mg/kg (*N*=4)	Total (*N*=16)
Age (years), mean ± s.d.	33.0 ± 12.4	33.8 ± 14.3	44.3 ± 6.7	38.5 ± 13.4	37.4 ± 11.8
Male gender, *n* (%)	1 (25.0)	3 (75.0)	1 (25.0)	3 (75.0)	8 (50.0)
Race, n *(%)*					
Black	0	2 (50.0)	1 (25.0)	0	3 (18.8)
White	4 (100.0)	2 (50.0)	3 (75.0)	4 (100.0)	13 (81.3)
Duration since FSGS diagnosis (years), mean ± s.d.	3.9 ± 3.4	1.8 ± 1.5	3.2 ± 1.2	3.0 ± 1.6	3.0 ± 2.1
Baseline Up/c ratio (mg/mmol), median	845.0	666.1	376.2	713.5	736.5
Baseline eGFR (ml/min per 1.73 m^2), median	36.2	38.8	39.3	62.4	38.6

Abbreviations: eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; Up/c, urine protein : creatinine ratio.
To convert mg/mmol to mg/mg, divide by 113.11.

Table 2 | Treatment-related adverse events

System organ class/preferred term	0.3 mg/kg (*N*=4), *n* (%)	1 mg/kg (*N*=4), *n* (%)	2 mg/kg (*N*=4), *n* (%)	4 mg/kg (*N*=4), *n* (%)	Total (*N*=16), *n* (%)
Any related adverse event (AE)	1 (25)	3 (75)	1 (25)	2 (50)	7 (43.8)
Gastrointestinal disorders	0	0	1 (25)	1 (25)	2 (12.5)
Gingival bleeding	0	0	1 (25)	0	1 (6.3)
Vomiting	0	0	0	1 (25)	1 (6.3)
Infections and infestations	0	0	0	2 (50)	2 (12.5)
Rash pustular	0	0	0	2 (50)	2 (12.5)
Musculoskeletal and connective tissue disorders	1 (25)	0	0	0	1 (6.3)
Growing pains	1 (25)	0	0	0	1 (6.3)
Renal and urinary disorders	0	1 (25)	0	0	1 (6.3)
Renal impairment	0	1 (25)	0	0	1 (6.3)
Respiratory, thoracic and mediastinal disorders	0	1 (25)	0	0	1 (6.3)
Cough	0	1 (25)	0	0	1 (6.3)
Skin and subcutaneous tissue disorders	0	1 (25)	0	1 (25)	2 (12.5)
Pruritus	0	0	0	1 (25)	1 (6.3)
Vitiligo	0	1 (25)	0	0	1 (6.3)

If a patient had more than one event for a particular system organ class or preferred term, he/she is counted only once for that system organ class of preferred term.

drug-related events were single occurrences experienced by individual patients. All related TEAEs resolved by the end of the patient's participation in the study. No patients experienced a serious TEAE or died during the study. Following completion of the study, 1 patient was diagnosed with a histologically confirmed primitive neuroectodermal tumor 2 years posttreatment.

No pattern in differences between dose cohorts was observed for vital signs, physical examination findings, gingival examination findings, electrocardiograph findings, or renal function. Changes from baseline in safety laboratory parameters fluctuated over the course of the study and no consistent differences between dose cohorts were noted for laboratory evaluation results.

In three patients (two patients in the 1 mg/kg group and one in the 2 mg/kg group), the anti-fresolimumab antibody assay showed positive results when fresolimumab was no longer detectable in the blood. No clinical consequences were noted in the patients with detectable antibody response to fresolimumab.

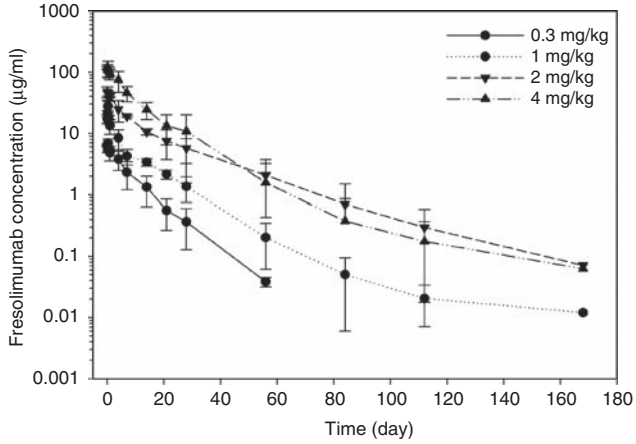

Figure 1 | Mean serum fresolimumab concentration over time.

Pharmacokinetics

Mean serum concentrations of fresolimumab over time are presented by dose in Figure 1. The pharmacokinetics of

Table 3 | Pharmacokinetic parameters after a single dose of fresolimumab

Dose (mg/kg)	C_{max} (μg/ml)	C_{max}/dose (μg/ml/mg)	AUC (ng*h/ml)	AUC/dose (ng*h/ml/mg)	$T_{1/2}\alpha$ (day)	$T_{1/2}\beta$ (day)	CL (ml/h)	Vss (ml)
0.3	5.70 ± 1.85	0.31 ± 0.11	1162 ± 492	63.9 ± 27.2	2.73 ± 1.15	10.9 ± 5.1	19.7 ± 13.0	4.48 ± 2.14
1	19.3 ± 3.59	0.27 ± 0.05	3484 ± 405	48.3 ± 9.0	2.73 ± 1.93	17.0 ± 8.7	21.1 ± 3.9	6.65 ± 2.06
2	47.5 ± 5.54	0.32 ± 0.03	13,016 ± 2814	88.1 ± 16.0	2.61 ± 1.73	17.7 ± 3.7	11.6 ± 2.2	5.17 ± 1.47
4	114 ± 16.3	0.37 ± 0.02	26,175 ± 7964	82.9 ± 19.7	2.92 ± 1.79	34.6 ± 47.7[a]	12.6 ± 2.8	3.56 ± 0.60

Abbreviations: AUC, area under the curve; CL, clearance; C_{max}, maximum concentration; $T_{1/2}\alpha$, distribution half-life; $T_{1/2}\beta$, elimination half-life; Vss, volume of distribution at steady state.
[a]Value appears to be elevated because of one patient. Median half-life was 12.5 days.
Values are presented as mean ± s.d.

Table 4 | Median change from baseline to final study visit in estimated glomerular filtration rate (eGFR), urine protein/creatinine ratio, urine albumin/creatinine ratio, serum creatinine, and serum albumin

	0.3 mg/kg (*N*=4)	1 mg/kg (*N*=4)	2 mg/kg (*N*=4)	4 mg/kg (*N*=4)	Total (*N*=16)
eGFR (ml/min per 1.73 m^2)	−5.65	−6.25	−3.05	−9.25	−5.85
Urine protein/creatinine ratio (mg/mmol)	−140.1	−331.2	77.4	−126.5	−133.3
Urine albumin/creatinine ratio (mg/mmol)	−93.8	−101.9	105.2	−122.3	−57.8
Serum creatinine (μmol/l)	7.0	23.5	16.0	27.0	17.5
Serum albumin (g/l)	0.0	1.5	2.0	2.0	1.5

To convert mg/mmol to mg/mg, divide by 113.11.

fresolimumab were best described using a two-compartment linear model. There was no difference in the pharmacokinetic behavior of fresolimumab in the FSGS cohort compared with the results observed in two other disease populations (idiopathic pulmonary fibrosis and advanced malignancy).[42,43] Patient weight was the only significant covariate identified as being predictive of pharmacokinetic variability. Derived pharmacokinetic parameters for fresolimumab in FSGS patients are presented in Table 3. The half-life of fresolimumab in this patient population was ~14 days. Values of mean dose-normalized C_{max} and area under the curve values ranged from 0.27 to 0.37 μg/ml/mg and from 48.3 to 88.1 ng*h/ml/mg, respectively, and appeared to be independent of dose (Table 3).

Efficacy measures

Proteinuria fluctuated during the study period and the median change from baseline to the final study visit in urine protein : creatinine ratio (PCR) for entire patient cohort was −133.31 mg/mmol (−1.2 mg/mg; Table 4). A graphic illustration of the time course of the response for each cohort is available as Figure 2. A clinically significant improvement in proteinuria was observed in the three Black patients (two from the fresolimumab 1 mg/kg cohort and one from the 2 mg/kg cohort). These three individuals demonstrated a mean decrease of 65% in urine PCR levels at the final study visit compared with baseline (Table 5).

The overall trend was for a slight decline in estimated glomerular filtration rate (eGFR): the median change from baseline at the final study visit was −5.85 ml/min per 1.73 m^2 (annualized: −19.0 ml/min per 1.73 m^2) with no dose-related differences. For the other efficacy measures assessed, results tended to fluctuate over the course of the study with no treatment-related changes noted (Table 4).

DISCUSSION

This report describes a phase 1, open-label study conducted in patients with treatment-resistant primary FSGS that was designed to assess the safety, tolerability, and pharmacokinetics of single-dose infusions of fresolimumab. The study agent was safely administered to four patients in each of four dose cohorts: 0.3, 1, 2 and 4 mg/kg body weight. All 16 patients who received a single dose of the antibody completed the study.

This trial represents the first description of the use of an anti-TGF-β antibody that has appeared in a peer-reviewed publication. It is important to emphasize that this phase I study involved patients with fairly advanced disease based on their resistance to previous treatments and the low eGFR criterion for eligibility. Thus, like patients with oncological diseases, the expectation of therapeutic benefit from a single dose of the anti-TGF-β antibody was low. The primary objective was to evaluate the safety and pharmacokinetics of fresolimumab. The assessment of clinical response was a secondary objective and the findings in this regard should be considered preliminary and proof-of-concept only.

There were no complications related to the infusion or that occurred within the 24-h observation period after administration of the antibody. Reporting of long-term adverse events and related adverse events was similar across the dose cohorts. All adverse events except one were of mild or moderate intensity. No patients experienced a serious TEAE, discontinued study participation because of an adverse event, or died during the study. The overall safety profile in these 16 patients can be added to the over 50 patients who have received single or multiple doses of fresolimumab in clinical trials of patients with idiopathic pulmonary fibrosis ($n=25$) and cancer ($n=29$) (refs 42, 43). Overall, no consistent differences between dose cohorts were noted

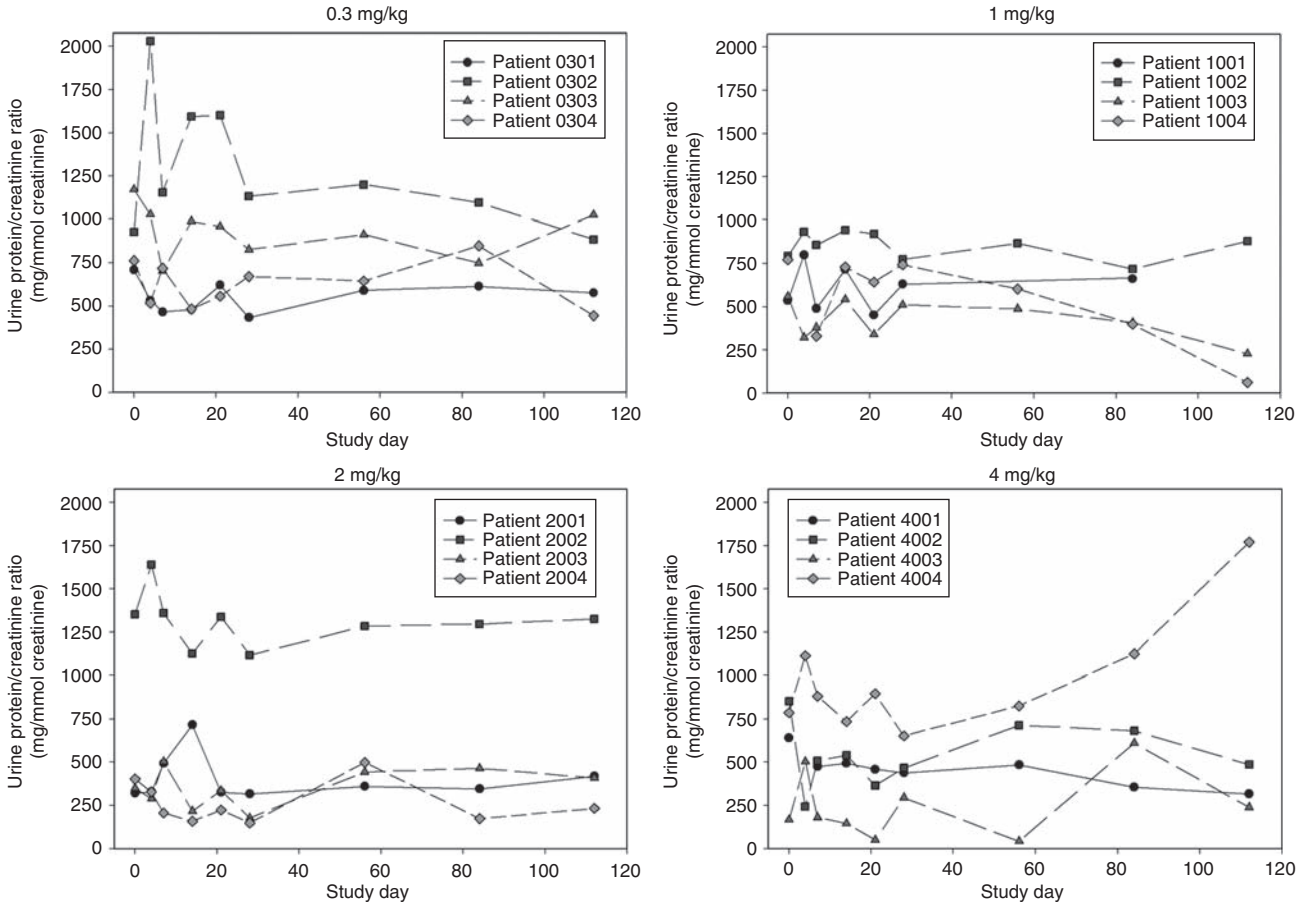

Figure 2 | Urine protein/creatinine ratio by cohort over time.

Table 5 | Urine protein to creatinine ratio for patients with a reduction from baseline

Patient	1003	1004	2004
Dose	1 mg/kg	1 mg/kg	2 mg/kg
Race	Black	Black	Black
Preinfusion (mg/mmol)	559.28	772.81	402.52
End of study (mg/mmol)	228.12	61.75	231.97

To convert mg/mmol to mg/mg, divide by 113.11.

for laboratory evaluation results, vital signs, physical examination including gingival examination findings, or electrocardiograph findings. There was one patient who was diagnosed with a primitive neuroectodermal tumor 2 years after receiving the single 1 mg/kg dose of fresolimumab. He was in complete remission from his FSGS, his kidney function was unaltered from the baseline, and fresolimumab was undetectable in his serum for >1 year before the diagnosis of the malignancy. In a repeat-dose oncology study with fresolimumab, squamous cell skin cancer was reported in two patients with advanced malignant melanoma who received multiple doses every 2 weeks (up to 15 mg/kg) of fresolimumab.[43] Although it is unlikely that fresolimumab was directly linked to the primitive neuroectodermal tumor, monitoring for both expected and unanticipated side effects, including infection, autoimmunity, and malignancy, should be incorporated into future clinical studies with this agent.

The pharmacokinetics of fresolimumab observed in this study were similar to that observed in studies of fresolimumab in patients with idiopathic pulmonary fibrosis and advanced malignancy. C_{max} and area under the curve were dose proportional over the range of doses tested, indicating that fresolimumab exhibits linear kinetics and that drug clearance was not saturable. The elimination half-life was ~14 days in patients with FSGS. This pharmacokinetic profile is consistent with the pharmacokinetics of monoclonal antibodies that neutralize soluble antigens (that is, cytokines).[44] The dosing interval to be used in future studies will be dependant on both the pharmacokinetic profile and the pharmacological activity of fresolimumab. A dosing interval of at least 2 weeks is expected to result in minimal accumulation of the antibody.

Exploratory clinical outcomes were evaluated; however, assessment of efficacy in treatment-resistant primary FSGS was not a primary objective of this phase I study. Moreover, the results are limited because of the small sample size, lack of a placebo-controlled comparison group, and a single-dose treatment design. Despite these limitations, the PCR declined in three patients. These patients, who were the only Blacks

enrolled in the study, demonstrated a clinically meaningful decrease in urine PCR levels. In one case, the patient achieved nearly a complete remission (PCR <0.3), in the second, there was nearly a partial remission (50% reduction in PCR to a level <2), and the third had a substantial 42% lowering in PCR. No other routine clinical or laboratory feature discriminated between those who had a reduction in proteinuria and those who manifested no short-term response to the experimental intervention. The finding is encouraging because reduction of proteinuria is critical to a patient's clinical course. Complete remission of primary FSGS is clearly linked to a favorable outcome, even if patients have a subsequent relapse.[26] Nonetheless, intermediate reductions in urine protein excretion are also associated with improved patient outcomes. This beneficial impact of treatments that reduce but do not normalize proteinuria has been documented in children and adults with FSGS and other glomerulopathies.[45–48] However, caution is advisable in interpreting the clinical findings in this small patient cohort.

The overall trend observed in GFR values in this study cohort was slightly downward, although sporadic, variable, and not dose related. This finding is consistent with that expected over a 4-month time period in patients with resistant FSGS.[47–48] There was no evidence that administration of the anti-TGF-β antibody accelerated the downward course of the disease. For the other clinical outcomes assessed, results tended to fluctuate over the course of the study with no treatment-related changes noted.

In summary, the results of this phase I clinical trial indicate that fresolimumab, administered as a single-dose infusion up to 4 mg/kg, is safe and well tolerated in patients with treatment-resistant primary FSGS. This provides justification for moving forward with larger randomized clinical trials to assess efficacy of this agent in patients with primary FSGS who are resistant to corticosteroids and other immunosuppressive agents.

PATIENTS AND METHODS

Patient selection

Patients with treatment-resistant primary FSGS aged $\geqslant 18$ years at nine centers in the United States, Germany, Italy, and the United Kingdom received a single fresolimumab infusion between May 2007 and August 2009. Patients with FSGS who in the investigator's opinion represented primary disease with eGFR $\geqslant 25$ ml/min per 1.73 m^2 and persistent proteinuria (urine PCR >200 mg/mmol (1.8 mg/mg)) and who had demonstrated resistance to steroid or other immunosuppressive agent were eligible for enrollment in the study, indicating that the patients were at high risk of progression to end-stage kidney disease. The patients did not have to manifest clinical evidence of nephrotic syndrome, namely edema, to qualify for the study. Biopsy slides were reviewed centrally to confirm the diagnosis and they were classified according to the Columbia scheme.[49] Secondary FSGS was excluded based on this internationally accepted FSGS classification. Patients were excluded from the study if they had clinical evidence of secondary FSGS including diabetes type 2, segmental hypoplasia, oligomeganephronia, unilateral renal agenesis, renal dysplasia, cortical necrosis, surgical renal ablation, chronic allograft nephropathy glomerulonephritis, atheroemboli or other vaso-occlusive processes, cyanotic congenital heart disease, sickle cell anemia, hypertension, infection, morbid obesity, reflux nephropathy, lymphomas, or other malignancies; sarcoidosis, radiation therapy, membranous nephropathy or IgA nephropathy viral infections (including hepatitis B, human immunodeficiency virus and parvovirus B19) or drug induced (for example, heroin, interferon A, lithium, pamidronate), current or history of any form of skin cancer; a history of other cancers or a known precancerous state; pre-existing oral-pharyngeal disease; a bleeding diathesis; any clinically significant medical condition including congestive heart failure, hepatic, or gastrointestinal disease; or were pregnant or lactating women.

The protocol and informed consent were reviewed and approved by an independent ethics committee at each participating site. All patients provided written, informed consent before the initiation of any study-related activities. This research was carried out in accordance with Good Clinical Practice guidelines and applicable regulations.

Study design

This was a phase 1, open-label, single-dose, dose-ranging study. Eligible patients were enrolled and allocated to one of four dosing cohorts. The original protocol specified that patients were to receive 1, 2, 4, or 8 mg/kg. However, based on preliminary results suggesting a response in patients who received the lowest dose (1 mg/kg), the dose range was changed to 0.3, 1, 2, and 4 mg/kg of fresolimumab to identify a minimal noneffective dose. As 0.3 mg/kg was lower than the starting dose of 1 mg/kg, the 0.3 and 4 mg/kg groups were run concurrently. Patients were randomized to either 0.3 or 4 mg/kg to mitigate any potential for bias.

In each cohort, patients received a single dose of fresolimumab infused over 30 min. Actual body weight was used to calculate the dose for patients with body mass index <30 kg/m^2. For patients with body mass index $\geqslant 30$ kg/m^2, ideal body weight calculation was used. Patients were followed periodically for 112 days (days 4, 7, 14, 21, 28, 56, 84, and 112) for safety evaluations, pharmacokinetics, and clinical outcome and biomarker assessments. Any patient whose blood level of fresolimumab was still detectable at study completion was to return every 4 weeks until no further fresolimumab was detected.

An independent DMC reviewed study safety data after each cohort in order to make a recommendation on continued dosing.

This clinical study (GC1008FSGS00505) was funded by Genzyme Corporation. The protocol was drafted by the sponsor, Genzyme, in conjunction with the team of investigators. The sponsor performed the data and analysis and the investigators reviewed and verified the results. The first draft of the manuscript was written by the sponsor together with the lead academic author. It was edited by all of

the coauthors, who vouch for the completeness of the data and the accuracy of the analysis. The decision to submit the manuscript represents a consensus of all representatives from the sponsor and the academic authors. The authors acknowledge Rasha Aguzzi, Sara Engstrand, Antony Jack, Joan Mannick, and Melissa Plone of Genzyme Corporation for assistance in study planning, implementation, and analysis and interpretation of the data.

Biochemical measurements

Fresolimumab concentrations were determined using a validated solid-phase enzyme-linked immunosorbent assay with colorimetric detection.

To detect antifresolimumab antibodies, serum samples were initially screened in a bridging enzyme-linked immunosorbent assay in a 1:30 final dilution. Samples with a result above the limit of detection were then titred in a confirmatory binding inhibition assay. Analyses for antifresolimumab antibodies were only conducted on serum samples without detectable fresolimumab, as it interferes with the immunogenicity assay.

Statistical analyses

Sample size. No formal sample size calculations were done for this study. The sample size was selected to allow for adequate assessment of pharmacokinetics at each dose level, and also to allow for an initial assessment of safety in this population and dose range.

Safety assessments. Adverse events were summarized by dose group. Changes in vital signs, weight, electrocardiograph, development of antibodies to fresolimumab, and shifts in laboratory assessments and physical examinations including gingival examinations, were assessed.

Pharmacokinetics. Pharmacokinetic data from the present trial were pooled with data from additional phase 1 trials conducted in patients with idiopathic pulmonary fibrosis and advanced malignancy[46,47] and analyzed using nonlinear mixed effect modeling methods. A population pharmacokinetic model that adequately characterizes the data was selected based on model development and was parameterized accordingly. Covariate models were tested to evaluate the effect of patient characteristics (for example, weight, age, gender, and disease state) on the pharmacokinetics of fresolimumab. Values for derived pharmacokinetic parameters, including C_{max}, area under the curve, distribution half-life ($T_{1/2}\alpha$), elimination half-life ($T_{1/2}\beta$), clearance (CL), and volume of distribution at steady state (Vss), were calculated for patients with FSGS using the final pharmacokinetic model and summarized by dose group using descriptive statistics.

Efficacy measures. Changes from baseline in urine PCR, eGFR, albumin/creatinine ratio, serum creatine, and albumin were summarized for each dose.

DISCLOSURE

JPWH, JS, MLH, and SRL are employees of Genzyme Corporation.

ACKNOWLEDGMENTS

The principal investigators who participated in this study included LC Rump, Düsseldorf, Germany; P Heering, Solingen, Germany; DRW Jayne, Cambridge, UK; H Peters, Berlin, Germany; N Perico, Ranica Bergamo, Italy; G Remuzzi, Bergamo, Italy; DS Gipson, Chapel Hill, NC, USA; H Trachtman, New Hyde Park, NY, USA; F Vincenti, San Francisco, CA, USA; FC Fervenza, Rochester, MN, USA. The members of the DMC included David Oliveira, London, UK (Chair); Neil Turner, Edinburgh, Scotland; Michael Robson, London, United Kingdom. We are grateful to the study coordinators and participating clinical units for their clinical, administrative, and organizational involvement; and especially the FSGS patients who graciously gave their time and energy on behalf of the study. This clinical study (GC1008FSGS00505) was funded by Genzyme Corporation.

SUPPLEMENTARY MATERIAL

Table S1. Clinical, laboratory, and histopathology data in patients receiving fresolimumab.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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