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A Phase 2, Double-Blind, Placebo-Controlled, Randomized Study of Fresolimumab in Patients With Steroid-Resistant Primary Focal Segmental Glomerulosclerosis

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Introduction: Steroid-resistant focal segmental glomerulosclerosis (SR-FSGS) is a common glomerulopathy associated with nephrotic range proteinuria. Treatment goals are reduction in proteinuria, which can delay end-stage renal disease.

Methods: Patients with SR-FSGS were enrolled in a randomized, double-blind placebo-controlled trial of fresolimumab, a monoclonal anti–transforming growth factor–β antibody, at 1 mg/kg or 4 mg/kg for 112 days, followed double-blind for 252 days (NCT01665391). The primary efficacy endpoint was the percentage of patients achieving partial (50% reduction) or complete (< 300 mg/g Cr) remission of proteinuria.

Results: Of 36 enrolled patients, 10, 14, and 12 patients received placebo, fresolimumab 1 mg/kg, and fresolimumab 4 mg/kg, respectively. The baseline estimated glomerular filtration rate (eGFR) and urinary protein/creatinine ratio were 63 ml/min/1.73 m^2 and 6190 mg/g, respectively. The study was closed before reaching its target of 88 randomized patients. None of the prespecified efficacy endpoints for proteinuria reduction were achieved; however, at day 112, the mean percent change in urinary protein/creatinine ratio (a secondary efficacy endpoint) was –18.5% ($P = 0.008$), +10.5% ($P = 0.52$), and +9.0% ($P = 0.91$) in patients treated with fresolimumab 1 mg/kg, fresolimumab 4 mg/kg, and placebo, respectively. There was a nonsignificant trend toward greater estimated glomerular filtration rate decline in the placebo group compared to either of the fresolimumab-treated arms up to day 252.

Discussion: The study was underpowered and did not meet the primary or secondary endpoints. However, fresolimumab was well tolerated and is appropriate for continued evaluation in larger studies with adequate power.

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Primary focal segmental glomerulosclerosis (FSGS) is a major cause of nephrotic syndrome in adults worldwide,[1] with a profound negative impact on quality of life, morbidity, and mortality. Nephrotic syndrome, which may be severe, can be both debilitating and associated with complications, including

anasarca, cardiovascular, and thromboembolic events. If untreated, FSGS typically results in a relatively rapid decline in renal function and end-stage renal disease (ESRD), with a need for either dialysis or transplantation in the majority of patients.

The incidence of FSGS is reported as being higher, and the rate of renal survival as being worse, in African Americans when compared with non−African Americans.[1] This risk has been linked to 2 allelic variants in the APOL1 gene, namely *APOL1 G1* and *APOL1 G2*, carried by patients of African but not European descent.[2] Irrespective of the racial predilection for FSGS, however, no proven treatment exists for FSGS, with failure to respond to empiric therapy with steroids or calcineurin inhibitors (CNIs) portending progression to ESRD and renal replacement therapy. To date, remission of proteinuria is the only factor that predicts delayed progression to ESRD.[3]

CNIs are used off-label as an alternative or second-line therapy to corticosteroids in FSGS patients[4] and in patients with steroid-resistant idiopathic nephrotic syndrome.[5] However, many patients fail to respond to CNIs, and the use of these agents is associated with a high relapse rate.[6] As such, there is a high unmet medical need for new therapies that can induce sustained remission of proteinuria in FSGS and slow progression to ESRD.[2,7–11]

Transforming growth factor−β (TGF-β) is a cytokine involved in normal homeostasis[12]; however, sustained overproduction of TGF-β has been implicated in the pathogenesis of fibrosis in many animal models,[13] and in humans with fibrotic kidney diseases, including FSGS.[14–16] Preclinical models of FSGS have demonstrated prevention of proteinuria and glomerular pathology, as well as reduction in proteinuria, with inhibition of TGF-β.[17] To this end, fresolimumab, an engineered human monoclonal Ig that neutralizes the 3 major isoforms of TGF-β, namely, β1, β2, and β3, was developed with the aim to slow and potentially reverse fibrosis. Thus, fresolimumab may represent a new therapy with a novel mechanism of action for fibrotic kidney disease, including primary FSGS.

The purpose of this clinical study was to evaluate the safety and efficacy of fresolimumab in patients with steroid-resistant FSGS (NCT01665391).

MATERIALS AND METHODS

Trial Design

This was a phase 2, multicenter, double-blind, parallel-dosing, randomized study of 4 i.v. infusions of fresolimumab or placebo on days 1, 28, 56, and 84 in patients with steroid-resistant FSGS. The study was divided into 3 periods: screening (visit 1, up to 6 weeks prior to day 1/visit 2); treatment period (day 1/visit 2, day 28/visit 3, day 56/visit 4, day 84/visit 5, and day 112/visit 6); and follow-up period (day 140/visit 7, day 168/visit 8, and day 252/visit 9). A patient was considered to have completed the treatment period when assessments scheduled for day 112 had been completed. Fresolimumab doses of 1 mg/kg and 4 mg/kg were selected to assess dose–response and dose–safety relationships in these patients, with 4 mg/kg selected as the highest dose, as it was the highest dose used in the phase 1 FSGS study that was expected to represent a pharmacodynamically active dose.[18] The dosing interval of 28 days was selected based on the ∼14-day terminal elimination half-life of fresolimumab seen in the phase 1 study to prevent significant accumulation while providing sustained exposure to the study drug.[18]

Randomization and Masking

At day 1, eligible patients who met all inclusion and exclusion criteria were randomly assigned, stratified by race (black versus nonblack) and prior CNI therapy (yes, no), to 1 of 3 treatment groups in a 3:3:2 allocation: fresolimumab 1 mg/kg total body weight; fresolimumab 4 mg/kg total body weight; or placebo, delivered via 30-minute i.v. infusions at 4-week intervals for a total of 4 doses. Patients were allowed treatment with immunosuppressive agents after day 112 at the clinicians' discretion and then were followed up for a total of 252 days, with patients and investigators remaining blinded to treatment assignment.

Ethical Considerations

The protocol complied with recommendations of the 18th World Health Congress (Helsinki, 1964) and all applicable amendments, and with the laws and regulations, as well as any applicable guidelines, of the countries in which the study was conducted. Informed consent was obtained prior to the conduct of any study-related procedures. The patient informed consent form was modified according to local regulations and requirements, and the protocol and consent forms were reviewed and approved by independent ethics committees and/or the institutional review board at each participating site. This research was carried out in approximately 40 centers in 5 countries in accordance with Good Clinical Practice guidelines and applicable regulations.

Inclusion and Exclusion Criteria

Inclusion and key exclusion criteria are listed in Table 1.

Table 1. Study inclusion and exclusion criteria

Inclusion criteria
Willing and able to provide signed informed consent
Age ≥18 yr
Renal biopsy results showing any histologic subtype of primary FSGS confirmed by a central renal pathologist
Estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73 m^2
Urinary total protein:creatinine ratio ≥ 3 mg protein/mg creatinine in ≥ 1 of the urine samples and an average urinary total protein: creatinine ratio ≥ 2 mg protein/mg creatinine in 2 samples collected at screening
Steroid-resistant FSGS, defined as lack of proteinuria response after treatment with a course of high-dose steroid therapy for ≥4 wk or clinically documented to be intolerant to steroids prior to the screening visit
Treated with angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers at a stable dose for ≥4 wk prior to visit 2 (treatment start) unless intolerant or contraindicated.
Key exclusion criteria
Prednisone use at a dose > 10 mg/d (or equivalent dose of an alternative glucocorticoid) within 4 wk
Any systemically administered nonglucocorticoid immunosuppressive drugs within 8 wk
Rituximab administration within 6 mo prior
History of systemic autoimmune disease such as lupus erythematosus or rheumatoid arthritis requiring systemic immunosuppressive therapy
History of organ transplantation
HIV, hepatitis B, or hepatitis C
Active infection
Patients currently pregnant or lactating
Unstable angina or myocardial infarction within 3 months
Known history of cancer or precancerous condition within 5 yr, other than curatively treated cervical intraepithelial neoplasia
Abnormal laboratory results of (i) hemoglobin < 9.0 g/dl, (ii) total bilirubin > 1.5 × upper limit of normal (ULN), (iii) ALT > 2.5 × ULN or (iv) AST > 2.5 × ULN
Active bleeding not due to a benign, self-limited cause
Any clinically significant unstable medical condition or other condition which, in the Study Investigator's opinion, prohibited the patient's participation in the study
Having received investigational drug administered as part of a clinical trial within 30 days

Outcome Measures

Efficacy

The prespecified per protocol primary efficacy endpoint was the percentage of patients in each treatment group versus the placebo group who achieved proteinuria remission, either a partial remission or a complete remission, at the conclusion of the treatment period (week 16/day 112/visit 6). Partial remission was defined as a ≥ 50% decline in urinary protein/creatinine ratio (Up/c ratio) compared to baseline to a level between ≥ 0.3 and ≤ 3.0 mg protein/mg creatinine. Complete remission was defined as a decline in Up/c ratio to a level less than 0.3 mg protein/mg creatinine. Because this trial was concluded before the original target of 88 randomized patients was reached, a *post hoc* definition of a "durable" clinical response was defined as (i) ≥ 2 partial remission events, (ii) 1 partial remission event with proteinuria remaining at ∼ 50% of baseline level, or (iii) a marked and consistently steep decline in Up/c over time.

Secondary efficacy endpoints were as follows: percentage of patients in each treatment group versus patients in the placebo group achieving complete remission in Up/c ratio at day 112/early termination (ET); percentage of patients in each treatment group versus patients in the placebo group achieving partial remission in Up/c ratio at day 112/ET; change in Up/c ratio and urinary protein excretion rate from baseline to day 112/ET; time to first partial remission or complete remission; change in estimated glomerular filtration rate (eGFR) from baseline to day 112/ET; and percentage of patients in each fresolimumab treatment group versus patients in the placebo group achieving partial remission or complete remission with stable eGFR (defined as < 35% reduction in eGFR) at day 112.

The exploratory efficacy endpoints were change in body weight, serum lipids (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides), and serum albumin from baseline to day 112/ET; change in blood and urine biomarkers from baseline to day 112/ET; and patient-reported outcome (PRO) questionnaire responses. The Kidney Disease Quality of Life Short Form (KDQOL-SF)[19] was assessed on day 1, day 56, day 112/ET, and day 252. The KDQOL-SF includes 43 kidney disease-targeted items as well as 36 items as the generic core. The scales (parameters) were summarized descriptively at each visit, and the responses had precoded numeric values.

Safety

Adverse events (AEs), serious adverse events (SAEs), and medical events of interest (MEOI), including herpes zoster, treatment-emergent skin lesions, cancers, bleeding events, and other events deemed of interest, were recorded, as well as changes in blood chemistry, hematology, and urinalysis assessments, vital signs (including temperature, heart rate, and blood pressure), and physical and skin examination findings. In addition, periodic skin examinations were to be conducted in all patients receiving fresolimumab, which would be discontinued in patients with a biopsy-confirmed diagnosis of skin cancer. Development of anti-fresolimumab antibodies was evaluated at day 252/visit 9. The Data Safety Monitoring Board (DSMB) meetings were held approximately every 3 months from the first patient randomized to the final dataset review.

Pharmacokinetics

Serum concentrations were summarized by treatment group using descriptive statistics.

Statistical Analysis

The study was originally planned to randomize 88 patients (33 fresolimumab 1 mg/kg patients, 33 fresolimumab 4 mg/kg patients, and 22 placebo patients), but was prematurely concluded at 36 randomized

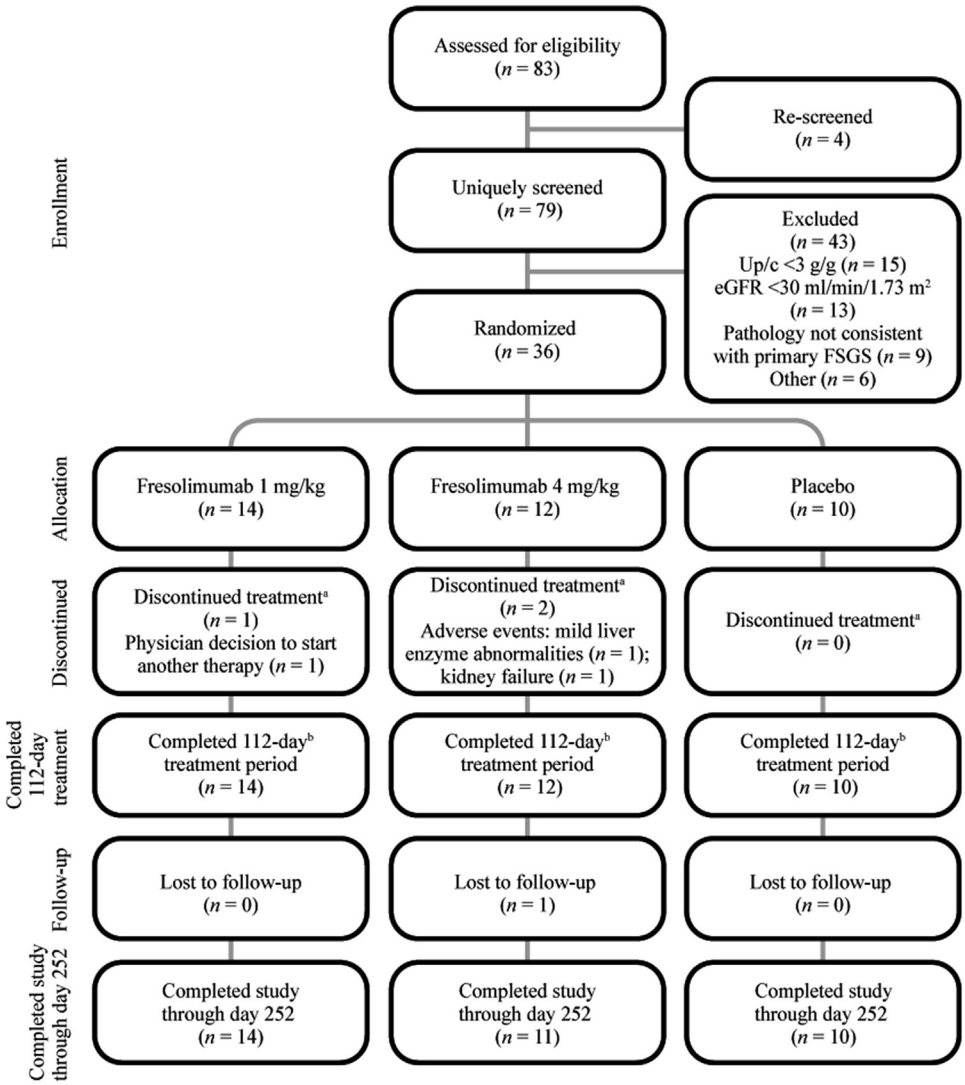
patients due to investigational drug expiration on 30 April 2014; consequently, a *post hoc* definition of a "durable" clinical response was defined as (i) ≥ 2 partial remission events, (ii) 1 partial remission event with proteinuria remaining at ~ 50% of baseline level, or (iii) a marked and consistently steep decline in Up/c over time (seen only in 1 patient in the 1 mg/kg fresolimumab arm: (patient number 4, a < 25 year-old black woman with collapsing variant; Supplementary Figure S1). No formal statistical analysis was performed regarding race/ethnicity or APOL1 status and clinical proteinuria response, because this was not in the original statistical analysis plan and also because the samples sizes were too small for a meaningful comparison. No statistical comparisons were performed for baseline characteristics for this randomized study. For some analyses, baseline eGFR was included as a covariate along with other baseline characteristics (see Supplementary Materials for further details).

RESULTS

A total of 36 patients with treatment-resistant FSGS were randomized to treatment with fresolimumab 1 mg/kg (n = 14), fresolimumab 4 mg/kg (n = 12), or placebo (n = 10) (Figure 1). All patients were included in the full analysis set. Demographic and clinical characteristics as well as baseline proteinuria and kidney function based on eGFR are shown in Table 2.

Renal History and Renal Biopsy Histology

The median time from initial diagnostic kidney biopsy to entry into the study was 3.0 years (range 0.1–16.8 years).



[a]Patients who "Discontinued treatment" but were not withdrawn from the study had their AEs recorded until they completed the study.

[b]Completed the study through day 112/visit 6 (primary efficacy assessment for proteinuria remission). A patient was considered as "Completed 112-day treatment period" when the assessments scheduled for the day 112 visit had been completed.

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram.

Table 2. Summary of demographic and baseline characteristics

Characteristic	Fresolimumab 1 mg/kg (n = 14)	Fresolimumab 4 mg/kg (n = 12)	Placebo (n = 10)	All randomized patients (n = 36)
Age (yr)	50.9 (22.7, 76.8)	38.1 (23.1, 64.8)	42.7 (19.2, 75.6)	41.4 (19.2, 76.8)
Male sex	7 (50%)	6 (50%)	6 (60%)	19 (52·8%)
Race/ethnicity				
White	12 (85.7%)	10 (83.3%)	8 (80%)	30 (83.3%)
Black	2 (14.3%)	2 (16.7%)	2 (20%)	6 (16.7%)
Ethnicity				
Hispanic or Latino	6 (42.9%)	2 (16.7%)	3 (30%)	11 (30.6%)
Not Hispanic or Latino	8 (57.1%)	10 (83.3%)	7 (70%)	25 (69.4%)
Weight (kg)	78.4 (55, 128.9)	75.0 (55, 114.8)	88.6 (58.1, 122.5)	77.4 (55, 128.9)
Height (cm)	164 (152, 185)	168.5 (157, 188)	174 (157, 187)	168 (152, 188)
BMI (kg/m^2)	29.4 (19.5, 50.4)	25.1 (21.2, 46.6)	29.5 (17.5, 43.7)	27.7 (17.5, 50.4)
Hypertension	9 (64.3%)	8 (66.7%)	7 (70%)	24 (66.7%)
History of thrombosis	3 (21.4%)	0 (0%)	0 (0%)	3 (8.3%)
Family history of renal disease	5 (35.7%)	2 (16.7%)	3 (30%)	10 (27.8%)
Time since FSGS diagnosis, yr	2.9 (0.2, 16.8)	3.02 (0.1, 12.2)	3.38 (0.1, 13.9)	3.02 (0.1, 16.8)
Renal function decline since FSGS diagnosis (yes)[a]	4 (28.6%)	5 (41.7%)	4 (40.0%)	13 (36.1%)
FSGS histologic subtypes				
NOS	8 (57.1%)	4 (33.3%)	6 (60%)	18 (50%)
Cellular	0 (0%)	2 (16.7%)	0 (0%)	2 (5.6%)
Collapsing	2 (14.3%)	3 (25%)	1 (10%)	6 (16.7%)
Perihilar	1 (7.1%)	0 (0%)	1 (10%)	2 (5.6%)
Tip	2 (14.3%)	3 (25%)	2 (20%)	7 (19.4%)
None[b]	1 (7.1%)	0 (0%)	0 (0%)	1 (2.8%)
Risk haplotype				
APOL1 G1 carrier	1 (7.1%)	0 (0%)	0 (0%)	1 (2.8%)
APOL1 homozygous G1	1 (7.1%)	0 (0%)	0 (0%)	1 (2.8%)
APOL1 G1/G2 carrier	0 (0%)	1 (8.3%)	1 (10.0%)	2 (5.6%)
APOL1 G2 carrier	0 (0%)	1 (8.3%)	0 (0%)	1 (2.8%)
No genetic testing	1 (7.1%)	0 (0%)	0 (0%)	1 (2.8%)
Negative	11 (78.5%)	10 (83.3%)	9 (90%)	30 (83.3%)
Patients with prior CNI therapy[c] (reported in eCRF)	8 (57.1%)	9 (75%)	9 (90%)	26 (72.2%)
Patients with prior CNI therapy[b] (used for stratification in IVRS)	6 (42.9%)	5 (41.7%)	4 (40%)	15 (41.7%)
Prior nonsteroid immunosuppressant treatments				
Cyclosporine	7 (50%)	7 (58.3%)	7 (70%)	21 (58.3%)
Tacrolimus	3 (21.4%)	6 (50%)	4 (40%)	13 (36.1%)
Mycophenolate (mofetil or acid)	4 (28.6%)	7 (58.3%)	4 (40%)	15 (41.7%)
Adalimumab (no. of patients)	0 (0%)	1 (8.3%)	0 (0%)	1 (2.8%)
Azathioprine (no. of patients)	0 (0%)	1 (8.3%)	0 (0%)	1 (2.8%)
Cyclophosphamide (no. of patients)	0 (0%)	2 (16.7%)	3 (30%)	5 (13.9%)
Rituximab (no. of patients)	0 (0%)	2 (16.7%)	2 (20%)	4 (11.1%)
Screening eGFR ($ml/min/1.73\ m^2$)	58 (32, 103)	68 (36, 146)	60 (33, 159)	63 (32,159)
Baseline (visit 2) Up/c ratio (mg protein/mg Cr)	5.92 (2.6, 17.3)	6.46 (1.3, 15.9)	6.41 (2.2, 13.7)	6.19 (1.3, 17.3)

CNI, calcineurin inhibitor; eCRF, electronic case report form; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; IVRS, interactive voice randomization system; NOS, not otherwise specified; Up/c ratio, urinary protein/creatinine ratio.
Results presented as median (minimum, maximum) or n (%). Some patients received multiple treatments, so percentages exceed 100% as well as total number of patients with prior CNI therapy.
[a]Investigators chose a "yes" or "no" response for each study subject in response to this question about renal functional decline since FSGS diagnosis.
[b]One patient was initially not considered to have primary FSGS due to some glomerular basement membrane abnormalities seen, but further discussion with clinician and central pathologist concluded that the clinical presentation was consistent with sudden onset nephrotic syndrome (not explained by basement membrane findings) and primary FSGS could not be definitively ruled out and patient was enrolled into the study; variant of FSGS lesions seen on biopsy were noted to be NOS.
[c]Previous CNI therapy status as recorded in the eCRF has been determined to be accurate and correlated with previous CNI medication use listed in past medications. BMI, body mass index.

Approximately 72% of patients had previously received CNIs for their FSGS disease. Prior medication use was similar across dose cohorts. Most patients had received multiple courses of different types of immunosuppressive medications in addition to high-dose corticosteroid therapy for ≥4 weeks as per study inclusion criteria; approximately two-thirds of treatments were reported as resulting in "no response," with the remainder being recorded as transient and/or partial responses (Supplementary Table S1).

Adherence to Treatment

The majority of patients (92%) completed all 4 study infusions, and no patients discontinued from the study during the treatment period (Figure 1).

One patient in the fresolimumab 1 mg/kg arm completed 3 infusions; 1 patient in the fresolimumab 4 mg/kg arm completed 3 infusions; and 1 patient in the fresolimumab 4 mg/kg arm completed 2 infusions. Reasons for not completing all study treatments included physician decision (to start another therapy) for the patient in the fresolimumab 1 mg/kg arm, and adverse events (AEs) of mild liver enzyme abnormalities and kidney failure (both assessed as not related to treatment) in the 2 patients in the fresolimumab 4 mg/kg arm. All 36 patients completed the 112-day treatment period. One patient in the fresolimumab 4 mg/kg arm did not complete the study and was lost to follow-up prior to conclusion of the study on day 252.

Primary Endpoint

Primary Efficacy Through Day 112/ET (Visit 6)

At day 112/visit 6, 2 of 14 patients receiving fresolimumab 1 mg/kg met the primary efficacy endpoint of partial remission ($P = 0.493$ vs. placebo [0%]) in contrast to 0 of 12 patients receiving fresolimumab 4 mg/kg. A total of 5 unique study patients (4 patients in the fresolimumab 1 mg/kg arm and 1 in the placebo arm) achieved partial remission at 1 or more visits between day 28/visit 3 and day 112/visit 6. No patients in the fresolimumab 4 mg/kg arm experienced a partial remission or complete remission.

At the day 112/ET (visit 6) time point, stratified analysis by race (black versus nonblack) or by previous CNI treatment did not reveal different responses to therapy in any of the treatment arms. On kidney biopsy, the 4 responders in the fresolimumab 1 mg/kg arm had FSGS NOS (n = 2) or collapsing variant (n = 2), whereas the 1 patient who met partial remission criteria in the placebo group had collapsing variant (n = 1).

Primary Efficacy Through Day 252/Visit 9

Because this study was prematurely concluded at 36 randomized patients instead of the target 88 patients, a definition of a "durable" clinical response was applied *post hoc* during patient-level analyses of the data (Supplementary Table S1; Supplementary Figures S1–S4). This definition of durable clinical response was applied based on the fact that proteinuria relapse is associated with a more rapid rate of renal function decline and worse renal survival.[20]

In examining the period between day 112 and day 252, 3 of 14 patients in the 1 mg/kg fresolimumab arm, 3 of 12 patients in the 4 mg/kg arm, and 2 of 10 patients in the placebo arm received immunosuppressive medications to treat proteinuria. As seen in Supplementary Figures S1 to S4, almost all of the patients receiving immunosuppression (represented by the dotted lines) did not have a response in decreased proteinuria to immunosuppression.

Considering only those patients who did not receive other additional immunosuppressant medications that may have lowered proteinuria, 4 of 14 patients (29%) in the 1 mg/kg treatment arm and 2 of 12 patients (17%) in the 4 mg/kg arm had a "durable" clinical response. In contrast, only 1 of 10 patients (10%) in the placebo arm (patient number P7) had partial remission events at visit 4 and visit 9 but otherwise maintained proteinuria near baseline levels.

Notably, all patients who had a "durable" clinical response were either black or Hispanic. There were a total of 6 black FSGS patients in the study, 2 assigned to each treatment arm. APOL-1 G1 and G2 haplotypes, which have been associated with increased risk for FSGS in persons of African descent,[8] were present in 6 study patients (5 black patients and 1 nonblack Hispanic patient). Among those patients who had a "durable" clinical response to fresolimumab therapy (either 1 mg/kg or 4 mg/kg), 1 patient was homozygous for G1 (patient number 1mg-13), 1 patient was a G1 carrier (patient number 1mg-6), 1 patient was a G2 carrier (patient number 4mg-3), and 1 patient was a G1/G2 carrier (patient number 4mg-2) (Supplementary Table S1, Supplementary Figures S1 and S4). One black patient (patient number 1mg-4) with a "durable" clinical response did not consent to genotyping data.

Besides the frequency of black and Hispanic patients, no clear trends in demographic or clinical characteristics were noted. Of the 6 patients with "durable" clinical response, 4 had previously received CNI therapy, all with either no response or transient partial remission in proteinuria reduction (Supplementary Table S1).

In examining the proportion of patients in each treatment arm who did not receive other immunosuppressive medications, which may have affected proteinuria and achievement of partial remission or complete remission, a statistically nonsignificant trend in those in the 1 mg/kg fresolimumab arm achieving partial remission was observed ($P = 0.224$ for the 1 mg/kg arm vs. placebo). No statistically significant differences between treatments in Kaplan–Meier or Cox proportional hazards analyses of time to remission event were noted (data not shown).

Secondary Endpoints

Percent Change in Up/c

Up through day 112/visit 6, the estimated mean percent change in Up/c from baseline to day 112/visit

6 was −18.5% (SE = 12.11; $P = 0.008$), 10.5% (SE = 13.06), and 9.0% (SE = 14.37) in the fresolimumab 1 mg/kg, fresolimumab 4 mg/kg, and placebo arms, respectively (Figure 2a). No statistically significant change was seen in any of the treatment arms through day 252/visit 9, but this was confounded by use of other immunosuppressant medications after day 112.

Percent Change in eGFR Over Time

Up through day 252/visit 9, the placebo arm showed a trend of declining eGFR including separation with nonoverlapping SE bars at day 140/visit 7 (Figure 2b) compared with either of the fresolimumab-treated arms, but P values were not statistically significant.

Exploratory Efficacy Endpoints

Up through day 112/visit 6, there was no clinically meaningful difference among the 3 treatment groups in any of the exploratory efficacy endpoints, including change in total cholesterol, serum albumin, body weight, or quality-of-life measures based on KDQOL-SF PRO questionnaire, either between baseline and day 112/visit 6 or over time.

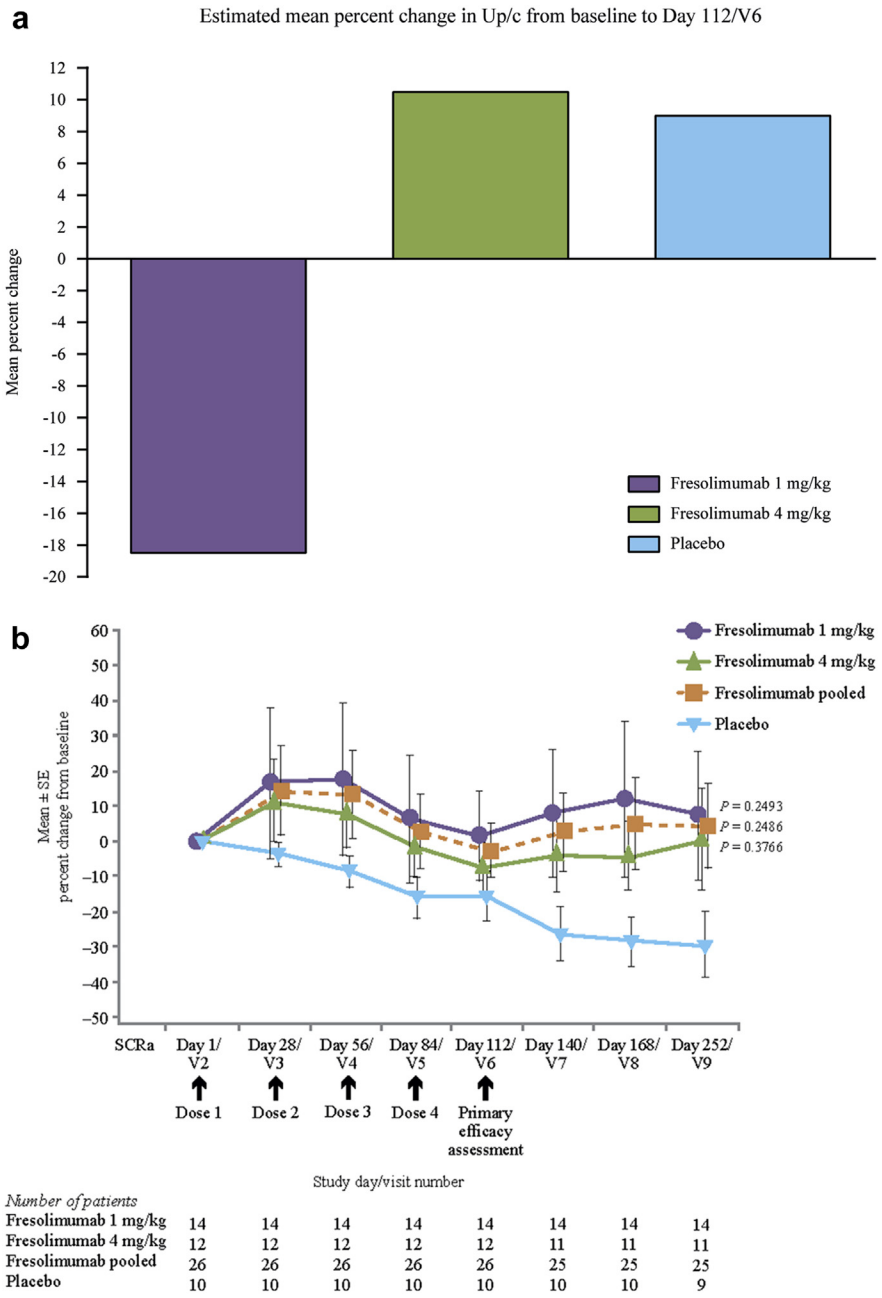


Figure 2. (a) Estimated mean percent change in urinary protein/creatinine ratio (Up/c) from baseline to day 112/visit 6 by treatment assignment. (b) Mean (± SE) percent change from baseline in estimated glomerular filtration rate (eGFR) over time by treatment assignment.

Similarly, up through day 252/visit 9, there were no clinically or statistically meaningful differences between the exploratory efficacy endpoints listed above among the 3 treatment arms.

Adverse Events

A summary of treatment-emergent adverse event (TEAEs), including those occurring in 3 or more patients, regardless of relationship to study drug, is shown in Table 3. Of the 36 patients treated in this study, 75% of patients experienced at least 1 TEAE. The majority (90%) of TEAEs in all treatment arms were nonserious and either mild or moderate in severity. The majority of the TEAEs were considered to be unrelated to the study drug.

There were no deaths in the study. SAEs were reported in 4 patients (11.1%): no patients (0.0%) in the fresolimumab 1 mg/kg arm, 3 patients (25.0%) in the fresolimumab 4 mg/kg arm, and 1 patient (10.0%) in the placebo group. No treatment-emergent SAEs were considered by the investigator as related to the study drug. Two patients, both in the 4 mg/kg arm, discontinued study treatment due to AEs during the 112-day treatment period; however, both AEs were assessed as not related to the study drug by the investigator. There were no drug-related or protocol-related SAEs reported during the follow-up period.

Table 3. Overview of patients with treatment-emergent adverse events

	Fresolimumab 1 mg/kg (n = 14)		Fresolimumab 4 mg/kg (n = 12)		Placebo (n = 10)	
AE category	Patients n (%)	Events n	Patients n (%)	Events n	Patients n (%)	Events n
Any TEAE	9 (64.3%)	39	11 (91.7%)	61	7 (70%)	33
Drug-related TEAEs	6 (42.9%)	15	5 (41.7)	13	2 (20%)	3
Protocol-related TEAEs	1 (7.1%)	2	0 (0%)	0	0 (0%)	0
Mild TEAEs	8 (57.1%)	37	4 (33.3%)	40	4 (40.0%)	19
Moderate TEAEs	1 (7.1%)	2	5 (41.7%)	11	2 (20%)	11
Severe TEAEs	0 (0%)	0	2 (16.7%)	10	1 (10%)	3
MEOIs	4 (28.6%)	9	4 (33.3%)	12	2 (20%)	2
Serious TEAEs	0 (0%)	0	3 (25%)	8	1 (10%)	5
Related serious TEAEs	0 (0%)	0	0 (0%)	0	0 (0%)	0
Deaths	0 (0%)	0	0 (0%)	0	0 (0%)	0
TEAEs leading to study drug discontinuation	0 (0%)	0	2 (16.7%)	2	0 (0%)	0
TEAEs occurring in ≥3 patients regardless of relationship to study drug						
Headache	3 (21.4%)		2 (16.7%)		1 (10.0%)	
Back pain	2 (14.3%)		1 (8.3%)		1 (10.0%)	
Gingival bleeding	2 (14.3%)[a]		1 (8.3%)		0 (0%)	
Pruritus	0 (0%)		1 (8.3%)		2 (20.0%)	
Rash	0 (0%)		2 (16.7%)		1 (10.0%)	
Upper RTI	1 (7.1%)		1 (8.3%)		1 (10.0%)	

AE, adverse event; MEOI, medical event of interest; RTI, respiratory tract infection; TEAE, treatment-emergent adverse event.
Note: Related TEAEs are defined as possibly related or related; not related TEAEs are defined as not related or unlikely related.
[a]One patient reported 1 event of gingival bleeding in the follow-up period.

Only 1, nonserious MEOI was reported during the follow-up period, in the 1 mg/kg group, namely, gingival bleeding of mild severity, assessed as possibly related to the study drug. Keratoacanthoma (KA) was observed in 1 patient in the 4 mg/kg arm; this event resolved spontaneously. No significant difference was noted in the adverse drug reactions reported between the 1 mg/kg and 4 mg/kg arms.

There were no consistent trends noted in laboratory values to suggest any study drug-related abnormalities. There were also no clinically significant findings or trends in vital signs.

Pharmacokinetic Results

Postinfusion serum levels of fresolimumab revealed approximate dose-proportionality from 1 mg/kg to 4 mg/kg (Supplementary Figure S5). There was no appreciable drug accumulation with repeated dosing at both dose groups (data not shown).

DISCUSSION

This report describes the results of a phase 2, multicenter, double-blinded, parallel dosing, randomized study of fresolimumab or placebo in patients with steroid-resistant primary SR-FSGS. Although the predefined primary and secondary efficacy endpoints were not met, there were 4 patients in the 1 mg/kg fresolimumab arm who had a "durable" clinical response (defined as part of a *post hoc* analysis), including a total of 7 partial remission events in 3 patients over the entire study period. In addition, there were 3 partial remission events in 2 patients assigned the 4 mg/kg fresolimumab arm versus only 1 patient in placebo arm who had at least 1 partial remission event. Although there were no statistical differences in eGFR between the groups, fresolimumab-treated patients showed a trend toward better preservation of renal function compared with placebo-treated patients. As the expected spontaneous remission rate in primary FSGS has been reported as ≤ 5%[21–25] or ≤ 6%,[3] the number of patient responders and total partial remission events in the treated arms may represent an efficacy signal. It is not clear why no dose response was seen in the lower versus higher doses of fresolimumab. It is possible that the short duration of treatment may account for the low rate of proteinuria remissions. Further studies with longer treatment duration are needed to provide additional clinical data.

Four of 6 patients treated with either low- or high-dose fresolimumab who had a clinically meaningful and consistent proteinuria response were black (2 of whom were Hispanic); 5 patients had APOL-1 risk haplotypes, and 1 patient was not genotyped (optional genetic testing consent not signed). The mechanism by which

APOL1 variants promote renal fibrosis remains unclear, with no direct, known linkage to regulation of TGF-β reported as of yet. The 2 black patients assigned to 4 mg/kg fresolimumab who had "durable" clinical responses experienced their partial remission events during the follow-up period (visit 7–9) after steadily declining proteinuria during the treatment period, whereas the 4 patient responders in the 1 mg/kg fresolimumab arm had most of their partial remission events during the treatment period (visit 3–6).

A potentially higher response rate to fresolimumab in black patients with FSGS may be consistent with previous reports that healthy black patients as well as black patients with ESRD and hypertension have significantly higher levels of circulating TGF-β when compared to white patients with comparable health status.[26] Furthermore, these signals of therapeutic response in the black patients harboring APOL1 risk alleles are noteworthy in light of the recent report from the NEPTUNE consortium showing that the APOL1 risk genotype in black patients with FSGS is associated with significantly greater interstitial fibrosis at the time of biopsy.[27] These trial findings, albeit preliminary, raise the possibility that black patients with APOL1 risk alleles may receive greater therapeutic benefit from fresolimumab than others. The phase 1 FSGS study also reported that black patients may have had higher rates of proteinuria responses to a single dose of fresolimumab.[18] The observation that not all patients responded to fresolimumab may not be surprising in view of the heterogeneity and racial predilection of FSGS.

Fresolimumab was generally well tolerated by study patients. The majority of TEAEs in all treatment groups were nonserious and either mild or moderate in severity. No significant difference was noted in the adverse drug reactions seen in the 1 mg/kg arm versus the 4 mg/kg arm. The MEOIs observed were consistent with what has been seen in the previous clinical studies on fresolimumab, specifically, events associated with bleeding and skin changes. The nature of the MEOIs was not significantly different between the 1 mg/kg and 4 mg/kg arms.

In previous clinical trials on fresolimumab, the relationship of the skin events (including keratoacanthoma and squamous cell carcinoma) to drug appeared to be associated with both dose and dose exposure, although the majority of skin events was reversible over time following discontinuation of treatment and did not cause significant morbidity. A recent study has linked mutations of TGFBR1 as the causative gene in self-healing squamous epithelioma.[28]

The relationship of fresolimumab to bleeding events remains unclear, but the majority of these events were grade 1, and, importantly, no study drug treatment-emergent abnormalities were observed in platelet number, platelet function, and coagulation parameters that might indicate a bleeding diathesis.

Intriguingly, in a *post hoc* exploratory analysis, a trend in eGFR stabilization that was not clearly related to the magnitude of proteinuria reductions or the presence of remission was observed in fresolimumab-treated patients compared to a decline in percent eGFR change in the placebo group over the total study period (252 days). This may reflect the effect of fresolimumab in slowing progression of kidney fibrosis and thereby stabilizing eGFR independent of proteinuria reduction. Although only a trend due to the limited patient numbers, this should be further explored in a larger and adequately powered study. The possibility of an anti−TGF-β treatment to stabilize the otherwise downward trajectory of the kidney's decline could be important for the overall prognosis of patients with FSGS.

Further studies of fresolimumab in FSGS in larger studies with adequate power are needed for validation to show whether this trend portends a statistically significant clinical benefit. In the future, biomarkers reflecting glomerular and tubular cell injury and/or repair may aid the selection of patients with refractory FSGS who will benefit from this novel therapeutic agent.

DISCLOSURE

KNC, MDE, FCF, LG, PN, MP, JR, LS, AS, DT-B, JT, FVV, and FV received research funding from Sanofi to their institution for incurred clinical study participation expenses. The following are interests unrelated to this clinical study: FV and LS have received research funding from Sanofi. FCF has received unrestricted research grants from Sanofi. KNC has received consultancy fees from Mallinckrodt Pharmaceuticals. LG has been a member of Advisory Boards for Baxter, Alexion, MedImmune, NxStage, Sandoz; has lectured, chaired lectures, or participated in symposia/panel discussions for Alexion and Otsuka; and has received research grants from Amgen-Dompé, Alexion, and Bellco. MP has received personal fees from AbbVie and Otsuka, grants and personal fees from Alexion, Astellas and Novartis, grants and nonfinancial support from Baxter, and grants, personal fees, and nonfinancial support from Fresenius; AS has been a member of a steering committee for GlaxoSmithKline; JT has received research funding from LaJolla, Lilly, and ZS Pharmaceuticals; SE, SL, JL, and JN are former employees of Sanofi. BA is an employee of Sanofi.

ROLE OF THE FUNDING SOURCE

The study sponsor, Sanofi, had multiple study design meetings with authors and physicians treating FSGS patients that established the study design and especially the inclusion/exclusion criteria decisions. The sponsor had final protocol authorship and decision-making responsibility during protocol reviews with regulatory agencies and institutional review boards. The sponsor funded an external clinical research organization for database hosting, programming, and management, clinical site monitoring, and statistical analysis. All sites entered their own patient data in the database, and the principal investigator verified the locked data fields for accuracy. The sponsor personnel drafted the clinical study report and the lead investigator, JT, was provided all datasets for the report review and authorization. The lead investigator decided to submit a publication with the sponsor's agreement. The sponsor funded an external medical writer to organize the multiple authors draft publication edits. The initial draft manuscript was written by an author, JL, and all authors were provided the clinical study report for their reviews and contributions. One active sponsor personnel, a statistician, contributed to the publication.

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SUPPLEMENTARY MATERIAL

Table S1. FSGS Study Group contributors.

Figure S1. Focal segmental glomerulosclerosis (FSGS) patients assigned to 1 mg/kg (n = 14).

Figure S2. Focal segmental glomerulosclerosis (FSGS) patients assigned to placebo (n = 10).

Figure S3. Focal segmental glomerulosclerosis (FSGS) patients assigned to 1 mg/kg with > 1 partial remission event (n = 6).

Figure S4. Focal segmental glomerulosclerosis (FSGS) patients assigned to 4 mg/kg (n = 12; 3 treated with other immunosuppressant medications).

Figure S5. Postinfusion fresolimumab serum concentrations (mean ± SE).

Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. D'Agati VD, Kaskel FJ, Falk RJ. Focal segmental glomerulosclerosis. *N Engl J Med*. 2011;365:2398–2411.
2. Genovese G, Tonna SJ, Knob AU, et al. A risk allele for focal segmental glomerulosclerosis in African Americans is located within a region containing APOL1 and MYH9. *Kidney Int*. 2010;78:698–704.
3. Korbet SM. Clinical picture and outcome of primary focal segmental glomerulosclerosis. *Nephrol Dial Transplant*. 1999;14(suppl 3):68–73.
4. Korbet SM. Treatment of primary FSGS in adults. *J Am Soc Nephrol*. 2012;23:1769–1776.
5. Ponticelli C, Rizzoni G, Edefonti A, et al. A randomized trial of cyclosporine in steroid-resistant idiopathic nephrotic syndrome. *Kidney Int*. 1993;43:1377–1384.
6. Cattran DC, Appel GB, Hebert LA, et al. A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. North America Nephrotic Syndrome Study Group. *Kidney Int*. 1999;56:2220–2226.
7. Crook ED, Habeeb D, Gowdy O, et al. Effects of steroids in focal segmental glomerulosclerosis in a predominantly African-American population. *Am J Med Sci*. 2005;330:19–24.
8. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*. 2010;329:841–845.
9. Freedman BI. APOL1 and nephropathy progression in populations of African ancestry. *Semin Nephrol*. 2013;33:425–432.

10. Freedman BI, Kopp JB, Langefeld CD, et al. The apolipoprotein L1 (APOL1) gene and nondiabetic nephropathy in African Americans. *J Am Soc Nephrol*. 2010;21:1422–1426.
11. Pollak MR. Familial FSGS. *Adv Chronic Kidney Dis*. 2014;21:422–425.
12. Lee HS. Mechanisms and consequences of TGF-ss overexpression by podocytes in progressive podocyte disease. *Cell Tissue Res*. 2012;347:129–140.
13. Schiffer M, Bitzer M, Roberts IS, et al. Apoptosis in podocytes induced by TGF-beta and Smad7. *J Clin Invest*. 2001;108:807–816.
14. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med*. 1994;331:1286–1292.
15. McCormick LL, Zhang Y, Tootell E, et al. Anti-TGF-beta treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: a model for human scleroderma. *J Immunol*. 1999;163:5693–5699.
16. Yamamoto T, Takagawa S, Katayama I, et al. Anti-sclerotic effect of transforming growth factor-beta antibody in a mouse model of bleomycin-induced scleroderma. *Clin Immunol*. 1999;92:6–13.
17. Trachtman H, Goyal S, Finn P, et al. Neutralizing TGF-β in fibrotic renal disorders: focus on fresolimumab. *Drugs Future*. 2012;37:787–794.
18. Trachtman H, Fervenza FC, Gipson DS, et al. A phase 1, single-dose study of fresolimumab, an anti-TGF-beta antibody, in treatment-resistant primary focal segmental glomerulosclerosis. *Kidney Int*. 2011;79:1236–1243.
19. Hays RD, Kallich JD, Mapes DL, et al. *Kidney Disease Quality of Life Short Form KDQOL-SF) Version 1.3. A Manual for Use and Scoring*. Santa Monica, CA: RAND; 1995.
20. Troyanov S, Wall CA, Miller JA, et al. Focal and segmental glomerulosclerosis: definition and relevance of a partial remission. *J Am Soc Nephrol*. 2005;16:1061–1068.
21. Korbet SM, Schwartz MM, Lewis EJ. The prognosis of focal segmental glomerular sclerosis of adulthood. *Medicine [Baltimore]*. 1986;65:304–311.
22. Pei Y, Cattran D, Delmore T, et al. Evidence suggesting undertreatment in adults with idiopathic focal segmental glomerulosclerosis. Regional Glomerulonephritis Registry Study. *Am J Med*. 1987;82:938–944.
23. Ponticelli C. Focal segmental glomerular sclerosis. To treat or not to treat? 1. It is worthwhile to give the adult patient with nephrotic syndrome the benefit of an adequate therapeutic trial? *Nephrol Dial Transplant*. 1995;10:2351–2354.
24. Rydel JJ, Korbet SM, Borok RZ, et al. Focal segmental glomerular sclerosis in adults: presentation, course, and response to treatment. *Am J Kidney Dis*. 1995;25:534–542.
25. Deegens JK, Assmann KJ, Steenbergen EJ, et al. Idiopathic focal segmental glomerulosclerosis: a favourable prognosis in untreated patients? *Neth J Med*. 2005;63:393–398.
26. August P, Sharma V, Ding R, et al. Transforming growth factor beta and excess burden of renal disease. *Trans Am Clin Climatol Assoc*. 2009;120:61–72.
27. Sampson MG, Robertson CC, Martini S, et al. Integrative genomics identifies novel associations with APOL1 risk genotypes in black NEPTUNE subjects. *J Am Soc Nephrol*. 2016;27:814–823.
28. Goudie DR, D'Alessandro M, Merriman B, et al. Multiple self-healing squamous epithelioma is caused by a disease-specific spectrum of mutations in TGFBR1. *Nat Genet*. 2011;43:365–369.