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ORIGINAL ARTICLE

A Phase 1 first-in-human study of the safety, tolerability, and pharmacokinetics of the ROBO2 fusion protein PF-06730512 in healthy participants

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Abstract

Proteinuria associated with podocyte effacement is a hallmark of focal segmental glomerulosclerosis (FSGS). Preclinical studies implicated ROBO2/SLIT2 signaling in the regulation of podocyte adhesion, and inhibition of this pathway is a novel target to slow FSGS disease progression. This first-in-human dose-escalation study evaluated the safety, tolerability, pharmacokinetics, and immunogenicity of PF-06730512, an Fc fusion protein that targets the ROBO2/SLIT2 pathway, in healthy adults. In this Phase 1, double-blind, sponsor-open study, single ascending dose (SAD) cohorts were randomized to receive up to 1000 mg or placebo intravenously (IV); multiple ascending dose (MAD) cohorts were randomized to receive up to 400 mg subcutaneous (SC) doses, 1000 mg IV dose, or matching placebo. Safety evaluations were performed up to 71 (SAD) and 113 (MAD) days after dosing; blood samples were collected to measure serum PF-06730512 concentrations and antidrug antibodies (ADA) to PF-06730512. Seventy-nine participants (SAD, 47; MAD, 32) were enrolled. There were 108 mild (SAD, 46; MAD, 62) and 21 moderate (SAD, 13; MAD, 8) treatment-emergent adverse events (TEAEs); no deaths, treatment-related serious AEs, severe TEAEs, or infusion reactions were reported. PF-06730512 exposure generally increased in an approximately dose-proportional manner; mean $t_{1/2}$ ranged from 12–15 days across 50–1000 mg doses. Immunogenicity incidence was low (SAD, 0 ADA+; MAD, 2 ADA+). In conclusion, single IV doses of PF-06730512 up to 1000 mg and multiple IV and SC dosing up to 1000 and 400 mg, respectively, were safe and well tolerated in healthy participants. Further trials in patients with FSGS are warranted.

Clinical trial registration: Clinicaltrials.gov: NCT03146065.

KEYWORDS

first-in-human, focal segmental glomerulosclerosis, pharmacokinetics, Phase 1, ROBO2

Abbreviations: FSGS, focal segmental glomerulosclerosis; IV, intravenously; MAD, multiple ascending dose; SAD, single ascending dose.

Gabriele E. Sonnenberg's at the time of the study: Pfizer Inc.

1 | INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) is the most common glomerular disease that leads to end-stage renal disease.[1,2] Many patients with FSGS will be treated with kidney transplantation each year, and unfortunately, the rate of disease recurrence in the transplant is approximately 30%–35%.[3] Most patients with FSGS have partial or no response to the standard of care, and patients who achieve a response often relapse.[2] FSGS is characterized by segmental scarring in a portion of glomeruli that disrupts the glomerular filtration barrier, which consists of fenestrated endothelial cells, glomerular basement membrane (GBM), and podocytes.[2,4] Podocytes are specialized kidney epithelial cells that extend foot processes to cover the outer surface of the GBM.[4] Interdigitating foot processes from neighboring podocytes create filtration slits that form the final filtration barrier.[4] Podocyte foot process effacement occurs in response to various stresses and can eventually result in proteinuria, which is a hallmark of FSGS.[2,4] Thus, therapies that improve or preserve podocyte function and thereby diminish proteinuria could be transformative for patients with FSGS by improving glomerular filtration and preserving kidney function.

Previous studies have suggested that the roundabout guidance receptor 2 (ROBO2) signaling pathway is associated with alteration of the podocyte foot process and slit diaphragm.[5,6] ROBO2 is a receptor for the slit guidance ligand 2 (SLIT2) and is localized to the basement membrane of podocytes.[5] SLIT2/ROBO2 signaling has been shown to destabilize the slit diaphragm and reduces podocyte adhesion to the GBM through inhibition of nephrin-induced actin polymerization and destabilization of podocyte focal adhesions and attachment to the GBM.[5,6] In preclinical animal models, loss of ROBO2 function has been shown to promote podocyte adhesion, reduce podocyte foot process effacement, and reduce proteinuria.[7]

PF-06730512 is a first-in-class, recombinant, ROBO2 human immunoglobulin G1 (IgG1) crystallized fragment (Fc) fusion protein in clinical development for the treatment of FSGS. By acting as a neutralizing ligand trap through preventing the interaction of SLIT2 with ROBO2, PF-06730512 may reduce downstream ROBO2/SLIT2 signaling and improve podocyte structure and function. This Phase 1, first-in-human clinical trial (NCT03146065) aimed to evaluate safety, tolerability, pharmacokinetics (PK), and immunogenicity of PF-06730512 in healthy adult participants following single and multiple ascending doses.

1. What is already known about this subject:

- Most patients with focal segmental glomerulosclerosis (FSGS) show partial or no response to treatment, and relapse is common.
- FSGS is characterized by podocyte injury and detachment.
- PF-06730512 is a recombinant human roundabout guidance receptor 2 crystallized fragment fusion protein developed for the treatment of FSGS that may improve the structural integrity of podocytes.

2. What this study adds:

- This first-in-human trial establishes the acceptable safety, tolerability, and pharmacokinetic profile of PF-06730512 in healthy participants after single and multiple ascending doses up to 1000 mg via intravenous or subcutaneous administration.
- Phase 2 clinical trials are warranted to investigate the efficacy and safety of PF-06730512 in patients with FSGS.

2 | MATERIALS AND METHODS

2.1 | Participants

This randomized, investigator- and participant-blinded (sponsor-open), placebo-controlled study was approved by the comité d'éthique hospitalo-facultaire Erasme and was conducted in compliance with the International Council for Harmonisation Guideline for Good Clinical Practice and the Declaration of Helsinki. All participants were required to provide written informed consent before any study-related procedures. Participants were enrolled at a single site in Belgium (Pfizer Clinical Research Unit Brussels), and the study was conducted between 10 May 2017 and 3 May 2018.

Healthy male and female participants of nonchildbearing potential (i.e., were postmenopausal, had undergone a documented hysterectomy and/or bilateral oophorectomy, or had medically confirmed ovarian failure), age 18–55 years (inclusive) with body mass index (BMI) between 17.5 and 30.5 kg/m^2, and a total body weight greater than 50 kg were eligible for the study. Key exclusion criteria were evidence or history of clinically significant hematologic, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease; urinary protein to creatinine ratio value ≥0.2 g/g or urinary albumin to creatinine ratio value ≥0.03 g/g; and estimated glomerular filtration rate <90 ml/min based on the Cockcroft–Gault formula.

2.2 | Study design

This study consisted of 10 randomized cohorts (6 single ascending dose [SAD] cohorts and 4 multiple ascending dose [MAD] cohorts [including an open-label investigation of the safety, tolerability, and PK of a single-dose intravenous (IV) administration of PF-06730512 in healthy Japanese male participants to support inclusion of Japanese participants in future clinical trials]) that were studied sequentially, with the requirement of review of available PK and safety data from the previous cohort before initiating dosing of the next cohort. An outline of the study design is provided in Figure S1.

In the SAD part of the study, sentinel dosing was implemented for cohorts 1–6 as a safety precaution to allow detection and mitigation of acute safety risks before dosing of additional participants. In addition, a two-step IV infusion approach was applied in which a small fraction of the IV dose was administered over the first hour, with the remaining dose administered over the second hour to further allow for assessment of safety before administration of the entire dose. Dose selection for this study was based on relevant information obtained from nonclinical pharmacology and toxicity studies and the projected efficacious dose. The planned starting dose of 5 mg for SAD cohorts was chosen to mitigate unknown or theoretical risks associated with PF-06730512 and its neutralization of SLIT2. At this planned starting dose, the projected safety margin was at least 500-fold relative to the exposures from the no-observed-adverse-effect level dose.

Participants in the SAD cohorts were randomized to receive one IV dose of 5, 15, 50, 150, 400 and 1000 mg, or placebo, and participants in the Japanese cohort received a single 1000 mg IV dose. Participants in the MAD cohorts received three subcutaneous (SC) doses of PF-06730512 (50, 150, or 400 mg) once a week (QW), IV doses of PF-06730512 (1000 mg) once every 2 weeks (Q2W), or matching placebo QW or Q2W. The sample sizes for SAD and MAD cohorts were selected as a compromise between the need to minimize exposure to PF-06730512 and the need to have sufficient participants randomized to provide adequate safety, tolerability, and PK information at each dose level. Dosing occurred within 28 days of participant screening for each cohort. Participants were admitted to the clinical research unit (CRU) on day −1 and remained confined to the CRU until discharge on day 8 (SAD) or day 22 (MAD). Follow-up visits occurred on day 71 (SAD) or day 113 (MAD).

2.3 | Study objectives

The primary objective was to evaluate the safety and tolerability of single and multiple ascending IV infusions or SC injections of PF-06730512 in healthy adult participants. Secondary objectives were to characterize the single- and multiple-dose PK and evaluate the immunogenicity profile of PF-06730512 following IV or SC administration. Tertiary objectives included evaluation of the safety, tolerability and PK of a single dose of PF-06730512 in healthy Japanese adult male participants.

2.4 | Safety and tolerability

Safety assessments included monitoring of adverse events (AEs), injection site reaction, vital signs, clinical laboratory tests (hematology, chemistry, urinalysis, and cytokines), physical examinations, and cardiac conduction intervals as assessed via 12-lead electrocardiogram. Continuous cardiac telemetry was also conducted for the SAD cohorts at least 2 h before dosing on day 1 and for 8 h following dose administration. AEs were monitored throughout the study and were evaluated by investigators in terms of severity, outcome, and association with the study drug. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) v.21.0.

2.5 | Pharmacokinetics

Blood samples were collected for analysis of PF-06730512 serum concentrations as outlined in Figure S2. Serum samples were analyzed for PF-06730512 concentrations by QPS, LLC (Newark, DE) using a validated sensitive and specific electrochemiluminescent (ECL) analytical assay. The lower limit of quantification for the assay was 25 ng/ml. During sample analysis, between-day assay accuracy, as assessed by percent relative error for quality control samples, ranged from −4.0% to 0.4%. Assay precision, expressed as the between-day coefficient of variation (%CV) of the mean estimated serum concentrations of quality control samples, was ≤10.5%. PK parameters were calculated using an internally validated noncompartmental analysis software. Actual sampling times were used for the calculation of PK parameters for individual participants. The PK parameters for SAD cohorts included maximum serum concentration (C_{max}), time for C_{max} (t_{max}), area under the concentration–time profile from time 0 extrapolated to infinite time (AUC_{inf}), terminal half-life ($t_{½}$), geometric mean volume of distribution at steady state (V_{ss}), and clearance (CL). PK parameters for MAD cohorts included C_{max}, t_{max}, $t_{½}$, V_{ss}, area under the concentration–time profile from time 0 to time τ (AUC_{τ}), apparent clearance (CL/F), and apparent volume of distribution (V_z/F) for the SC cohorts, and CL and V_{ss} for the IV cohort.

2.6 | Immunogenicity

Blood samples were collected for immunogenicity analysis on days −1, 8, 29, and 71 for the SAD cohorts and days −1, 8, 15, 29, 57, 85, and 113 for the MAD cohorts. Samples were analyzed at QPS, LLC (Newark, DE) for the presence or absence of antidrug antibodies (ADA) to PF-06730512, with a tiered approach to screening, confirmation, and titer/quantification using a validated ECL immunoassay. Samples that were positive for ADA were further characterized for neutralizing antibodies (NAb) to PF-06730512 using a validated competitive ligand binding ECL assay. Assay precision, expressed as the between day %CV, for both the positive control endpoint titer and the negative control (used to calculate cut point) were less than 10% for the ADA assay. For the NAb assay, between-day %CV was not applicable for the positive control endpoint titer, as only a single assay was performed to assess the three serum samples, and was <10% for the negative control (used to calculate cut point).

2.7 | Statistical analyses

No formal statistical analyses were planned or conducted for the primary safety analyses. All participants who received at least one

dose of study medication were included in the safety analyses. Data were reported in accordance with the sponsor reporting standards, which included summarizing AEs, safety laboratory abnormalities, vital signs, electrocardiogram data, and immunogenicity by treatment group. Descriptive summary statistics were calculated for PK parameters and included arithmetic and geometric means, %CV, median, minimum, maximum, and geometric %CV.

3 | RESULTS

3.1 | Participants

In the SAD cohorts, 47 participants were randomized to receive PF-06730512 (n = 35) or placebo (n = 12). Three discontinued due to participant withdrawal (one each in the placebo, 400 and 1000 mg [Japanese] cohorts). In the MAD cohorts, 32 participants were randomized to receive PF-06730512 (n = 24) or placebo (n = 8). Three participants discontinued; two due to participant withdrawal (one each in the placebo SC and the 1000 mg IV cohorts) and one due to AEs (400 mg SC cohort). Demographic characteristics are presented in Table 1. All participants were male, and the majority were white (SAD, 38/47; MAD, 30/32). All participants were between 19 and 55 years of age.

3.2 | Safety

All participants who received study treatment (PF-06730512 or placebo) were included in the safety evaluation. Overall, the higher frequency of AEs reported in the PF-06730512 group compared with the placebo group did not follow a dose-dependent relationship and is unlikely to be mechanism-based. The most commonly reported all-causality treatment-emergent AEs (TEAEs) by system organ class in the SAD cohorts were infections and infestations (n = 11, PF-06730512; n = 1, placebo) and general disorders and administration site conditions (n = 7, PF-06730512; n = 1, placebo; Table 2). The most commonly reported all-causality TEAEs by system organ class in the MAD cohorts were skin and subcutaneous tissue disorders (n = 7, PF-06730512 SC or IV; n = 2, placebo) and general disorders and administration site conditions (n = 5, PF-06730512 SC or IV; n = 3, placebo; Table 2). In the SAD cohorts, the most frequently reported treatment-related TEAE was headache (n = 4, PF-06730512; n = 2, placebo; Table S1). In the MAD cohorts, the most frequently reported treatment-related TEAEs were dry skin (n = 5, PF-06730512 SC or IV) and headache (n = 3, PF-06730512 SC; n = 1, placebo IV; Table S1). In all cohorts, most TEAEs were mild or moderate in severity (108 and 21, respectively). One participant in cohort 9 (MAD, 400 mg SC) was permanently discontinued due to a treatment-related TEAE of generalized rash. One participant in cohort 6 (SAD, 1000 mg IV) reported a serious AE of contusion after a fall, which was not treatment-related. There were no deaths, severe TEAEs or infusion reactions, or treatment-related serious AEs, and

TABLE 1 Participant demographics

	SAD								MAD					
Characteristic	Placebo IV	5 mg IV	15 mg IV	50 mg IV	150 mg IV	400 mg IV	1000 mg IV	1000 mg IV Japanese	Placebo SC QW	50 mg SC QW	150 mg SC QW	400 mg SC QW	Placebo IV Q2W	1000 mg IV Q2W
Participants, n	12	4	4	4	6	6	6	5	6	6	6	6	2	6
Male, n (%)	12 (100)	4 (100)	4 (100)	4 (100)	6 (100)	6 (100)	6 (100)	5 (100)	6 (100)	6 (100)	6 (100)	6 (100)	2 (100)	6 (100)
Age, mean (SD), y	36.0 (8.1)	28.3 (5.7)	28.8 (7.2)	28.5 (4.5)	28.5 (8.4)	26.5 (8.2)	36.2 (9.7)	30.8 (3.3)	37.7 (12.8)	30.8 (8.3)	36.5 (10.6)	33.2 (11.4)	32.5 (14.9)	28.2 (7.2)
Weight, mean (SD), kg	81.0 (12.1)	81.2 (14.2)	77.6 (12.7)	74.5 (5.1)	81.3 (10.8)	78.2 (7.8)	80.0 (12.1)	71.0 (9.0)	80.6 (10.9)	76.1 (13.6)	78.6 (6.7)	79.6 (12.0)	79.8 (8.2)	72.7 (3.9)
BMI, mean (SD), kg/m^2	25.1 (3.1)	24.9 (3.5)	23.5 (2.5)	22.9 (2.8)	24.9 (3.4)	23.3 (1.4)	25.6 (2.5)	22.9 (2.7)	25.6 (2.4)	23.6 (3.6)	25.0 (1.5)	24.7 (4.3)	26.1 (0.6)	23.2 (1.5)
Race, n (%)														
White	10 (83)	4 (100)	4 (100)	4 (100)	6 (100)	4 (67)	6 (100)	0	6 (100)	6 (100)	5 (83)	5 (83)	2 (100)	6 (100)
Black/African–American	1 (8)	0	0	0	0	2 (33)	0	0	0	0	1 (7)	0	0	0
Other	1 (8)	0	0	0	0	0	0	5 (100)	0	0	0	1 (17)	0	0

Abbreviations: BMI, body mass index; IV, intravenous; MAD, multiple ascending dose; Q2W, once every 2 weeks; QW, once weekly; SAD, single ascending dose; SC, subcutaneous.

TABLE 2 Incidence of treatment-emergent adverse events by system organ class – all causality

	SAD								MAD					
n (%)	Placebo IV (*n* = 12)	5 mg IV (*n* = 4)	15 mg IV (*n* = 4)	50 mg IV (*n* = 4)	150 mg IV (*n* = 6)	400 mg IV (*n* = 6)	1000 mg IV (*n* = 6)	1000 mg IV Japanese (*n* = 5)	Placebo SC QW (*n* = 6)	50 mg SC QW (*n* = 6)	150 mg SC QW (*n* = 6)	400 mg SC QW (*n* = 6)	Placebo IV Q2W (*n* = 2)	1000 mg IV Q2W (*n* = 6)
Eye disorders	0	0	0	0	0	0	0	0	0	0	3 (50.0)	0	0	0
Gastrointestinal disorders	2 (16.7)	0	0	1 (25.0)	0	0	1 (16.7)	0	0	1 (16.7)	1 (16.7)	0	1 (50.0)	2 (33.3)
General disorders and administration site conditions	1 (8.3)	1 (25.0)	1 (25.0)	1 (25.0)	2 (33.3)	1 (16.7)	1 (16.7)	0	3 (50.0)	1 (16.7)	1 (16.7)	2 (33.3)	0	1 (16.7)
Infections and infestations	1 (8.3)	1 (25.0)	1 (25.0)	1 (25.0)	3 (50.0)	2 (33.3)	2 (33.3)	1 (20.0)	0	1 (16.7)	2 (33.3)	2 (33.3)	1 (50.0)	0
Injury, poisoning, and procedural complications	0	0	1 (25.0)	1 (25.0)	1 (16.7)	1 (16.7)	1 (16.7)	1 (20.0)	1 (16.7)	0	1 (16.7)	0	0	0
Metabolism and nutrition disorders	0	0	0	0	0	0	0	0	1 (16.7)	0	0	0	0	0
Musculoskeletal and connective tissue disorders	2 (16.7)	2 (50.0)	1 (25.0)	0	0	1 (16.7)	1 (16.7)	0	0	0	3 (50.0)	0	0	3 (50.0)
Nervous system disorders	2 (16.7)	0	0	0	0	3 (50.0)	2 (33.3)	0	1 (16.7)	0	1 (16.7)	2 (33.3)	2 (100.0)	1 (16.7)
Psychiatric disorders	0	1 (25.0)	0	0	0	0	0	0	0	0	3 (50.0)	0	1 (50.0)	0
Renal and urinary disorders	0	0	0	0	0	1 (16.7)	0	0	0	0	0	0	0	0
Respiratory, thoracic and mediastinal disorders	0	1 (25.0)	0	0	0	0	0	1 (20.0)	1 (16.7)	2 (33.3)	1 (16.7)	0	0	0
Skin and subcutaneous tissue disorders	1 (8.3)	1 (25.0)	0	0	1 (16.7)	1 (16.7)	2 (33.3)	1 (20.0)	1 (16.7)	0	4 (66.7)	2 (33.3)	1 (50.0)	1 (16.7)
Vascular disorders	0	1 (25.0)	0	0	0	0	0	0	0	0	0	0	0	2 (33.3)

Abbreviations: AE, adverse event; IV, intravenous; MAD, multiple ascending dose; Q2W, once every 2 weeks; QW, once weekly; SAD, single ascending dose; SC, subcutaneous.

none of the laboratory abnormalities in the SAD or MAD cohorts were considered clinically significant. The maximum-tolerated dose of PF-06730512 was not established in this study.

3.3 | Pharmacokinetics

Following single IV administration of PF-06730512 at doses ranging from 5 to 1000 mg in healthy participants (SAD cohorts), C_{max} was observed shortly after the end of the infusion (Table 3), and concentrations exhibited a biphasic decline over time (Figure 1A). Mean $t_{½}$ in non-Japanese participants ranged from 5.3 to 13 days, with a shorter $t_{½}$ observed at the lower doses, likely due to sensitivity limitation of the bioanalytical assay (i.e., lack of quantifiable concentrations at later time points at lower doses). Following IV administration in non-Japanese participants, geometric mean CL values ranged from 0.034 to 0.054 L/h across doses, and geometric mean V_{ss} values ranged from 9.3 to 14 L. Serum PF-06730512 AUC_{inf} generally increased proportionally from 5 to 50 mg and from 150 to 1000 mg, whereas C_{max} increased in an apparent dose-proportional manner across 5–1000 mg in non-Japanese participants (Figure 1A).

Overall, serum PF-06730512 PK was comparable between non-Japanese participants and Japanese participants following a single 1000 mg IV dose. Mean $t_{½}$ values for non-Japanese and Japanese participants were similar at 13 and 15 days, respectively. Geometric mean AUC_{inf} and C_{max} values for Japanese participants were slightly higher (<1.25-fold relative to non-Japanese participants), which may be due to the effect of body weight on clearance and volume of distribution.

Serum PF-06730512 PK parameters for IV (Q2W) and SC (QW) dosing in MAD cohorts were determined following the first (QW and Q2W, day 1), second (Q2W, day 15), and third (QW, day 15) dose. Serum PF-06730512 concentration–time profiles on day 15 following IV and SC multiple-dose administration are presented in Figure 1B. PK parameters for day 15 following IV and SC multiple-dose administration are summarized descriptively in Table 3.

Following multiple-dose SC administration of PF-06730512 at 50, 150, or 400 mg QW for a total of three doses, C_{max} was reached with a median t_{max} of 66–84 h after the first SC dose on day 1 and 36–84 h following the third dose on day 15. The mean $t_{½}$ values for SC doses on day 15 ranged from 13 to 15 days. Geometric mean values of CL/*F* and V_z/F across the 50–400 mg SC doses were similar, ranging

TABLE 3 Summary of serum PF-06730512 PK parameters

SAD							
Parameter[a]	**5 mg IV (*n* = 4)**	**15 mg IV (*n* = 4)**	**50 mg IV (*n* = 4)**	**150 mg IV (*n* = 6)**	**400 mg IV (*n* = 5)[b]**	**1000 mg IV (*n* = 6)**	**1000 mg IV Japanese (*n* = 4)[c]**
AUC_{inf}, μg·h/ml	92 (18)	338 (16)	1160 (22)	4348 (15)	10,970 (18)	29,030 (17)	35,340 (13)
C_{max}, μg/ml	0.9 (17)	3.3 (15)	8.9 (10)	34.7 (12)	76.0 (22)	235.1 (20)	274.2 (16)
t_{max}, h	2.13 (2.12–3.00)	3.00 (2.13–8.00)	2.12 (2.10–6.00)	2.56 (2.12–3.00)	2.57 (2.08–3.02)	2.14 (2.08–3.00)	3.00 (2.13–3.00)
$t_{½}$, d	5.34 ± 0.53	9.78 ± 1.54	11.70 ± 2.17	11.51 ± 1.63	11.98 ± 2.05	13.02 ± 0.81	15.25 ± 1.95
V_{ss}, L	9.26 (14)	10.06 (10)	13.86 (15)	9.62 (11)	11.49 (16)	11.56 (22)	11.40 (18)
CL, L/h	0.05428 (18)	0.04441 (16)	0.04317 (22)	0.03453 (15)	0.03650 (18)	0.03446 (17)	0.02827 (13)

MAD (Day 15)				
Parameter[a]	**50 mg SC QW (*n* = 6)**	**150 mg SC QW (*n* = 6)**	**400 mg SC QW (*n* = 5)**	**1000 mg IV Q2W (*n* = 5)**
$AUC_{τ}$, μg·h/ml	643 (30)	2068 (37)	5643 (32)	25,540 (15)
C_{max}, μg/ml	4.4 (29)	13.9 (41)	41.3 (32)	246.4 (8)
t_{max}, h	84.0 (60.0–168.0)	54.0 (24.0–72.0)	36.0 (36.0–84.0)	2.1 (2.1–3.0)
CL/*F*, L/h	0.07785 (30)	0.07257 (37)	0.07092 (32)	N/A
CL, L/h	N/A	N/A	N/A	0.03916 (15)
$t_{½}$, d	13.98 ± 1.31	13.38 ± 1.00	14.72 ± 0.97	15.02 ± 1.77
V_z/F, L	37.58 (30)	33.54 (38)	36.06 (30)	N/A
V_{ss}, L	N/A	N/A	N/A	13.53 (17)

Abbreviations: %CV, percent coefficient of variation; AUC_{inf}, area under the concentration–time profile from time 0 extrapolated to infinite time; $AUC_{τ}$, area under the concentration–time profile from time 0 to time tau (τ), the dosing interval, where tau, 168 h for once-weekly dosing and 336 h for every-other-week dosing; CL, clearance; CL/*F*, apparent clearance; C_{max}, maximum serum concentration; IV, intravenous; MAD, multiple ascending dose; N/A, not applicable; PK, pharmacokinetics; Q2W, once every 2 weeks; QW, once weekly; SAD, single ascending dose; SC, subcutaneous; $t_{½}$, terminal half-life; t_{max}, time to C_{max}; V_{ss}, volume of distribution at steady state; V_z/F, apparent volume of distribution.

[a]Geometric mean (geometric %CV) for all except: median (range) for t_{max}; arithmetic mean ± SD for $t_{½}$.

[b]*n* =6 for C_{max} and t_{max} and 5 for all other parameters due to incomplete PK profile.

[c]*n* =5 for C_{max} and t_{max} and 4 for all other parameters due to incomplete PK profile.

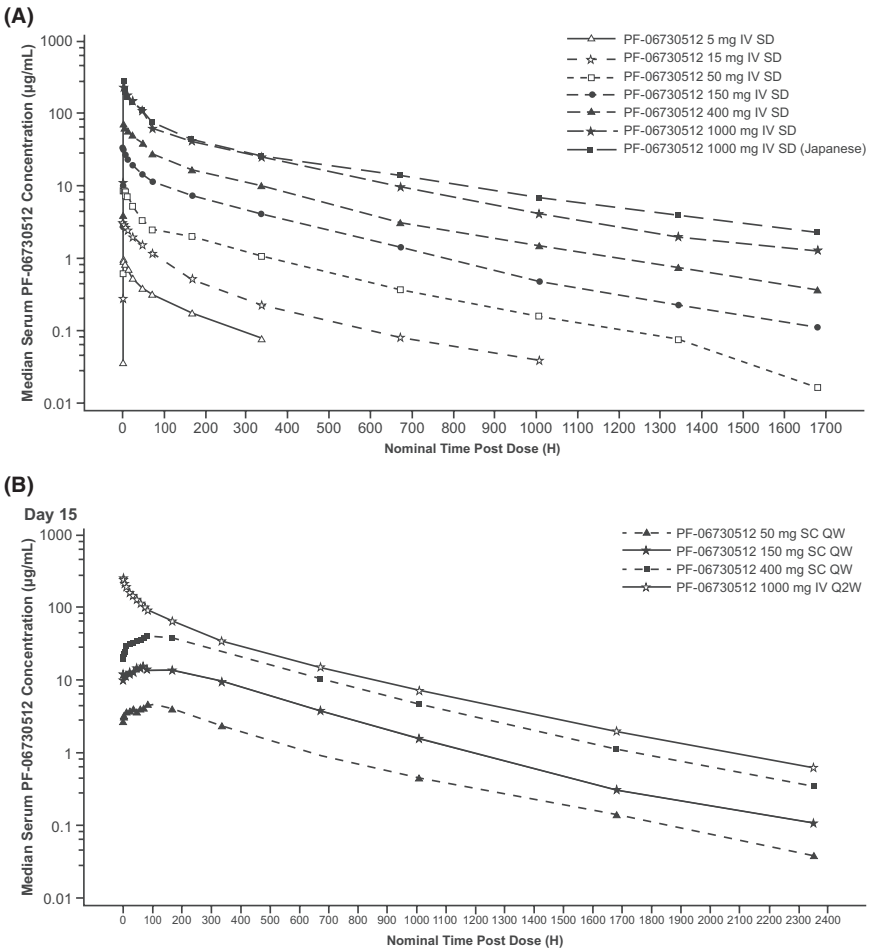

FIGURE 1 Median serum PF-06730512 concentration–time profiles. (A) SAD (B) MAD. Summary statistics were calculated by setting concentration values below the LLOQ to 0. The LLOQ was 0.025 µg/ml. IV, intravenous; LLOQ, lower limit of quantification; MAD, multiple ascending dose; Q2W, once every 2 weeks; QW, once weekly; SAD, single ascending dose; SC, subcutaneous; SD, single dose

approximately from 0.071 to 0.078 L/h for CL/*F* and 34–38 L for V_z/F. Serum PF-06730512 exposure based on AUC_τ and C_{max} increased approximately proportionally with increasing dose on days 1 and 15 following multiple-dose administration. The geometric mean observed accumulation ratio (R_{ac}) on day 15 ranged from 2.8 to 3.7 based on AUC_τ, and 2.4–3.4 based on C_{max}, indicating the magnitude of accumulation following SC dosing QW for a total of three doses. Estimated bioavailability based on AUC_τ for SC doses on day 15 in the MAD cohorts relative to the AUC_{inf} of corresponding reference IV doses in SAD cohorts is presented in Table 4. For the 50, 150, and 400 mg SC MAD cohorts, bioavailability was approximately 55%, 48%, and 51%, respectively. As AUC_τ is not expected to be at steady state by day 15, these values are likely an underestimation of bioavailability.

Following multiple IV administration at 1000 mg Q2W for a total of two doses, C_{max} values on days 1 and 15 were observed, slightly after the end of a 2-h infusion (median t_{max} ranged from 2.1 to 2.6 h across dosing days). On day 15, geometric mean CL and V_{ss} values were approximately 0.039 L/h and 14 L, respectively. Mean $t_{½}$ following the second dose was approximately 15 days, similar to $t_{½}$ values observed following a single 1000 mg IV administration to non-Japanese and Japanese participants in the SAD cohorts. Variability in PF-06730512 exposure on day 15 for the SC and IV doses based on geometric %CV ranged from 15% to 37% for AUC_τ and 8%–41% for C_{max}, with higher variability observed for SC dosing.

TABLE 4 Summary of PF-06730512 estimated bioavailability of SC doses (MAD) relative to corresponding single IV infusion doses (SAD)

Treatment group	Ratio of geometric means of AUC_τ (µg·h/ml) / AUC_{inf} (µg·h/ml)	*F* (%)
50 mg SC QW	0.554	55.4
150 mg SC QW	0.476	47.6
400 mg SC QW	0.514	51.4

Estimated *F* (%) was calculated based on ratio of geometric mean AUC_τ following last SC dose in MAD cohorts (estimate of AUC_τ at steady state) to geometric mean AUC_{inf} following single IV dose in SAD cohorts. AUC_τ values (SC, last dose) are nonsteady-state values.
Abbreviations: AUC_{inf}, area under the concentration–time profile from time 0 extrapolated to infinite time; AUC_τ, area under the concentration–time profile from time 0 to time tau (τ), the dosing interval; *F*, bioavailability; IV, intravenous; MAD, multiple ascending dose; QW, once weekly; SAD, single ascending dose; SC, subcutaneous.

3.4 | Immunogenicity

Overall, the incidence of immunogenicity in this study was low. There were no ADA-positive (ADA+) samples in the SAD cohorts. In the MAD cohorts, two participants had ADA + samples (both in the

400 mg SC cohort). Of these two participants, one had a positive NAb result (log 10 titer ≥1.0), and the other was negative for NAb. All three ADA + samples from these two participants occurred at the last few visits (day 85 and/or day 113) and tested positive for specificity to ROBO2.

4 | DISCUSSION

In this first-in-human study, PF-06730512 was generally safe and well tolerated at doses up to 1000 mg IV in the SAD cohorts and 1000 mg IV and 400 mg SC in the MAD cohorts in healthy participants. At the highest doses administered in the study, the limits of safety and tolerability were not reached; hence, the maximum tolerated dose was not determined. There were no deaths, treatment-related serious AEs, or severe TEAEs or infusion reactions. Most TEAEs were mild, and there was no dose-dependent increase in TEAE frequency. These findings demonstrate the favorable safety and tolerability profiles of PF-06730512 and support further development of PF-06730512 in Phase 2 studies to further investigate its safety and efficacy in patients with FSGS.

Following single- and multiple-dose administration, serum PF-06730512 C_{max} generally increased in an apparent dose-proportional manner across the dose range studied, and AUC increased in an apparent dose-proportional manner from 5 to 50 mg and from 150 to 1000 mg after a single IV dose and across the multiple SC doses studied. PF-06730512 PK parameters were comparable for single and repeated 1000 mg IV administration suggesting no time-dependent changes in PK in this study.

Fc fusion proteins generated to date tend to have shorter $t_{½}$ in comparison to intact IgG, ranging from 4 days (etanercept) to 17 days (abatacept).[8,9] This has been attributed to lower binding affinity to F_cR_n, glycan-mediated disposition, receptor-mediated clearance, and higher-order structure provided by F_{ab} arms of antibodies.[10,11] In this study, we observed that PF-06730512 $t_{1/2}$ was fairly long (approximately 13–15 days in the MAD cohorts), allowing it to be administered less frequently than medications with a shorter $t_{1/2}$ and potentially providing more convenience for patients. The volume of distribution observed for PF-06730512 was comparable to values reported for IgG monoclonal antibodies, indicating that distribution was generally limited to vascular and interstitial spaces.[12,13] Similarly, the clearance of PF-06730512 was slightly higher than but generally comparable to the clearance values reported for IgG monoclonal antibodies.[12]

Whereas renal clearance is usually considered insignificant for monoclonal antibodies and other biologics with a molecular weight larger than the glomerular filtration threshold, the impact of renal impairment in patients with proteinuria (e.g., patients with FSGS) is unclear.[12,14] For endogenous high-molecular-weight proteins such as IgG, reports in patients with FSGS have suggested fractional excretion of approximately 9%.[15,16] In a Phase 1 study conducted in patients with FSGS, the PK of fresolimumab was reported to be similar to that observed in idiopathic pulmonary fibrosis (IPF) and advanced malignancy patient populations.[17] However, it remains unclear if nephrotic syndrome was a comorbidity in the IPF and advanced malignancy patient populations and if the comparison may have been confounded by other factors such as body weight and immunogenicity. In the combined population PK analysis of belimumab in patients with systemic lupus erythematosus, proteinuria was explored as a covariate on clearance and was estimated to increase clearance by 8.7% per g/day of proteinuria.[18] Although proteinuria was a statistically significant covariate, the small proportion of patients with proteinuria (5.1%, >2 g/day; 1.5%, >4 g/day) and limited range of proteinuria in the analysis limits interpretation and application.[18] Further investigation is needed to determine the impact of proteinuria on clearance of PF-06730512 in the FSGS patient population.

Fc fusion proteins have been utilized in drug development to treat a variety of diseases due to their ability to stabilize peptides, with promising results. In Phase 1 trials of trebananib, in development for solid tumors[19]; KH902, in development for macular degeneration[20]; and rFIXFc, in development for hemophilia B patients,[21] Fc fusion proteins were tolerated and had acceptable safety profiles at the doses tested. Immunogenicity was generally low.[19,20] In this study, the Fc fusion protein PF-06730512 was well tolerated with a low observed incidence of immunogenicity after relatively short-term dosing in healthy participants.

Due to the inherent limitation of a Phase 1 study in healthy participants, the relatively short duration and number of participants in this study do not permit full characterization of the incidence and impact of immunogenicity on PK and safety. Longer term studies in patients with FSGS will be required for further characterization of the incidence and impact of immunogenicity in the intended population. Additionally, the maximum SC dose was limited to 400 mg due to the number of required injections, hence, the 1000 mg dose in the MAD cohort was administered intravenously. Another potential limitation is that all participants in this study were male and the age range was relatively narrow, which is not reflective of the sex and age distribution in the FSGS patient population.[2] Although sex may have some effect on absorption in the case of SC route of administration, to our knowledge there is no evidence of clinically significant sex-related PK exposure differences for biologics after accounting for body weight.[22] Further understanding of the effect of sex and other covariates, such as body weight, disease severity, and age, on PK will be examined in future studies.

In summary, single doses of PF-06730512 up to 1000 mg (IV) and multiple doses of PF-06730512 up to 1000 mg (IV) and 400 mg (SC) were safe and well tolerated in healthy participants in this study. Further studies in larger, Phase 2 clinical trials are warranted to investigate the efficacy and safety of PF-06730512 in patients with FSGS.

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DISCLOSURES

Chay Ngee Lim, Donal Gorman, and Stephen Berasi are employees of and stockholders in Pfizer Inc. Constantino Kantaridis and Isabelle Huyghe are employees of and stockholders in Pfizer N.V. – S.A. Gabriele E. Sonnenberg was an employee of Pfizer Inc at the time of the study.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the study conception and design, data analysis and interpretation, and critical revision of the manuscript and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the manuscript.

ETHICS APPROVAL STATEMENT

This study was approved by the comité d'éthique hospitalo-facultaire Erasme and was conducted in compliance with the International Council for Harmonisation Guideline for Good Clinical Practice and the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

All participants were required to provide written informed consent before any study-related procedures.

PERMISSION TO REPRODUCE MATERIALS FROM OTHER SOURCES

Not applicable.

DATA AVAILABILITY STATEMENT

Upon request, and subject to certain criteria, conditions and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines, and medical devices (1) for indications that have been approved in the US and/or EU or (2) in programs that have been terminated (i.e., development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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