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ORIGINAL ARTICLE

Trial design and baseline characteristics of CaLIPSO: a randomized, double-blind placebo-controlled trial of SNF472 in patients receiving haemodialysis with cardiovascular calcification

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ABSTRACT

Background. The objective of CaLIPSO, a Phase 2b, randomized, double-blind, placebo-controlled clinical trial, is to test the hypothesis that myo-inositol hexaphosphate (SNF472) attenuates the progression of cardiovascular calcification in patients receiving maintenance haemodialysis. Here we report the trial design and baseline characteristics of trial participants.

Methods. Adult patients on maintenance haemodialysis (≥6 months) with an Agatston coronary artery calcium score, as measured by a multidetector computed tomography scanner, of 100–3500 U were enrolled. Patients were stratified by Agatston score (100–<400, 400–1000 or >1000 U) and randomized in a 1:1:1 ratio to receive placebo, SNF472 300 mg or SNF472 600 mg administered intravenously three times weekly during each haemodialysis session.

Results. Overall, 274 patients were randomized. The mean age of trial participants was 63.6 (standard deviation 8.9) years and 39% were women. The coronary artery, aorta and aortic valve median (25th-75th percentile) Agatston scores at baseline were 730 U (315–1435), 1728 U (625–4978) and 103 U (31–262), respectively, and the median (25th–75th percentile) calcium

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volume scores at baseline were 666 (310–1234), 1418 (536–4052) and 107 (38–278), respectively. Older age and diabetes mellitus were associated with higher calcium scores at baseline.

Conclusions. The CaLIPSO trial enrolled patients on haemodialysis with pre-existent cardiovascular calcification to test the hypothesis that SNF472 attenuates its progression in the coronary arteries, aorta and aortic valve.

Keywords: chronic kidney disease, coronary artery calcification, randomized clinical trial, SNF472, vascular calcification

INTRODUCTION

Chronic kidney disease (CKD) is associated with significant morbidity, premature mortality, reduced quality of life and high health care expenditures [1, 2]. Traditional risk factors for the development and progression of CKD include advanced age, cardiovascular disease, diabetes mellitus, hypertension and obesity [3]. As kidney function declines, a variety of metabolic and hormonal changes occur, many of which result in disordered mineral metabolism, existing within a state of chronic inflammation and multiple organ dysfunction [4–8]. Disorders of mineral metabolism in CKD include phosphate retention and elevated serum concentrations of phosphaturic hormones, including parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). A large body of evidence indicates that soft tissue calcifications, particularly cardiovascular calcification, contribute to cardiovascular morbidity and mortality in patients with CKD [9, 10]. Cardiovascular calcification is an actively regulated process controlled by multiple cellular and humoral mechanisms, including the loss of inhibitors of mineralization typically expressed by vascular cells and the overexpression of osteogenic processes operating within vascular lesions, including those that promote hydroxyapatite nucleation and crystal growth [4].

The reported prevalence of coronary artery calcification (CAC) increases from ~40% in patients with moderate to advanced (Stage 3/4) CKD [11–13] to ~60% in patients at haemodialysis initiation and up to 80% in patients receiving maintenance haemodialysis [14]. Cardiovascular calcification may be a modifiable phenomenon [15]. Thus management guidelines for CKD mineral and bone disorder (CKD-MBD) recommend the diagnosis and management of cardiovascular calcification in all patients with at least moderately decreased kidney function (Stage 3a or higher CKD) [16]. In these patients, computed tomography (CT) may be used to determine the presence or absence of cardiovascular calcification; lateral abdominal radiograph for vascular calcification and echocardiogram for valvular calcification are reasonable alternatives [16]. Recommended management strategies to reduce cardiovascular calcification include lowering elevated serum phosphate while avoiding calcium-based phosphate binders to prevent net positive calcium balance [16].

Several large, randomized clinical trials in patients receiving dialysis have evaluated a number of therapeutic interventions to reduce cardiovascular events, but they required large sample sizes [17–20]. These and other studies did not show a cardiovascular benefit; thus a significant unmet need remains.

SNF472 is an intravenous formulation of the hexasodium salt of myo-inositol hexaphosphate (IP6, phytate), a compound that targets vascular calcification by binding to the growth sites of the hydroxyapatite crystal and inhibiting hydroxyapatite nucleation and crystal growth [21, 22]. IP6 is a naturally occurring substance found in beans, brown rice, corn, sesame seeds, wheat bran and other high-fibre foods; however, oral absorption is low. IP6 is normally present in minimal concentrations (in the micromolar range) in body fluids [21, 22].

CaLIPSO is a Phase 2b, multicentre, randomized, double-blind, placebo-controlled clinical trial testing the hypothesis that SNF472 compared with placebo attenuates the progression of cardiovascular calcification in patients receiving maintenance haemodialysis. In this report we present the design of the CaLIPSO trial and summarize baseline characteristics of the trial participants.

MATERIALS AND METHODS

Trial participants

Adult men and women (18–80 years) receiving haemodialysis for ≥6 months were screened for eligibility. Initially the trial recruited patients with an Agatston CAC score between 100 and 2000 U measured by a multidetector CT scanner (MDCT). An amendment to the protocol increased the upper limit to 3500 U, which is more fully representative (and inclusive) of this complex patient population (Table 1). Ethical approval was obtained in accordance with the local/national processes for each study site. All participants provided written informed consent prior to enrolment. The trial, which was conducted according to the principles of the Declaration of Helsinki, was registered at EudraCT (2016-002834-59) and ClinicalTrials.gov (NCT02966028).

Overview of trial design

CaLIPSO consisted of three different phases: screening for 28 ± 3 days, double-blind treatment for 52 weeks and safety assessment 4 weeks after administration of the last dose of study drug (Figure 1). Patients who met eligibility criteria were randomized on Day 1 in a 1:1:1 ratio to receive placebo, SNF472 300 mg or SNF472 600 mg, three times weekly during haemodialysis sessions. In view of the overall association of Agatston CAC score with progression of cardiovascular calcification, randomization was stratified by baseline Agatston CAC score, divided into three categories: 100–<400, 400–1000 and >1000 U.

Study visits in CaLIPSO were scheduled for screening, baseline (Week 1) and Weeks 2, 4, 6, 10, 16, 22, 28, 34, 40, 46, 52 (last dose) and 56. If the patient terminated study treatment earlier than planned, then the assessments scheduled for the Week 52 visit were conducted at an early termination visit.

At screening and Week 52, imaging of the coronary arteries, aorta and aortic valve was performed using MDCT, with a minimum of 64 slices. Contiguous 3 mm tomographic slices from above the aortic arch to the diaphragm were obtained at end-expiration and reconstructed during diastole or at the time of least motion. All scans were centrally reviewed and each calcified area was quantified using both volume [23] and Agatston score [24]. The Agatston score is more sensitive to the calcium content of a plaque, but it is less reproducible than the volume score [25], thus the calcium volume score was selected as the primary endpoint.

Total hip and femoral neck bone mineral density (BMD) were measured at screening and Week 52 by dual-energy X-ray absorptiometry. Imaging modality, anatomical positioning,

Table 1. Trial eligibility criteria

Inclusion criteria
Female or male patients
CAC score of 100–3500 U inclusive within a 4-week period prior to randomization, as measured by an MDCT scanner
Patients 18–54 years of age at randomization with a history of diabetes mellitus or 55–80 years of age at randomization (with or without a history of diabetes mellitus)
Patients receiving haemodialysis for ≥6 months prior to randomization
Exclusion criteria
Scheduled date for kidney transplant from a known living donor
Weight >300 lb (136 kg)
Hospitalization in the previous 3 months prior to randomization for unstable angina, myocardial infarction, stroke, transient ischeamic attack, amputation or peripheral or coronary bypass surgery
History of unstable heart failure in the previous 3 months, defined as an unplanned presentation to a hospital or dialysis treatment facility with signs/symptoms of acute pulmonary oedema and requiring ultrafiltration therapy
History of cancer that has been in remission for <5 years prior to randomization. A history of basal cell carcinoma or Stage 1 squamous cell carcinoma of the skin is allowed
Pregnant or trying to become pregnant, currently breastfeeding or of childbearing potential (including perimenopausal women who have had a menstrual period within 1 year) and not willing to practice birth control using a double-barrier method (criteria apply to women only) at least 30 days after the last dose of study medication
Hypocalcaemia, defined as serum calcium <8.0 mg/dL (or 2.0 mmol/L) for the serum calcium most proximal to screening per patient's medical records
Extreme elevation in serum phosphorous, defined as phosphorous >10 mg/dL (or 3.23 mmol/L) within the last 2 months proximal to screening per patient's medical records
Uncontrolled hypertension, defined as any two or more consecutive post-dialysis diastolic blood pressure measurements >100 mmHg within the last 2 months proximal to screening
Expected survival <2 years in the investigator's medical opinion
Known active drug or alcohol abuse within 1 year of randomization
Use of other investigational drugs within 30 days of randomization
Non-compliance with dialysis treatment that, in the opinion of the investigator, as evidenced by either repeated missed dialysis treatments or significant non-compliance with the patient's medication regimen
Inability to comply with all required study procedures and schedules, inability to speak and read in the protocol-derived language of that patient's clinical site or unwillingness or inability to give written informed consent

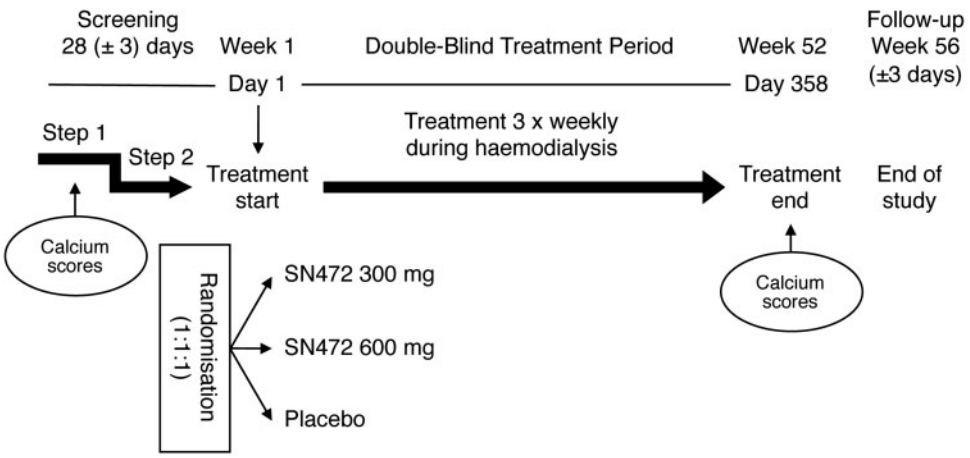

FIGURE 1: CaLIPSO trial flow chart. In Step 1, potential study participants who satisfied the inclusion and exclusion criteria underwent an assessment by MDCT scanner to determine the Agatston score for the coronary artery, as well as dual-energy X-ray absorptiometry for BMD of the total hip and femoral neck. Patients with confirmed calcification of the coronary artery (initially 100–2000 U; later 100–3500 U) at Step 1 entered Step 2 to complete all other screening assessments and confirm all eligibility criteria were met.

coverage and parameter were consistent across both imaging visits for each patient. The left femur was scanned at both visits; if a fracture, prosthesis, deformity or problem prohibited scanning of the left femur, then the right femur was scanned at both visits. A foot positioner was used to keep the femoral shaft straight and parallel to the edge of the scanned image, with the greater trochanter centred vertically in the window. The entire femoral head was visible, with a 25° internal rotation of the hip showing minimal or no lesser trochanter on the scanned image. For the Hologic scanner (Marlborough, MA, USA), the array mode was used for each image, while for the Prodigy Lunar scanner (GE Healthcare, Chicago, IL, USA) automatically chose thin (<13 cm), standard (13–25 cm) or thick (>25 cm) depending on the body thickness and height/weight of the patient.

Samples for pharmacokinetic and biomarker analyses were collected from a subset of participants at selected sites before

and after the infusion of SNF472/placebo at baseline and Weeks 10, 22 and 52. Laboratory analyses, including haematology, coagulation and chemistry, were performed at baseline and Weeks 10, 28, 40 and 52 via a central laboratory.

A Data and Safety Monitoring Board (DSMB) was formed to monitor patient safety and data integrity during the study. In January 2019, a futility analysis was performed based on data from the first 136 patients and the DSMB recommended continuation of the study.

Statistical analysis

In this report we describe baseline characteristics of patients enrolled in the study, including demographics, medical history, current and prior medications and calcium scores (Agatston and calcium volume). We calculated the Spearman's correlation between the Agatston CAC score and CAC calcium volume score at baseline. We used an exploratory multiple linear regression model to identify associations with the log-transformed CAC volume score at baseline. Based on prior research [26], candidate variables in the initial model were sex (male/female), age (years), dialysis vintage (months), PTH, magnesium, diabetes (yes/no), non-calcium-based phosphate-binder use (yes/no), statin use (yes/no), warfarin use (yes/no) and history of overt atherosclerotic cardiovascular disease (yes/no). Stepwise regression was used to identify factors associated with baseline log-transformed CAC volume score with 0.10 significance level for entry into the model and 0.10 significance level for remaining in the model. Analyses were conducted using SAS version 9.3 or above (SAS Institute, Cary, NC, USA). Results for body mass index were not included in this baseline analysis.

The primary endpoint of the study will be the change in log coronary artery calcium volume score from baseline to Week 52 for the combined dose groups versus placebo. Secondary endpoints are change from baseline in log Agatston CAC score at Week 52; proportion of patients with <15% progression in Agatston CAC score at Week 52; change in thoracic aorta calcium score from baseline to Week 52; change in aortic valve calcium score from baseline to Week 52; incidence of composite safety endpoint that includes death from cardiovascular causes, myocardial infarction, stroke or heart failure; mortality rate (all-cause and cardiovascular); change from baseline in levels of selected biomarkers including C-reactive protein, fetuin-A, FGF23, matrix gla protein (MGP), sclerostin and growth differentiation factor 15; change in BMD between baseline and 52 weeks and safety. Exploratory endpoints include change from baseline in pulse pressure, systolic blood pressure and diastolic blood pressure at Weeks 28 and 52 in all patients.

The modified intention-to-treat analysis population is used for all primary and secondary efficacy analyses. This population consists of all randomized patients who receive at least one dose of SNF472 or placebo and have at least one post-randomization evaluable CT scan with a CAC score. The safety population will include all randomized patients who receive at least one dose of SNF472 or placebo. The CAC volume score is log-transformed prior to analysis. The primary analysis model is an analysis of covariance (ANCOVA) with the change in log score (log[Week 52] – log[baseline]) as the dependent variable, and a fixed effects term for the randomized treatment group as well as log(baseline) as a covariate; the model is stratified by baseline Agatston CAC score. For secondary efficacy outcomes, ANCOVA is used to compare combined SNF472 doses versus placebo and SNF472 300- and 600-mg doses versus placebo individually. For the event-driven secondary outcomes, Kaplan–Meier product limit estimates are used to display the data and proportional hazards (Cox) regression is used to compare SNF472 to placebo, stratified by baseline Agatston CAC score.

The planned sample size for CaLIPSO was approximately 270 patients. Based on available data [26], assuming a standard deviation (SD) of the mean change in CAC volume score of 0.30 on the log scale (from baseline to Week 52) and a dropout rate of 25%, the sample size (i.e. 90 patients per group) was selected to provide 80% power to test the hypothesis that the log-transformed true difference in progression between the combined SNF472 doses and the placebo group is 0.126, corresponding to a true ratio of 1.134, or 19% progression for the average of the SNF472 doses and 35% progression for the placebo group.

RESULTS

Enrolment

Of 645 patients from 72 centres who were screened for enrolment, 239 did not meet the inclusion criteria, most of whom ($n = 231$) did not have an Agatston CAC score within the required range at screening. Other reasons for not entering the randomization are shown in Table 2. A total of 274 patients were randomized to one of the three treatment groups. Of these, 82 (30%) had an Agatston CAC score of 100–399 U, 77 (28%) had a score of 400–1000 U and 115 (42%) had a score >1000 U.

Demographics and clinical and laboratory characteristics at baseline

The mean age of the 274 randomized patients was 63.6 years (SD 8.9) and 39% were women (Table 3). Demographic characteristics and vital signs were similar across the Agatston CAC score strata. Most patients (93%) had hypertension, 62% had diabetes mellitus, 22% had clinical history of coronary artery disease and 16% had congestive heart failure. Patients with higher Agatston CAC scores had a numerically higher prevalence of diabetes mellitus, coronary artery disease and congestive heart failure than those with lower Agatston CAC scores. The median duration of haemodialysis was 42.4 months.

Laboratory parameters were generally similar across the Agatston CAC score strata (Table 4). In each treatment group, serum PTH was well above the normal range (10–65 pg/mL) and magnesium was slightly above the normal range (1.7–2.2 mg/dL). However, serum PTH concentrations were consistent with Kidney Disease: Improving Global Outcomes (KDIGO) recommendations for patients with kidney failure [16]. Serum calcium and albumin were in the normal range in each treatment group.

Approximately one-third of the patients were treated with a calcium-based phosphate binder and half were treated with the non-calcium-based phosphate binder, sevelamer; 7% were

Table 2. Summary of reasons for screening failure

Reason patient did not meet entry criteria	All screened ($N = 645$)
Inclusion criteria	239
Exclusion criteria	30
Both inclusion and exclusion	1
Withdrew consent	42
Lost to follow-up	0
Other	59
CT not completed/not evaluable	19
Kidney transplant	2
Screening/enrolment closed	32
Other (reason not specified)	6

Table 3. Demographic and baseline characteristics

Characteristic	Coronary artery calcium score (Agatston) category			
	100–399 U ($n = 82$)	400–1000 U ($n = 77$)	>1000 U ($n = 115$)	Total ($N = 274$)
Age (years), mean ± SD	61.0 ± 9.1	62.9 ± 8.7	65.9 ± 8.3	63.6 ± 8.9
Sex, *n* (%)				
Male	51 (62)	42 (55)	74 (64)	167 (61)
Female	31 (38)	35 (45)	41 (36)	107 (39)
Race, *n* (%)				
White	54 (66)	50 (65)	84 (73)	188 (69)
Black or African American	21 (26)	19 (25)	21 (18)	61 (22)
Asian	4 (5)	4 (5)	2 (2)	10 (4)
Other	0	0	4 (4)	4 (1)
Not reported	3 (4)	4 (5)	5 (4)	12 (4)
Ethnicity, *n* (%)				
Hispanic or Latino	31 (38)	29 (38)	40 (35)	100 (36)
Body mass index (kg/m^2)	28.4 ± 6.7	29.6 ± 6.2	29.0 ± 5.6	29.0 ± 6.1
Vital signs, mean ± SD				
Systolic blood pressure (mmHg)	138.8 ± 21.5	136.1 ± 25.6	136.4 ± 26.6	137.0 ± 24.9
Diastolic blood pressure (mmHg)	72.3 ± 11.6	70.1 ± 13.6	65.5 ± 13.6	68.8 ± 13.3
Medical history, *n* (%)				
Diabetes mellitus	48 (59)	47 (61)	75 (65)	170 (62)
Hypertension	77 (94)	71 (92)	107 (93)	255 (93)
Peripheral vascular disease	13 (16)	9 (12)	14 (12)	36 (13)
Cerebrovascular accident	11 (13)	6 (8)	15 (13)	32 (12)
Myocardial infarction	9 (11)	6 (8)	16 (14)	31 (11)
Coronary artery disease	12 (15)	15 (19)	34 (30)	61 (22)
Congestive heart failure	10 (12)	12 (16)	21 (18)	43 (16)
Time on haemodialysis (months)				
Median (25th–75th percentile)	37.9 (19.1–66.1)	44.3 (19.5–77.0)	43.4 (20.4–74.8)	42.4 (19.5–74.8)
<12 months, *n* (%)	9 (11)	7 (9)	15 (13)	31 (11)
12–36 months, *n* (%)	31 (38)	26 (34)	36 (31)	93 (34)
>36 months, *n* (%)	42 (51)	44 (57)	64 (56)	150 (55)

Table 4. Baseline mineral metabolism parameters

Baseline laboratory parameter	Coronary artery calcium score (Agatston) category			
	100–399 U ($N = 82$)	400–1000 U ($n = 77$)	>1000 U ($n = 115$)	Total ($N = 274$)
Parathyroid hormone (pg/mL), median (25th–75th percentile)	312 (240–482)	318 (213–501)	362 (238–587)	335 (231–530)
Magnesium (mg/dL), mean ± SD	2.5 ± 0.4	2.5 ± 0.4	2.4 ± 0.4	2.5 ± 0.4
Albumin (g/dL), mean ± SD	4.0 ± 0.3	4.0 ± 0.3	3.9 ± 0.3	4.0 ± 0.3
Uncorrected serum calcium (mg/dL), mean ± SD	8.7 ± 0.8	9.0 ± 0.8	8.9 ± 0.6	8.8 ± 0.7
Corrected serum calcium[a] (mg/dL), mean ± SD	8.7 ± 0.7	9.0 ± 0.7	8.9 ± 0.7	8.9 ± 0.7
Alkaline phosphatase (U/L), mean ± SD	110.8 ± 48.7	112.6 ± 62.0	118.6 ± 81.8	114.7 ± 68.1

[a]Corrected serum calcium = uncorrected serum calcium + 0.8 (4 – serum albumin).

treated with lanthanum and 12% with another non-calcium-based phosphate binder (Table 5). Statins were used by 62% of patients, calcimimetics by 28%, warfarin by 7% and activated vitamin D by 53%. More patients with the highest Agatston CAC scores at baseline (>1000 U) were being treated with non-calcium-based phosphate binders, calcimimetics, statins or warfarin, whereas patients with lower Agatston CAC scores were more likely to be treated with calcium-based binders.

Measures of cardiovascular calcification

Baseline calcium scores (Agatston and volume) for coronary artery, aorta and aortic valve were not normally distributed. Thus summary statistics at each location are presented as medians (25th–75th percentile) and geometric means (95% confidence interval). Median Agatston scores and calcium volume scores, respectively, were 730 U and 666 for the coronary arteries, 1728 U and 1418 for the aorta and 103 U and 107 for the aortic valve (Table 6 and Figure 2). For all patients combined, the correlation at baseline between Agatston CAC scores and CAC calcium volume scores was 0.968 ($P < 0.001$). As expected, median Agatston scores and calcium volume scores in the aorta and the aortic valve were numerically higher in patients with a baseline Agatston CAC score >1000 U than in those with a baseline score of 100–399 U. Among the 115 patients with an Agatston CAC score >1000 U at baseline, the score at the aorta and aortic arch was >100 U for 110 (96%) and 44 (38%) patients, respectively.

Table 5. Baseline medication use

Baseline medication	Coronary artery calcium score (Agatston) category			
	100–399 U ($n = 82$)	400–1000 U ($n = 77$)	>1000 U ($n = 115$)	Total ($N = 274$)
Calcium-based phosphate binders, *n* (%)	27 (33)	20 (26)	35 (30)	82 (30)
Non-calcium-based phosphate binders, *n* (%)				
Sevelamer	37 (45)	39 (51)	61 (53)	137 (50)
Lanthanum	4 (5)	3 (4)	12 (10)	19 (7)
Other	10 (12)	6 (8)	18 (16)	34 (12)
Calcimimetics, *n* (%)	20 (24)	21 (27)	37 (32)	78 (28)
Statins, *n* (%)	47 (57)	44 (57)	78 (68)	169 (62)
Warfarin, *n* (%)	3 (4)	3 (4)	12 (10)	18 (7)
Activated vitamin D, *n* (%)	47 (57)	39 (51)	58 (50)	144 (53)

Table 6. Baseline calcification assessments using Agatston and volume methods

Location and baseline Agatston score	Agatston calcium score		Calcium volume score	
	Median (25th–75th percentile)	Geometric mean ± SE	Median (25th–75th percentile)	Geometric mean ± SE
Coronary artery				
100–399 U ($n = 82$)	210 (149–285)	207 ± 9	204 (163–278)	212 ± 11
400–1000 U ($n = 77$)	598 (479–757)	602 ± 19	596 (441–692)	564 ± 17
>1000 U ($n = 115$)	1617 (1222–1903)	1600 ± 52	1378 (1068–1640)	1324 ± 44
Total ($n = 274$)	730 (315–1435)	659 ± 45	666 (310–1234)	602 ± 37
Aorta				
100–399 U ($n = 77$)	984 (427–2429)	926 ± 465	807 (344–2096)	830 ± 368
400–1000 U ($n = 72$)	1446 (411–3904)	1102 ± 570	1217 (360–3040)	969 ± 440
>1000 U ($n = 113$)	3620 (1305–7302)	2617 ± 785	2837 (1099–6063)	2219 ± 605
Total ($n = 262$)	1728 (625–4978)	1520 ± 407	1418 (536–4052)	1323 ± 315
Aortic valve				
100–399 U ($n = 42$)	73 (31–192)	60 ± 31	55 (37–209)	69 ± 27
400–1000 U ($n = 41$)	76 (31–231)	89 ± 46	86 (46–219)	92 ± 37
>1000 U ($n = 74$)	137 (32–395)	102 ± 73	136 (37–353)	113 ± 57
Total ($n = 157$)	103 (31–262)	85 ± 38	107 (38–278)	94 ± 30

SE, standard error.

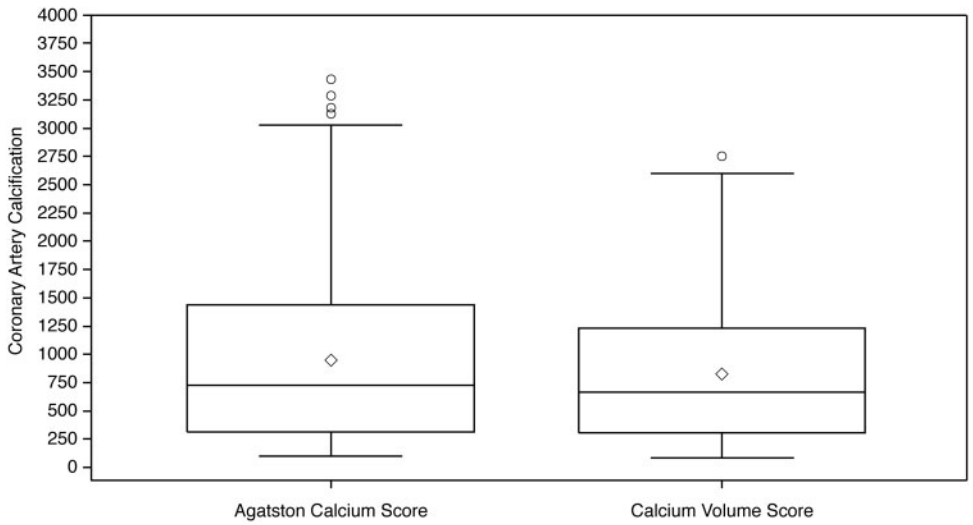

FIGURE 2: Distribution of baseline coronary artery calcium scores (Agatston and calcium volume).

Table 7. Linear regression analysis of predictors of log-transformed coronary artery calcium volume scores at baseline

Variable	Parameter estimate	Standard error	P-value	Model R^2
Intercept	4.875	0.409	<0.001	–
Age (years)	0.022	0.006	<0.001	0.039
Coexistent diabetes	0.255	0.111	0.022	0.060

Candidate variables in the initial model were sex (male/female), age (years), dialysis vintage (months), PTH, magnesium, diabetes (yes/no), non-calcium-based phosphate binder use (yes/no), statin use (yes/no), warfarin use (yes/no) and history of overt atherosclerotic cardiovascular disease (yes/no). Stepwise regression was used to identify factors associated with baseline log-transformed CAC volume score with 0.10 significance level for entry into the model and 0.10 significance level for remaining in the model.

Factors associated with CAC score at baseline

In the multivariable-adjusted linear regression analysis that required a 0.10 significance level for variables to remain in the model, older age ($P < 0.001$) and diabetes mellitus ($P = 0.022$) were independently associated with higher baseline CAC volume scores (Table 7).

DISCUSSION

Cardiovascular calcification is highly prevalent in patients with impaired kidney function; at least 80% of patients receiving maintenance haemodialysis exhibit some degree of cardiovascular calcification [14, 27]. Cardiovascular calcification is also associated with adverse outcomes [28–31]. Among 181 consecutive patients with non-dialysis-requiring CKD, baseline CAC score and CAC score progression predicted the occurrence of cardiac death and/or myocardial infarction [32]. Numerous observations support the contribution of valvular and vascular calcification to morbidity and mortality in CKD [33]. CAC has been associated with increased arterial stiffness [34, 35], left ventricular hypertrophy [36], epicardial adipose tissue thickness and volume, myocardial ischaemia [37] and pro-arrhythmic electrocardiographic abnormalities [35]. Valvular calcification causes restriction of leaflet motion [38, 39], increased transvalvular gradient [38, 39] and left atrium enlargement [38, 39] and is associated with reduced survival [40].

In this study, patients with higher Agatston CAC scores at baseline were more likely to be treated with a non-calcium-based phosphate binder, calcimimetics, statin or warfarin. These management strategies were consistent with recent KDIGO CKD-MBD guidelines [16]. Intense research has been undertaken in recent years to identify modifiable risk factors that induce calcification, as well as drugs that may reduce its progression, in the hope of reducing the incidence of cardiovascular events and mortality in patients with impaired kidney function [16]. Multiple prior studies have tested the effects of a variety of interventions that modify mineral metabolism on calcification of the coronary arteries, aorta and cardiac valves. Treatment with non-calcium-based phosphate binders has been shown to attenuate CAC progression compared with calcium-based phosphate binders [41]. Similarly, in a randomized clinical trial (ADVANCE), cinacalcet was compared with placebo on top of standard care including calcitriol or vitamin D analogues in patients receiving haemodialysis with moderate-to-severe secondary hyperparathyroidism. Cinacalcet was associated with slower progression of calcification from baseline to Week 52 in the thoracic aorta (cinacalcet 19%, control 33%; $P = 0.055$), aortic valve (cinacalcet 6%, control 52%; $P = 0.014$) and mitral valve (cinacalcet 12%, control 54%; $P = 0.053$), but the trial did not meet its primary endpoint for mean change in Agatston CAC score (cinacalcet 24%, control 31%; $P = 0.073$) [26]. EVOLVE, a large event-driven randomized clinical trial comparing cinacalcet with placebo on top of standard care, showed generally favourable effects on cardiovascular events and fracture, but the primary endpoint was also not met [42].

The pathogenesis of cardiovascular calcification in CKD is complex and only partly understood [10]. Irrespective of the triggering factors, calcium–phosphate crystals may deposit in the soft tissues if there is an imbalance of calcification inhibitors and promoters [43]. In the presence of both high and low mineral turnover, it has been postulated that bone cannot accommodate additional calcium and phosphate in the matrix, leading to excess circulating ions and eventually precipitation of these minerals in soft tissue [44]. Cardiovascular calcification has often been described as a passive degenerative process due to 'wear and tear' and progressive senescence [5, 45]. However, active cellular mechanisms are principally responsible for ectopic calcification [6, 10, 45]. Laboratory experiments show that excess calcium and/or phosphate, along with decreased levels of calcification inhibitors, induce vascular smooth muscle cells to differentiate and assume an osteoblastic phenotype. These cells initiate deposition of collagen, which becomes calcified in the arterial wall [43]. Micronutrients such as magnesium and phosphorus may have a similar role in valvular calcification [45]. Further stimulus towards the accumulation of soft tissue calcification in uraemia is provided by the frequent depletion of inhibitors of calcification [46]. For example, low levels of fetuin-A, osteoprotegerin, MGP, vitamin K and pyrophosphate have been consistently described in patients with CKD and associated with vascular calcification [10].

To date, there are no approved therapies to directly slow the progression of cardiovascular calcification. Although a number of therapies potentially target the calcification process, such as pyrophosphate, bisphosphonates, thiosulphate and vitamin K, none of these compounds have been shown to be effective in or approved for the treatment or prevention of cardiovascular calcification. Current guidelines consider patients with CKD and cardiovascular calcification to be at the highest cardiovascular risk and suggest managing the biochemical abnormalities observed in patients with CKD as a therapeutic strategy to mitigate progression of cardiovascular calcification [16].

Preclinical data showed that SNF472 reduced development of cardiovascular calcification by up to 80% [47]. In early clinical trials, SNF472 was well tolerated in humans [48, 49]. The CaLIPSO study was designed to test the hypothesis that SNF472 slows the progression of calcification in the coronary arteries, aorta and aortic valve. CaLIPSO was powered to detect a clinically meaningful reduction in CAC as measured by CAC volume scores between the combined SNF472 dose groups versus placebo after 52 weeks of treatment.

The CaLIPSO study has limitations. This Phase 2 trial was not powered to evaluate a reduction in cardiovascular mortality and morbidity. Future appropriately powered studies will be needed to assess the effects of SNF472 on cardiovascular

outcomes. The relatively short follow-up (52 weeks) and the use of different CT scanners at study sites are other potential limitations. To increase the quality and comparability of evaluations, a common procedure for CT scanning acquisition was used and CT scans were evaluated in a central core laboratory by readers blinded to treatment assignment using common methods for calcium quantitation in the coronary arteries, including Agatston and volume scores. Although multiple factors may affect the progression of cardiovascular calcification, patients recruited in the CaLIPSO study were only stratified according to baseline Agatston CAC score, a powerful predictor of CAC progression. Serum phosphate, which was collected in a subset of patients, was not available for this analysis. Data on dialysis session duration and dialysate calcium concentration were not collected.

In summary, the CaLIPSO trial was designed to investigate the inhibitory effect of SNF472 on the progression of cardiovascular calcification in patients on maintenance haemodialysis. The results will determine further evaluation of SNF472 in managing cardiovascular disease in this patient population.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the research idea and study design. M.R. and S.S. contributed to the acquisition of data. All authors contributed to the analysis and interpretation of data. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy and integrity of any portion of the work are appropriately investigated and resolved. A.B. takes responsibility that this study has been reported honestly, accurately and transparently; that no important aspects of the study have been omitted and that any discrepancies from the study as planned and registered have been explained.

CONFLICT OF INTEREST STATEMENT

A.B. received personal fees from Sanifit. P.R. received other compensation from Sanifit. J.B. received personal fees from Sanifit, Sanofi-Genzyme, Vifor-Fresenius-Renal Pharma, AbbVie, Amgen and Shire. D.A.B. received personal fees from Sanifit, Tricida, Relypsa/Vifor/Fresenius, Sanofi/Genzyme and Amgen and grants from the National Institutes of Health and Renal Research Institute. G.M.C. received personal fees from Akebia, AMAG, Amgen, Ardelyx, AstraZeneca, Gilead, Reata, Sanifit and Vertex; has an ownership interest in Ardelyx, CloudCath, Cricket, Durect, Outset, PuraCath and Physiowave and received research funding from Amgen and Janssen. M.K. received personal fees from Sanifit, Amgen, Medice, Sanofi and Vifor. M.R. received personal fees from Amgen, Sanofi, Vifor, and Sanifit. S.S. received personal fees from Sanifit, Vifor Fresenius and Napp. C.S. and J.P. are employees and shareholders of Sanifit Therapeutics S.A., and have patents related to SNF472. R.G. and A.G. are employees and shareholders of Sanifit Therapeutics S.A.

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