Clinical Study Protocol

A double-blind, randomised, placebo-controlled study to assess the effect of SNF472 on progression of cardiovascular calcification on top of standard of care in end-stage-renal-disease (ESRD) patients on haemodialysis (HD)

SNFCT2015-05

Development Phase: Phase 2b

Investigational Product: SNF472

Indication: CVC in ESRD

Sponsor: Laboratoris Sanifit
Palma de Mallorca, Spain

EudraCT Number 2016-002834-59

IND Number 116437

Protocol Date: Version 05, 01-MAR-2019

Amendment History:

Date	Amendment Number	Amendment Type	Region
26-JUL-2016	Initial Protocol	Not applicable	Global
31-JUL-2017	01	Substantial	Global
31-OCT-2017	1.1	Country-specific	US
29-MAR-2018	02	Substantial	Global
27-JUN-2018	03	Non-substantial	Global
22-OCT-2018	04	Substantial	Global
01-MAR-2019	05	Substantial	Global

Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Council for Harmonisation Guidelines on Good Clinical Practice (ICH E6 GCP) and regulatory requirements as applicable.

CONFIDENTIAL INFORMATION

SPONSOR'S PROTOCOL APPROVAL SIGNATURE PAGE
Amendment 5

Sponsor: Laboratoris Sanifit

I have read and understand the contents of this clinical protocol for Study No. SNFCT2015-05 and agree to meet all obligations of the Sponsor as detailed in all applicable regulations and according to Good Clinical Practice (ICH E6 GCP).

[redacted]
Chief Medical Officer
Laboratoris Sanifit

Signature

Date (DD-MMM-YYYY)

PRINCIPAL/COORDINATING INVESTIGATOR'S AGREEMENT AMENDMENT 5

I have read and understand the contents of this clinical protocol for Study No. SNFCT2015-05 dated and will adhere to the study requirements as presented, including all statements regarding confidentiality. In addition, I will conduct the study in accordance with current International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline for Good Clinical Practice and applicable regulatory requirements:

Principal/Coordinating Investigator Name

Principal/Coordinating Investigator Signature **Date (DD-MMM-YYYY)**

PROTOCOL AMENDMENT 5 SUMMARY OF CHANGES

1) Section 10.7: Additional analyses of the dataset used for the futility analyses will be conducted in the event of an equivocal result of the futility analysis indicating borderline conditional power of the study. These analyses will include primary and secondary endpoints, demographic and background characteristics, key subgroups, and PK/PD correlations with efficacy. Details will be provided in the SAP.

   Rationale: To assist the Sponsor in decision making regarding future clinical development of SNF472.

2) New Appendix 7 containing the summary of changes for the previous version of the protocol (Amendment 4).

3) Revisions of amendment number and date throughout.

PROTOCOL SYNOPSIS

Sponsor: Laboratoris Sanifit	**Investigational Product:** SNF472	**Developmental Phase:** Phase 2b
Title of Study: A double-blind, randomised, placebo-controlled study to assess the effect of SNF472 on progression of cardiovascular calcification on top of standard of care in end-stage-renal-disease (ESRD) patients on haemodialysis (HD)		
Protocol Number: SNFCT2015-05		
EudraCT: 2016-002834-59		
IND Number: 116437		
Number of Patients and Study Centre(s): Approximately 270 patients in approximately 75 centers in 3 countries		
Indication: Cardiovascular Calcification (CVC) in ESRD		
Objectives: The primary objective is to assess the effect of 2 dose levels of SNF472 (300 mg and 600 mg) compared to placebo on the progression of coronary artery calcium volume score over a 12-month (52 weeks) period in ESRD patients on HD. The secondary objectives are to: • assess change from baseline (Week 1, Day 1) in coronary artery calcium (CAC)/Agatston score • assess the number of patients with <15% progression in CAC/Agatston score • assess the change from baseline in thoracic aorta calcification score • assess the change from baseline in aortic valve calcification score • assess the occurrence of the composite safety endpoint: death from cardiovascular causes, myocardial infarction (MI), stroke, or heart failure • assess change in biomarkers as signals for treatment efficacy/response • assess changes in bone mineral density (BMD) • describe the long-term safety profile of SNF472 in this target population The exploratory objective is to assess changes from baseline in pulse pressure, systolic blood pressure (SBP), and diastolic blood pressure (DBP).		
Methodology: This is a randomised, double-blind, placebo-controlled, phase 2b study to evaluate the effects of 2 dose levels of SNF472 on progression of cardiovascular calcification		

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(CVC) as measured by calcium volume and CAC scores in ESRD patients on HD. The randomization will be stratified by baseline CAC score category.

Endpoints:

The primary endpoint is the change in coronary artery calcium volume scores between baseline (Week 1, Day 1) and Week 52 measured by computed tomography (CT) scan.

The secondary endpoints are:

- change from baseline in CAC/Agatston score at Week 52
- number of patients with <15% progression in CAC/Agatston at Week 52
- change from baseline in thoracic aorta calcification score at Week 52
- change from baseline in aortic valve calcification score at Week 52
- incidence of composite safety endpoint that include death from cardiovascular causes, MI, stroke, or heart failure
- mortality rate (all-cause and CV)
- change from baseline in levels of selected biomarkers including C-reactive protein (CRP)
- changes in BMD between baseline and 52 weeks
- safety of SNF472 in terms of incidences of adverse events (AE) and serious adverse events (SAE) and clinically relevant changes from baseline in laboratory and ECG parameters

The exploratory endpoints are changes from baseline in pulse pressure, SBP, and DBP at Week 28 and Week 52 in all patients and in the subgroup of patients with hypertension at baseline.

Criteria for Inclusion:

1. female or male patients, 18 to 80 years (inclusive) of age at randomisation
2. CAC score of 100 to 3500 AU (Agatston Units) inclusive within a 4-week period prior to randomisation as measured by a multi-detector CT scanner
3. patients who are EITHER ≥ 55 years OR have a history of diabetes mellitus at randomisation
4. patients on HD for ≥ 6 months prior to randomisation
5. willing and able to understand and sign the informed consent

Criteria for Exclusion:

1. scheduled date for kidney transplant from a known living donor

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2. weight above 300 lbs (136 kg)
3. hospitalisation in the previous 3 months prior to randomisation for unstable angina, MI, stroke, transient ischaemic attack, amputation or peripheral or coronary bypass surgery
4. 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 edema and requiring ultrafiltration therapy
5. history of cancer that has been in remission for < 5 years prior to randomisation. A history of basal cell carcinoma or Stage 1 squamous cell carcinoma of the skin is allowed
6. pregnant or trying to become pregnant, currently breast-feeding, or of child-bearing potential (including peri-menopausal women who have had a menstrual period within one year) and not willing to practice birth control using a double barrier method (criteria apply to women only) at least 30 days post last dose of study medication
7. hypocalcaemia defined as a serum calcium below 8.0 mg/dL (or 2.0 mmol/L) for the serum calcium most proximal to screening per patient's medical records
8. extreme elevation in serum phosphorous, defined as a serum phosphorous above 10 mg/dL (or 3.23 mmol/L) within the last 2 months proximal to screening per patient's medical records
9. uncontrolled hypertension defined as any 2 or more consecutive post-dialysis diastolic blood pressure (DBP) > 100 mmHg within the last 2 months proximal to screening
10. expected survival < 2 years in the Investigator's medical opinion
11. known active drug or alcohol abuse within 1 year of randomisation
12. use of other investigational drugs within 30 days of randomisation
13. non-compliance with dialysis treatment which, in the opinion of the Investigator, evidenced by either repeated missed dialysis treatments or significant non-compliance with the patient's medication regimen
14. Inability to comply with all required study procedures and schedule, 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

Test Product, Dose and Mode of Administration:

A single intravenous (IV) dose of one of two dose levels of SNF472 (300 mg and 600 mg) delivered in conjunction with each HD session (3 times weekly) over 52 weeks.

Reference Therapy, Dose and Mode of Administration:

Placebo, administered as SNF472

Study Duration: 28 + 3 days screening; 52-week treatment period; follow-up at Week 56.

Statistical Methods:

Approximately 270 patients are planned to be randomised.

Sponsor: Laboratoris Sanifit	**Investigational Product:** SNF472	**Developmental Phase:** Phase 2b
The primary endpoint is the absolute change in coronary artery calcium volume score from baseline to Week 52. The primary analysis will use an analysis of covariance (ANCOVA) model to compare each of the 2 dose levels of SNF472 to the placebo group. The dependent variable will be the difference between log 52-week and log baseline calcium volume scores, with log baseline calcium volume score and treatment group as explanatory variables. This analysis will be stratified for the randomization stratification factor, i.e. baseline CAC/Agatston score. A non-binding interim futility analysis will be conducted when approximately N=120 subjects (63% of N=190) have provided Week 52 data on the primary endpoint. PK and PD analyses will also be conducted at the time of the interim analysis.		

SCHEDULE OF ASSESSMENTS

A total of 159 visits (2 screening visits followed by 3 dialysis sessions per week x 52 weeks and 1 follow-up visit 1 month post-dose) are scheduled throughout the study. Table 1.1 shows key visits where intensive data collection is performed. For other visits, standard of care assessments should be performed. ***If also participating in Sub-Study, please see additional Schedule of Sub-Study Assessments in Appendix 1.***

Table 1.1 Schedule of Assessments and Study Activities at Key Visits

Procedures	**Screening[a] 28 + 3 days**		**Double Blind Treatment Period**												**Wk 56 or 1 month after last dose**
			<u>156 VISITS</u>: Study Drug Infusion Administered at Each HD Session (3x Weekly)[k]												
	Key Evaluation Visits (Preferably at Mid-Week Dialysis Sessions)														
Week	**Step 1[a]**	**Step 2[a]**	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 10**	**Wk 16**	**Wk 22**	**Wk 28**	**Wk 34**	**Wk 40**	**Wk 46**	**Wk 52/ET[m]**	
Day			**1**	**8 (±3 d)**	**22 (±3 d)**	**36 (±3 d)**	**64 (±3 d)**	**106 (±3 d)**	**148 (±3 d)**	**190 (±3 d)**	**232 (±3 d)**	**274 (±3 d)**	**316 (±3 d)**	**358 (±3 d)**	
Informed consent[b]	X														
Eligibility, inclusion/exclusion criteria[c]	X	X	X												
CT scan for calcium scores for screening[d] and endpoints	X													X	
DEXA for BMD[e]	X													X	
Evaluation of CT scan at Step 1 must be completed before proceeding to Step 2															
Medical history, demographics		X													
Physical exam, including height and weight		X												X	

Procedures	Screening[a] 28 + 3 days		Double Blind Treatment Period												Wk 56 or 1 month after last dose
			156 VISITS: Study Drug Infusion Administered at Each HD Session (3x Weekly)[k]												
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Week	Step 1[a]	Step 2[a]	Wk 1	Wk 2	Wk 4	Wk 6	Wk 10	Wk 16	Wk 22	Wk 28	Wk 34	Wk 40	Wk 46	Wk 52/ET[m]	
Day			1	8 (±3 d)	22 (±3 d)	36 (±3 d)	64 (±3 d)	106 (±3 d)	148 (±3 d)	190 (±3 d)	232 (±3 d)	274 (±3 d)	316 (±3 d)	358 (±3 d)	
Treatment history, current and prior medication		X													
Pregnancy[f]		X	X			X	X	X	X	X	X	X	X	X	
12-lead ECG (including QTc)[g]		X					X			X		X		X	
Vital signs[h]		X	X	X		X		X		X		X		X	
Randomisation			X												
Haematology, coagulation, and chemistry[i]			X				X			X		X		X	
PTH, TSAT, ferritin			X											X	
Plasma PK sampling[j]			X				X		X					X	
Biomarkers sampling[j]			X				X		X					X	
Blinded study drug infusion (3x weekly)[k]			X	X	X	X	X	X	X	X	X	X	X	X	
Post-dialysis weight			X				X			X		X		X	
Change in concomitant medications[l]			X		X		X			X				X	
AE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X (SAEs only)

AE: adverse event; BL: Baseline; BMD: bone mineral density; CAC: coronary artery calcium; CT: computed tomography; DEXA: dual-energy x-ray absorptiometry; ECG: electrocardiogram; ET: early termination; PK: pharmacokinetic(s); PTH: parathyroid hormone; SAEs: serious adverse events; TSAT: transferrin saturation; Wk: week

[a] Screening will proceed in 2 steps: Step 1 will involve informed consent followed by the CT scan for CAC score and BMD by DEXA for Screening. Only those patients who are eligible in Step1 can proceed to Step 2.

[b] Patients will provide written informed consent before any clinical study-specific procedures are performed.

[c] The inclusion/exclusion criteria will be assessed for each patient at Screening and will be confirmed again on Day 1 prior to randomisation.

[d] CT scan of coronary artery using a multi-detector CT with at least 64 slices to quantify calcium scores for Step 1 screening. Only patients who meet the inclusion criterion of CAC score of 100 to 3500 AU (Agatston Units) will proceed to Screening Step 2.

[e] BMD will be measured by DEXA at the same visits at the CT scan.

[f] For females of child-bearing potential, serum pregnancy test.

[g] ECG will be performed at Screening and **post-dialysis** at the visits indicated.

[h] Vital signs include heart rate, respiratory rate, blood pressure and body temperature **post-dialysis**. These are recorded for the study at key visits only. Standard of care vital signs assessments are not being recorded into study database at each HD visit.

[i] Blood samples will be collected **pre-dose** via the dialysis port for haematology, coagulation, and chemistry tests.

[j] Only for patients at participating sites, the PK and biomarker samples will be collected via the dialysis port at **pre-dose and at 3 hrs (~10 minutes before end of infusion)** on the assessment day. Biomarkers may include but are not limited to fetuin A, FGF23, MGP, sclerostin, phosphorus (phosphate), pharmacodynamics (PD), and GDF15. **No genetic testing will be performed**.

[k] The treatment period is 52 weeks, and study treatment is administered at each HD session (3x weekly following the standard procedure established by the nephrologist). Intravenous doses of either SNF472 or matching placebo will be administered to patients in conjunction with each HD session (see Section 5.5). **Except for Baseline (Day 1), intensive data collection visits are recommended to be done on the 2nd dialysis session of each week (mid-week HD session).**

[l] Record change in concomitant medications relative to previous assessment visit; only prescription medications are to be recorded.

[m] The early termination (ET) visit will replace the Week 52 visit if the patient terminated the study earlier than planned. PK and biomarker samples will be collected during this visit if the post-baseline samples haven't been collected prior to early termination. A CT scan for calcium quantification and BMD by DEXA should be done. If not feasible for this visit, the site must make all efforts to set an appointment for a CT scan and DEXA. In addition, sites must encourage patients to report for the follow-up visit.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine transaminase (SGPT)
ANCOVA	analysis of covariance
AST	aspartate transaminase (SGOT)
ATC	Anatomical Therapeutic Chemical
AU	Agatston units
AUC	area under curve
BP	blood pressure
BMD	bone mineral density
BUN	blood urea nitrogen
BW	body weight
CAC	coronary artery calcium
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHF	congestive heart failure
CKD	chronic kidney disease
C_{max}	observed maximum concentration
C_{min}	minimum observed concentration
CRA	clinical research associates
CRO	contract research organisation
CRP	C-reactive protein
CSP	clinical study protocol
CSR	clinical study report
CT	computed tomography
CTCAE	common terminology criteria for adverse events
CV	cardiovascular
CVC	cardiovascular calcification
DBP	diastolic blood pressure
DEXA	dual-energy x-ray absorptiometry
DSMB	Data and Safety Monitoring Board
EBCT	electron beam computed tomography
ECG	electrocardiogram

Abbreviation	Definition
eCRF	electronic case report form
EDC	electronic data capture
eDMP	electronic data management plan
ESRD	end-stage renal disease
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	gamma-glutamyl transpeptidase
GRAS	generally recognised as safe
h	hour
HAP	hydroxyapatite
HD	haemodialysis
HIPAA	Health Insurance Portability and Accountability Act
HNR	Heinz Nixdorf Recall
HR	heart rate
IC_{50}	half maximal inhibitory concentration
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IMP	investigational medicinal product
INR	international normalised ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	intravenous
KDOQI	Kidney Disease Outcomes Quality Initiative
LDH	lactic acid dehydrogenase
LLOQ	lowest level of quantification
LOCF	last observation carried forward
MBD	mineral and bone disease
MDCT	multi-detector CT scanners
MedDRA	Medical Dictionary for Regulatory Activities
MESA	Multi-Ethnic Study of Atherosclerosis
mg	milligram
mg/kg	milligram/kilogram
MI	myocardial infarction

Abbreviation	Definition
MMRM	mixed model repeated measures
mITT	Modified intent-to-treat
NOAEL	no-observed adverse effect level
PD	pharmacodynamics
PK	pharmacokinetics
pmp	per million population
PP	per protocol
PT	prothrombin time
PTH	parathyroid hormone
PTT	partial thromboplastin time
QTcB	QTc corrected by Bazett's formula
RR	respiratory rate
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
SOC	system organ class
SSR	sample size re-estimation
SUSAR	suspected unexpected serious adverse reactions
TEAE	treatment-emergent adverse events
TSAT	transferrin saturation
WBC	white blood cell
WHO	World Health Organisation

1 INTRODUCTION

1.1 INDICATION/BACKGROUND TO THE DISEASE

1.1.1 Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD)

Chronic kidney disease (CKD) is a serious condition associated with significant morbidity, premature mortality, decreased quality of life, and increased health-care expenditures. Risk factors for CKD include cardiovascular (CV) disease, diabetes mellitus, hypertension, and obesity. As the kidneys progressively fail, a variety of metabolic and hormonal abnormalities accumulate, eventually leading to a chronic state of inflammation, malnutrition, disordered mineral metabolism, anemia, and electrolyte disturbances. These conditions contribute further to the burden of CV disease in the CKD population; increasingly it has been recognized that extra-osseous soft tissue calcification, particularly cardiovascular calcification (CVC), plays an important role in the clinical manifestation of CV disease in these patients.

Patients who reach the most severe stage of CKD, end-stage renal disease (ESRD), require replacement of renal function either through dialysis treatment or renal transplantation. ESRD patients receiving dialysis therapy have all-cause and CV mortality rates that are at least 15-20 times higher than the general age- and gender-matched population (USRDS 2015, Baigent 2000). This burden of CV disease is evident upon the initiation of renal replacement therapy. Forty percent of patients starting dialysis already have evidence of coronary heart disease and only 15% are considered to have normal left ventricular structure and function by echocardiographic criteria (USRDS 2015, Bansal 2013). As described below, the development of CVC during CKD leads to this reduction in cardiac function by the time patients reach ESRD and predisposes to arrhythmia and sudden cardiac death, the most prevalent form of CV mortality in this patient population.

The occurrence of ESRD, as reflected by 2013 incidence rates for renal replacement therapy, varies by region, with countries in Europe generally ranging between 90-170 per million population (pmp) and a much higher incidence in the United States (363 pmp) driven by a greater U.S. incidence of diabetic ESRD (ERA-EDTA 2015, USRDS 2015).

The economic burden of ESRD is far greater than its proportionate incidence. U.S. Medicare costs for ESRD exceed $32B annually, representing over 7% of total Medicare expenditures for a population that represents only 1.5% of Medicare patients (USRDS 2015). Similarly, in European countries like Italy, 2% of the annual healthcare budget is utilized for ESRD, which totals only approximately 0.1% of the general population (Pontoriero 2007).

It is evident that for the ESRD population, investigation of a novel therapy to reduce the progression of CVC is an appropriate endeavor to lower the burden of morbidity and mortality in these disproportionately affected patients. Similarly, a reduction in the clinical consequences of CVC and CV disease would be expected to result in significant healthcare savings globally.

1.1.2 Vascular Calcification

Bone serves as the storage unit for 99% of total body calcium, predominantly in the form of a calcium-phosphate moiety called hydroxyapatite (HAP). Calcium deposition outside of bone is broadly termed extraskeletal calcification and is induced in the setting of cellular necrosis, damaged proteins, or transformation of cells to an osteoblastic phenotype. An important example of the latter occurs with vascular smooth muscle cell transformation resulting in mineralization of collagen and non-collagenous proteins within the arterial wall and other CV structures. Pathologic calcification of these CV structures is known as vascular calcification. In blood vessels, calcified deposits are found in distinct layers of the blood vessel and are related to underlying pathology. Intimal calcification occurs in atherosclerotic lesions, whereas medial calcification (also known as Monckeberg's medial sclerosis) is associated with vascular stiffening and arteriosclerosis observed with age and metabolic disorders including diabetes and end-stage renal disease (ESRD). While intimal and medial calcification may each occur independently, patients with ESRD exhibit a mixture of both intimal and medial calcification.

Vascular calcification is considered an actively regulated process that may arise via multiple cellular mechanisms, including the loss of inhibitors of mineralization that are typically expressed by vascular cells and osteogenic mechanisms operating within vascular lesions. Ultimately, no matter the initiating mechanism for vascular calcification, CKD and ESRD patients have additional exacerbating 'passive' mechanisms of elevated calcium and phosphorous, which combine to promote HAP nucleation and calcification crystal growth. Furthermore, evidence suggests that calcium and phosphorus may additionally have direct effects on vascular cells that predispose to mineralization.

Computed tomography (CT) scanning using electron beam, spiral, or multi-detector scanners is the most common method for quantifying CVC. Common methods for calcium quantitation in the coronary arteries include the Agatston CAC score (in Agatston units or AU) and the newer calcium volume and mass (mineral) scores. The coronary artery calcium (CAC) score is an independent risk factor for adverse CV clinical outcomes in the general population (Madhavan 2014) and has been shown to be more predictive of CV disease events than carotid intima-media thickness measurement (Folsom 2008). In contrast to the CAC score, the calcium volume score represents an actual measured volume of CAC and has been reported to exhibit reduced variability between scans. A previous clinical trial evaluating the impact of an unrelated therapy on CVC progression in HD patients demonstrated a more significant impact on calcium volume score than on the CAC score (Raggi 2011).

1.1.3 Cardiovascular Calcification in CKD and ESRD

As described above, the metabolic and hormonal disorders resulting from the progression of CKD to ESRD result in an environment within the circulatory system that is ripe for the development of CVC. It has been long recognized that patients with CKD exhibit more vascular calcification than arteries from age-matched healthy individuals. Seminal findings by Goodman (2000) and additional work by other groups demonstrates that coronary artery calcification occurs in young patients with ESRD decades before this pathology is observed in the normal population. Moreover, adult patients on receiving HD therapy are reported to

have calcification scores over 5-fold higher than age- and sex-matched individuals with established coronary artery disease, but normal kidney function. These HD patients displayed a disturbing tendency for progression of calcification over one year. Risk factors for greater progression of CVC in HD patients include age, diabetes mellitus, time since initiation of renal replacement therapy, and elevated levels of serum phosphorous and inflammatory markers. In addition to the magnitude of the CVC score, the rate of progression of vascular calcification also appears to be an important risk factor for CV events including mortality.

The major clinical consequences of CVC in ESRD patients include medial artery calcification and cardiac valvular calcification. Medial calcification contributes to vascular stiffness, resulting in increased pulse-wave velocity, reduced diastolic blood pressure (DBP), and increased systolic blood pressure (SBP). These changes lead to increases in cardiac afterload and reductions in diastolic coronary artery perfusion pressures, both of which lead to left ventricular hypertrophy, diastolic dysfunction and, ultimately cardiac failure with a predisposition to arrhythmia and sudden cardiac death. Cardiac valve calcification is a major mechanism for valve failure and predisposes to stroke, atrial fibrillation, heart failure and sudden cardiac death. CVC is also found in locations of atherosclerotic vascular lesions, although the impact of calcification on plaque rupture or stabilization in the general population has been questioned recently. Regardless, increased density of calcium in atherosclerotic plaques was associated with increased all-cause mortality in a cohort of HD patients.

Thus, the presence and progression of CVC in ESRD patients receiving HD therapy is increased compared the general population. Evidence suggests that this elevated CVC burden plays an important mechanistic role in the development of CV diseases, including heart failure, myocardial infarction (MI), stroke, and both CV-specific and all-cause death. The preponderance of data strongly suggests that CVC is an attractive target for an appropriate intervention to reduce the growth and propagation of calcification and potentially improve CV outcomes in the at-risk ESRD population treated with HD therapy.

1.1.4 Current Treatments

There are no FDA approved therapies indicated for the treatment or reduction of CVC. A number of potential therapies putatively target the calcification process, but these are currently experimental, and there are few controlled studies on which to base therapeutic decisions. Compounds that have been investigated include pyrophosphate and bisphosphonates, thiosulfate, and vitamin K (O'Neill 2010). Each of these therapies has significant issues precluding either widespread use or specifically use in ESRD patients.

Due to the lack of available therapy, treatment is currently focused on managing bone mineral metabolism (eg, normalizing calcium, phosphorus, and parathyroid hormone [PTH]), including either minimizing vitamin D use or considering cinacalcet therapy for secondary hyperparathyroidism in patients with known vascular calcification. Current treatment guidelines for the management of bone mineral metabolism disorders and related complications in CKD and ESRD patients are detailed in the Kidney Disease Outcomes Quality Initiative (KDOQI, National Kidney Foundation).

CV disease is the most common cause of mortality in patients with CKD and ESRD. CVC induced by calcium and phosphate excess and uraemia is a major risk factor and is independently associated with CV events and death (Toussaint 2007).

Coronary artery calcification is a risk factor for adverse clinical outcomes in the general population (Madhavan 2014). Patients on dialysis exhibit more severe vascular calcification than age and sex-matched individuals without CKD. Moreover, patients on HD are reported to have CAC scores as evaluated by electron beam computed tomography (EBCT) over 5-fold higher than age- and sex-matched individuals with established coronary artery disease, but normal kidney function. In addition to the magnitude of the CAC score, the rate of progression of vascular calcification also appears to be an important risk factor for CV events including mortality.

In patients with CKD and diabetes mellitus, the risk of CVC increases compared to CKD patients without diabetes (Anand 2006). Prolonged exposure to hyperglycemia is a major factor in the pathogenesis of atherosclerosis in diabetes. It induces a large number of alterations at the cellular level of vascular tissue that potentially accelerate the atherosclerotic process including calcification (Aronson 2008).

1.1.5 Current Treatment Guidelines

Current treatment guidelines for CKD and related complications are detailed in the KDOQI (National Kidney Foundation). There are a number of potential therapies that directly target the calcification process. These include pyrophosphate and bisphosphonates, thiosulfate, and vitamin K (O'Neill 2010). However, most of these therapies are considered experimental, and there are few controlled studies on which to base therapeutic decisions. There are no therapies indicated for treating vascular calcification and therapy is currently focused on managing bone mineral metabolism (eg, normalizing calcium, phosphorus, and PTH), including either minimizing vitamin D use or considering cinacalcet therapy for secondary hyperparathyroidism in patients with known vascular calcification.

1.2 INVESTIGATIONAL PRODUCT SNF472

In an effort to address the significant medical challenges of CVC, SNF472 is being developed for the treatment of CVC and calciphylaxis in ESRD patients undergoing HD.

SNF472 is an intravenous (IV) formulation of the hexasodium salt of myo-inositol hexaphosphate (IP6, phytate), a compound that inhibits calcification by binding to the growth sites of the HAP crystal. The molecular weight of SNF472 is 791.93 g/mol or daltons.

IP6 is a naturally occurring substance found in beans, brown rice, corn, sesame seeds, wheat bran and other high-fibre foods. It is also present in mammalian cells and tissues at concentrations in the µM range (Shears 2001). The calcium salt of IP6 is listed by the US FDA as Generally Recognised as Safe (GRAS). It is highly polar and poorly absorbed when given orally. When high oral doses are administered, IP6 has chelating properties in the gastrointestinal lumen preventing the absorption of cations such as calcium, magnesium and iron (Davies 1979; Forbes 1984, Pallauf 1999). Due to this property, it has been regarded for

almost 50 years as an anti-nutrient, because it can reduce the gastrointestinal absorption of mineral oligoelements.

The physicochemical properties of IP6 in biological systems make it undetectable with most conventional analytical techniques. Its presence in human tissues and body fluids was only shown at the end of the 1990s, after the development of sensitive assay methods (March 1998, Grases 2004, Perelló 2004). Evidence of the potential health benefits of IP6 have emerged in parallel with advances in the development of analytical tools for its determination in biological fluids and tissues. These benefits include including antitumor, antioxidant, antiproliferative and antiplatelet properties.

A new extracellular role for IP6 as an inhibitor of CVC is claimed by Sanifit, which is the rationale for the development of SNF472.

SNF472 inhibits the final common pathway of CVC (Grases 2008) by inhibiting growth and formation of calcium crystals. This effect appears to be independent of the etiology of the CVC and is present at any plasma calcium and/or phosphate levels (Grases 1999).

Based on current information, it is hypothesised that SNF472 is able to slow the process of CVC by:

- blocking the formation and growth of HAP crystals in the blood vessels. SNF472 is a polyphosphate with a high affinity for solid calcium salts. It quickly binds to the surface of a forming nucleus or on the faces of a growing crystal. It acts as an inhibitor of crystallisation at substoichiometric levels, since it specifically binds to the growth sites (not the entire crystal surface required) blocking the calcification process. An effect on the circulating colloidal calciprotein particles in the blood stream is also likely. SNF472 might be adsorbed on the surface of these nanoparticles, giving them negative charge and avoiding its aggregation to form the solid HAP crystal.
- avoiding bone mass loss (a source of circulating calcium). As treatment, it could be used at serum concentrations far below free calcium concentrations. The target is not the free calcium in solution, but solid calcium being deposited on the vessels walls. From preclinical data, calcium chelation does not occur at therapeutic concentrations, suggesting that there will be no risk of producing hypocalcaemia with therapeutic doses.

1.2.1 Nonclinical Data

SNF472 has been extensively studied in nonclinical models for efficacy, safety, pharmacokinetics (PK) and toxicity, and showed a promising profile that supported progression to studies in humans. The results of nonclinical studies are summarised in the Investigator's Brochure.

In vitro and *in vivo* studies show that SNF472 inhibits the formation of HAP through binding to the growing sites of the HAP, thus preventing the progression of crystallisation in vessels and soft tissues in IC_{50} in the range of 2-3 µM.

A vitamin D model was also used to evaluate the progression of already established calcification. SNF472 was able to stop the progression of calcification when it was administered in the presence of an established calcification.

In a uremic model in rats (adenine model), SNF472 showed an efficacy of 80% when given intravenously infused for 4h with a plasma concentration of 6.5 µM in free concentration.

SNF472 prevented peripheral (skin) calcification in rats in 3 models of calcinosis cutis.

A pharmacodynamic (PD) assay to measure the crystallisation potential of blood has been set up to use as a biomarker to predict SNF472 activity. The first validation was performed in rats where the addition of SNF472 to plasma samples, *in vitro* or *in vivo*, reduced the HAP crystallisation rate. Subcutaneous administration of SNF472 in rats reduced the HAP crystallisation potential of plasma by up to 70%. The *ex vivo* HAP crystallisation rate is being used as a PD measurement of the CVC reduction of SNF472 in clinical trials.

Pre-clinical safety and toxicology work has been completed, including up to 6 months in rats and 9 months repeated toxicology in dogs. No-observed adverse effect levels (NOAELs) obtained establish a wide safety margin that provides appropriate toxicology support to proceed with exposure in long-term clinical trials.

1.2.2 Pharmacology

In vitro and *in vivo* pharmacological characterisation has been carried out for SNF472. Phase 1 clinical trial results of the key studies are summarised in the Investigator's Brochure.

1.2.3 Clinical Experience

Two Phase 1 studies designed to characterise the pharmacology, efficacy, and safety of SNF472 have been carried out, as listed below:

- SNFCT2012-03 Part A was a double-blind, randomised, phase I, single-dose, tolerability and PK study of SNF472 in healthy volunteers.
- SNFCT2012-03 Part C was a double-blind, randomised, phase I, single-dose, tolerability and PK study of SNF472 in HD patients.
- SNFCT2014-03 was a double-blind, randomised, phase 1b/2a, tolerability and PK study of repeated doses up to 1 month of SNF472 in HD patients.

In Part A, 20 healthy male volunteers received single ascending doses of SNF472 ranging from 0.5 mg/kg up to 12.5 mg/kg (19 volunteers) and/or placebo (8 volunteers) via IV infusion, under fasting conditions. Due to the cross-over design of the study, some of the subjects received both SNF472 and/or placebo.

In Part C, 8 male HD patients were enrolled in the study and received SNF472 (9 mg/kg) or placebo during the dialysis session over a 4-h period under fasting conditions.

In total, 18 HD patients were enrolled in the Phase 1b/2a study (SNFCT2014-03). In Cohort 1, 10 patients were enrolled. Each patient received 5 different doses of SNF472 ranging from 1 to 20 mg/kg, or placebo. Each dose was infused 3 times a week during HD sessions, in treatment periods of 1 week each. In Cohort 2, 8 HD patients were enrolled and received 10 mg/kg of SNF472 or placebo, infused 3 times a week, for 4 weeks during HD sessions.

The details of these studies are provided in their respective clinical study reports (CSRs) and summarised in the Investigator's Brochure.

The Sponsor has also conducted SNFCT2015_04 (EudraCT 2014-004313-25), a Phase 2 open-label, single arm, repeat dose study to assess the effect of SNF472 on wound healing in uraemic calciphylaxis patients. Follow-up of subjects has been completed with full results pending.

1.2.4 Potential Risks

As of 13-Feb-2016, a total of 39 healthy volunteers and patients have been exposed to SNF472.

No serious adverse reactions or suspected unexpected serious adverse reactions (SUSARs) were reported and no clinically relevant safety concerns were identified. One serious adverse event (SAE) of "polycystic kidney infection" was reported in the Phase 1b/2a study. The event was not considered to be related to SNF472 and resolved without sequelae.

SNF472 has been shown to be safe and well tolerated in clinical trials to date, with the exception of non-serious mild to moderate local infusion site irritations in healthy volunteers. Infusion site irritations are not expected to be an issue in HD patients as SNF472 is infused into the dialysis catheter and passed through a volume of blood of at least 200 mL before reaching the patient's blood vessels.

Treatment-emergent adverse events (TEAEs), assessed as probably related to the SNF472, were infusion site pain, infusion site erythema, infusion site hypoesthesia, and infusion site swelling in healthy volunteers. TEAEs assessed as possibly related to the SNF472 were injection site bruising, headache, dizziness, pain in extremity, eyelid injury, and myalgia in healthy volunteers.

No clinically significant effects of SNF472 were observed for vital signs or laboratory values. Electrocardiogram (ECG) analysis revealed no clinically significant effects of SNF472. Mild QTcB (QTc corrected by Bazett's formula) prolongation was observed in healthy volunteers, although all QTcB values were under the upper limit of normal values. A limited number of ionised calcium values were available for analysis in this study; however, the available data suggested that mild hypocalcemia was the causative factor for modest prolongation in QTcB. In HD patients exposed to SNF472, no impact on QTcB was observed, potentially related to the lack of observable hypocalcemia among these patients receiving dialysis therapy. Coincident with SNF472 administration, the continuous systemic

exposure to dialysate calcium levels during the dialysis procedure is anticipated to negate any potential for mild reductions in serum calcium related to SNF472.

Based on the totality of preclinical and clinical data generated to date, no clinically relevant safety concerns have been identified that preclude continued human investigation.

1.3 CORONARY ARTERY CALCIFICATION SCORES

Coronary artery calcium level is a well-documented marker for coronary atherosclerosis (Budoff 2008). There are several methods used in the quantification of the amount of calcium in the coronary arteries. CT is the only noninvasive test with high sensitivity and specificity for calcium detection and is capable of quantifying calcification. Because of the strong correlation between the presence of coronary calcification and underlying coronary atherosclerosis, CT imaging of coronary calcium has been determined to be a good predictor of cardiac events (Madhavan 2014).

A variety of imaging results can be produced with a CT scanner, including calcium area, volume and mass. Two widely used scoring methods include the calcium volume (or volumetric) score and the CAC/Agatston score. The original work (Agatston 1990) was based on EBCT which has now evolved in today's imaging arena to high-speed, multi-detector CT scanners.

The CAC score in Agatston units (AU) is calculated using a weighted value assigned to the highest density calcification within the coronary artery being measured. The measurement is obtained in Hounsfield units and a score assigned based on area in square millimeters and a weighted score. The sum of these measurements is then calculated to give the CAC score. It is well established that patients with CAC score > 400 AU have an increased occurrence of coronary events (Budoff 2006, Greenland 2007). Several cohort studies, including the Multi-Ethnic Study of Atherosclerosis ([MESA]; McClelland 2006, 2015), the Heinz Nixdorf Recall ([HNR]; Budoff 2013, Erbel 2014) study and the ADVANCE study (Floege 2010, Raggi 2011) have used calcium scoring, specifically CAC score as primary outcome measures.

The calcium volume score is another measure of CVC using CT scans. This measure has been used in several studies and has the advantage over the CAC/Agatston score in reducing variability between scans, thus improving reproducibility (Detrano 2005, McEvoy 2010, Criqui 2014). For this reason, the calcium volume score may be a more sensitive and accurate measure of CVC progression over time. Similar to the CAC score, clinical investigations demonstrate that calcium volume is positively and independently associated with the occurrence of clinically significant CV events (Criqui 2014).

1.4 RATIONALE FOR THE STUDY

Reducing the progression of CVC in HD patients may improve the severe burden of CV disease related to the underlying ESRD. As no therapy is currently indicated to target CVC, there is a need to investigate the ability of SNF472 to reduce CVC progression and, ultimately, to improve CV outcomes in HD patients. This phase 2b double-blind,

randomised, placebo-controlled study is designed as a proof-of-concept trial to assess the effect of SNF472 on the progression of CVC as measured by calcium volume and CAC/Agatston scores in ESRD patients receiving HD. The study hypothesis is that administration of SNF472 over 52 weeks can slow the progression of CVC in this patient population compared to placebo.

2 STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective is to assess the effect of 2 dose levels of SNF472 (300 mg and 600 mg) compared to placebo on the progression of coronary artery calcium volume score over a 12-month (52 weeks) period in ESRD patients on HD.

2.2 SECONDARY OBJECTIVES

The secondary objectives of the study are to:

- assess change from baseline (Week 1, Day 1) in coronary artery calcium (CAC)/Agatston score
- assess the number of patients with <15% progression in CAC/Agatston score
- assess the change from baseline in thoracic aorta calcification score
- assess the change from baseline in aortic valve calcification score
- assess the occurrence of the composite safety endpoint: death from cardiovascular causes, myocardial infarction (MI), stroke, or heart failure
- assess changes in biomarkers as signals for treatment efficacy/response
- assess changes in bone mineral density (BMD)
- describe the long-term safety profile of SNF472 in this target population

2.3 EXPLORATORY OBJECTIVE

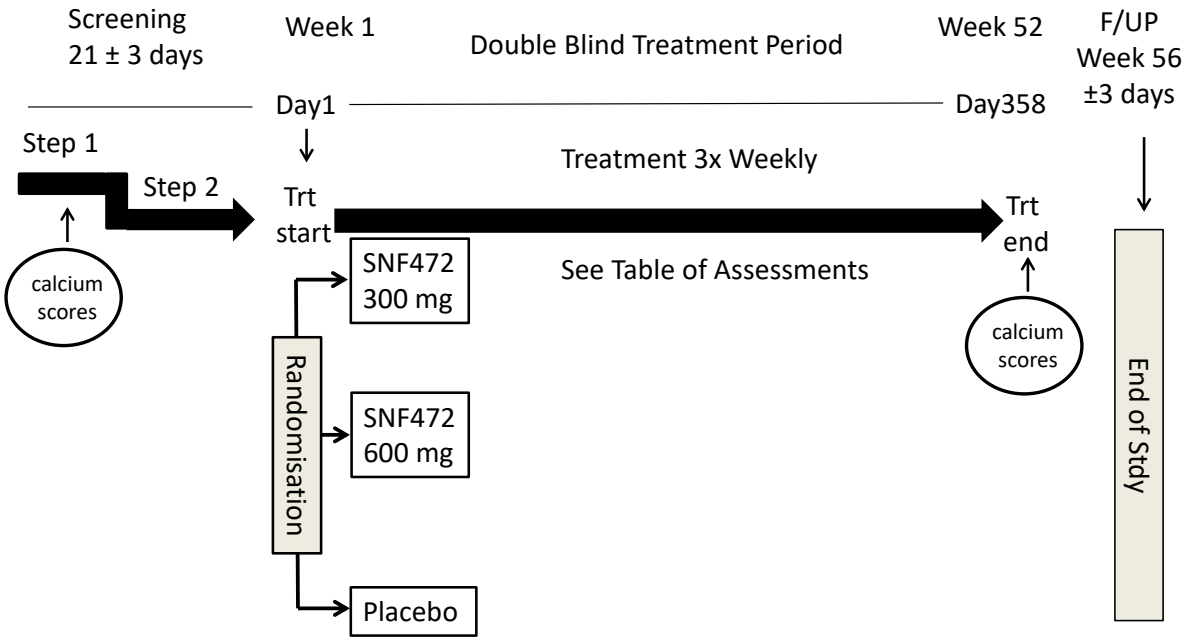
The exploratory objective is to assess changes from baseline in pulse pressure, systolic blood pressure (SBP), and diastolic blood pressure (DBP).

3 STUDY DESIGN

3.1 STUDY DESIGN OVERVIEW

This study is designed as multicentre, double-blind, randomised, placebo-controlled, phase 2b study. Approximately 75 sites in 3 countries will enroll approximately 270 patients. The study will consist of a screening period, a 52-week double-blind treatment period, and a follow-up visit. A study flow chart is provided in Figure 3.1.

Figure 3.1 Study Flowchart



The study treatment duration of 52 weeks has been selected as an appropriate duration likely to provide clinically meaningful changes in the primary outcome.

3.2 STUDY ENDPOINTS

3.2.1 Primary Endpoint

The primary endpoint is the change in log coronary artery calcium volume scores between baseline (Week 1, Day 1) and Week 52 measured by CT scan.

3.2.2 Secondary Endpoints

The secondary endpoints are

- change from baseline in CAC/Agatston score at Week 52

- number of patients with <15% progression in CAC/Agatston score at Week 52
- change from baseline in thoracic aorta calcification score at Week 52
- change from baseline in aortic valve calcification score at Week 52
- incidence of composite safety endpoint that include death from cardiovascular causes, MI, stroke, or heart failure
- mortality rate (all-cause and CV)
- change from baseline in levels of selected biomarkers, including C-reactive protein (CRP)

The endpoints above will be analysed as efficacy as well as safety endpoints.

The following secondary safety endpoints will be analysed:

- changes in BMD levels between baseline and Week 52
- safety of SNF472 in terms of incidences of adverse events (AE) and serious adverse events (SAE) and clinically relevant changes from baseline in laboratory and ECG parameters

3.2.3 Exploratory Endpoints

The exploratory endpoints are changes from baseline in pulse pressure, SBP, and DBP at Week 28 and Week 52 in all patients and in the subgroup of patients with hypertension at baseline.

4 SELECTION OF PATIENTS

Approximately 270 patients from approximately 75 sites in 3 countries are planned to be enrolled.

Patients will be required to meet all the inclusion and none of the exclusion criteria detailed in subsequent sections to be enrolled in the study. Screening is scheduled 28 + 3 days prior to randomisation.

4.1 INCLUSION CRITERIA

Patients meeting all of the following criteria will be considered for enrollment in the study:

1. female or male patients, 18 to 80 years (inclusive) of age at randomisation
2. CAC score of 100 to 3500 AU inclusive within a 4-week period prior to randomisation, as measured by a multi-detector CT scanner
3. patients who are EITHER ≥ 55 years OR have a history of diabetes mellitus at randomisation
4. patients on HD for ≥ 6 months prior to randomisation

5. willing and able to understand and sign the informed consent

4.2 EXCLUSION CRITERIA

Patients presenting with any of the following will not be included in the study:

1. scheduled date for kidney transplant from a known living donor
2. weight above 300 lbs (136 kg)
3. hospitalisation in the previous 3 months prior to randomisation for unstable angina, MI, stroke, transient ischaemic attack, amputation or peripheral or coronary bypass surgery
4. 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 edema and requiring ultrafiltration therapy
5. history of cancer that has been in remission for < 5 years prior to randomisation. A history of basal cell carcinoma or Stage 1 squamous cell carcinoma of the skin is allowed
6. pregnant or trying to become pregnant, currently breast-feeding, or of child-bearing potential (including peri-menopausal women who have had a menstrual period within one year) and not willing to practice birth control using a double barrier method (criteria apply to women only) at least 30 days post last dose of study medication
7. hypocalcaemia defined as a serum calcium below 8.0 mg/dL (or 2.0 mmol/L) for the serum calcium most proximal to screening per patient's medical records
8. extreme elevation in serum phosphorous, defined as a phosphorous above 10 mg/dL (or 3.23 mmol/L) within the last 2 months proximal to screening per patient's medical records
9. uncontrolled hypertension defined as any 2 or more consecutive post-dialysis DBP > 100 mmHg within the last 2 months proximal to screening
10. expected survival < 2 years in the Investigator's medical opinion
11. known active drug or alcohol abuse within 1 year of randomisation
12. use of other investigational drugs within 30 days of randomisation
13. non-compliance with dialysis treatment which, in the opinion of the Investigator, evidenced by either repeated missed dialysis treatments or significant non-compliance with the patient's medication regimen
14. Inability to comply with all required study procedures and schedule, 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

4.3 RE-SCREENING AND SCREEN FAILURES

Rescreening will be performed on a case-by-case basis after discussion with the Medical Monitor.

5 STUDY TREATMENTS

5.1 INVESTIGATIONAL DRUGS

Description of the investigational medicinal product (IMP) is provided below in Table 5.1.

Table 5.1 Identity of the Investigational Medical Products

	Active Treatment	Matching Placebo
Code name	SNF472	Not applicable
Description	Clear, colourless solution free from visible particles	Clear, colourless solution free from visible particles
Composition	SNF472, 30 mg per 1 mL of physiological saline (0.9% sodium chloride)	Physiological saline (0.9% sodium chloride)
Dosage form	Solution for IV infusion	Solution for IV infusion
Strength	30 mg/mL	Identical vials containing physiological saline
Dose based on 85 kg body weight (BW)	300 mg (3.5 mg/kg) 600 mg (7.0 mg/kg)	Identical vials are used containing only physiological saline

SNF472 is prepared in 10 mL sterile vials of one strength, 30 mg/mL (300 mg/vial). The placebo will be supplied to be identical in appearance to SNF472. The full volume of the IMP will be administered as a constant rate IV infusion using one 100 mL bag of saline connected to an infusion pump, which will be connected directly to the dialysis machine via an IV giving set and an accessory heparin line. The infusion should be initiated approximately 30 min after the start of the dialysis treatment procedure and should take 2.5 hrs.

NOTE FOR UNITED STATES SITES ONLY: The preferred physiological saline bag size for dilution of the IMP is 100 mL. However, if 100 ml bags of physiological saline are not available, 150 mL or 250 mL bags may be used as described in Appendix 3.

All patients will receive 2 identical vials of 10 mL:

- Placebo arm: 2 vials of physiological saline
- Dose 1 arm (300 mg): 1 vial of physiological saline and 1 vial of active (10 mL SNF472 at 30 mg/mL)
- Dose 2 arm (600 mg): 2 vials of active (10 mL SNF472 at 30 mg/mL)

5.2 DOSE RATIONALE

The planned doses for this study are 300 mg (2.5-4 mg/kg for 75-120 kg bodyweight) and 600 mg (5-8 mg/kg for 75-120 kg bodyweight) to be administered at every dialysis session during the treatment period. Administration is detailed in Section 5.5.

The dose response relationship with respect to vascular calcification in HD patients has been estimated using preclinical models of calcification along with a biomarker assay designed to provide an estimate of the PD activity of SNF472. These separate lines of evidence suggest a very steep dose response relationship with a narrow range between initial evidence of efficacy and asymptotic maximal efficacy.

The biomarker assay results using both rat and human HD patient plasma samples suggest that SNF472 is able to inhibit systemic blood calcification when plasma levels are above the calculated IC_{50}, estimated at plasma concentrations of ~5000 ng/mL (achieved with dosing of ~ 2 mg/kg in humans). The PK-PD relationship estimated by the biomarker assay demonstrates a very steep dose-response curve, with ~ 70% inhibitory activity of SNF472 at 3 mg/kg and a plateau of just over 80% inhibitory activity at 5 mg/kg and higher.

Multiple preclinical models of accelerated cardiac and aorta calcification in rats demonstrate that doses below 1 mg/kg produce no effect; doses of 1 mg/kg can produce ~60% inhibition of calcification; and the maximal effect of 80% inhibition is seen at doses of 3 mg/kg and higher. Thus, a steep dose response curve is also demonstrated in these *in vivo* models.

Pre-clinical safety and toxicology studies suggest an appropriate margin of safety for the doses tested in this clinical trial. Repeated dose administration of SNF472 was studied in rats for up to 6 months and up to 9 months in dogs. Hypocalcaemia, accompanied by QTc change, is the main adverse effect noted in rats and dogs when treatment is administered by bolus injection. This effect, thought to be due to chelation of plasma calcium in the presence of SNF472, is not seen when treatment is administered by infusion. Local injection site reaction, and associated thrombus formation, was observed in the 28 day and 6 month studies in rats but not in dogs when dosed up to 9 months. This event is believed to be related to local irritation when SNF472 is injected into a small caliber vein. Injection site reactions were observed in the healthy volunteer study, where treatment was given into a peripheral vein, but not in HD patients who received the treatment as part of the dialysis procedure.

In this clinical trial, SNF472 is being administered via slow infusion and diluted within dialyses tubing by at least 200 mL volume of blood, thus ensuring lower C_{max} concentration. Additionally, avoidance of hypocalcaemia is expected based on the constant exposure to a standard calcium concentration gradient across the dialysis membrane, which allows free exchange of calcium from dialysate to plasma compartments. SNF472 has been dosed for up to 1 month at 10 mg/kg in HD patients with no evidence of hypocalcaemia, local injection site irritation or other drug related toxicity.

Taking into consideration the steep pharmacological activity observed with SNF472, doses of 300 mg (2.5-4 mg/kg for 75-120 kg bodyweight) and 600 mg (5-8 mg/kg for 75-120 kg bodyweight) are considered appropriate options to evaluate clinical efficacy and are well within the anticipated safety and toxicity margins for the drug.

5.3 TREATMENT ASSIGNMENT

Patients will be randomised to either one of two active drug dose levels or placebo (1:1:1).

Each patient's treatment will be given a unique code number, traceable to the batch number of the IMP. The SNF472 and matched placebo treatments will be identical in appearance. To maintain the blinded nature of the clinical trial, the same volume of solution will be administered to each patient in a treatment group.

5.3.1 Randomisation

Randomisation will be used to avoid bias and to enhance the validity of statistical comparisons. Blinded treatment (active and matched placebo) will be used to reduce potential bias during data collection and evaluation of clinical endpoints.

Randomisation should occur on Day 1 after all pre-dose procedures have been performed and eligibility for the clinical trial has been confirmed. However, it is acceptable for first dose to occur on a later day than randomisation to accommodate for pharmacy/IMP preparation needs. For clarity, Day 1 Week 1 is the first dose date. Randomisation will be performed using a centralised electronic randomisation system.

Due to the strong association of baseline CAC score on subsequent calcification progression, baseline CAC score will be used as a randomization stratification factor by breaking into three categories (100-399, 400-1000 and >1000). In keeping with the ICH E9 guidance, baseline CAC score will be included in the primary efficacy analysis.

5.3.2 Patient Identification

At screening, each patient will be assigned a unique 4-digit screening number which will be used as the patient identification (ID) number all throughout the study. The numbers will be sequential. The patient ID should be used on all study documents relating to the patient from screening and throughout the study. No recognisable personal patient data (names, initials, etc.) will be collected.

5.4 BLINDING, PACKAGING, AND LABELING

5.4.1 Blinding

The clinical trial will be performed in a double-blind manner, i.e., the Investigator, all clinical staff, the volunteers, and the Clinical Monitor involved in the trial will remain blinded throughout the clinical trial, unless safety concerns or a regulatory requirement make unblinding necessary.

5.4.2 Unblinding

The clinical trial blind should not be broken except in a medical emergency (where knowledge of the IMP administered would affect the treatment of the emergency) or regulatory requirement (e.g., for SAEs).

The decision to break the blind can only be made by the Investigator or other persons duly registered in the clinical trial file as sub-Investigator after consulting with the Medical Monitor. Unblinded staff members will not convey information regarding treatment

assignments in the clinical trial, whether informally or formally, to any other person, unless required for medical reasons. If the blind is broken, the date, time, and reason must be recorded and included in any associated AE report.

If an emergency unblinding becomes necessary, the Investigator should notify the Sponsor/Medical Monitor prior to unblinding, if possible, unless identification of the IMP is required for emergency therapeutic measures.

If either an Investigator or a patient is unblinded, the patient must be withdrawn from the clinical trial and procedures accompanying early termination visit will have to be performed.

5.4.3 Packaging

SNF472 will be packaged, labeled, and supplied to study sites by:

The IMP [redacted] will be stored in accordance with the manufacturer's instructions (-20ºC). The IMP will be labelled according to the applicable local health authority, FDA and EMA requirements. [redacted] will send the IMP to the local depots and these will then send the IMP to the Clinical Unit. All IMP shipments will be carried out on dry ice.

All supplies of SNF472 must be stored on site at 5±3ºC. The preparation of the patient doses will be described in the Pharmacy Manual.

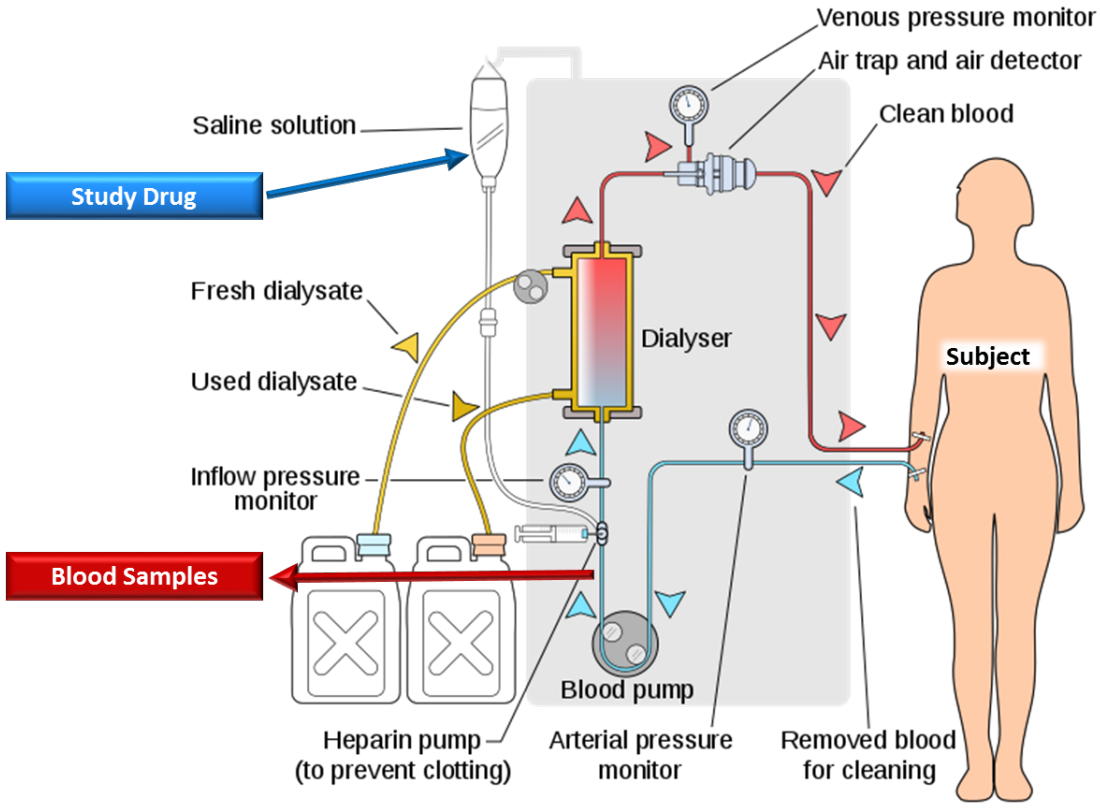
5.5 ADMINISTRATION OF INVESTIGATIONAL MEDICINAL PRODUCTS

The IMP will be administered 3 times weekly in conjunction with the patient's dialysis sessions. The IMP administration will consist of one of 2 doses of SNF472 or placebo administered intravenously during HD. The infusion should be initiated approximately 30 min after the start of the HD procedure and should take 2.5 hrs.

The Principal Investigator or designees will administer/oversee the administration of all IMPs. The exact infusion starting and stopping times will be recorded in the electronic case report form (eCRF).

The appropriate volume of the IMP will be administered as a constant rate IV infusion over 2.5 hrs using a 100 mL bag of saline connected to an infusion pump, which will be connected directly to the dialysis circuit before the dialyzer via an IV giving set.

Figure 5.1 Method of Administration



5.6 SUPPLIES AND ACCOUNTABILITY

IMP will be sent to the sites from [redacted] where it will be stored prior to the initiation of the study. The Investigators are responsible for maintaining accurate accountability records of the IMP throughout the clinical trial.

Pharmaceutical services at the sites will keep cumulative inventory and dispensing records as appropriate throughout the clinical trial. Each administration of the IMP will be documented in the eCRF.

Unused IMP will be destroyed following each site's Drug Destruction Policy. The IMP destruction will only occur after drug accountability has been performed, after any discrepancies have been resolved, and after receiving approval of destruction by the Sponsor or designee.

5.7 COMPLIANCE

The Principal Investigator or designees will administer/oversee the administration of all IMPs. In addition, the following measures will be taken to ensure treatment compliance:

- The Investigator will attempt to enroll patients that are able to understand and comply with the clinical trial requirements.
- The exact day and the starting and stopping times of the IMP infusion will be recorded in the eCRF.
- The Investigator will maintain accountability records showing the quantities of IMPs received at the site and dispensed to each patient.

Any empty or partially used containers will be accounted for and destroyed according to each site's drug destruction policy. Any unused IMP, will be accounted for and destroyed according to each site's drug destruction policy after obtaining written Sponsor approval.

6 STUDY PROCEDURES AND SCHEDULE

The timing of assessments required during the study is outlined in Table 1.1. Data will be collected using eCRF and an appropriate Electronic Data Capture (EDC) system, as described in Section 12.

6.1 DESCRIPTION OF STUDY DAYS

Table 1.1 Schedule of Assessments and Study Activities describes the different activities per study visit, the assessments on intensive data collection visits. ***If also participating in Sub-Study, please see additional Schedule of Sub-Study Assessments in Appendix 1.***

6.1.1 Screening (28 + 3 days)

Screening will be conducted in two steps.

6.1.1.1 Step 1

- Obtain written informed consent.
- Determine patient eligibility according to the inclusion and exclusion criteria defined in Section 4.
- Obtain a CT scan of the coronary artery using a multi-detector CT with at least 64 slices to measure calcium volume and CAC scores. The CAC score will be used for eligibility determination (only patients with CAC score of 100 to 3500 AU are eligible for the study), the volume and CAC scores for the endpoints. Refer to the Imaging Manual for detailed procedure.
- In conjunction with the CT scan, BMD will be measured by dual-energy x-ray absorptiometry (DEXA). Refer to the Imaging Manual for detailed procedure.
- Ensure that the patient meets the CAC score eligibility criterion of 100 – 3500 AU before proceeding to Step 2.

6.1.1.2 Step 2

- Once the CAC score inclusion criterion is met, continue with the evaluation of patient eligibility according to the inclusion and exclusion criteria defined in Section 4.

- Record medical history and demographics.
- Perform physical examination, including height and weight.
- Record treatment history and current and prior medications (prescription drugs only taken 30 days prior to Screening), therapies and surgical procedures.
- Draw blood samples via the dialysis port for serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Measure 12-lead ECG, including QTc interval (post-dialysis if done during a dialysis session).
- Measure and record vital signs (heart rate [HR], respiratory rate [RR], blood pressure [BP], and body temperature [degree C]) (post-dialysis if done during a dialysis session).

6.1.2 Treatment Period

6.1.2.1 Week 1

- Review and confirm patient eligibility according to the inclusion and exclusion criteria pre-dose as defined in Section 4.
- Complete the randomisation process.
- Draw blood samples via the dialysis port pre-dose for safety laboratory assessments (Table 6.1), including serum pregnancy testing for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Draw blood samples via the dialysis port pre-dose for PTH, ferritin, transferrin saturation (TSAT). Blood volumes are defined in the laboratory manual.
- **For participating sites**, draw blood samples for PK and biomarkers via the dialysis port pre-dose **and at 3 hrs (~10 minutes before end of infusion)**. Blood volumes are defined in the bioanalytical manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Measure post-dialysis weight.
- Record change in concomitant medications (only prescription drugs), relative to previous visit.
- Assess and record AEs.

6.1.2.2 Weeks 1 to 52 (3x weekly)

- Administer study treatment 3x week during each HD session in the dialysis unit as described in Section 5.5.
- Perform all standard of care assessments.

6.1.2.3 Week 2 (preferably at mid-week HD session)

- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Assess and record any new AEs relative to previous visit.

6.1.2.4 Week 4 (preferably at mid-week HD session)

- Administer study treatment in the dialysis unit as described in Section 5.5.
- Record change in concomitant medications (only prescription drugs), relative to previous visit. For administration of iron preparations, see Section 6.2.6.
- Assess and record any new AEs relative to previous visit.

6.1.2.5 Week 6 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Assess and record any new AEs relative to previous visit.

6.1.2.6 Week 10 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for safety laboratory assessments (Table 6.1), including serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- **For participating sites**, draw blood samples for PK and biomarkers via the dialysis port pre-dose and **at 3 hrs (~10 minutes before end of infusion)**. Blood volumes are defined in the bioanalytical manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure 12-lead ECG, including QTc interval post-dialysis.
- Measure post-dialysis weight.
- Record change in concomitant medications (only prescription drugs), relative to previous visit. For administration of iron preparations, see Section 6.2.6.
- Assess and record any new AEs relative to previous visit.

6.1.2.7 Week 16 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.

- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Assess and record any new AEs relative to previous visit.

6.1.2.8 Week 22 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- **For participating sites**, draw blood samples for PK and biomarkers via the dialysis port pre-dose and at **3 hrs (~10 minutes before end of infusion)**. Blood volumes are defined in the bioanalytical manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Assess and record any new AEs relative to previous visit.

6.1.2.9 Week 28 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for safety laboratory assessments (Table 6.1), including serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure 12-lead ECG, including QTc interval post-dialysis.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Measure post-dialysis weight.
- Record change in concomitant medications (only prescription drugs), relative to previous visit. For administration of iron preparations, see Section 6.2.6.
- Assess and record any new AEs relative to previous visit.

6.1.2.10 Week 34 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Assess and record any new AEs relative to previous visit.

6.1.2.11 Week 40 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for safety laboratory assessments (Table 6.1), including serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.

- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure 12-lead ECG, including QTc interval post-dialysis.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Measure post-dialysis weight.
- Assess and record any new AEs relative to previous visit.

6.1.2.12 Week 46 (preferably at mid-week HD session)

- Draw blood samples pre-dose via the dialysis port for serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Assess and record any new AEs relative to previous visit.

6.1.2.13 Week 52 (preferably at mid-week HD session) / Early Termination

- Obtain a CT scan of the coronary artery using a multi-detector CT with at least 64 slices to measure calcium volume and CAC scores. Refer to the Imaging Manual for detailed procedure.
- In conjunction with the CT scan, BMD will be measured by DEXA. Refer to the Imaging Manual for detailed procedure.
- Perform physical examination, including weight.
- Draw blood samples pre-dose via the dialysis port for safety laboratory assessments (Table 6.1), including serum pregnancy test for female patients of childbearing potential. Blood volumes are defined in the laboratory manual.
- Draw blood samples pre-dose via the dialysis port for PTH, ferritin and TSAT. Blood volumes are defined in the laboratory manual.
- **For participating sites**, draw blood samples for PK and biomarkers via the dialysis port pre-dose and **at 3 hrs (~10 minutes before end of infusion)**. Blood volumes are defined in the bioanalytical manual.
- Administer study treatment in the dialysis unit as described in Section 5.5.
- Measure 12-lead ECG, including QTc interval post-dialysis.
- Measure and record vital signs (HR, RR, BP and body temperature [degree C]) post-dialysis.
- Measure post-dialysis weight.
- Record change in concomitant medications (only prescription drugs), relative to previous visit. For administration of iron preparations, see Section 6.2.6.
- Assess and record any new AEs relative to previous visit.

6.1.2.14 Early Termination

Assessments are identical to those scheduled for Week 52. PK and biomarker samples will be collected during this visit if the post-baseline samples have not been collected prior to early termination. A CT scan for calcium quantification and DEXA for BMD should be done. If this is not possible for this visit, the site must make all efforts to set an appointment for a CT scan and DEXA. In addition, sites must encourage patients to report for a follow-up visit (see Section 6.1.3).

6.1.3 Follow-up Visit (Week 56 or 1 month after last dose)

- Assess and record SAEs only.

6.2 METHODS OF EVALUATION

6.2.1 Efficacy Assessments

6.2.1.1 CT Scan

A CT scan of the thoracic area will be performed using a multi-detector CT scanner with at least 64 slices at Screening (Step 1) and at Week 52 as shown in Table 1.1. The scan should be performed onsite or in a nearby facility. The methodology is described in detail in the Imaging Manual.

6.2.1.2 Calcium Scores

From the CT scans, the following scores will be calculated:

- calcium volume score
- CAC/Agatston score in AUs
- thoracic aorta calcification score
- aortic valve calcification score

The calculations are described in detail in the Imaging Manual.

6.2.1.3 Cardiovascular Events and Mortality

Composite safety outcomes that include death from cardiovascular causes, MI, stroke, or heart failure will be assessed. Heart failure is defined as an unplanned presentation to a hospital or dialysis treatment facility with signs/symptoms of acute pulmonary edema and requiring ultrafiltration therapy.

In addition, all-cause and CV mortality will also be assessed and recorded.

6.2.2 Pharmacokinetics

Samples for PK analyses will be collected from subjects at participating sites which have a -70°C freezer for sample storage or, if requested by Sponsor, samples may be shipped on

dry ice on the same day as collection. Samples will be collected via the dialysis port at pre-dose and **at 3 hrs (~10 minutes before end of infusion)** on the assessment day.

The schedule of PK sampling is detailed in Table 1.1. The actual date and time of each blood sample collected will be recorded. PK analyses will be performed in central laboratories using a bioanalytical laboratory manual.

For sites, PK samples (see also Section 6.2.3) will be stored at -70°C freezer and/or be packed on dry ice and shipped by courier as requested by the Sponsor to a qualified specimen storage depot/laboratory to hold until analysis is performed.

When requested by Sponsor, all EU samples will be shipped by courier [redacted] for analysis. ALL PK samples will be analysed at:

[redacted]

6.2.3 Biomarkers

Samples for biomarker analyses will be collected from a subset of patients from participating sites which have a -70°C freezer for sample storage or as requested by Sponsor for same day dry ice shipment.. The samples will be collected via the dialysis port at pre-dose and **at 3 hrs (~10 minutes before end of infusion)** on the assessment day. The schedule of biomarker sampling is detailed in Table 1.1. The actual date and time of each blood sample collected will be recorded.

CRP will be measured with the high sensitivity CRP test (hs-CRP). Additional biomarkers may include but are not limited to fetuin A, FGF23, MGP, sclerostin, phosphorus (phosphate), PD, and GDF15. No genetic testing will be conducted.

For sites, serum samples for biomarkers analysis will be stored at -70°C freezer (see also Section 6.2.2), and/or will be packed on dry ice and shipped by courier as requested by the Sponsor to a qualified specimen storage depot/laboratory to hold until analysis is performed.

Biomarker analyses will be performed in central laboratories using a laboratory manual.

6.2.4 Safety Assessments

6.2.4.1 Adverse Events

The Investigator will carefully monitor each patient for AEs at each key evaluation visit as shown in Table 1.1. In addition, the information on AEs will be obtained by regular questioning of each patient by the clinical trial staff. No leading questions should be asked. When an AE occurs, the Investigator will decide whether to withdraw the patient from the clinical trial and/or initiate appropriate treatment. Generally, withdrawal is not warranted

unless there is clinical reason why this would benefit the patient. However, if the withdrawal does occur, the investigator will ensure that the patient is given appropriate medical care.

In the case of any event requiring medical intervention occurring during the Treatment Period, the Investigator will institute general supportive measures including, where necessary, respiratory assistance and cardiopulmonary resuscitation.

AEs are discussed in detail in Section 7. HD-related AEs, as listed in Section 7.2, should be recorded and indicated as such in the eCRF.

6.2.4.2 Bone Mineral Density

BMD will be measured by DEXA at Screening (Step 1) and Week 52/ET visit. The methodology is described in detail in the Imaging Manual.

6.2.4.3 Medical History

At Screening (Step 2), the patient's medical history will be recorded in the eCRF under the medical history section. The medical history will comprise of previous illnesses and history of drug and alcohol abuse.

6.2.4.4 Physical Examination

A physical examination (including weight and height) will include an examination of general appearance, skin, head and neck, chest, lungs, heart, abdomen, extremities and basic nervous system evaluation. If any physical findings are abnormal, the Investigator must document the abnormality as non-clinically significant or clinically significant. Any abnormality assessed as 'CS' must be recorded as an AE if not explained by a coexisting condition (documented in the medical history). Weight will be measured post-dialysis as part of the physical examination.

Information about the physical examination must be present in the source documentation at the study site. Significant findings must be reported on the eCRF.

6.2.4.5 Vital Signs

Vital signs (SBP and DBP, HR, RR, and body temperature) will be assessed post-dialysis at each assessment visit after the patient has been sitting for 5 min. The data are recorded for the study at key visits only. Standard of care vital signs assessments are not being recorded into study database at each HD visit.

6.2.4.6 Electrocardiograms

Standard 12-lead ECGs (including QTc) will be performed at Screening and post-dialysis assessment visits as scheduled in Table 1.1.

6.2.4.7 Laboratory Evaluations

The list of safety laboratory evaluations is provided in Table 6.1. Blood samples will be collected pre-dose via the dialysis port for haematology, coagulation, and chemistry tests.

Table 6.1 Safety Laboratory Assessments

Haematology	haematocrit haemoglobin platelet count	white blood cell (WBC) count (total and differential)
Coagulation	international normalised ratio (INR) partial thromboplastin time (PTT) prothrombin time (PT)	
Biochemistry	alanine transaminase (ALT) albumin alkaline phosphatase (ALP) amylase aspartate transaminase (AST) bicarbonate blood urea nitrogen (BUN) calcium chloride creatinine gamma-glutamyl transpeptidase (GGT)	glucose lactic acid dehydrogenase (LDH) magnesium phosphorus (phosphate) potassium sodium total bilirubin total protein uric acid

The following safety parameters will also be analysed:

- PTH level is an indicator of mineral metabolism status and will be monitored as safety laboratory.
- Ferritin and TSAT are indicators of iron level status and will be monitored as safety laboratory.

6.2.4.8 Pregnancy

All female patients of childbearing potential must use 2 reliable methods of contraception (see Section 6.3.2) until study completion and for at least 30 days following their final dose of study medication.

For female patients of childbearing potential, serum pregnancy tests will be performed at the visits indicated in Table 1.1.

Female subjects who become pregnant during the trial or within 1 month after the last dose of IMP will be followed for safety during the pregnancy through 1 month after delivery or termination of the pregnancy.

6.2.5 Collection Schedule for Biological Samples

6.2.5.1 Blood

All blood samples will be drawn via the dialysis port following the schedule in Table 1.1. The volumes of the blood draws will be specified in the respective laboratory manual.

6.2.5.2 Sample Handling, Shipment and Retention

Collection, storage and handling of biological samples are described in the laboratory manual. See Sections 6.2.2 and 6.2.3 for storage and handling of PK and biomarkers samples, respectively.

6.2.6 Prior and Concomitant Treatments

Only prescription drugs other than the IMP will be considered as prior and concomitant medications. At Screening, the patient's treatment history of the past 30 days will be recorded in the eCRF under the prior concomitant medications section. Changes in prior medications relative to Screening will be documented prior to the first dose of IMP. Medications taken with or after the first dose of IMP will be considered concomitant medication. Changes in concomitant medications relative to the previous visit will be documented following the schedule in Table 1.1; only prescription concomitant medications are to be recorded.

The use of iron preparations as part of standard of care is allowed. The timing of iron preparation administration relative to the IMP treatment is at the discretion of the site but should be documented in the eCRF.

6.3 RESTRICTIONS AND PRECAUTIONS

6.3.1 Dietary and Fluid Restrictions

Food and fluids allowed will be in accordance with the dietary requirements for the HD patients.

6.3.2 Contraception Requirements

To prevent pregnancy in a female patient or partner of a patient, or to prevent fertilisation of female participants in the study, they should be non-fertile, ie, post-menopausal females, surgically sterilised, or must practice an effective contraceptive method to prevent pregnancies.

Effective contraceptive methods include:

- Sexual abstinence starting before the first administration of the IMP until 30 days after final administration of the IMP.
  - Note: Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- Female patients of child bearing potential should use an oral or injectable hormonal contraceptives, contraceptive patch, intrauterine devices, vaginal hormonal rings, vaginal diaphragm or cervical caps from before the first administration of the IMP until 30 days after final administration of the IMP.
- Male patients should use a condom in addition to having their female partner use another acceptable method (see bullet point above).

- Note: Male patients must not have unprotected sexual intercourse with a female who is pregnant or breastfeeding during the clinical trial.

- Male patient's female sexual partner is of non-childbearing potential, ie, post-menopausal or surgically sterilised (eg, tubal ligation, hysterectomy in medical history).

7 ADVERSE EVENTS

7.1 DEFINITIONS

7.1.1 Adverse Event

An AE is defined by the International Council for Harmonization (ICH) Guideline for Good Clinical Practice (ICH E6 GCP) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.

A TEAE is defined as an AE that begins or that worsens in severity after at least one dose of the IMP has been administered.

AEs fall into the categories "non serious" and "serious" (see Section 7.1.2).

Surgical procedures or laboratory values themselves are not AEs; they are therapeutic measures for conditions that require surgery or diagnose a condition. The condition for which the surgery or laboratory value is indicative for is an AE if it occurs or is detected during the study period. Planned surgical measures and the condition(s) leading to these measures are not AEs if the condition(s) was (were) known before the period of observation (see Section 7.2). In the latter case the condition should be reported as medical history.

7.1.2 Serious Adverse Event

A SAE is one that at any dose (including overdose):

- results in death
- is life-threatening[1]
- requires inpatient hospitalisation or prolongation of existing hospitalisation[2]
- results in persistent or significant disability or incapacity[3]
- is a congenital anomaly or birth defect
- is an important medical event[4]

1. "Life-threatening" means that the patient was at immediate risk of death at the time of the SAE; it does not refer to a SAE that hypothetically might have caused death if it were more severe.
2. This means that hospital inpatient admission or prolongation of hospital stay was required for the treatment of the AE, or that one or the other occurred as a consequence of the event.
3. "Persistent or significant disability or incapacity" means a permanent or significant and substantial disruption of a person's ability to carry out normal life functions.

4. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious.

7.1.3 Adverse Event by Severity or Intensity

Assessment of severity of an AE will be rated according to the criteria in Table 7.1.

Table 7.1 Definitions of Adverse Events Severity

Grade 1 (Mild)	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. The AE does not interfere with routine activities. The patient may experience slight discomfort.
Grade 2 (Moderate)	Moderate; minimal, local or noninvasive intervention indicated. The AE interferes with routine activities. The patient may experience significant discomfort.
Grade 3 (Severe)	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated. The patient is unable perform routine activities. The patient may experience intolerable discomfort or pain.
Grade 4 (Life-Threatening)	Life-threatening consequences; urgent intervention indicated.
Grade 5 (Fatal)	Death related to AE

Based on the Common Terminology Criteria for Adverse Events v4.0 (CTCAE)

7.1.4 Relationship between Adverse Events and Investigational Product

Determination of the relationship (if any) between the AE and the study drug will be made using the guidelines below:

Table 7.2 Guidelines for Determining the Relationship (if any) Between Adverse Event and the Study Drug

Definite:	This causal relationship is assigned if the AE starts a reasonable time after the administration of study drug, stops/improves when the study drug is stopped, and could reasonably be explained by known characteristics of the study drug.
Probable:	This causal relationship is assigned when the AE starts a reasonable time after the administration of study drug, stops/improves when the study drug is stopped, and could not be reasonably explained by known characteristics of the patient's clinical state.
Possible:	This causal relationship is assigned when the AE starts a reasonable time after the administration of study drug, but could be produced by the patient's clinical state or other modes of therapy administered to the patient.
Not Related:	This causal relationship is assigned when the time association or the patient's clinical state is such that the study drug was not likely to have had an association with the observed AE.

7.2 HAEMODIALYSIS-RELATED EVENTS

The following events are known to occur during HD treatments:

- hypotension
- muscle cramps
- disequilibrium syndrome
- nausea and vomiting
- headache
- chest pain
- itching
- fever and chills
- pyrogen reaction
- hypertension

Occurrence of these events should be recorded in the eCRF and indicated as HD-related events.

The cannula inserted in a forearm vein for the collection of blood samples can lead to superficial irritation of the vein, inflammation or clot formation. This will be appropriately treated by the clinical trial staff and not reported as an AE in the eCRF or the CSR.

7.3 PERIOD OF OBSERVATION

The AEs assessment will be carried out by the Investigator throughout the study period, ie, from baseline (first dose of IMP) until Week 56 (Follow-up Visit).

All AEs experienced by a patient, irrespective of the suspected causality, will be monitored until the event has resolved, any abnormal laboratory values have returned to baseline or stabilised at a level acceptable to the Investigator and Medical Monitor, until there is a satisfactory explanation for the changes observed, or until the patient is lost to follow-up.

7.4 DOCUMENTATION AND REPORTING OF ADVERSE EVENTS

7.4.1 Documentation and Reporting of Adverse Events by Investigator

7.4.1.1 Recording of Adverse Events

AEs will be collected from the first time a patient receives IMP until Week 52/ET; during the 1-month follow up after last dose of IMP (Week 56), only SAEs will be documented. All AEs after informed consent signature but before first IMP administration should be captured as medical history.

All AEs, regardless of the relationship to IMP, will be recorded in the eCRF and documents provided by the Sponsor. All AE reports should contain a brief description of the event, date, and time of onset, date and time of resolution, intensity, type of AE whether serious or non-serious, treatment required, causal relationship to IMP, action taken with the IMP, and outcome.

7.4.1.2 Reporting of Adverse Events

All SAEs that occur during the period of observation (from the first administration of the IMP until Week 56 [or 1 month after last dose]) whether considered to be associated with the IMP or not, must be entered into the EDC system for reporting within 24 h to the Safety Contact (see Section 9.1). An alternative method of reporting will be provided to sites should they not have access to the EDC system.

The Investigator should not delay initial SAE report entry due to missing data. As soon as the minimum information is available, and no later than 24 h after becoming aware of the SAE event, the initial SAE report will be entered into eCRF. The eCRF will automatically generate a notification e-mail that will inform the Safety Associate at the Contract Research Organisation (CRO) and at the Sponsor that an SAE has been reported.

Each initial SAE report should contain at least the following information:

- sender of report (name, address of Investigator)
- patient identification (screening/randomisation number, NO names, NO initials)
- protocol number
- details of the IMP
- Investigator's identification
- AE (description, start date, outcome on the day of the report)
- Investigator's assessment of seriousness and intensity (severity)
- Investigator's assessment of causal relationship to study drug and study procedures

If necessary, the Investigator will provide follow-up reports in a timely manner after knowledge of further relevant information.

A 24h- emergency contact will be available during the conduct of the clinical trial. For medical emergencies, phone numbers will be available for contacting the Medical Monitor in each participating country.

After receipt of the initial report, the Safety Centre will review the information and, if necessary, contact the Investigator to obtain further information for assessment of the event. The Investigator will be responsible for informing the Institutional Review Board (IRB) or Independent Ethics Committee (IEC), and the Sponsor will be responsible for informing the Regulatory Authority of the SAE as per local requirements.

If considered necessary, the Investigator may decide to withdraw patients from the study as a consequence of SAEs or AEs.

If a patient is withdrawn from the study due to an AE at the end of the Treatment Period, the patient should be followed-up until an outcome of the event can be defined. If this is not possible due to clinical or organisational reasons, an outcome assessment has to be performed by the Investigator. A final examination should be performed.

AEs will be coded by data management using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) and displayed in tables and listings using MedDRA system organ class (SOC) and preferred term.

7.4.2 Reporting of Adverse Events by Sponsor

The Sponsor or their designee will provide annual safety reports to the Regulatory Authorities and the Independent Ethics Committee (IEC) responsible for the clinical trial, as applicable. These updates will include information on SUSARs and other relevant safety findings.

Any AE that is serious, unexpected, and has evidence suggesting a causal relationship to IMP (ie, a SUSAR) has additional reporting requirements. Reporting of SUSARs will follow all applicable local safety reporting requirements.

The Sponsor or their designee will notify the Investigators of relevant information about SUSARs that could adversely affect the safety of patients in a timely fashion. Follow-up information may be submitted if necessary.

7.5 DATA AND SAFETY MONITORING BOARD

A Data and Safety Monitoring Board (DSMB) will monitor patient safety and data integrity. The roles and responsibilities of the DSMB are defined in a DSMB Charter.

8 DISCONTINUATION AND WITHDRAWALS

8.1 REMOVAL OF PATIENTS FROM THE STUDY

After discussion with the Medical Monitor a patient may be discontinued from study medication or withdrawn from the study for reasons including but not limited to

- AE
- patient request/withdrawn consent
- Investigator’s decision
- study termination by the Sponsor

All patients who discontinued prematurely will continue with their standard of care dialysis sessions. They will report for an early termination visit, as described in Table 1.1 and

Section 6.1.2.14. The sites will make every effort to get post-baseline CT scans and DEXA and to have patients return for a follow-up visit.

If considered necessary, the Investigator may decide to withdraw patients from the study for safety reasons.

8.2 REPLACEMENT OF PATIENTS

Patients withdrawn from the study will not be replaced.

8.3 WITHDRAWAL OF SAMPLES

Blood samples collected will be stored under the control of Laboratoris Sanifit for a maximum of 1 year after study end and then destroyed. In case of early discontinuation, no additional samples will be collected from the patient. The patient has the right to withdraw any remaining biological samples or have them destroyed.

9 EMERGENCY PROCEDURES

9.1 EMERGENCY SPONSOR CONTACT

All SAEs that occur during the study must be reported immediately (within 24 hrs of awareness). Detailed SAE reporting instructions and training will be provided to study site personnel.

9.2 EMERGENCY IDENTIFICATION OF INVESTIGATIONAL PRODUCTS

See unblinding procedures in Section 5.4.2.

10 STATISTICAL METHODS

The primary endpoint of coronary artery calcium volume score values will be log-transformed prior to analysis. The primary analysis will use an ANCOVA model with the change in log score (log 52 week score – log week1/day1 score] as the dependent variable and with a fixed effect term for randomised treatment group and log coronary artery calcium volume score at baseline as a covariate; the model will also be stratified by the randomization stratification factor, i.e. baseline CAC/Agatston score.

10.1 GENERAL STATISTICAL CONSIDERATIONS

10.1.1 Analysis Sets

The following analysis populations will be defined: Safety, modified Intent-to-Treat (mITT), and Per Protocol (PP).

The Safety population will consist of all randomised patients who receive at least one dose of IMP. Patients will be analysed according to the treatment they received. The Safety population will be used for analyses of AEs and safety endpoints.

The mITT population will consist of all enrolled patients who are randomised, receive at least one dose of IMP, and have at least one post-randomization efficacy evaluation. Patients will be analysed according to the treatment to which they were randomised. The mITT population will be handled as the primary efficacy analysis population.

The PP population is the subset of the mITT population who do not have any major protocol violations or deviations (both of which will be determined prior to final data lock and unblinding). The PP population will be used for secondary/supportive efficacy analyses.

10.1.2 Subgroups and Subsets

10.1.3 Covariates and Strata

The primary efficacy analysis will be adjusted for log coronary artery calcium volume score at baseline as a covariate. The analysis will be stratified for baseline CAC score (as this is a stratification variable in the randomization).

As exploratory analysis, clinically relevant covariates will be included in the analysis model, including but not limited to: age, use of non-calcium containing phosphate binder at baseline, diabetes status at baseline, and dialysis vintage. If any covariates are considered to be imbalanced across the treatment arms, then those covariates will also be included in the covariate-adjusted model to determine if they have an impact on calcification progression.

10.2 DEMOGRAPHICS AND BACKGROUND CHARACTERISTICS

The treatment groups will be compared for baseline demographic and disease history variables.

10.2.1 Demographics

Demographic and baseline characteristic data will be summarised with descriptive statistics across the 3 treatment arms. Demographic variables will include sex, race, ethnicity, age, and diabetes status.

10.2.2 Disease Characteristics

Disease characteristics will be summarised with descriptive statistics across the 3 treatment arms. Disease characteristic variables will include diabetes status, baseline CAC score, baseline calcium volume score, years since primary ESRD diagnosis, use of non-calcium containing phosphate binder at baseline, and dialysis vintage.

10.2.3 Disposition

Patient disposition over time will be presented overall as well as separately for each treatment arm, showing the number of patients screened, enrolled, randomised, treated, etc. Reasons for early discontinuation from study will be presented with frequencies and percentages for all categories.

10.2.4 Exposure and Compliance

Study treatment dosing compliance will be summarised with descriptive statistics by treatment group.

10.2.5 Concomitant Therapies

Concomitant medications will be coded with the World Health Organisation (WHO) drug dictionary. Concomitant medications will be summarised with descriptive statistics by drug Anatomical Therapeutic Chemical (ATC) Classification System (level one), generic name, and treatment group.

Dialysis calcium ion concentrations will be recorded as concomitant medications.

10.3 EFFICACY ANALYSES

The mITT analysis population will be considered primary for all primary and secondary efficacy analyses. Unless specified otherwise, all baseline assessments will be based upon the Week 1, Day 1 assessment.

10.3.1 Primary Efficacy Analysis

The primary endpoint of coronary artery calcium volume score values will be log-transformed prior to analysis. The primary analysis will use an ANCOVA model with the change in log score (log 52-week score – log week1/day1 score] as the dependent variable and with a fixed effect term for randomised treatment group and log coronary artery calcium volume score at baseline as a covariate; the model will also be stratified by the randomization stratification factor, i.e. baseline CAC/Agatston score. LS means for each of the 3 treatment groups will be estimated and back transformed prior to presentation. The main contrasts of interest to assess treatment effect will be the difference in LS means between each dose and placebo and between the average of the two doses vs placebo. These contrasts and their estimated 95% CIs will be back transformed prior to presentation. P-value for each contrast will also be provided.

To facilitate data interpretation, the preceding ANCOVA will be re-run but with the log of the 52-week coronary artery calcium volume score as the dependent variable (i.e., without subtracting log baseline score). This will allow estimation of the absolute 52-week score by back transformation as well as the common baseline score so that the degree of absolute change in coronary artery calcium volume score can be assessed in a fashion that is consistent with the preceding analysis of the change in log score.

The secondary endpoint of change from baseline in CAC/Agatston score at Week 52 will be analyses in the same manner as the primary endpoint.

Underlying details of the other secondary analyses will be specified in the Statistical Analysis Plan (SAP).

10.3.2 Missing Data

The primary efficacy analysis will impute missing Week 52 calcium volume score via last observation carried forward (LOCF) imputation; per Table 1.1, patients who discontinue early will have a calcium volume assessment at the time of discontinuation – for those patients, that last calcium volume value will be used for the missing Week 52. In addition to LOCF imputation, additional analyses using multiple imputation will be implemented to explore the impact of missing data. Firstly, missing data will be imputed within each arm using distribution implied by the non-missing patient data for that arm. Secondly, missing data will be imputed for all arms using distribution implied by the non-missing patient data within the placebo arm. Further, tipping point analyses may be performed to assess how extreme a departure in the distribution of data from patients with missing values would have to be to render the primary endpoint analysis non-significant.

10.3.3 Multiple Comparisons / Multiplicity

The trial will not be adjusted for multiple comparisons. The primary efficacy analysis (high-dose active to control) will be assessed at the 0.05 significance level. Secondary efficacy analyses will all be examined at the 0.05 significance level.

10.3.4 Secondary and Exploratory Analyses

Secondary efficacy analyses will be implemented to assess the treatment effect on a variety of efficacy endpoints. Continuous variables will be analysed via ANCOVA; categorical variables will be analysed by chi-square. All-cause mortality will be displayed descriptively using the Kaplan-Meier curves, censoring subjects as of the last known contact.

Secondary efficacy variables include:

- change from baseline (Week 1, Day 1) in CAC/Agatston score at Week 52
- number of patients with <15% progression in CAC/Agatston score at Week 52
- change from baseline (Week 1, Day 1) in thoracic aorta calcification score at Week 52
- change from baseline (Week 1, Day 1) in aortic valve calcification score at Week 52
- incidence of composite safety endpoint that include death from cardiovascular causes, MI, stroke, or heart failure
- mortality rate (all-cause and CV)
- change from baseline (Week 1, Day 1) in levels of CRP and other selected biomarkers
- changes in BMD between baseline (Week 1, Day 1) and Week 52
- safety of SNF472 in terms of incidences of adverse events (AE) and serious adverse events (SAE) and clinically relevant changes from baseline in laboratory and ECG parameters

The exploratory efficacy variables are changes from baseline in pulse pressure, systolic blood pressure (SBP), and diastolic blood pressure (DBP) at Week 28 and Week 52.

The final list of biomarkers will be defined prior to database lock. The underlying details of the secondary and exploratory analyses will be specified in the SAP.

10.4 SAFETY ANALYSES

Safety analyses will be performed on the Safety analysis population.

10.4.1 Adverse Events

All reported terms (Investigator descriptions) for AEs will be coded using the most recent version of the MedDRA and summarised with frequencies and percentages by treatment group, SOC classification, and preferred term.

Additionally, AE summaries will be broken out by all AEs occurring during treatment and follow-up periods, all TEAEs, Sponsor-identified AEs of special interest, AEs leading to discontinuation from the study, AEs possibly or probably related to study treatment, and AEs by Common Terminology Criteria for Adverse Events (CTCAE) severity. For all AE summaries, events will be counted only once for a given patient by primary SOC and preferred term. When an AE occurs more than once for a patient, the maximum severity and causality will be used.

All AEs will be included in comprehensive data listings. A separate listing will be included for AEs leading to discontinuation from study.

10.4.2 Bone Mineral Density

BMD is an indication of bone health and CKD patients may develop mineral and bone disease (CKD-MBD). To assess whether this occurs, BMD will be assessed at baseline (Week 1, Day 1) and Week 52. BMD will be analysed using the same analysis method as the primary efficacy model – an ANCOVA of Week 52 BMD T-score adjusted for baseline (Week 1, Day 1) score and treatment group.

10.4.3 Clinical Laboratory Evaluation

Laboratory assessments and change from baseline will be summarised with descriptive statistics by panel, test, treatment group, and time point. Additionally, abnormal results will be summarised with frequencies and percentages by clinical significance, panel, test, treatment group, and time point.

10.4.4 Vital Signs

The results of change from baseline of vital signs (including HR, RR, SBP and DBP, body temperature, and weight) will be summarised with descriptive statistics by treatment group and time point.

10.4.5 ECG

ECG findings will be summarised with frequencies and percentages by treatment group and time point.

10.4.6 Physical Findings and Other Observations Related to Safety

The results of physical examinations will be summarised with frequencies and percentages by category, treatment group, and time point.

10.5 OTHER STATISTICAL ANALYSES

Additional statistical analyses and more detailed descriptions of the methods presented in this protocol will be included in a separate SAP.

10.6 DETERMINATION OF SAMPLE SIZE

The hypothesized values for calcium volume score at baseline and Week 52 for placebo (standard of care) are provided in the table below. These data are derived from the ADVANCE trial (Raggi 2010). The standard deviation of the change from baseline to Week 52 in calcium volume scores is estimated to be 0.30 on the log scale (Raggi 2010).

Hypothesized values for calcium volume score at baseline and Week 52 for placebo

	Placebo
Assumed percentage change in calcium volume score progression from baseline to Week 52	35%
Expected raw calcium volume score at Week 52 assuming a raw calcium volume score of 380 at baseline (Week 1, Day 1)	513
Expected change in log-transformed calcium volume score progression from baseline to Week 52	0.30
Estimated standard deviation of change in log-transformed calcium volume scores from baseline to Week 52	0.30

With N=270 subjects randomized (ie, 90 per group) and 200 patients expected to provide Week 52 data, and assuming a 35% progression in CAC score on placebo at Week 52, this trial has 80% power with a 1-sided alpha level of 2.5% to test the hypothesis that the true difference between SNF472 high dose vs placebo in log CAC progression scores is 0.146. This corresponds to a true ratio of CAC progression scores, SNF472 high dose vs placebo, of 1.157 which, in turn, corresponds to a 16.7% progression score on SNF472 high dose vs 35% on placebo.

The corresponding value for the average of the two SNF472 doses vs placebo to provide 80% power to test the hypothesis that the true difference between SNF472 average dose vs placebo alone in log CAC progression scores is 0.126. This corresponds to a true ratio of CAC progression scores, SNF472 average dose vs placebo of 1.134 which, in turn, corresponds to a 19.0% progression score on SNF472 average dose vs 35% on placebo.

10.7 INTERIM ANALYSIS

Approximately 270 subjects were planned to be randomized with N=190 expected to provide Week 52 data on the primary endpoint in the final analysis. A non-binding interim futility analysis will be conducted when approximately N=120 subjects (63% of N=190) have provided Week 52 data. The purpose of this interim analysis is to ascertain if the conditional power for achieving a statistically significant result in the final analysis of the two SNF472 doses combined would be low, ≤ 5%; if so the study may be declared futile and subject follow-up may consequently cease. There is no plan or intent to curtail follow-up at this interim for a positive efficacy finding. Conditional power is to be computed under the result observed at the interim as per Mehta and Pocock (2011).

In the event of an equivocal result of the futility analysis indicating borderline conditional power of the study, additional analyses of the same dataset will be conducted to support forward decision making regarding clinical development of SNF472. Additional analyses

will include primary and secondary endpoints, demographic and background characteristics, key subgroups, and PK/PD correlations with efficacy. Further details will be provided in the SAP.
In addition, PD and PK analyses will be performed at the time of the interim analysis. PD as the percent inhibition of HAP crystallization and PK as the EOI plasma levels (Cmax) may be analyzed by timepoint (Week 1, 10, 22, and 52) or by pooling all timepoints. The percent inhibition and Cmax may be then used to calculate a PK/PD correlation between both parameters by timepoint and by pooling all timepoints. Further details will be provided in the SAP.

10.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSIS

Any changes to the protocol-specified analyses will be described and explained in the SAP and/or CSR.

11 REGULATORY, ETHICAL AND LEGAL OBLIGATIONS

11.1 GOOD CLINICAL PRACTICE

The procedures set out in this Clinical Trial Protocol are designed to ensure that the Sponsor and the Investigator abide by the principles of the guidelines provided by the ICH E6 GCP and the Declaration of Helsinki and applicable amendments. The clinical trial will also be carried out according to the national and local legal requirements.

11.2 INFORMED CONSENT

All patients will be required to participate in the consent process. During the consent process, the person obtaining consent will inform the patient of all elements of informed consent. No protocol specific procedures, including screening procedures will be performed until the patient has signed and dated an IRB/IEC - approved informed consent form (ICF). Study participation will start with the signing and dating of the ICF.

The Investigator must ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the clinical trial. Patients must also be notified that they are free to withdraw from the clinical trial at any time without prejudice to future care. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

In case of protocol amendment, the Subject Information Sheet and ICF need to be revised to reflect the changes, if needed. Also, if the Subject Information Sheet and ICF are revised, they must be reviewed and approved by the responsible IRB/IEC, and signed by all patients subsequently enrolled in the clinical trial as well as those currently enrolled in the clinical trial.

11.3 PATIENT CONFIDENTIALITY AND DISCLOSURE

All clinical trial findings and documents will be regarded as confidential. The Investigator and members of his research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating patients must be maintained. All patient data will be identified only by a patient identification number. Documents that identify the subject (e.g., the signed Subject Information Sheet and ICF) must be maintained in confidence by the Investigator. However, in compliance with federal guidelines regarding the monitoring of clinical studies and in fulfillment of his/her obligations to the Sponsor, the Investigator must permit the study monitor, Sponsor representative or auditor, and/or FDA representative or other regulatory authority to review the portion of the patient's medical record that is directly related to the study. This shall include all study-relevant documentation including medical history to verify eligibility, admission/discharge summaries for hospital stays occurring while the patient is enrolled in the study and autopsy reports if a death occurs during the study.

11.4 PROTOCOL AMENDMENTS

To alter the Clinical Study Protocol (CSP), amendments must be written. Administrative changes may be made without the need for a formal CSP Amendment, but will also be mentioned in the integrated CSR. All CSP Amendments will be distributed to all CSP recipients, with appropriate instructions.

11.5 APPROVAL OF THE CLINICAL STUDY PROTOCOL AND AMENDMENTS

Before the start of the clinical study, the CSP and other relevant documents will be approved by the IRB/IEC/Regulatory Authorities in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first patient is enrolled in the clinical trial. This CSP should be followed exactly.

The CSP Amendments must be released by the responsible staff and receive IRB/IEC/Regulatory Authority approval prior to implementation (as appropriate). The only circumstance in which an amendment may be initiated without prior IRB/IEC approval is to eliminate an apparent immediate hazard to a patient or patients. In such a case, however, the Investigator must notify the Sponsor immediately and the IRB/EC within 5 working days after implementation or according to the Investigator's IRB/IEC requirements.

11.6 ONGOING INFORMATION FOR INDEPENDENT ETHICS COMMITTEE/ INSTITUTIONAL REVIEW BOARD

If a safety issue of clinical relevance is identified, from review of any data, then the Sponsor will issue prompt notification to all parties – Investigator and IRB/IEC/Regulatory Authorities.

A safety issue of clinical relevance is one that has a relevant impact on the course of the clinical trial or program (including the potential for suspension of the clinical trial program or amendments to the CSP) or warrants immediate update of the Subject Information Sheet and ICF.

11.7 CLOSURE OF THE STUDY

The Investigator is required to submit eCRFs and all other relevant data and records to the Sponsor. The Investigator will complete and report (submission of eCRFs) his/her study in satisfactory compliance with the protocol as soon as possible after the completion of the study.

The Investigator must submit a final report as required by their IRB/IEC of study completion or early termination.

11.8 RECORD RETENTION

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. However, these documents should be retained for a longer period if required by the applicable legal requirements.

12 DATA MANAGEMENT, MONITORING AND AUDITS

12.1 STUDY MONITORING

The CRO will be responsible for monitoring the trial according to GCP guidelines.

Clinical Research Associates (CRAs) will conduct both central monitoring and monitoring on site.

Central monitoring will include data review by the CRA via the EDC off-site, with a focus on incomplete fields and fields where potential errors could exist, based on the CRA's understanding of the data specification.

On site, the CRA will review the eCRF data entry against the source documents. Handling of queries and clarification/reconciliation of queries between the CRA and the site will be described in the eData Management Plan (eDMP).

12.2 DATA CAPTURE AND VERIFICATION

Data capture and management will be conducted using the CRO's clinical management and EDC/eCRF system. The EDC/eCRF system will be compliant with the Health Insurance Portability and Accountability Act (HIPAA) and meet all requirements for 21 CFR Part 11. The processes and responsibilities of data collection, management and quality assurance will be specified in the eDMP.

All applicable study data collected on each subject will be entered by approved site personnel into the eCRF. Instructions for the completion and submission of eCRFs will be provided to the sites in a separate document.

Authorised personnel will verify all data entered into eCRFs for completeness and accuracy with reference to the source documents and records and will issue data queries to correct missing data or discrepancies found against the source within the EDC system. Data validation will consist of automated and manual edit checks that are created directly in the EDC system. Edit checks will be executed on all data points defined and documented by the study team and data management will be able to issue manual queries as needed to the eCRF. Study metrics will be reported from the EDC system.

Only authorised site personnel will be able to enter/modify/correct data in the eCRF. Once all data have been verified by approved staff at the CRO, an Investigator or sub-Investigator listed on the statement of Investigators (Form FDA 1572) must sign the eCRF.

12.3 ON-SITE AUDITS

During the course of the study and/or after it has been completed, 1 or more study sites may be audited by authorised representatives of the Sponsor. The purpose of the audit is to determine whether or not the study is being conducted and monitored in compliance with recognised GCP/ICH guidelines and regulations.

Additionally, the study may be inspected by regulatory authorities. These inspections may take place at any time during or after completion of the study and are based on local regulations.

13 DOCUMENTATION AND USE OF STUDY FINDINGS

13.1 DOCUMENTATION OF STUDY FINDINGS

An eCRF must be completed and submitted for each subject enrolled, including those discontinued from the study for any reason. For screen failures, only baseline demographics and the reason for discontinuation will be recorded. The eCRFs must be kept current to reflect the subject's status at each phase during the course of the study. Subjects are not to be identified on eCRFs by name; appropriately coded by subject identification number. The Investigator must keep a separate log of the subjects' names and addresses.

Source documents such as the clinic chart are to be maintained separately from the eCRF to allow data verification. Because of the potential for errors, inaccuracies, and illegibility in transcribing data onto eCRFs, originals of laboratory and other test results must be kept on file at the site. Source documents and copies of test results must be available at all times for inspection by the CRA.

13.2 USE OF STUDY FINDINGS

By signing the CSP, the Investigator agrees with the use of results of the clinical trial for the purposes of national and international registration, publication, and information for medical

and pharmaceutical professionals. If necessary, the Regulatory Authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement.

An Investigator shall not publish, or present for publication any articles or papers, or make any presentations, nor assist any other person in publishing any articles or papers, or making any presentations, or making any public declaration relating or referring to the clinical trial, the results of the clinical trial, in whole or in part, without the prior written consent of the Sponsor.

13.3 STUDY REPORT, PUBLICATION POLICY AND ARCHIVING OF STUDY DOCUMENTATION

13.3.1 Study Documents

The Investigator must maintain primary source documents supporting significant data for each subject's medical notes. These documents, which are considered 'source data', should include documentation of:

- demographic information
- evidence supporting the diagnosis/condition for which the patient is being studied
- general information supporting the subject's participation in the study
- general history and physical findings
- CT scans
- DEXA scans
- ECG tracings
- laboratory test results
- Investigator's prescriptions/concomitant medications for the respective subject related to this study
- unscheduled laboratory test or other investigation, if done
- hospitalisation or Emergency Room records (if applicable)
- each study visit by date, including any relevant findings/notes by the Investigator(s), occurrence (or lack) of AEs, and changes in medication usage, including the date the IMP was started and stopped
- any additional visits during the study
- any relevant telephone conversations with the subject regarding the study or possible AEs

The Investigator must also retain all subject-specific printouts/reports of tests and procedures performed as a requirement of the study. During monitoring visits the CRA will validate eCRF entries against these sources of data.

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APPENDIX 1 Sub-Study Protocol: For Opt-In Sites Only

SNFCT2015-05 Sub-Study

Sub-Study to Assess the Effects of SNF472 on Arterial Stiffness and Cardiovascular Function in ESRD Patients on Hemodialysis

Investigational Product:	SNF472
Indication:	CVC in ESRD
Sponsor:	Laboratoris Sanifit Palma de Mallorca, Spain
EudraCT Number	2016-002834-59
IND Number	116437

Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Council for Harmonisation Guidelines on Good Clinical Practice (ICH E6 GCP) and regulatory requirements as applicable.

SUB-STUDY PROTOCOL SYNOPSIS

Sponsor: Laboratoris Sanifit	**Investigational Product:** SNF472	**Developmental Phase:** Phase 2b
Title of Study: Sub-Study to Assess the Effects of SNF472 on Arterial Stiffness and Cardiovascular Function in ESRD Patients on Hemodialysis		
Protocol Number: SNFCT2015-05 Sub-Study		
EudraCT: 2016-002834-59		
IND Number: 116437		
Number of Patients and Study Centre(s): It is estimated that up to 50% of the sites participating in the main study may opt into participating in the Sub-Study in order to enroll up to 200 subjects.		
Indication: Cardiovascular calcification (CVC) in ESRD		
Objectives: The primary objective of this Sub-Study is to assess the effect of 2 dose levels of SNF472 (300 mg and 600 mg) compared to placebo on the progression of arterial stiffness as measured by an absolute change in pulse wave velocity (PWV) over a 52-week period in ESRD patients on HD. The secondary objectives of this Sub-Study are to assess: • Change from Sub-Study Entry in left ventricular mass index (LVMI) • Change from Sub-Study Entry in left ventricular (LV) function parameters The exploratory objectives of this Sub-Study are to assess: • Change from Sub-Study Entry in left atrial (LA) function and change in parameters over time • Change from Sub-Study Entry in aortic valve gradient		
Methodology: Patients enrolled in the main study will be eligible to participate in this Sub-Study and will be requested to undergo additional assessments as outlined in the Sub-Study Schedule of Assessments.		
Endpoints: The primary endpoint is the absolute change in aortic PWV (m/s) from Sub-Study Entry to Week 52 by 2-dimensional (2D) echocardiography. The secondary endpoints will be assessed by 2-D echocardiography and are: • Absolute change in LVMI from Sub-Study Entry to Week 52. • Absolute change in LV function from Sub-Study Entry to Week 52: • Absolute change in the ratio of mitral inflow early diastolic to mitral annular velocity (E/e' ratio)		

Sponsor: Laboratoris Sanifit	**Investigational Product:** SNF472	**Developmental Phase:** Phase 2b

- Absolute change in mitral annular early diastolic tissue velocity (e') measured with tissue Doppler (average of septal and lateral values)
- Absolute change in LV global longitudinal systolic strain (%) measured by speckle tracking echocardiography

The exploratory endpoints are:

- Absolute change in additional measures of LA volume (mL) and LA ejection fraction from Sub-Study Entry to Week 52:
- Absolute change in PWV, LVMI and other parameters of LV and LA function over time (Weeks 28 and 52).
- Absolute change in aortic valve gradient from Sub-Study Entry to Week 28 and Week 52) in all subjects and in the subgroup of subjects with evidence of aortic stenosis at Sub-Study Entry

Criteria for Inclusion:

1. Participant in the main study
2. Willing and able to understand and sign the additional Sub-Study informed consent

Criteria for Exclusion:

1. Presence of current atrial fibrillation, atrial flutter with variable atrioventricular conduction, or very frequent premature beats (premature ventricular contractions [PVCs])
2. A diagnosis of primary hypertrophic obstructive cardiomyopathy, or cardiac infiltrative disease (such as cardiac amyloidosis).
3. Severe cardiac valve disease

Test Product, Dose and Mode of Administration: Per main protocol

Reference Therapy, Dose and Mode of Administration: Per main protocol

Study Duration: Per main protocol

Statistical Methods:

The primary Sub-Study endpoint will be summarized using descriptive statistics (by treatment arm) and analyzed using an ANCOVA model to compare the 2 active treatment groups (pooled) to the control group, with the Week 52 value as the response variable, and Sub-Study Entry value and treatment group as explanatory variables. This comparison will be evaluated using a 95% 1-sided level of significance and will be conducted in the Sub-Study ITT and PP populations. Pairwise comparisons, relative to the control, will be presented as 90% two-sided confidence intervals.

The secondary and exploratory endpoints will be summarized using descriptive statistics by treatment arm and analyzed using methods similar to the primary Sub-Study endpoint.

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LIST OF IN-TEXT TABLES

LIST OF ABBREVIATIONS NOT USED IN THE MAIN PROTOCOL

Abbreviation	Definition
2D	2-dimensional
4D	Deutsche Diabetes Dialyse studies
AASK	African-American Study of Kidney Disease
CF	carotid-femoral
CRIC	Chronic Renal Insufficiency Cohort
DCOR	Dialysis Clinical Outcomes Revisited study
EACVI	European Association of Cardiovascular Imaging
e’	mitral annular early diastolic tissue velocity
E/e’	ratio of mitral inflow early diastolic to mitral annular velocity
EF	ejection fraction
eGFR	estimated glomerular filtration rate
E_{inc}	incremental elastic modulus
ET	early termination
EVOLVE	Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events trial
GLS	global longitudinal strain
HEMO	Hemodialysis Study
LA	left atrial
LAVI	left atrial volume index
LIFE	Losartan Intervention For Endpoint study
LV	left ventricular
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
MACE	Major Cardiovascular Events
PVC	premature ventricular contraction
PWV	pulse wave velocity

1 INTRODUCTION

This exploratory Sub-Study is offered to only those sites and subjects participating in the main study, Study SNFC2015-05, A double-blind, randomised, placebo-controlled study to assess the effect of SNF472 on progression of cardiovascular calcification on top of standard of care in end-stage-renal-disease patients on haemodialysis. The Sub-Study is optional to main study sites. Assessments will be performed only in subjects consenting to participate in the Sub-Study at sites that opt-in to participate in the Sub-Study. The main study will continue as per protocol. The Sub-Study will require assessments to measure the functional consequence of a reduction in progression of CVC on arterial stiffness as well as cardiac structure and function as assessed by pulse wave velocity (PWV), left ventricular mass index (LVMI), diastolic function, myocardial strain, and other echocardiographic parameters.

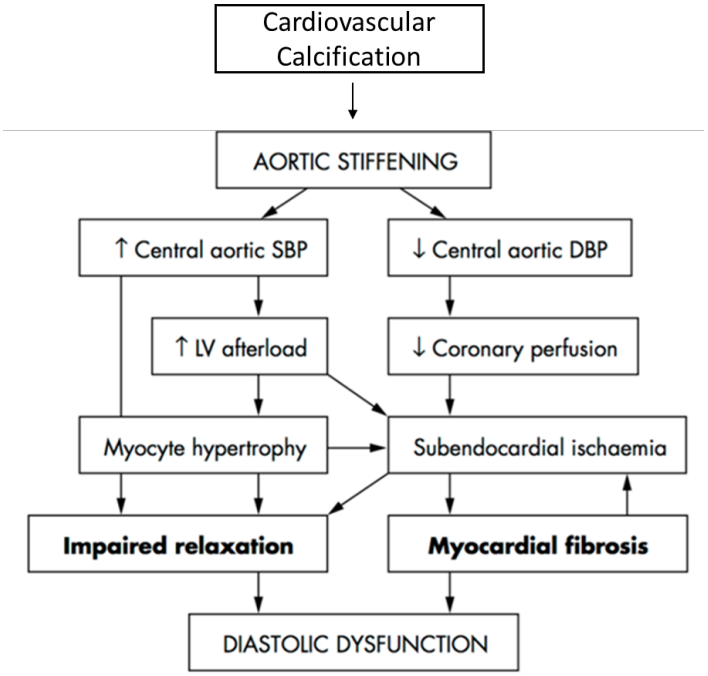
1.1 RATIONALE FOR THE SUB-STUDY

1.1.1 Background

Cardiovascular disease is a significant contributor to morbidity and mortality in patients with ESRD on dialysis (USRDS, 2015; Bansal et al, 2013; Baigent et al, 2000). No drugs have received regulatory approval for the reduction of CV events in ESRD patients on dialysis. Large CV trials designed to show benefit of a given therapeutic intervention in dialysis patients such as Deutsche Diabetes Dialyse Studies (4D), the Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events (EVOLVE) trial, the Hemodialysis (HEMO) Study, and the Dialysis Clinical Outcomes Revisited (DCOR) have utilized traditional endpoints such as all-cause mortality and Major Cardiovascular Events (MACE) requiring large sample sizes and significant time to enroll and complete these studies (Wanner et al, 2005; EVOLVE Trial Investigators, 2012; St. Peter et al, 2008; Eknoyan et al, 2002). These and other similar studies unfortunately have failed to show CV benefit with the studied intervention in the dialysis population and thus, a significant unmet need remains. Identifying and assessing surrogate CV functional endpoints that are reasonably likely to predict clinical benefit ahead of large CV outcomes trials may be important to hasten development of therapeutics in this area and increase the probability of success.

The main objective of the Phase 2b study is to demonstrate that SNF472 can slow the progression of CVC. If SNF472 demonstrates a significant reduction in progression of CVC in dialysis patients, it is unknown whether this reduction will translate into improvement of CV outcomes in subsequent studies. The inclusion of additional cardiovascular surrogate endpoints should inform the likelihood that reducing CVC will translate to an improvement in CV outcomes prior to subsequent larger CV outcomes trials. The selection of these surrogate endpoints is guided by an understanding of the initial role that calcification may play in contributing to CV disease, particularly aortic stiffening as measured by PWV and the resultant pathophysiologic changes as illustrated in Figure S.1.1.

Figure S.1.1 **Pathological Pathways through which Aortic Stiffness may Contribute to the Development of Diastolic Dysfunction**



Adapted from Mottram et al, 2005.

As shown, progressive aortic stiffening leads to a widening of pulse pressure, changes in coronary perfusion, and an increase in left ventricular (LV) afterload that results in structural and function changes in the heart and consequent diastolic dysfunction. A number of these surrogate clinical endpoints have been well studied in the dialysis population and have been demonstrated to be relevant predictors of CV morbidity and mortality including left ventricular mass (LVM), diastolic function, myocardial strain, left atrial (LA) size and function, and PWV, the latter as a measure of arterial wall stiffness.

The hypothesis for this Sub-Study is that improving the burden and progression of CVC will slow the progressive aortic stiffening and also improve left ventricular hypertrophy (LVH) and diastolic dysfunction. To explore these hypotheses, additional surrogate endpoints are being evaluated in the Sub-Study, namely PWV, an indicator of aortic stiffness, LVMI, diastolic function, and LA structure and function. A scientific rationale and justification for inclusion of these endpoints is provided below.

1.1.2 Considerations in the Selection of Cardiovascular Endpoints

The use of surrogate structural and/or functional CV measures in this Sub-Study provides an opportunity to assess the effect of SNF472 on arterial and LV structure and function (Rubin et al, 2011). Whereas it is recognized that CV structural/functional surrogate markers are not equivalent to clinical outcomes, the evaluation of the impact of SNF472 on these surrogate endpoints should provide valuable information about potential CV effects of the drug and will guide the design of future trials. In general, we selected endpoints which are:

1. Plausibly modified by SNF472 via the drug's hypothesized effects on reducing vascular calcification, arterial stiffness, and secondary effects on LV afterload;
2. Considered clinically relevant, and have been previously studied in patients with ESRD in particular. The clinical relevance was established based on the following considerations:
   a) Positive effects on these endpoints can reasonably be expected to mediate a potential clinical CV benefit of SNF472;
   b) Endpoints represent quantitative traits that can be obtained non-invasively, accurately, reproducibly, and with minimal risk to trial subjects;
   c) Endpoints are independently predictive of prospective CV risk in various populations, particularly among patients with ESRD;
   d) Whenever possible, available data demonstrates that modification of the endpoint by an intervention reflects the ultimate benefit of that intervention on CV outcomes.

Aortic PWV

Large artery stiffness is a highly relevant phenotype in CKD. Arterial stiffening impairs the ability of conduit arteries to accommodate the stroke volume intermittently ejected by the LV during systole, thus increasing the pulsatile hydraulic load to the LV (Chirinos and Segers, 2010a; Chirinos and Segers, 2010b). In addition, given the effect of arterial stiffness on pulsatile hemodynamics, increased large artery stiffness can also promote excessive penetration of pressure and flow pulsatility into the kidney and the brain, leading or contributing to target organ damage (O'Rourke and Safar, 2005).

PWV is a functional parameter affected by the stiffness of the arterial wall, which is intimately related to the incremental elastic modulus (E_{inc}) of the wall material, as demonstrated by the Moens-Korteweg equation (Chirinos, 2012).

Arterial stiffness increases with aging and various disease states, including kidney disease (Payne et al, 2010). Large artery stiffness increases with declining kidney function (Hermans et al, 2007; Mourad et al, 2001). In the Chronic Renal Insufficiency Cohort (CRIC) study unadjusted analysis indicated that each 10 mL/min/1.73m^2 decrease in estimated glomerular filtration rate (eGFR) was associated cross-sectionally with a 0.5 m/s increase in aortic PWV (Kaminski et al, 2011; Townsend et al, 2010).

Available studies demonstrate that carotid-femoral (CF) PWV, an index of large artery stiffness, independently predicts the risk of incident CV events across a number of patient populations, including high risk populations with hypertension, diabetes, and ESRD (Chirinos et al, 2014; Vlachopoulos et al, 2010; Mattace-Raso et al, 2006; Boutouyrie et al,

2002). In a recent meta-analysis of 17 longitudinal studies across a number of different patient populations, each 1 m/s increase in aortic PWV resulted in an age-, sex-, and risk factor adjusted increase of 14% in total CV events and 15% in CV mortality. Each 1 standard deviation increase in PWV, which averaged about 3 m/s, was associated with a 47% increase in total CV events and a 47% increase in CV mortality (Nakanishi et al, 2017). Importantly, among these 17 studies were four studies of ESRD populations, which showed increased risk for CV events and mortality consistent with the other populations from studies included in the meta-analyses. Available evidence also demonstrates the prognostic value of PWV across the spectrum of CKD severity (Sulemane et al, 2017). In subjects with CKD stages 4 and 5, aortic PWV is independently related to renal function decline (Chen et al, 2013a; Tripepi et al, 2007). CF-PWV is also an independent predictor of incident heart failure in CKD (Chirinos et al, 2014).

Importantly, among patients on hemodialysis, longitudinal changes in measures of aortic PWV in response to an intervention were predictive of future risk for mortality. In a study of 150 hemodialysis patients reported by Guerin et al, mean aortic PWV was higher at baseline (11 m/s compared with 9.5 m/s) and ***rose*** over time in those who died during follow up, despite antihypertensive treatment compared with those patients in the study who survived in which a fall in PWV and a parallel reduction in mean arterial pressure was observed over time (Guerin et al, 2001).

A number of studies haves established an important link between arterial calcification and PWV across populations, including those with ESRD. Arterial calcification is an independent correlate of increased pulse pressure (a surrogate of aortic stiffness) in ESRD (Kraus et al, 2015). Aortic and superficial femoral artery calcification is associated with increased aortic stiffness in healthy individuals (McEniery et al, 2009) and in patients with CKD (Toussaint et al, 2008; Sigrist et al, 2006). In a rodent model of CKD, aortic calcium content measured by atomic absorption spectrophotometry was related to an increase in PWV (Ng et al, 2011). Among 48 patients with CKD (GFR 17-55 mL/min), abdominal aortic and superficial femoral artery calcification, aortic vascular calcification correlated positively with PWV (Toussaint et al, 2008). Similarly, among 134 subjects with advanced CKD (60 hemodialysis patients, 28 peritoneal dialysis and 46 patients with CKD stage 4) increase in calcification of the superficial femoral artery as measured by CT was associated with increased CF-PWV and increased pulse pressure (Sigrist et al, 2006). Observational studies suggest that higher dialysate calcium is associated with a transient increase in PWV, probably from a smooth muscle effect (Mac-Way et al, 2011); however, one study suggested that a higher dialysate calcium was associated with an increased progression of PWV over a 6-month period (Mac-Way et al, 2011; LeBeuf et al, 2011).

Left ventricular mass

LVH is associated with CKD (Hee et al, 2014). Approximately 3 out of 4 patients initiating dialysis have LVH. LVH is also highly prevalent in earlier stages of kidney disease and is more prevalent with worsening renal function. Levin et al reported prevalence rates of 27%, 31% and 45% of LVH among CKD patients with a creatinine clearance >50 mL/min, 25-50 mL/min and <25 mL/min, respectively. The major correlates of LVH in this study were systolic hypertension and anemia (Levin, 2003). LVH is a common cardiac abnormality

in patients with ESRD with varying incidence of 36% to >90% in incident and prevalent dialysis patient populations (Di Lullo et al, 2011; Foley et al, 2010; Foley et al, 1995; Takeda et al, 2008; Hayashi et al, 2013; Satyan et al, 2007; Madsen et al, 2007; Dimitrijevic et al, 2009; Virga et al, 2006; Kutlay et al, 2006).

LVH is a strong independent predictor of the risk of adverse CV events (such as coronary events, heart failure and death) in the general population and in patients with CKD (Dubin et al, 2017; Gardin et al, 2004; Gardin et al, 1997; Koren et al, 1991; Levy et al, 1990). The regression or worsening in LVH is strongly predictive of subsequent cardiac events among patients on dialysis (Paoletti et al, 2004; Zoccali et al, 2004). In addition, regression of LVH in response to therapy independently predicts a favorable prognosis (Lantelme et al, 2008; Levin et al, 1998), supporting the use of LVM as a useful surrogate endpoint of the clinical benefit of therapeutic interventions (Wachtell et al, 2007; Devereaux et al, 2004). In the Losartan Intervention For Endpoint (LIFE) study, a 11% reduction in LVMI with therapy was associated with a 15% reduction in relative risk of cardiovascular events, which was independent of the degree of blood pressure lowering (Wachtell et al, 2007; Devereaux et al, 2004). Importantly for this particular trial, a 10% reduction in LVM achieved by multiple interventions was associated with a relative risk of 0.72 for CV death among patients with advanced hypertensive kidney disease (London et al, 2001).

Although the pathophysiology of LVH in CKD is complex, hemodynamic load is a key factor contributing to LVH in this population. In the presence of a normal aortic valve, LV afterload is largely dependent on the properties of the arterial tree (Desai et al, 2011; Nicholas, 2011) including aortic stiffness. Among 49 patients on chronic hemodialysis, LVM has been shown to be associated with increased PWV, systolic blood pressure and abdominal aortic calcification quantified by CT (Nitta et al, 2004). Furthermore, longitudinal changes in LVM in 55 patients with stage 3/4 CKD were shown to be independently associated with aortic stiffness. Over 2 years, among patients with initial normal LV indices, 68% showed no appreciable change with time while 32% developed LVH, whereas among those with initial LVH, 50% maintained elevated LV indices and 22% regressed. Factors to maintaining or achieving normal LVM included age, a lower pulse pressure and a higher GFR (McMahon et al, 2004). Interestingly, PWV has been shown to be independently associated with LVH in ESRD even in the absence of hypertension, consistent with the fact that hemodynamic load, as opposed to blood pressure per se, is related to LVH in this population (Lin et al, 2002).

LV diastolic function and filling pressures

LV diastolic dysfunction is highly prevalent (~85%) in advanced CKD, with greater than 1/3 of patients demonstrating grade 2 or higher diastolic dysfunction. Diastolic dysfunction is a strong predictor of mortality in patients with stage 4-5 CKD (Farshid et al, 2013). Among patients with ESRD, the ratio of mitral inflow early diastolic peak flow velocity (E) to mitral annular velocity (e') is indicative of an increased LV filling pressure and predicted all-cause mortality and CV death independently of LVM and LV ejection fraction (LVEF) (Wang et al, 2008; Sharma et al, 2006).

In other studies that included patients with stage 3-5 CKD, a high E/e' was independently associated with CV events, mortality and progression to dialysis (Kim et al, 2013; Chen et al,

2013b). Similarly, among 578 patients enrolled in the African-American Study of Kidney Disease (AASK) cohort, LV hypertrophy and diastolic function parameters predicted cardiovascular events requiring hospitalization or causing death; a renal composite outcome of doubling of serum creatinine or ESRD; and heart failure. After adjustment for LVM and clinical variables, lower systolic tissue Doppler velocities were significantly associated with future heart failure events (Peterson et al, 2013). However, in the CRIC study, which included 3939 racially and ethnically diverse individuals between the ages of 21–74 years old with eGFR between 20 and 70 mL/min per 1.73 m^2, diastolic dysfunction assessed by echocardiography was not significantly associated with heart failure or death (Dubin et al, 2017). The differences between these 2 studies may be due to differences in the study cohorts. In the AASK trial, all subjects were African American, diabetes was an exclusion criterion and 50% of participants in the AASK had a history of prior CV disease. In contrast, the CRIC study included an ethnically diverse cohort with diabetes and excluded those who self-reported heart failure.

Diastolic function is also correlated with aortic stiffness and PWV and calcification (Fujiu et al, 2008; Weber et al, 2008; Borlaug et al, 2007). In a study that included 142 hemodialysis patients, aortic arch calcification volume measured by CT and arterial stiffness estimated by brachial-ankle PWV were independently associated with the E/e' ratio (Fujiu et al, 2008).

In summary, diastolic function is an independent predictor of outcomes in ESRD. Further, given the association with PWV and vascular calcification, improvement in these parameters may translate into improvements in diastolic function.

LV longitudinal strain and strain rate

Myocardial deformation (strain) is a highly sensitive measure of myocardial function, even in patients with preserved ejection fraction (EF). Strain is defined as the percent change in length, relative to a reference length, a given direction. For the LV, the end-diastolic length is used as the reference length. LV strain in the radial, circumferential and longitudinal directions can be measured with 2D-echocardiography, using speckle-tracking echocardiography. In speckle tracking echocardiography, small stable myocardial footprints (“speckles”) are tracked throughout the cardiac cycle using digital echocardiographic images. Strain in the longitudinal direction has been shown to be the most clinically significant parameter in a variety of clinical conditions (Collier et al, 2017).

Longitudinal LV strain measured with speckle-tracking echocardiography has been shown to be reduced in CKD (Pressman et al, 2015; Chen et al, 2014; Wang et al, 2012) even in the absence of overt systolic dysfunction measured by LVEF. Reduced LV longitudinal strain demonstrates a progressive reduction with worsening stages of renal dysfunction (Panoulas et al, 2015; Krishnasamy et al, 2014; Liu et al, 2011). Reduced LV strain has also been associated with CKD even in patients with established heart failure with preserved EF (Unger et al, 2016).

Multiple studies demonstrate the high clinical relevance of LV strain in CKD. Among 108 patients with stage 3/4 CKD, reduced (i.e., less negative) global longitudinal strain (GLS) is associated with a reduced submaximal exercise capacity (6-minute walk distance)

and health-related quality of life (Krishnasamy et al, 2016). Among 106 patients with CKD, no history of CV disease, and normal LVEF, a reduced GLS was predictive of a composite endpoint of all-cause mortality, acute coronary syndrome, stable angina requiring revascularization, hospitalization for heart failure and stroke, over a median follow up period of 49 months, independently of other predictors and CF-PWV (Sulemane et al, 2017). Among 88 stable hemodialysis patients with preserved LVEF followed-up for 25.6±9.9 months, a reduced GLS was an independent predictor of all-cause mortality (Liu et al, 2013). Across the spectrum of CKD severity (stages 1-5), GLS is also predictive of all-cause mortality (Krishnasamy et al, 2014) and CV events (Panoulas et al, 2015).

Importantly, strain is a more sensitive marker of myocardial function in CKD than the LVEF, and predicts incident events independently of EF and measures of diastolic function. In a study including 48 African Americans with ESRD and preserved LVEF, GLS was independently predictive of all-cause mortality during a median follow-up of 1.9 years, whereas LVEF was not. The association between GLS and mortality persisted after adjustment for multiple potential confounders, including LVEF (Pressman et al, 2015). The prognostic value and clinical significance of GLS in patients with CKD above and beyond LV EF is further supported by a study among 183 patients with stage 4-5 CKD (63% on dialysis). During a median follow up of 7.8±4.4, GLS was a significant predictor of all-cause and CV mortality after adjustment for relevant clinical variables including LVMI and EF. GLS also had greater predictive power for both all-cause and CV mortality compared to EF. Furthermore, reduced GLS (less negative than -16%) was associated with a 5.6-fold increased unadjusted risk of CV mortality in patients with preserved EF (Krishnasamy et al, 2015a). In another study that included 89 patients with advanced CKD (43 non-dialysis patients and 43 dialysis patients) followed for 70.2±35 months, reduced GLS was the only significant echocardiographic predictor of survival in non-dialysis CKD patients, whereas among dialysis patients, GLS and the E/e' ratio were significant predictors of survival (Valocikova et al, 2016).

Importantly, reduced longitudinal systolic function is associated with arterial stiffness in patients with CKD. Among 136 patients with moderate CKD (stages 3 and 4), GLS measured with speckle-tracking echocardiography was independently associated with CF-PWV (Krishnasamy et al, 2015b). This is consistent with the known effect of afterload on LV longitudinal function, even in patients with preserved EF (Chirinos, 2017; Chirinos and Segers, 2010a; Chirinos and Segers, 2010b; Weber et al, 2008). Therefore, it is highly plausible that an intervention that reduces aortic stiffness can have a positive impact on LV strain.

Whereas one study suggests that GLS demonstrates little sensitivity to removal of volume during hemodialysis (Panoulas et al, 2015), another study demonstrated a ~1.5% absolute reduction in GLS with hemodialysis (Choi et al, 2008). These data have implications regarding the timing of the dialysis session and volume status of the dialysis patient. Therefore, to the extent possible we will standardize the time of the echocardiogram relative to dialysis session in the trial.

LA size and function

LA size is a well-established marker of CV risk in general and among patients with CKD in particular. Among 200 patients with creatinine clearance <60 mL/min followed-up for 3.7 (3.5-4.0) years, LA size was independently predictive of CV mortality (Chan et al, 2008). Among 289 unselected consecutive patients referred for transthoracic echocardiography, patients with stage 3 or 4 CKD (n=49) demonstrated a higher LA volume index (LAVI) than controls. Furthermore, CKD, hypertension and a larger LAVI were independent predictors of a composite endpoint of cardiac death, myocardial infarction, and congestive cardiac failure (Hee et al, 2014). LA size has also been shown to predict CKD progression (Chen et al, 2013a). Importantly, longitudinal changes in LA size have been shown to predict subsequent fatal and nonfatal CV events in patients on hemodialysis, independently of baseline LA volume and LVM (Tripepi et al, 2007).

LA size is independently associated with PWV (Lantelme et al, 2008), consistent with the effects of aortic stiffness on LV afterload and diastolic function. Given that methods for the assessment of LA strain are relatively novel, data regarding the correlates of reduced LA strain in ESRD are not yet available. However, given the importance of diastolic dysfunction on LA function, it is likely that aortic stiffness can influence LA strain through effects on LV load.

2 SUB-STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of this Sub-Study is to assess the effect of 2 dose levels of SNF472 (300 mg and 600 mg) compared to placebo on the progression of arterial stiffness as measured by an absolute change in PWV over a 52-week period in ESRD patients on HD.

2.2 SECONDARY OBJECTIVES

The secondary objectives of this Sub-Study are to assess:

- Change from Sub-Study Entry in LVMI
- Change from Sub-Study Entry in LV function parameters

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives of this Sub-Study are to assess:

- Change from Sub-Study Entry in LA function and change in parameters over time
- Change from Sub-Study Entry in aortic valve gradient

3 STUDY DESIGN

3.1 STUDY DESIGN OVERVIEW

Patients enrolled in the main study will be eligible to participate in this Sub-Study and will be requested to undergo additional assessments as outlined in the Sub-Study Schedule of Assessments (Table S.5.1).

3.2 STUDY ENDPOINTS

3.2.1 Primary Endpoint

The primary endpoint is the absolute change in aortic PWV (m/s) from Sub-Study Entry to Week 52 by 2-dimensional (2D) echocardiography.

3.2.2 Secondary Endpoints

The secondary endpoints will be assessed by 2D echocardiography and are:

- Absolute change in LVMI from Sub-Study Entry to Week 52
- Absolute change in LV function from Sub-Study Entry to Week 52:
    - Absolute change in the E/e' ratio
    - Absolute change in mitral annular early diastolic tissue velocity (e') measured with tissue Doppler (average of septal and lateral values)
    - Absolute change in LV global longitudinal systolic strain (%) measured by speckle tracking echocardiography

3.2.3 Exploratory Endpoints

The exploratory endpoints are:

- Absolute change in additional measures of LA volume and LA ejection fraction from Sub-Study Entry to Week 52:
- Absolute change in PWV, LVMI and other parameters of LV and LA function over time (Weeks 28 and 52).
- Absolute change in aortic valve gradient from Sub-Study Entry to Week 28 and Week 52 in all subjects and in the subgroup of subjects with evidence of aortic stenosis at Sub-Study Entry

4 SELECTION OF PATIENTS

It is estimated that up to 50% of the sites will participate in order to enroll up to 200 subjects.

4.1 INCLUSION CRITERIA

Patients meeting all of the following criteria will be considered for enrollment in this Sub-Study:

1. Participant in the main study
2. Willing and able to understand and sign the additional Sub-Study informed consent

4.2 EXCLUSION CRITERIA

Patients presenting with any of the following based on medical history will not be included in this Sub-Study:

1. Presence of current atrial fibrillation, atrial flutter with variable atrioventricular conduction, or very frequent premature beats (premature ventricular contractions [PVCs])
2. A diagnosis of primary hypertrophic obstructive cardiomyopathy, or cardiac infiltrative disease (such as cardiac amyloidosis).
3. Severe cardiac valvular disease

5 STUDY PROCEDURES AND SCHEDULE

5.1 SCHEDULE OF ASSESSMENTS

For subjects participating in the Sub-Study, echocardiographic measures will be done at Sub-Study Entry, Week 28, and Week 52 or early termination (ET), whichever comes first. Table S.5.1 shows key visits as well as timing for echocardiographic assessments for the Sub-Study. Refer to the Echocardiography Manual for detailed procedures.

Table S.5.1 Schedule of Sub-Study Assessments and Visits

Procedures	Screening[a]		Double-Blind Treatment Period										Wk 56 or 1 month after last dose
			156 VISITS: Study Drug Infusion Administered at Each HD Session (3x Weekly)										
	Key Evaluation Visits (On Day After Dialysis)												
Week	Step 1[a]	Step 2[a]	Sub-Study Entry Wk 1 – Wk 4[b]	Wk 6	Wk 10	Wk 16	Wk 22	Wk 28	Wk 34	Wk 40	Wk 46	Wk 52/ET[c]	
Day			1 – 28[b]	36 (±3 d)	64 (±3 d)	106 (±3 d)	148 (±3 d)	190 (±2 wks)	232 (±3 d)	274 (±3 d)	316 (±3 d)	358 (±2 wks)	
Sub-Study informed consent[d]		X											
Sub-Study Eligibility, inclusion/exclusion criteria[e]		X											
Sub-Study-Specific Assessments[f]													
Echocardiographic assessments[f]			X[b]					X[g]				X[g]	

d: day; ET: early termination; HD: hemodialysis; Wk: week

[a] Screening for the main study as described in the main study protocol will proceed in 2 steps: Step 1 will involve informed consent followed by the CT scan for coronary artery calcium score and bone mineral density by Dexa for Screening. Only those patients who are eligible in Step 1 can proceed to Step 2.

[b] Sub-Study Entry echocardiographic assessments can be completed from Screening Step 2 up to 4 weeks after initiation (first dose) of study drug.

[c] The ET visit will replace the Week 52 visit if the patient terminates the study earlier than planned.

[d] Sub-Study informed consent can be obtained from Step 2 of Screening and up to Week 4 post first dose. Patients will provide written informed consent before any clinical Sub-Study-specific procedures are performed.

[e] The Sub-Study inclusion/exclusion criteria can be assessed from Step 2 of Screening and up to Week 4.

[f] It is recommended that the echocardiographic assessments be performed on the day after dialysis (see Study Echocardiographic Manual for further details).

[g] For the echocardiographic assessments at Weeks 28 and 52/ET, a window of ±2 weeks is allowed to complete the assessments.

5.2 DESCRIPTION OF STUDY DAYS

5.2.1 Screening (up to Week 4)

Following completion of Step 1 of the Screening activities for the main study:

- Obtain written informed consent for the Sub-Study.
- Determine patient eligibility for the Sub-Study according to the inclusion and exclusion criteria defined in Section 4.

5.2.2 Treatment Period

Day 1 is the first day of study treatment per the main protocol.

5.2.2.1 Sub-Study Entry (up to 4 weeks after first dose)

- Review and confirm patient eligibility for the Sub-Study according to the inclusion and exclusion criteria pre-dose as defined in Section 4
- Perform echocardiographic assessments up to 4 weeks after the first dose

5.2.2.2 Week 28 (±2 weeks)

- Perform echocardiographic assessments (a window of ±2 weeks is allowed to complete the assessments)

5.2.2.3 Week 52/ET (±2 weeks)

- Perform echocardiographic assessments (a window of ±2 weeks is allowed to complete the assessments)

5.3 METHODS OF EVALUATION

5.3.1 Efficacy Assessments

5.3.1.1 Echocardiographic Assessments

Two-dimensional echocardiography will be performed at Sub-Study Entry, Week 28, and Week 52 by dedicated technicians. It is recommended that the echocardiographic assessments be performed on non-dialysis days (see Echocardiography Manual) since this period permits better control of hydration status and is associated with the least intravascular volume.

The echocardiographic assessment for Sub-Study Entry can be performed up to 4 weeks after initiation of study drug. While performing the initial echocardiographic assessments after randomization and up to the first 4 weeks of treatment may not be characterized as a true “baseline” assessment, it is not expected that clinically significant changes in the progression of vascular calcification will occur in treatment arms during this short time period. Therefore, the overall assessment of change in PWV and other echocardiographic measures from the time period of the initial intervention at study entry to end of study should not be affected by this approach. Therefore, to increase subject participation and the feasibility of performing the Sub-Study, a window of time from weeks 1-4 will be allowed to consent subjects for Sub-Study participation and to complete the initial echocardiographic assessments. Refer to

the Echocardiography Manual for detailed procedures and description of assessments of PWV, LVMI, LV function, LA function, and other parameters.

6 STATISTICAL METHODS

6.1 GENERAL STATISTICAL CONSIDERATIONS

6.1.1 Analysis Sets

The Sub-Study analysis sets will be derived from the primary modified ITT (mITT) and PP sets for the main study. The Sub-Study mITT will be made of subjects in the overall mITT set who consented to participate in the Sub-Study. Similarly, the Sub-Study PP set will be made of subjects in the overall PP set who consented to participate in the Sub-Study.

6.2 DEMOGRAPHICS AND BACKGROUND CHARACTERISTICS

The treatment groups in the Sub-Study will be descriptively summarized for baseline (main study) demographic and disease history variables using the Sub-Study mITT set. The baseline characteristics and diagnostic factors of those subjects included in the Sub-Study will be informally compared to the baseline characteristics and diagnostic factors of those subjects not included to ensure there are no major differences of clinical relevance.

6.2.1 Demographics

Demographic and baseline characteristic data for the Sub-Study will be summarized with descriptive statistics across the 3 treatment arms. Demographic variables will include sex, race, ethnicity, age, and diabetes status.

6.2.2 Disease Characteristics

Disease characteristics for the Sub-Study will be summarized with descriptive statistics across the 3 treatment arms. Disease characteristic variables will include diabetes status and baseline CAC score, baseline calcium volume score, years since primary ESRD diagnosis, use of non-calcium containing phosphate binder at baseline, and dialysis vintage.

6.3 EFFICACY ANALYSES

6.3.1 Primary Efficacy Analysis

The primary Sub-Study endpoint, absolute change in PWV (m/s) from Sub-Study Entry to Week 52 measured by 2D echocardiography, will be summarized using descriptive statistics (by treatment arm) and analyzed using an ANCOVA model with the Week 52 value as the dependent variable and randomized treatment group as an explanatory fixed effect and the Sub-Study Entry value as a covariate. The analysis will be stratified by baseline CAC score category and conducted in the Sub-Study mITT and PP populations. The main contrasts of interest to assess treatment effect will be the difference in LS means between each dose and placebo and between the average of the two doses vs placebo. These contrasts and their estimated 2-sided 90% CIs will be back transformed prior to presentation. The 1-sided P-value for each contrast will also be provided.

As recommended by current guidelines (Townsend et al, 2015), additional analyses will be performed after adjustment for mean arterial pressure and heart rate.

6.3.2 Missing Data

The efficacy analyses for the Sub-Study will impute missing Week 52 values with the latest post-Sub-Study Entry value. If no such value is available multiple imputation will be implemented. by imputing missing data within each arm using distribution implied by the non-missing patient data for that arm. As an additional analysis, missing data will be imputed for all arms using distribution implied by the non-missing patient data within the placebo arm.

6.3.3 Multiple Comparisons / Multiplicity

The analyses for the Sub-Study are viewed as exploratory and no adjustments for multiple comparisons/multiplicity are planned.

6.3.4 Secondary and Exploratory Efficacy Analyses

The secondary endpoints of this Sub-Study are provided in Section 3.2.2.

The secondary and exploratory endpoints will be summarized using descriptive statistics by treatment arm and analyzed using methods similar to the primary Sub-Study endpoint; ANCOVA with the value at Sub-Study Entry and treatment as the explanatory variables. The results will be summarized both in tabular form and graphically as forest plots (2-sided 90% confidence intervals for the pairwise treatment comparisons to control). As these analyses are viewed as supportive, no adjustment for multiple testing is planned.

For the endpoint related to aortic valve gradient, evidence of aortic stenosis at Sub-Study Entry will be defined as subjects with a mean aortic valve gradient >20 mm Hg at Sub-Study Entry.

6.3.5 Sensitivity Analyses

Given the potential effect of co-existing valvular disease on LV strain, LV diastolic function parameters and LA strain, we will perform a sensitivity analysis excluding subjects who demonstrate more than moderate left sided regurgitant valvular disease (aortic regurgitation, mitral regurgitation) and/or more than mild mitral or aortic stenosis at baseline. These criteria for sensitivity analyses will not apply for assessment of aortic PWV.

6.4 DETERMINATION OF SAMPLE SIZE

The sample size (up to approximately 65/placebo and 130/active) is considered adequate to provide preliminary estimates of the change from Sub-Study Entry for the treatment arms and the treatment effect (placebo vs. the pooled active arms) of the primary endpoint for the Sub-Study. Assuming a standard deviation in the change from Sub-Study Entry of 1.75 m/s in PWV, the test of the difference between the placebo and active arms (pooled) would have approximately 85% power to detect a true difference of 0.75 m/s (1-sided alpha level of 0.05 for the proof-of-concept objective) for these sample sizes. This threshold is considered clinically significant based on results of previous studies discussed in Section 1.1.2.

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APPENDIX 2. PROTOCOL AMENDMENT 1: SUMMARY OF CHANGES

This section describes the changes in reference to the Protocol Incorporating Amendment 1.

The primary purpose of this amendment is to add the Sub-Study, an opt-in Investigator addition of echocardiographic assessments, to measure reduction in progression of CVC on arterial stiffness for exploratory analyses on subjects participating in the main study and willing to provide additional informed consent. Additionally, this protocol was amended for administrative updates and to clarify procedural details. The justification for the each of the changes to the protocol is listed below.

1. Addition of opt-in Sub-Study echocardiographic assessments at 3 time points: Sub-Study Entry, Week 28, and Week 52/Early Termination. **See addition of Appendix 1 for complete details and endpoints.**

   Justification: The main objective of the Phase 2b study is to demonstrate that SNF472 can slow the progression of CVC. If SNF472 demonstrates a significant reduction in progression of CVC in dialysis patients, it is unknown whether this reduction will translate into improvement of CV outcomes in subsequent studies. The inclusion of additional cardiovascular surrogate endpoints should inform the likelihood that reducing CVC will translate to an improvement in CV outcomes prior to subsequent larger CV outcomes trials. The selection of these surrogate endpoints via echocardiographic assessments is guided by an understanding of the initial role that calcification may play in contributing to CV disease.

2. Increase the Screening Period duration from 21 days +/- 3 days to 28 days +3 days.

   Justification: To allow sites more time to schedule subjects for required screening CT and Dexa scans with radiology facility/personnel for inclusion into the study.

3. Changed number of participating countries from 5 to 3.

   Justification: Updated to reflect actual number of countries participating: USA, UK, Spain

4. Changed “Indication” being studied from ESRD to Cardiovascular Calcification (CVC) in ESRD.

   Justification: More accurately reflects patient population under study.

5. Clarified PK and Biomarker sample collection information. Should be done ~10 minutes before end of infusion and participating sites do NOT need to have a -70 freezer.

   Justification: Clarification to time of collection from previous “end of infusion” was not specific enough. Sites now can be provided dry ice for same day sample shipments, as needed.

6. Updated section 5.3.1 Randomisation to allow the act of randomisation to treatment assignment in the study system and first day of dosing to be on different days, if needed.

   Justification: To accommodate for pharmacy/IMP preparation time needs. For clarity, Day 1 Week 1 is the first dose date.

7. Updated 5.4.3 Packaging supplier name.

   Justification: Former “B&C” supplier was acquired by CSM Europe SA.

8. Deleted B&C Group to ship and store of biomarker samples.

   Justification: Central laboratory being used for the study is providing storage.

9. Section 9.1 Emergency Sponsor Contact updated.

   Justification: Provided more specific safety contact information for EU and USA sites.

APPENDIX 3. PROTOCOL AMENDMENT 1.1 UNITED STATES ONLY

Section 5 specifies that the study drug is to be added to a 100 mL bag of physiological saline (0.9% sodium chloride) and administered via an infusion pump which is connected directly to the dialysis machine via an IV giving set and an accessory heparin line. The infusion should be given at a constant rate over 2.5 hours.

Due to the shortage of 100 mL saline bags, sites that are unable to obtain 100 mL saline bags can use saline bags with a volume of 150 mL or 250 mL. The preparation and administration of the study drug will be the same, however, the infusion rate will be adjusted to allow the infusion to be completed in 2.5 hours (see table below).

Additional fluid should be removed as necessary according to the site's standards of care.

Bag Volume (mL)	**IMP Volume (mL)**	**Total Volume (mL)**	**Infusion Rate (mL/hr)**
100 mL	20 mL	120 mL	48 mL/hr
150 mL	20 mL	170 mL	68 mL/hr
250 mL	20 mL	270 mL	108 mL/hr

APPENDIX 4. PROTOCOL AMENDMENT 1.1 UNITED STATES ONLY: Summary of Changes

The purpose of this US only amendment is to allow sites who are unable to obtain 100 ml bags of physiological saline to use larger bags. The amendment also specifies the flow volume to be used with each bag size. The locations of changes in this amendment are detailed in the table below.

SECTION	AMENDMENT 1	AMENDMENT 1.1
APPENDIX 2. Protocol Amendment 1: Summary of Changes	none	Protocol Amendment 1 Summary of Changes
APPENDIX 3. Protocol Amendment 1.1 United States Only	none	Protocol Amendment 1.1 US Only Allows use of bags larger than 100 mL and specifies adjusted flow rate

APPENDIX 5. PROTOCOL AMENDMENT 2 SUMMARY OF CHANGES

The main goals of this amendment are to increase the upper limit of the CAC score allowed for enrollment from 2000 AU to 3500 AU, to clarify the planned sample size re-estimation, to clarify the endpoint descriptions, and provide additional details on planned statistical analyses, including those in the substudy. Additional description and rationale for the changes is provided below.

1) Minor revision to primary objective and primary endpoint to remove “absolute change”.

Rationale: Results will be analyzed on a log scale to give results that can be presented as absolute change or percent change.

Sections: Synopsis, Section 2.1, Section 10.3

2) The secondary endpoint “percent change from baseline in calcium volume score at Week 52” was deleted.

Rationale: This information will be provided as part of the primary endpoint.

Sections: Synopsis, Section 10.3.4.

3) The secondary endpoints “absolute change from baseline in CAC/Agatston score at Week 52” and “percent change from baseline in CAC/Agatston score at Week 52” were consolidated to “change from baseline in CAC/Agatston score at Week 52”.

Rationale: Results will be analyzed on a log scale to give results that can be presented as absolute change or percent change.

Sections: Synopsis, Section 10.3.4.

4) Description of the composite safety endpoint “death from cardiovascular causes, MI, stroke, or heart failure” was standardized throughout the protocol to clarify and specify that it included deaths only from cardiovascular causes and not all-cause mortality.

Sections: Synopsis, Sections 3.2.2, 6.2.1.3, 10.3.4

5) It is now specified that C-reactive protein (CRP) is one of the biomarkers that will be assessed.

Sections: Synopsis, Section 6.2.3

6) The safety endpoint was revised to add “clinically relevant changes from baseline in laboratory and ECG parameters.”

Sections: Synopsis, Section 10.3.4.

7) A blood pressure exploratory objective and endpoint were added.

Sections: Synopsis, Sections 2.3, 3.2.3, and 10.3.4

8) Inclusion Criterion 2: The maximum allowable CAC score has been increased from 2000 AU to 3500 AU: "CAC score of 100 to ~~2000~~ 3500 AU".

Rationale: After additional literature review and consultation with medical experts the Sponsor believes that subjects with higher CAC scores are at risk of cardiovascular events and may benefit from therapeutic intervention. Raising the CAC limit to 3500 AU will allow recruitment of a more representative sample of ESRD patients.

Sections: Synopsis, Sections 4.1 and 6.1.1.1

9) Revision of the primary endpoint analysis method to, "The dependent variable will be the difference between log 52-week and log baseline calcium volume scores, with log baseline calcium volume score and treatment group as explanatory variables. This analysis will be stratified for the randomization stratification factor, i.e. baseline CAC/Agatston score."

Rationale: Clarification of methods and stratification factor

Section: Synopsis and Section 10.3.1

10) Figure 5.1 was revised to more accurately show the location of study drug infusion.

11) Correction of the definition of concomitant medications in Section 6.2.6 to "Medications taken with or after the first dose of IMP will be considered concomitant medication."

12) Conditions for removal of patients from the study (Section 8.1) has been revised so that patients will not be withdrawn from the study for missed doses. The following sentence was deleted, "Patients who missed 12 consecutive doses (4 weeks) of the IMP or 24 aggregate doses will be considered as non-compliant and will be withdrawn from the study." In addition, Investigators are asked to contact the Medical Monitor prior to discontinuing a patient from study medication or withdrawing a subject from the study.

13) Statistical Methods: Clarified that the primary efficacy analysis population is a *modified* ITT (Section 10.1.1 and Appendix 1 Section 6.1.1). Methods for imputation of missing data were added (Section 10.3.2). Details and clarifications on blinded sample size re-estimation procedure were provided and a nonbinding futility analysis was added (Section 10.6). Additional methods and clarifications were provided throughout Section 10.

14) The objective "Change from Sub-Study Entry in aortic valve gradient" and corresponding endpoint of "Absolute change in aortic valve gradient from Sub-Study Entry to Week 28 and Week 52 in all subjects and in subjects with evidence of aortic stenosis at Sub-Study Entry" were added.

Sections: Appendix 1, Substudy Sections 2.3, 3.2.3, 6.3.4

15) Clarification was provided that the first echocardiogram can occur within 4 weeks of the first dose of study drug (Section 5.2.2.1).

16) Investigational Drugs. Notation was added that US SITES ONLY may use 150 mL or 250 mL bags of physiological saline for dilution of the IMP if 100 mL bags are not available. The preferred bag size for dilution of the IMP remains 100 mL. This allowance was previously communicated to US Sites as Protocol Amendment 1.1 as described in Appendices 3 and 4.

17) Additional minor administrative changes and changes to version numbering and dates were made throughout.

APPENDIX 6. Protocol Amendment 3 Summary of Changes

The amendment provides a revised sample size calculation which led to a reduction in planned enrollment from approximately 450 to approximately 270. There are no changes in study visits or procedures. Additional details are provided below.

1) Changed planned sample size from approximately 450 to approximately 270.

Sections: Synopsis, Sections 3.1, 4, 10.6

Rationale: To expose the minimum number of subjects needed to achieve 80% power based on the revised sample size calculation.

2) Removed details of planned sample size re-estimation (SSRE, Section 10.6).

Rationale: Because of the revised sample size, the previous considerations regarding SSRE are no longer germane.

3) Revised sample size calculation (Section 10.6).

Rationale: The previous versions of the protocol based sample size on the anticipated Per Protocol Population, however, the primary efficacy analysis will be carried out on the modified ITT Population (Section 10.1.1) with last observation carried forward (LOCF) imputation for missing data (Section 10.3.2). To establish consistency the sample size calculation has been revised to more appropriately use the modified ITT Population. In addition, the Sponsor has determined that 80% power with 2-sided significance level of 0.05 is acceptable for a Phase 2 study, in contrast to the previously planned 85% power.

4) Clarified the definition of the Per Protocol population (Section 10.1.1). The PP population is the subset of the mITT population who do not have any major protocol violations or deviations.

5) New Appendix 5 containing the summary of changes for Amendment 2

6) Revisions of amendment number and date throughout.

APPENDIX 7. Protocol Amendment 4 Summary of Changes

1) Added phosphorus (phosphate) to the list of analytes in the safety laboratory assessments (Table 6.1) and the list of biomarkers Section 6.2.3.

Rationale: Corrects omission of a standard analyte and clarifies list of biomarkers.

2) Clarification that IMP must be added to the dialysis circuit before the dialyzer (Section 5.5).

Rationale: Clarification

3) Addition of a non-binding interim futility analysis to be conducted when approximately N=120 subjects (63% of N=190) have provided Week 52 data on the primary endpoint. PK and PD analyses will also be conducted at the time of the interim analysis (Synopsis, Section 10.7, Section 14).

Rationale: To allow the Sponsor to assess whether there is a low probability of success of the trial and to determine that the PK and PD characteristics of SNF472 are as expected.

4) New Appendix 6 containing the summary of changes for Amendment 3

5) Revisions of amendment number and date throughout.