



STATISTICAL ANALYSIS PLAN (Version 1.08)

Protocol Title: A randomised, double-blind, placebo-controlled trial to assess the effect of phosphate reduction with lanthanum carbonate on arterial compliance and vascular calcification in patients with chronic kidney disease stages 3b-4 (Version 3.0)

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APPROVALS

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PREFACE

This Statistical Analysis Plan (SAP) describes the planned analyses and reporting for The Australasian Kidney Trials Network (AKTN) protocol 10.01, A randomised, double-blind, placebo-controlled trial to assess the effect of phosphate reduction with lanthanum carbonate on arterial compliance and vascular calcification in patients with chronic kidney disease stages 3b-4: The IMPROVE-CKD study.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Guidance on Statistical Principles in Clinical Trials (1). All work planned and reported for this SAP will follow national and international guidelines for statistical practice (2, 3)

The planned analyses identified in this SAP will be included in future manuscripts. Exploratory analyses not necessarily identified in this SAP may be performed to support planned analyses. Any post-hoc or unplanned analyses not specified in this SAP will be clearly identified as such in the Final Statistical Report (FSR) and manuscripts for publication.

This SAP was written and reviewed by a statistician and clinical investigators from the IMPROVE-CKD Trial Steering Committee (TSC). All contributors were blinded to treatment allocations and treatment-related study results and will remain so until the central database is locked and the final data are extracted for analysis. To ensure and maintain blinding, treatment allocations and statistical code for generating them are stored electronically in a separate location accessible only by the designated un-blinded AKTN statistician.

The following documents were reviewed when preparing this SAP:

- Clinical Research Protocol for AKTN Trial Number 10.01 (4).
- Electronic case report forms (CRFs) for AKTN Trial Number 10.01.
- Data Safety Monitoring Board (DSMB) Terms of Reference for AKTN Trial Number 10.01 (5).
- ICH Harmonised Tripartite Guideline on Statistical Principles for Clinical Trials (1).
- ICH Harmonised Tripartite Guideline on Estimands and Sensitivity Analysis in Clinical Trials (6).
- ICH Harmonised Tripartite Guideline on Structure and Content of Clinical Study Reports (7).

Readers of this SAP are encouraged to read the Clinical Research Protocol for details on the conduct of this study and the operational aspects of clinical assessments and timing for completing a patient in this study.

ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
ADR	Adverse Drug Reaction
ANZDATA	Australian and New Zealand Dialysis and Transplant Registry
AKTN	Australasian Kidney Trials Network
BMD	Bone Mineral Density
BMI	Body Mass Index
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease-Epidemiology Collaboration
CT	Computed Tomography
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ESKD	End-Stage Kidney Disease
FSR	Final Statistical Report
ICH	International Committee on Harmonisation
ITT	Intention-To-Treat
LVM	Left Ventricular Mass
MACE	Major Adverse Cardiovascular Events
MAR	Missing at Random
MNAR	Missing Not at Random
MRI	Magnetic Resonance Imaging
PCR	Protein to Creatinine Ratio
PWV	Pulse Wave Velocity
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TSC	Trial Steering Committee
ULN	Upper Limit of Normal
WHO	World Health Organisation

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1. INTRODUCTION

1.1 Background

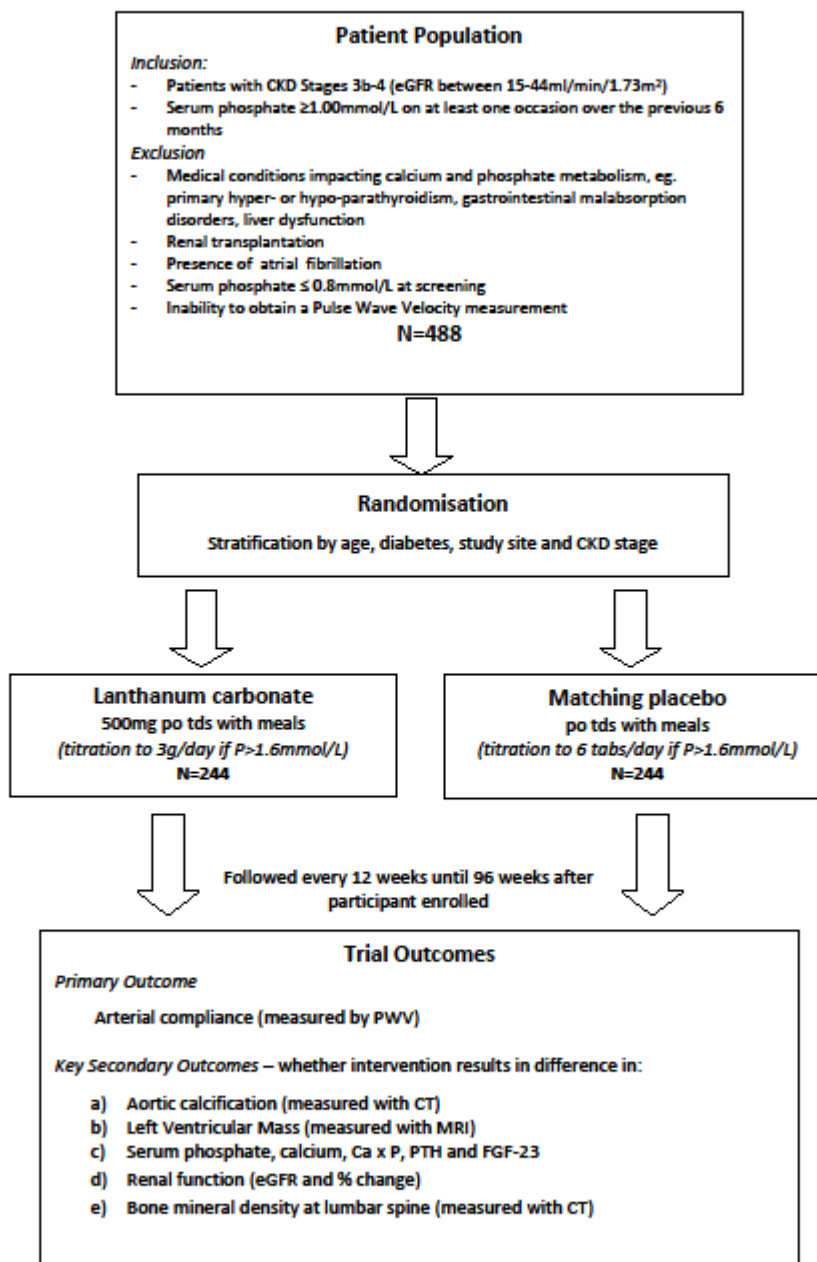
Chronic kidney disease (CKD) is recognised globally as a major public health problem. CKD is defined as abnormalities of kidney structure and/or function, present for at least 3 months, and the prevalence of CKD is about 10% worldwide (8). Cardiovascular disease is the leading cause of morbidity and mortality in patients with CKD (9, 10) and, compared to the general population, patients with CKD have a 3- to 30-fold increase in mortality, depending on the age-group. Cardiovascular disease accounts for over half of all deaths among CKD stage 5 patients on dialysis, with myocardial infarction, ischaemic cardiomyopathy, stroke and peripheral vascular disease making up the majority of deaths. Much of this problem may relate to non-traditional cardiovascular risk factors such as vascular calcification and arterial stiffness, precipitated by abnormal mineral metabolism (mainly calcium and phosphate) in CKD (11, 12). CKD itself is a strong independent risk factor for cardiovascular disease, and patients with CKD stages 3b and 4 are much more likely to die from cardiovascular mortality than to require dialysis or transplantation (by up to a 20-fold increased risk) (13, 14).

Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD) is a clinical entity which encompasses the intimately related abnormalities of mineral homeostasis, bone turnover and mineralisation, and vascular and soft tissue calcification, which are almost universal in patients with advanced CKD. Vascular calcification is the pathological distribution of mineral in the vascular system as a result of imbalances in mineral metabolism and is associated with enhanced bone resorption. A mediated complex of factors is also involved in the pathogenesis of vascular calcification, including local and systemic calcium-regulatory proteins and a number of inhibitory extra-cellular factors such as fetuin-A and matrix Gla protein, but the normal balance becomes dysregulated in CKD towards the development of vascular calcification (15).

Arterial stiffness represents the functional disturbance of vascular calcification, with reduced compliance of large conductance arteries, and predominantly results from greater medial calcification (15). Arterial stiffness is an independent predictor of all-cause and cardiovascular mortality and comprises non-occlusive arterial remodelling, different from the atherosclerotic plaques with intimal damage. As with increased vascular calcification, patients with CKD have greater arterial stiffness resulting in the principal consequences of left ventricular hypertrophy and altered coronary perfusion. Vascular calcification and arterial stiffness can be measured and monitored through non-invasive techniques involving computed tomography (CT), ultrasound, echocardiography, and pulse wave velocity (PWV).

Evidence in the general population and those with CKD support a strong independent association between higher phosphate levels (even at the upper level of the normal range) and cardiovascular disease and mortality (16-25). There is also evidence demonstrating that higher phosphate levels are associated with increased progression of CKD (26). Whilst observational studies show associations however, causality has never been demonstrated. Serum phosphate provides a poor measure of overall phosphate balance or the risk of soft tissue calcification in patients with CKD, and benefits of phosphate lowering are yet to be demonstrated. Lanthanum carbonate is an effective phosphate binder with a low side-effect profile. The use of lanthanum carbonate to lower serum phosphate in patients with CKD stages 3b and 4 may reduce the burden of cardiovascular disease already associated with this population and with minimal adverse effects.

Figure 1. Study schema



1.2 Study synopsis

The IMPROVE-CKD (**IM** pact of **P**hosphate **R**eduction **O**n **V**ascular **E**nd-points in **C**hronic **K**idney **D**isease) trial is a phase III multi-centre, prospective, double-blinded, randomised, placebo-controlled clinical trial designed to assess the effect of lanthanum carbonate, a non-calcium-based phosphate binder, on surrogate markers of cardiovascular disease in patients with CKD stages 3b and 4 (4). The primary outcome is arterial compliance measured as PWV. Figure 1 displays the study schema.

Eligible patients were randomly assigned to receive either lanthanum carbonate (500mg po tds with meals; titration to 3g/day if serum phosphate >1.6 mmol/L) or placebo (po tds with meals; titration to 6 tablets/day if serum phosphate >1.6 mmol/L). The planned recruitment target was 488 patients (includes adjustment for anticipated rates of treatment drop-out and non-compliance; see below for assumptions and estimation). The trial randomised its first participant on 15 March 2012 and the last participant was randomised on 20 January 2017. The trial stopped recruiting at the end of January 2017 before reaching the recruitment target. A total of 278 participants from 17 clinical centres in Australia, New Zealand and Malaysia were randomised, which is 57% of the planned recruitment target. The last observation on the last participant was entered into the central REDCapTM database on 24 January 2019 and the database is expected to be ready for analysis in the second quarter of 2019.

1.3 Sub-studies

There are two sub-studies associated with the IMPROVE-CKD trial: the cardiac magnetic resonance imaging (MRI) sub-study and the dietary sub-study. The cardiac MRI sub-study will assess change in several measures of heart structure and function and associations between these and other measures of cardiovascular and kidney disease. While one measure, change in left ventricular mass (LVM) from baseline to 96 weeks after randomisation, is a secondary outcome, all MRI measures will be reported as part of the sub-study. The dietary sub-study will collect dietary phosphate information from a questionnaire and interviews and examine associations with other trial variables. Analyses for these sub-studies will be documented separately.

2. STUDY DESIGN ISSUES

2.1 Overview

The study is a prospective, multi-centre, double-blind, randomised, placebo-controlled phase-III trial.

2.2 Study population

The population of interest is non-dialysis CKD patients attending renal units (outpatients and dialysis) throughout Australia and New Zealand and in selected centres in Malaysia. Patients who met all inclusion and no exclusion criteria were considered eligible to participate in the trial after undergoing a two-week washout period immediately prior to enrolment.

2.2.1 Inclusion criteria

Patients were eligible for inclusion in the study if all the following criteria were met:

1. CKD stages 3b and 4 (estimated glomerular filtration rate [eGFR] from 15 to 44 ml/min/1.73m² where eGFR was determined using the CKD-Epidemiology Collaboration (CKD-EPI) equation) (27)
2. Serum phosphate \geq 1.00 mmol/L on at least one occasion in the previous 6 months
3. 18 years of age or over
4. Able to give informed consent

2.2.2 Exclusion criteria

Patients were excluded from the study if any one of the following conditions was met:

1. History of psychological illness or condition which interferes with their ability to understand or comply with the requirements of the study
2. Renal transplantation
3. Recent (within 1 month) hospitalisation or cardiovascular event
4. Pregnancy or breast feeding
5. Medical conditions that impacts on phosphate metabolism (apart from CKD), e.g., primary hyperparathyroidism or hypoparathyroidism; previous subtotal parathyroidectomy; gastrointestinal malabsorption disorders such as Crohn's disease, ulcerative colitis, coeliac disease or severe liver dysfunction
6. Malnutrition, defined as serum albumin $<$ 30 g/L
7. Atrial fibrillation as documented on electrocardiogram (ECG) performed at screening
8. Inability to obtain a measure of PWV

2.2.3 Protocol amendments to eligibility criteria

Several modifications were made to eligibility criteria after the trial commenced. Details of the amendments and reasons for them are given in Lioufas et al. (28). Below is a summary:

1. Inclusion criterion removed: An initial inclusion criterion was urine albumin/creatinine ratio (ACR) $>$ 10 mg/mmol or protein/creatinine ratio (PCR) $>$ 15mg/mmol. This was removed from version 2.0 of the protocol.
2. Inclusion criterion amended: The original inclusion criterion for serum phosphate was a value $>$ 1.2 mmol/L (3.72 mg/dL) at screening. This was initially changed to $>$ 1.2 mmol/L on at least one occasion over the previous 6 months (protocol version 2.0), and subsequently to $>$ 1.0 mmol/L (3.10 mg/dL) on at least one occasion over the previous 6 months (protocol version 3.0).
3. Exclusion criterion added: Inability to obtain a measure of PWV was added to the list of exclusion criteria for version 3.0 of the protocol.

2.3 Study design and treatment allocation

Participants were randomised to receive either lanthanum carbonate (Fosrenol®, Shire Pharmaceuticals) 500 mg three times daily with meals or matching placebo three times daily with meals for 96 weeks. Study medication was up-titrated to a total dose of six tablets daily (3000 mg/day lanthanum carbonate) if serum phosphate remained persistently greater than

1.60 mmol/L (i.e., for > 3 months and decided by treating clinicians). Allocation to an intervention arm used minimisation, a covariate adaptive algorithm with age (<60, ≥60), presence of diabetes (no, yes), study site (17 sites) and CKD stage (3b, 4) as minimisation variables. The minimisation algorithm was implemented via web-based access to a central electronic randomisation system provided by The George Institute in Sydney, Australia.

2.4. Sample size

The study was designed to detect a clinically meaningful difference of 1 m/s in PWV between the lanthanum carbonate and placebo groups at the 96-weeks study follow-up visit. Assuming a within group standard deviation of 2.9 m/s, a sample size of 356 patients would detect a 1 m/s difference in PWV at the 5% significance level with 90% statistical power. To account for an estimated 10% study withdrawal rate and 10% non-adherence rate, recruitment of 488 participants is anticipated to be required. No provision was made for drop-ins given there is no provision for lanthanum carbonate to be accessed in the pre-dialysis CKD population in Australia, New Zealand and Malaysia.

2.5 Treatment blinding and allocation concealment

Investigators and participants were blinded to treatment assignment. Biochemistry staff in local laboratories who performed outcome assessments were blinded to the patient's assigned treatment. To ensure concealment of treatment allocation, randomisation was performed using a central web-based randomisation system provided by The George Institute in Sydney, Australia.

2.6 Schedule of assessments

2.6.1 Clinical Assessments

Table 1. Clinical assessments by study visit

			Weeks post-baseline (Study visit window is ±14 days of due date from baseline)							
			Week 12	week 24	week 36	week 48	week 60	week 72	week 84	week 96
			Visit 1 Month 3	Visit 2 Month 6	Visit 3 Month 9	Visit 4 Month 12	Visit 5 Month 15	Visit 6 Month 18	Visit 7 Month 21	Visit 8 Month 24
Visits	Screening/ Randomisation	Baseline								
Visit Assessments										
Informed Consent	X									
Participant randomised	X									
Electrocardiogram (ECG)	X									
Demographics and Medical History		X								
Physical Examination		X		X		X		X		X
Concomitant Medications		X	X	X	X	X	X	X	X	X
CT scan – multi-slice, non-contrast *		X								X
Pulse Wave Velocity and Augmentation Index**	X			X		X		X		X
Cardiac MRI * (for participating sites)		X								X
Medication dispensed		X	X	X	X	X	X	X	X	
Medication returned			X	X	X	X	X	X	X	X
Adverse Events recorded (SAEs and ADRs)			X	X	X	X	X	X	X	X
Dietitian assisted food record^ (for participating sites)		X				X				X

* if participants withdraw from the study after 12 months and prior to 24 months they will be requested to do a 'close-out' CT and MRI scan. Some sites will also perform additional MRI scans to investigate diastolic dysfunction

** Data from screening to be used for baseline measure.

^A for participants enrolled in the dietary component of the study

2.6.2 Laboratory assessments

Table 2. Laboratory assessments by study visit

			Weeks post-baseline (Laboratory test window is ± 14 days from visit date)							
			Week 12	week 24	week 36	week 48	week 60	week 72	week 84	week 96
Visits	Screening/ Randomisation	Baseline	Visit 1 Month 3	Visit 2 Month 6	Visit 3 Month 9	Visit 4 Month 12	Visit 5 Month 15	Visit 6 Month 18	Visit 7 Month 21	Visit 8 Month 24
Locally Tested Laboratory Assessments										
Full Blood Count		X	X	X	X	X	X	X	X	X
Biochemistry, Renal and Liver Function Tests		X	X	X	X	X	X	X	X	X
C-reactive Protein (CRP)		X	X	X	X	X	X	X	X	X
Fasting Serum Lipid Profile		X		X		X		X		X
Fasting Glucose		X		X		X		X		X
HbA1c *		X		X		X		X		X
Intact Parathyroid Hormone (iPTH)		X		X		X		X		X
Other Liver Function Tests		X				X				X
Iron studies		X				X				X
Vitamin D (25-D)		X				X				X
Spot urine (ACR, PCR and Phosphate:creatinine ratio)		X				X				X
24 Hour Urine collection – timed excretion		X				X				X
Centrally Tested Laboratory Assessments										
Fibroblast growth factor-23 (FGF-23)		X		X		X		X		X
Asymmetrical dimethyl arginine (ADMA)		X				X				X
Calcification inhibitors (OPG, MGP, fetuin-A)		X		X		X		X		X
Vitamin D binding protein		X				X				X
Spot Urine		X				X				X

* Only performed in patients with diabetes

3. STUDY OUTCOME VARIABLES

3.1 Primary efficacy outcome

The primary outcome is large arterial compliance at 96 weeks after randomisation, where large arterial compliance is measured as the mean of two carotid-femoral PWVs obtained at the same study visit. Carotid-femoral PWV measures the time interval between pulse waves at the carotid and femoral arteries, with larger values representing stiffer vessels. Measures of carotid-femoral PWV for a CKD population are expected to fall within the range of 3 to 45 metres/second (29). PWV is being measured at baseline and 6-monthly to 96 weeks post randomisation using SphygmoCor devices (AtCor, PWC Inc., Westmead, Sydney, Australia).

The SphygmoCor devices store the carotid and femoral waveforms from which PWV measures are derived (30). These waveforms are being evaluated independently by two cardiologists blinded to treatment allocations and all other characteristics associated with the PWV measures, including the actual PWV value. Based on the waveforms, PWV measures are being rated as “usable” or “not usable”. Where the two cardiologists disagree on the usability of a PWV measure, a consensus decision will be reached. Only those PWV measures with an agreed rating of “usable” will be included in the main analysis of PWV.

3.2 Secondary outcomes

Data are being collected on a range of secondary outcome variables. While substantially the same, the list of secondary outcomes has been refined since writing the original protocol and comprises the following outcomes:

1. Abdominal aortic calcification, measured by CT scan at baseline and the 96-week study visit. Agatston scores measuring the extent of aortic calcification will be calculated from the CT images (L2 to L4).
2. Left ventricular mass (LVM), measured by cardiac magnetic resonance imaging (MRI) at baseline and the 96-week study visit. LVM will be indexed to body surface area measured in metres squared.
3. Serum phosphate (mmol/L), measured at baseline and 3-monthly to 96 weeks.
4. Serum calcium (mmol/L), measured at baseline and 3-monthly to 96 weeks.
5. Serum calcium-phosphate product (mmol^2/L^2), measured as per the components described above.
6. Parathyroid hormone (PTH) levels (pmol/L), measured at baseline and 6-monthly to 96 weeks.
7. Development of secondary hyperparathyroidism (SHPT), defined as PTH >65 pg/mL which is measured at baseline and 6-monthly to 96 weeks.
8. Fibroblast growth factor 23 (FGF-23) levels (pg/ml)
 - a. Intact FGF-23, measured at baseline, weeks 24, 48 and 96
 - b. C-terminal FGF-23, measured at baseline, weeks 48 and 96.
 - c. Intact/C-terminal FGF-23 ratio, measured at baseline, weeks 48 and 96
9. Urinary phosphate excretion (mmol/L), measured at baseline, 48 and 96 weeks.
10. Bone mineral density (BMD) at the lumbar spine, measured by CT scan at baseline and 96 weeks and recorded in Hounsfield Units.

11. Phosphate binder use additional to study medication (especially calcium-based binders).
12. Rate of renal progression, defined as
 - a. creatinine clearance (ml/min), measured at baseline, 48 and 96 weeks.
 - b. estimated glomerular filtration rate (eGFR, ml/min/1.73m²), calculated using the CKD-EPI formula and recorded at baseline and 3-monthly to 96 weeks.
 - c. a binary composite outcome: a 30% drop in eGFR from baseline or requirement for dialysis or renal transplantation.
13. Proteinuria, defined as urine protein to creatinine ratio (PCR) >30 mg/mmol, both variables measured at baseline, 48 and 96 weeks.

3.3 Exploratory outcomes

Central analysis of blood samples collected at baseline and 6-monthly to 96 weeks will generate the following exploratory outcome variables:

1. Soluble α -Klotho levels
2. Calciprotein particles (CPPs)
3. Calcification inhibitors:
 - a. Fetuin-A protein
 - b. Matrix Gla protein (MGP)
 - c. Osteoprotegerin (OPG)

Additional exploratory outcomes will be formed by dichotomising four continuous secondary outcomes as less than or equal to the upper limit of normal (ULN) versus above this limit:

1. Serum phosphate (ULN=1.5 mmol/L), measured at baseline and 3-monthly to 96 weeks.
2. Serum calcium (ULN=2.6 mmol/L), measured at baseline and 3-monthly to 96 weeks.
3. Serum calcium-phosphate product (ULN=3.9 mmol²/L²), measured as per the components described above.
4. PTH levels (ULN=6.9 pmol/L), measured at baseline and 6-monthly to 96 weeks.

3.4 Safety outcomes

The following safety outcome variables have been recorded:

1. Major adverse cardiovascular events (MACE): non-fatal stroke, non-fatal myocardial infarction, heart failure hospital admissions, ischaemic cardiovascular events, peripheral vascular disease admissions and cardiovascular death)
2. Any treatment-emergent adverse events (i.e., events either not present at baseline or present at baseline but of increased severity)
3. Any serious adverse event (SAE)
 - a. Death due to any cause
 - b. Any life-threatening event
 - c. Any initial or prolonged inpatient hospitalisation
 - d. Any persistent or significant disability/incapacity
 - e. Any important medical event
 - f. Any congenital abnormality/birth defect

4. SAE organ systems (cardiovascular, respiratory, gastrointestinal, renal, neurological, musculoskeletal, endocrine, cancer/neoplasm, haematology, skin)
5. SAE relationship to study medication (none, unlikely, possible, probable)
6. Any adverse drug reaction (ADR)
 - a. Any bleeding event
 - b. Any gastrointestinal event
 - c. Other event
7. ADR severity (mild, moderate, severe, life-threatening)
8. ADR relationship to study medication (possible, probable)

4. SEQUENCE OF PLANNED ANALYSES

4.1 Interim analyses

An independent Data and Safety Monitoring Board (DSMB) comprising experts in clinical trials, biostatistics, and nephrology was in charge of reviewing un-blinded data on patient characteristics, treatment compliance, data quality on the primary and key secondary outcomes, and safety outcomes. The DSMB held an orientation meeting (11 September 2013) and five data and safety review meetings (19 June 2014 to 20 February 2018) at which un-blinded trial results were assessed. The DSMB recommended continuation of the trial without modification on each occasion. Only DSMB members and the statistician compiling closed-session reports for DSMB meetings had access to un-blinded interim data and results.

4.2 Blinded reviews of primary outcome

An unplanned blinded review of the primary outcome data was conducted in March 2014 to check that the assumptions underlying the original sample size calculations remained plausible. This review was performed at the request of Shire Pharmaceuticals, supplier of the active study drug. Baseline PWV measurements were more variable than assumed in the sample size calculations performed at trial planning and the review suggested the need to recruit more participants to achieve 90% power to detect a between-group difference in PWV of 1 m/s but a similar number (520) to achieve 80% power.

Additional reviews of sample size assumptions and PWV data quality were conducted at irregular intervals by a statistician and clinicians blinded to treatment allocation. Results from these reviews were presented to the TSC. In addition to reviewing assumptions on which the sample size calculations were based, these reviews checked the validity of individual PWV measurements (within plausible range of values for a CKD population and meeting SphygmoCor quality criteria) and pairs of PWV measurements (within 1 m/s of each other).

4.3 Final analyses and reporting

Planned analyses identified in the trial protocol and this SAP will be performed only after the last patient has completed the 96-week follow-up assessment visit, the REDCapTM database has been cleaned and locked, and results from the central laboratory and CT scans, which will be received in password-protected Excel spreadsheets, have been cleaned and declared final. Blinded data review meetings will be held before locking the REDCapTM database and, again, before declaring final electronic copies of the data in Excel spreadsheets. There will be no un-

blinded review and analyses will not commence until this SAP has been approved by the Lead Principal Investigators and Trial Statistician and reviewed and approved by the TSC. Key statistics and trial results from the final analyses will be presented to the TSC for discussion prior to completion of the FSR and subsequent manuscripts. Any post-hoc exploratory analyses performed to provide support for planned analyses but not identified in this SAP will be documented and reported in appendices to the FSR and clearly identified as unplanned analyses. All analyses and their interpretation will be conducted independently of the trial funders: the National Health and Medical Research Council of Australia; and the manufacturer of lanthanum carbonate (FosrenolTM), Shire Pharmaceuticals Pty Ltd.

5. CHANGES TO STATISTICAL INFORMATION IN THE PROTOCOL

The following amendments have been made to statistical information given in the study protocol and publication of the study protocol (4, 28):

1. Due to a substantial amount of missing data on the primary outcome, the proposed analysis has been changed from analysis of covariance (ANCOVA) to mixed effects model for repeated measures (MMRM). The former could still be performed with multiple imputation but it was decided to use a mixed model approach and implicitly rather than explicitly impute missing values.
2. The lists of secondary and exploratory outcomes have been amended to include more and newer biomarkers and dichotomisations using ULN of routinely measured biomarkers that are recognised risk factors for cardiovascular disease and mortality.
3. Subgroups were not described in the protocol but three subgroup variables are described in the SAP based on minimisation variables in the treatment allocation algorithm: CKD stage (stage 3b vs. stage 4), age group (age < 65 vs age ≥ 65), and diabetes mellitus (no, yes).

6. ANALYSIS PRINCIPLES

6.1 Intention-to-treat principle and analysis dataset

Tests of the effects of treatment on the primary outcome and secondary outcomes will be conducted as close as possible to the intention-to-treat (ITT) ideal. For the primary outcome, all randomised patients who provided at least one usable (as determined by expert review of waveforms) post-randomisation PWV measure will be included in the “full analysis set” and analysed in the group to which they were randomly allocated regardless of whether they received the assigned treatment and irrespective of any protocol deviations or violations. Missing values will be assumed to be missing at random (MAR). Analyses of other efficacy outcome variables will be based on the same criteria and assumptions. All study discontinuations will be captured in a figure illustrating participant progression through the trial.

6.2 Multicentre study and heterogeneity

Seventeen centres recruited between five and 51 participants. Although included as a variable in the randomisation scheme, study centre will not be adjusted for in the main analyses of primary and key secondary efficacy outcomes because some centres were small (≤ 5

participants). However, if there is a positive treatment effect on the primary outcome, study centres will be combined within four Australian states, within Malaysia, and within New Zealand (six regional strata in total) to assess the homogeneity of the treatment effect across regions. Potential heterogeneity will be tested by including a treatment-by-region interaction term in a multivariable model with treatment and region as main effects.

6.3 Multiple comparisons and multiplicity

There will be no adjustments for multiplicity as there is a pre-defined hierarchy of importance of outcome variables and study objectives and the influence of individual results on the overall interpretation of the trial will reflect their level within this hierarchy. Further, there are only three subgroup variables, all of which are pre-specified. All statistical tests of significance will be two-sided.

6.4 Covariate adjustment of main analyses

The main statistical analyses of primary and secondary efficacy outcomes comparing lanthanum carbonate with placebo will be adjusted for baseline measures of the outcome where one is available (continuous outcomes) but otherwise unadjusted. Supporting analyses will be adjusted for regional location of the clinical site (6 regions) and baseline variables used in the minimisation algorithm: CKD stage (stage 3b, stage 4), age group (age < 65, age ≥ 65), and diabetes mellitus (no, yes).

6.5 Missing data

6.5.1 Missing outcome data

For repeatedly measured outcomes, defined as outcomes with at least two post-baseline measurements, missing outcome data will be addressed using likelihood-based statistical models that allow inclusion of all randomised participants in analyses of the primary and secondary outcomes who have at least one post-randomisation outcome measure. For outcomes measured on one occasion after baseline and where more than 5% of outcome data are missing, multiple imputation assuming MAR will be used to impute missing observations.

6.5.2 Missing baseline covariate data

Missing values on baseline variables used as covariates in covariate-adjusted analyses of the treatment effect on the primary outcome will be replaced using mean imputation. While mean imputation can bias statistical estimates in observational studies, this is not the case in randomised trials where randomisation ensures baseline variables are independent of treatment group (31, 32). Mean values will be calculated from the non-missing values for the baseline variable using pooled data from both treatment groups. For binary (coded 0 or 1) variables, the imputed mean will be rounded up to 1 or down to 0, whichever is nearest. For computed variables such as Body Mass Index (BMI), mean imputation will be performed at the level of the component variables of height and weight. Imputation will be performed using the median rather than the mean for skewed continuous variables that are not to be transformed. The number (percentage) of missing values will be reported for all baseline

covariates with missing data. Covariate adjusted statistical models will include a missing value indicator (0=observed, 1=missing) for each covariate with missing data (31, 32)

7. STATISTICAL METHODS

7.1 Analysis of the primary outcome

The primary objective of the IMPROVE-CKD trial is to estimate and test treatment differences in PWV at 96 weeks after commencement of treatment with lanthanum carbonate or placebo. A likelihood-based mixed effects model with repeated measurements (MMRM) will be used as the method for the primary analysis of PWV (33).

7.1.1 Primary comparison

Measurements of PWV over time will be analysed with an MMRM analysis using a full multivariate approach with unstructured modelling of time (4 occasions), the treatment-by-time interaction, and within-patient errors. The specific model will have fixed effects for treatment, categorical time, the treatment-by-time interaction, baseline measurements of PWV, and an interaction between baseline PWV and time. An unstructured variance-covariance matrix will be used to model the within-patient correlation structure. The primary result will be the treatment effect estimate at 96 weeks and 95% confidence limits.

This specification of the MMRM model is free from assumptions due to the unstructured modelling of treatment effects over time and unstructured within-patient error correlations. Due to this lack of structure, the model is not parsimonious and may suffer from lack of convergence. If the unstructured pattern fails to converge, more parsimonious within-patient error structures will be tested, starting with a heterogeneous Toeplitz pattern, then considering a Toeplitz pattern and finally compound symmetry with robust standard errors.

7.1.2 Treatment effect heterogeneity

Heterogeneity of the effect of lanthanum carbonate on PWV will be tested by adding a treatment-by-region interaction effect to the primary and robustness models (see 7.1.3). These analyses will be performed regardless of the p-value associated with the treatment effect in the original models.

7.1.3 Supporting analyses adjusted for randomisation variables

Robustness of the estimate of the lanthanum carbonate treatment effect relative to placebo will be assessed by an MMRM analysis which adjusts for region (4 Australian states, New Zealand, and Malaysia) and three patient baseline characteristics used in the treatment allocation algorithm: CKD stage (stage 3b vs. stage 4), age group (age < 65 vs age ≥ 65), and presence-absence of diabetes mellitus. No interaction between these covariates and time will be included in the MMRM models assessing sensitivity. This adjusted analysis will be viewed as supportive, providing additional context for interpreting the primary unadjusted (except for baseline PWV) analysis.

7.1.4 Sensitivity to the quality of PWV measurements

All PWV measures were assessed for usability (usable, not usable) by two experts in the area of arterial compliance who examined the quality of the carotid and femoral waveforms associated with the PWV values. Only those PWV values judged usable will be included in the main analysis. A supporting analysis will include PWV values that meet the SphygmoCor inbuilt quality criteria.

7.1.5 Sensitivity to missing data mechanism

The MMRM statistical model for analysis of the primary outcome and longitudinally-measured secondary outcomes assumes data are missing at random (MAR). While MAR is likely to be plausible for the majority of missing values, sensitivity to the MAR assumption will be assessed by analysing data with models that assume plausible MNAR assumptions.

7.1.6 Subgroup analyses

Three subgroups will be formed by the following baseline characteristics which were included as minimisation variables in the treatment allocation: CKD stage (stage 3b vs. stage 4), age group (age < 65 vs age ≥ 65), and diabetes mellitus (no, yes). The main subgroup analyses will be tests of treatment-by-subgroup variable interactions in MMRM models. These analyses will be performed regardless of the result of the MMRM model for the primary outcome comparison. Regression coefficients for treatment effect on the final PWV measurement at 96 weeks within each sub-group will be reported along with their 95% confidence limits.

7.1.7 Other comparisons

An on-treatment subset of participants will be identified, where on-treatment is defined as at least 80% compliant with randomised treatment. Treatment differences in PWV will be assessed using the MMRM model specified for the primary treatment comparison. This treatment compliant subset will also be used to compare treatment differences on four secondary outcomes (abdominal aortic calcification, serum phosphate, and intact and C-terminal FGF-23) using the methods described below.

7.2 Analysis of secondary outcomes

Secondary outcome variables are continuous or binary variables with a single post-baseline measurement per participant or repeatedly measured (at least two post-randomisation values) continuous or binary outcomes. Binary outcomes with a single follow-up measure will be analysed using a log binomial regression model to obtain estimates of risk ratios and 95% confidence intervals and continuous outcomes with a single follow-up measure will be analysed using analysis of covariance (ANCOVA) adjusted for the baseline measure of the variable. Repeatedly measured continuous outcomes will be analysed using the same likelihood-based MMRM model used to analyse the primary outcome, starting with an unstructured variance-covariance pattern for within patient errors, and stepping through the same sequence of back-up patterns if the unstructured pattern fails to converge. Repeatedly measured binary outcomes will be analysed using a generalised version of the MMRM approach which fits the treatment and other fixed effects by a logistic model.

7.3 Analysis of exploratory outcomes

Exploratory outcome variables that are not part of a designated sub-study are five biomarkers: soluble α -Klotho levels, CPPs, fetuin-A protein, MGP, and OPG. All five biomarkers are continuous outcomes measured at baseline and repeatedly over four study follow-up visits separated by at least six months. If distributional characteristics allow (either with or without transformation), these exploratory outcomes will be analysed using MMRM models as per the primary and secondary outcomes, adopting the same sequential approach to selection of an appropriate variance-covariance structure for the repeated measurements.

7.4 Analysis of safety outcomes

The relationship of any MACE event, treatment-emergent AE, any SAE and each category of SAE and ADR (all present versus absent) to treatment group will be summarised by frequencies and percentages and analysed with chi-square tests. The relationship between treatment group and SAE organ system (cardiovascular, respiratory, gastrointestinal, renal, neurological, musculoskeletal, endocrine, cancer/neoplasm, haematology, skin), SAE relationship to study medication (none, unlikely, possible, probable), ADR severity (mild, moderate, severe, life-threatening), and ADR relationship to study medication (possible, probable) will be summarised by frequencies and percentages and analysed with chi-square tests.

7.5 Data manipulation and computing

Most of the trial data are stored in a central REDCapTM database. Measurements obtained from MRI, CT scans, and some measurements obtained from samples analysed by a central laboratory at the end of the trial will be saved in Excel spreadsheets and save on a server in a folder with restricted access. All data manipulation, tables, figures, listings and analyses will be documented in SAS[®] or Stata[®] programs and performed using SAS version 9.4 or Stata version 15.

8. REPORTING

All results described above as well as tables, listings and figures (TLFs) listed below will be presented in the FSR.

8.1 Trial profile

All patients who provide informed consent will be accounted for in the FSR. A CONSORT-style flow diagram will illustrate patient progression through the trial from initial screening for eligibility to completion of the final primary outcome assessment. Number (percentage) of participants randomised to each treatment group will be given for all randomised patients along with reasons for study withdrawal by treatment group.

8.2 Patient characteristics and baseline comparisons

Demographic and other baseline characteristics, including laboratory investigations, will be summarised by assigned treatment group. Categorical variables will be summarised by

frequencies and percentages. Percentages will be calculated according to the number of patients for whom data are available. Where values are missing, the denominator, which will be less than the number of patients assigned to the treatment group, will be reported either in the body or a footnote in the summary table. Continuous variables will be summarised by mean and standard deviation as well as quartiles.

8.3 TABLES, LISTINGS, AND FIGURES (TLFs)

Templates for all planned TLFs are in a separate document titled IMPROVE-CKD FSR Tables, Listings and Figures.

8.3.1 Planned tables

The following are planned summary tables:

- Table 1. Enrolment by study centre stratified by country
- Table 2. Treatment group allocations by study centre
- Table 3. Minimisation variables by treatment group
- Table 4. Protocol deviations by treatment group (stratified by deviation category)
- Table 5. Study medications dispensed by treatment group across study visits
- Table 6. Study medication compliance (pill counts) by treatment group across study visits
- Table 7. Study medication withdrawals by treatment group across study visits
- Table 8. Baseline concomitant medications by treatment groups
- Table 9. Baseline demographic and clinical characteristics by treatment group
- Table 10. Baseline blood investigations by treatment group
- Table 11. Baseline urine investigations by treatment group
- Table 12. Physical examinations by treatment group across study visits
- Table 13. PWV mean values and standard deviations by treatment group across study visits (overall and by 3 dichotomous minimisation variables age-group, CKD stage, and diabetes mellitus)
- Table 14. Estimates, confidence intervals and p-values from a MMRM model analysis of PWV (table parts a, b, etc. for main and supporting analyses)
- Table 15. Estimates, confidence intervals and p-values from ANCOVA model analyses of abdominal aortic calcification (part a), LVM (part b), and BMD (part c)
- Table 16. Serum phosphate, serum calcium and serum calcium-phosphate product mean values and standard deviations by treatment group across 8 study visits (table parts a, b, and c)
- Table 17. Estimates, confidence intervals and p-values from MMRM model analyses of serum phosphate, serum calcium and serum calcium-phosphate product (table parts a, b, and c)
- Table 18. PTH mean values and standard deviations by treatment group across 8 study visits (table parts a and b)
- Table 19. Estimates, confidence intervals and p-values from MMRM model analyses of PTH
- Table 20. Estimates, confidence intervals and p-values from a generalised MMRM model analysis of SHPT
- Table 21. Intact FGF-23 and C-terminal FGF-23 mean values and standard deviations by treatment group across study visits (table parts a and b)
- Table 22. Estimates, confidence intervals and p-values from MMRM model analyses of Intact FGF-23 and C-terminal FGF-23 (table parts a and b)

- Table 23. Urinary phosphate excretion mean values and standard deviations by treatment group across study visits
- Table 24. Estimates, confidence intervals and p-values from a MMRM model analysis of urinary phosphate excretion
- Table 25. Estimates, confidence intervals and p-values from log-binomial regression analysis of (a) use of phosphate binders and (b) renal progression defined as a binary composite outcome
- Table 26. Creatinine clearance mean values and standard deviations by treatment group across study visits
- Table 27. Estimates, confidence intervals and p-values from a MMRM model analysis of creatinine clearance
- Table 28. eGFR mean values and standard deviations by treatment group across study visits
- Table 29. Estimates, confidence intervals and p-values from a MMRM model analysis of eGFR
- Table 30. Estimates, confidence intervals and p-values from a generalised MMRM model analysis of proteinuria.
- Table 31. Continuous exploratory outcome mean values and standard deviations by treatment group across study visits (parts a to e for 5 outcomes)
- Table 32. Estimates, confidence intervals and p-values from MMRM model analysis of continuous exploratory outcomes visits (parts a to e for 5 outcomes)
- Table 33. Estimates, confidence intervals and p-values from a generalised MMRM model analysis of binary exploratory outcomes (parts a to d for 4 outcomes)
- Table 34. Any ADR and ADR categories by treatment group
- Table 35. ADR severity by treatment group
- Table 36. ADR relationship to study medication by treatment group
- Table 37. Any MACE and any treatment-emergent AE by treatment group
- Table 38. Any SAE and SAE categories by treatment group
- Table 39. Relationship of SAE to study medication by treatment group
- Table 40. SAE body system by treatment group

8.3.2 Planned listings

The following are planned data and patient listings:

- Listing 1. Reasons for participants withdrawing from the study
- Listing 2. Medication compliance problems requiring participant to come off trial
- Listing 3. Deaths and life threatening events

8.3.3 Planned figures

The following are planned summary figures:

- Figure 1. Monthly and cumulative entry of participants into the study
- Figure 2. Flowchart of patient progression through the study
- Figure 3. Forest plot of mean differences, 95% confidence intervals and interaction test p-values for subgroup analyses on the primary outcome (PWV)
- Figure 4. Primary outcome (PWV) across visits by treatment group (baseline & 4 post-randomisation visits)

8.3.4 Supplementary TLFs

Results from supporting and sensitivity analyses will be presented in supplementary tables. Missing data on the primary outcome will be summarised by treatment groups and presented in supplementary tables and figures as appropriate.

8.4 General reporting conventions

All TLFs will be presented in portrait orientation, unless landscape orientation suggests that the information is easier to view. Legends will be used for all figures with more than one variable or item displayed. Figure lines should be wide enough to see the line after being copied.

All titles will be centred on a page. The first title line will be the number of the table, figure, or data listing. The second (and if required, third) line will be the description of the table, figure, or data listing. The ICH numbering convention will be used for all TLFs (7).

All tables, figures, and data listings will have the name of the relevant SAS (or Stata) program and a date-time stamp on the bottom of each output. All analysis programs developed for a table, figure, or data listing will be self-contained to facilitate transfer of programs to multiple computing environments. A separate analysis program will be written to produce each table.

8.5 Statistical summary conventions

For tables, sample sizes for each treatment group will be presented as totals in the column header (N=xxx), where appropriate. Sample sizes shown with summary statistics are the number (n) of patients with non-missing values.

Summaries for categorical variables will include only categories that patients had a response in. Percentages corresponding to null categories (cells) will be suppressed. All summaries for continuous variables will include: N, mean, and SD. Other summaries (e.g. median, quartiles, 5%, 95% intervals, coefficient of variation (CV) or %CV will be used as appropriate. All percentages should be rounded and reported to a single decimal place (xx.x%). If percentages are reported as integers, percentages greater than 0% but <1% will be reported as <1%, whereas percentages greater than 99% but <100% will be reported as >99%. A percentage of 100% will be reported as 100%. No value of 0% should be reported. Any computation of percent that results in 0% is to be reported as a blank. Summaries that include p-values will report the p-value to three decimal places with a leading zero (0.001). P-values <0.001 will be reported as <0.001.

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