Summary Table of Study Protocol

Title	Characterizing the management of hypocalcemia among European hemodialysis patients receiving cinacalcet
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Date of last version of the protocol	19 December 2016
EU Post Authorisation Study (PAS) Register No	EUPAS15875
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Procedure Number	N/A
Marketing Authorisation Holder(s)	Amgen Europe B.V.

Research Question and Objectives	**Primary Objectives** Hypocalcemia HD patients with cinacalcet prescription (*Cohort 1*) 1. To determine the rate of cinacalcet discontinuation following hypocalcemia event 2. To describe prescribing patterns of cinacalcet at initiation, at time of hypocalcemia event, and after hypocalcemia event 3. To describe prescribing patterns of any treatment intervention (e.g. vitamin D, phosphate binders and cardiovascular disease (CVD) medications) prior to cinacalcet initation, at time of cinacalcet initation and after hypocalcemia event 4. To identify factors associated with changes to any treatment intervention following a hypocalcemic event **Secondary objectives** HD patients with cinacalcet prescription and serum calcium >8.4 mg/dL (*Cohort B)* 5. To determine the incidence of hypocalcemia in SHPT patients with chronic kidney disease (CKD) on hemodialysis (HD) following cinacalcet initiation 6. To determine the frequency of hypocalcemia events in cinacalcet patients 7. To determine time to first hypocalcemia event 8. To describe characteristics of cinacalcet patients who develop and who do not develop hypocalcemia 9. To identify factors associated with time to hypocalcemia event in cinacalcet patients Hypocalcemia HD patients who discontinued cinacalcet prescription (*Cohort C)* 10. To determine time to cinacalcet re-initiation 11. To determine the rate of cinacalcet re-initiation among patients who discontinuee cinacalcet) following hypocalcemia event 12. To describe factors associated with time to cinacalcet re-initiation event in patients who discontinued cinacalcet following hypocalcemia event 13. To describe treatment patterns (e.g. vitamin D, phosphate binders and CVD medications) at time of hypocalcemia event, at time of discontinuation, and at time of cinacalcet re-initiation 14. To describe the trajectory of PTH, calcium and phosphate values following cinacalcet initiation, following hypocalcemia event and following cinacalcet reinitiation of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels

	Exploratory objectives: 1. To explore the number of HD patients who initiate cinacalcet (i.e. first prescription) with hypocalcemia (*Cohort A)* HD patients with cinacalcet prescription (*Cohort B)* 2. To explore the incidence of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels 3. To explore the frequency of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels Hypocalcemia HD patients with cinacalcet prescription (*Cohort 1)* 4. To explore kinetics of hypocalcemia events in cinacalcet patients with more than one episode of hypocalcemia 5. To explore the impact of hypocalcemia treatment on serum calcium level 6. To explore the impact of hypocalcemia on cardiovascular (CV) events or death
Country(-ies) of Study	Czech Republic, France, Hungary, Ireland, Italy, Poland, Portugal, Romania, Russia, Serbia, Slovak Republic, Slovenia, Spain, Turkey, and the United Kingdom
Author	Karly Louie, PhD, Amgen Inc. Bruno Fouqueray, MD, Amgen Inc. The ARO Study Investigators, EU-FMC Jürgen Flöege, University Hospital, RWTH Aachen

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Investigator's Agreement

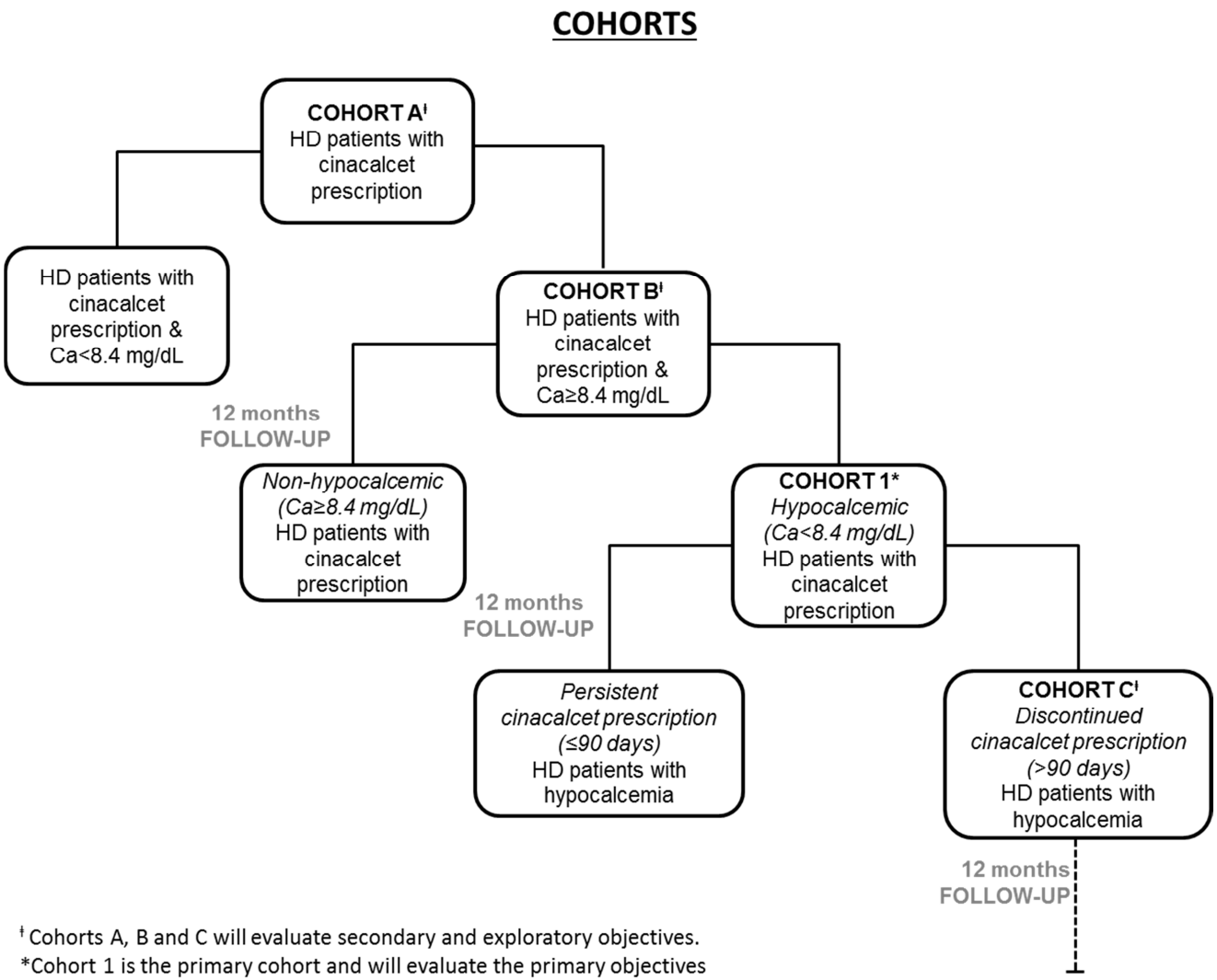
I have read the attached protocol entitled "Characterizing the management of calcium reductions among European hemodialysis patients receiving cinacalcet", dated 19 December 2016, and agree to abide by all provisions set forth therein.

Signature

Name of Investigator

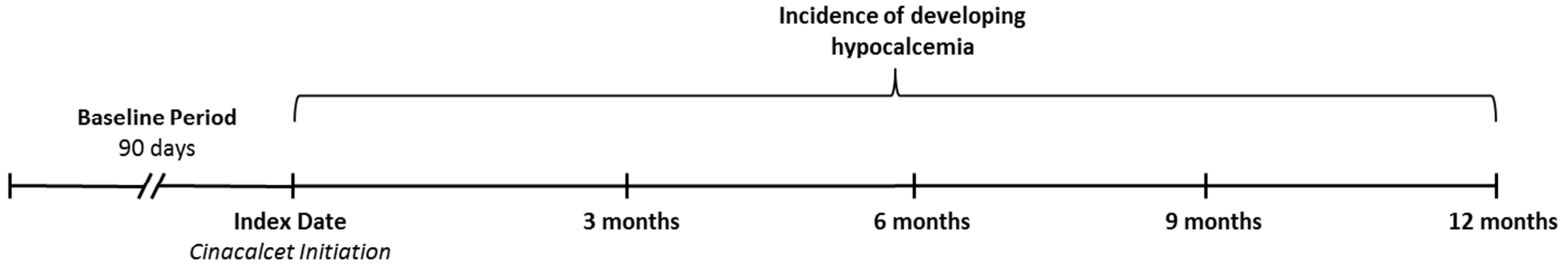
Date (DD Month YYYY)

Study Design Schema

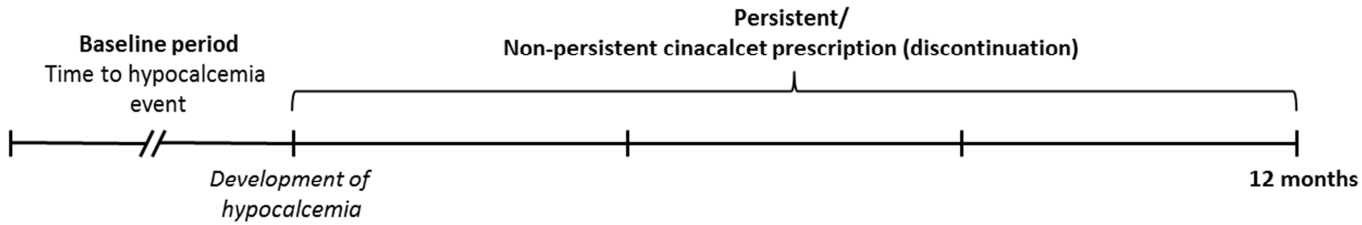


† Cohorts A, B and C will evaluate secondary and exploratory objectives.
*Cohort 1 is the primary cohort and will evaluate the primary objectives

COHORT B – HD patients with cinacalcet prescription and serum calcium >8.4 mg/dL

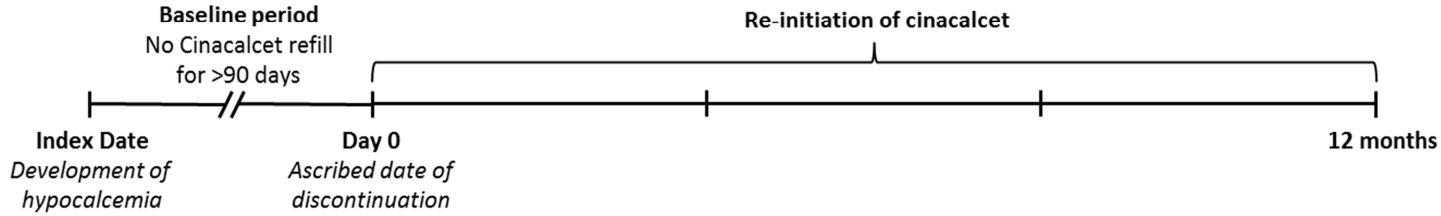


COHORT 1 – Hypocalcemia HD patients with cinacalcet prescription



*Sub-set of Cohort B patients who developed hypocalcemia

COHORT C – Hypocalcemia HD patients who discontinued cinacalcet prescription



*Sub-set of Cohort 1 patients with non-persistent cinacalcet prescription

1. Table of Contents

2. List of Abbreviations

Abbreviation	Full Term
ANOVA	Analysis of variance
ARO	Analyzing Data, Recognizing Excellence and Optimizing Outcome
Ca	Calcium
CaSR	Calcium sensing receptors
CKD	Chronic Kidney Disease
CKD-MBD	CKD-mineral bone disorder
EPRC	European Protocol Review Committee
ESRD	End-stage renal disease
EuCliD	European Clinical Database
FMC	Fresenius Medical Care
HCP	Healthcare provider
HCPCS	Healthcare Common Procedure Coding System
HD	Hemodialysis
IV	Intravenous
MBD	Mineral Bone Disease
NDC	National Drug Code
P	Phosphate
PTH	Parathyroid Hormone
RRT	Renal replacement therapy
SAP	Statistical analysis plan
SD	Standard deviation
SHPT	Secondary hyperparathyroidism
SmPC	Summary of product characteristics

3. Responsible Parties

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4. Abstract

- **Study Title**

Characterizing the management of hypocalcemia among European hemodialysis patients receiving cinacalcet

- **Study Background and Rationale**

Hypocalcemia is a common adverse event in hemodialysis (HD) patients with secondary hyperparathyroidism (SHPT) receiving cinacalcet as first-line treatment. Patients using cinacalcet are six times more likely to experience hypocalcemia (total calcium <8.4 mg/dl) compared to those who do not use it. Any side effects such as hypocalcemia could adversely affect a patient's level of medication compliance and/or adherence or changes in treatment strategies (e.g. dose reduction or discontinuation of cinacalcet). Calcium-containing phosphate binders, vitamin D sterols and/or adjustment of dialysis fluid calcium concentrations can be used to raise serum calcium according to clinical judgement. Dose reduction or cinacalcet discontinuation may negatively affect SHPT control. Data are needed to inform appropriate treatment guidance on the management of hypocalcemia among SHPT patients using cinacalcet.

To date, treatment strategies to manage cinacalcet-induced hypocalcemia have not been well-characterized in a European HD population. In addition, it is unclear whether patients who recover from hypocalcemia will achieve adequate SHPT control. Therefore, we propose to characterize clinician treatment and management practices of hypocalcemia in a retrospective observational cohort study (AROii database) of incident European HD patients with sHPT who were prescribed cinacalcet.

- **Research Question and Objective(s):**
  - **Primary Objectives**

Hypocalcemia HD patients with cinacalcet prescription (*Cohort 1*)

1. To determine the rate of cinacalcet discontinuation following hypocalcemia event
2. To describe prescribing patterns of cinacalcet at initiation, at time of hypocalcemia event, and after hypocalcemia event
3. To describe treatment patterns (e.g. vitamin D, phosphate binders and cardiovascular disease (CVD) medications) prior to cinacalcet initation, at time of cinacalcet initation and after hypocalcemia event
4. To identify factors associated with changes to any treatment intervention

- **Secondary objectives**

HD patients with cinacalcet prescription (*Cohort B)*

5. To determine the incidence of hypocalcemia in SHPT patients with chronic kidney disease (CKD) on HD following cinacalcet initiation
6. To determine the frequency of hypocalcemia events in cinacalcet patients
7. To determine time to first hypocalcemia event
8. To describe characteristics of cinacalcet patients who develop and who do not develop hypocalcemia
9. To identify factors associated with time to hypocalcemia event in cinacalcet patients

Hypocalcemia HD patients who discontinued cinacalcet (*Cohort C)*

10. To determine time to cinacalcet re-initiation
11. To determine the rate of cinacalcet re-initiation among patients who discontinued cinacalcet following hypocalcemia event
12. To describe factors associated with time to cinacalcet re-initiation event in patients who discontinued cinacalcet following hypocalcemia event
13. To describe treatment patterns (e.g. vitamin D, phosphate binders and CVD medications) at time of hypocalcemia event, at time of discontinuation, and at time of cinacalcet re-initiation
14. To describe the trajectory of PTH, calcium and phosphate values following cinacalcet initiation, following hypocalcemia event and following cinacalcet reinitiation

- **Exploratory objectives**

1. To explore the number of HD patients who initiate cinacalcet (i.e. first prescription) with hypocalcemia (*Cohort A)*

HD patients with cinacalcet prescription (*Cohort B)*

2. To explore the incidence of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels
3. To explore the frequency of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels

Hypocalcemia HD patients with cinacalcet prescription (*Cohort 1)*

4. To explore kinetics of hypocalcemia events in cinacalcet patients with more than one episode of hypocalcemia
5. To explore the impact of hypocalcemia treatment on serum calcium level
6. To explore the impact of hypocalcemia on cardiovascular (CV) events or death

- **Hypothesis(es)/Estimation**

No formal hypothesis will be tested.

- **Study Design/Type**

A retrospective cohort study using electronic medical records of HD patients prescribed cinacalcet in Europe

- **Study Population or Data Resource**

The source population for this study is the AROii cohort, which comprises adult subjects presenting at one of 304 participating FMC facilities in 14 European countires enrolled between 01 January 2007 and 31 December 2009. AROii data collection covers until the end of 2014 will reflect current practice patterns. Patients are chronic (ten or more contiguous dialysis sessions) incident (<183 days since commencing renal replacement therapy (RRT)) HD patients with no history of renal transplantation or peritoneal dialysis.

- **Eligibility Criteria**

Inclusion crtieria: HD patients in AROii cohort i) aged ≥18, ii) filled a cinacalcet prescription after 01 January 2007, iii) enrolled in AROii for at least 90 days prior to cinacalcet initiation and have at least 90 days follow-up following cinacalcet initiation (*Cohort A)*. HD patients who have serum Ca ≥8.4mg/dL at time of cinacalcet initation will be included in subsequent analyses (*Cohort B)*. A sub-set of HD patients who develop hypocalcemia (serum Ca <8.4 mg/dL) will define *Cohort 1* which will be used to evaluate the primary objectives. HD patients with hypocalcemia who discontinued cinacalcet will be included in further sub-analyses (*Cohort* C).

Exclusion crtieria: Patients with i) parathyroidectomy up to and including the first 90 days of follow-up, ii) a prescription for cinacalcet is less than 15 days (2 weeks) with no further prescriptions.

- **Follow-up**

Follow-up will commence on the date patients initiate a cinacalcet prescription and will continue for at least 90 days and up to 12 months. Among patients who develop hypocalcemia, follow-up will continue for 12 months. And among patients who discontinued cinacalcet, follow-up will continue for 12 months.

- **Variables**
  - **Outcome Variables:** Incidence of hypocalcemia; frequency of hypocalcemia; time to first hypocalcemia event; time to any treatment intervention, rate of cinacalcet discontinuation; cinacalcet prescribing patterns; prescribing patterns for vitamin D, phosphate binders and CVD medications; time to cinacalcet re-

initiation following discontinuation, rate of cinacalcet re-initation; kinetics of hypocalcemia; and serum calcium levels.

- **Exposure Variable:** cinacalcet initation, development of hypocalcemia, and cinacalcet discontinuation
- **Other Covariates:** Demographic (age, gender, country, exposure pre-KDIGO); clinical (hospitalization, BMI, pre-dialysis systolic blood pressure, smoking status); medical history (CKD aetiology); medical events (diabetes, cancer, CVD, fracture); dialysis parameters (vascular access, dialysis vintage, calcium dialysate concentration, net ultrafiltration, actual blood flow); laboratory parameters (hemoglobin, ferritin, CRP, serum albumin, creatinine, total calcium, phosphate, alkaline phosphatase, parathyroid hormone (PTH)); and medication use (cinacalcet, paricalcitol, other active vitamin D (AVD), phosphate binder, and cardiovascular medication)

- **Study Sample Size**

  Since this is a study with descriptive objectives, sample size calculations are based on the level of precision (i.e. half width) of the 95% confidence interval (CI) around the point estimate of the proportion discontinuing cinacalcet treatment (p) following development of hypocalcemia. Based on previous analysis by de Francisco et al (de Francisco *et al*, 2016), approximately 1245 HD patients in AROii were prescribed cinacalcet. Based on observational data (Brunelli *et al*, 2015), approximately 47% of patient on cinacalcet are expected to develop hypocalcaemia, so *Cohort 1* is expected to contain approximately 600 patients. The maximum half width of the 95% confidence interval for a proportion based on 600 patients is 0.040.

- **Data analysis**

Primary analysis

Point estimates and 95% confidence intervals will be derived to determine the rate of cinacalcet discontinuation and changes to disease related prescribing patterns following a hypocalcemic event

Time to first hypocalcemia event, time to any treatment intervention following hypocalcemia event, and time to cinacalcet re-initiation will be estimated using Kaplan Meier methods according to baseline calcium levels.

Cox regression models will be used to identify baseline covariates associated with time

to first occurrence of hypocalcemia during the first 12 months of the study, time to any treatment intervention during the 12 months following hypocalcemia event and time to cinacalcet re-iniation during the 12 months following cinacalcet discontinuation. Hazard ratios and 95% confidence intervals will be calculated.

Exploratory analysis
Point estimates and 95% confidence intervals will be derived to determine the rate of hypocalcemia according to PTH levels during the 12 months following cinacalcet initiation. Frequency of hypocalcemia events according to PTH levels will also be described.

To explore the kinetics of hypocalcemia events, we will plot serum calcium level after first hypocalcemia event according to the treatment intervention employed to manage the hypocalcemia. These will be considered separately by treatment and in combination (i.e. any treatment). Average % change in serum calcium from the value at the time of hypocalcemia occurrence will also be summarized.

Point estimates and 95% confidence intervals will be derived to determine the rate of CV events during the 12 months following cinacalcet initiation.

5. Amendments and Updates

None

6. Milestones

The study will carry out secondary data analysis of the AROii cohort.

Milestone	Planned date
Start of data collection (*start analysis)*	1 January 2017
End of data collection (*complete analysis)*	Q4 2017
Registration in the EU PAS register	1 January 2017
Final report of study results	Q1 2018

7. Rationale and Background

7.1 Diseases and Therapeutic Area

Secondary hyperparathyroidism (SHPT) is a common complication of chronic kidney diasese (CKD) (Goodman, 2004). As kidney function declines this causes disturbances

in the balance of calcium, phosphorous and vitamin D and consequently cause CKD-related mineral and bone disorders (CKD-MBD). These biochemical imbalances of increased calcium, phosphate lead to an overproduction of parathyroid hormone (PTH) and parathyroid gland hyperplasia which characterizes SHPT. Elevated levels of PTH can develop at early stages of CKD even when calcium and phosphorous are within normal range limits (Martinez *et al*, 1997). The impact of SHPT on progressive CKD results in bone mass reduction and an impaired rate of bone remodeling. This inadequate control of SHPT can consequently lead to increased risks of vascular calcification, fractures and cardiovascular morbidity and mortality (Horl, 2004). Approximately 30-47% of severe CKD patients on dialysis in Europe are affected with SHPT (PTH>300 pg/mL) (Hedgeman *et al*, 2015).

Prevention and treatment of SHPT remains a challenge although successful management can be partially achieved with standard treatment with dietary phosphate restriction, phosphate binders, vitamin D and parathyroidectomy. However, the introduction of calcimimetic agents has provided an alternative therapeutic option to manage SHPT. Calcimimetics acts as an allosteric activator by selectively binding to calcium-sensing receptors (CaSR) in the parathyroid gland which lowers the CaSR action of extracellular calcium ions on PTH secretion; and thereby reducing PTH levels and accompanying serum calcium levels (Rodriguez *et al*, 2015).

To date, Sensipar/Mimpara® (or cinacalcet) is the only currently approved calcimimetic for treatment of SHPT among patients with CKD undergoing dialysis. Cinacalcet has shown to improve metabolic abnormalities in CKD-MBD and recommended in clinical guidelines (Kidney Disease: Improving Global Outcomes, 2009). In a recent Cochrane systematic review of 11 randomized controlled trials (RCT) (n=2853 participants) (Ballinger *et al*, 2014; Block *et al*, 2004; Charytan *et al*, 2005; El-Shafey *et al*, 2011; Fishbane *et al*, 2008; Fukagawa *et al*, 2008; Ketteler *et al*, 2012; Lindberg *et al*, 2003; Messa *et al*, 2008; Quarles *et al*, 2003), the majority of trials (10 out of 11 except (Ketteler *et al*, 2012)) showed that participants assigned cinacalcet plus standard treatment vs. placebo/no standard treatment were more likely to achieve the study's PTH reduction target value of 30% or more from baseline or a reduction to below 250 pg/mL, 279 pg/mL or 300 pg/mL or a target value between 150 to 300 pg/mL (relative risk, RR=3.06; 95% CI: 1.89-4.98). Besides PTH reduction, cinacalcet treatment has also shown a reduction in the incidence of parathyroidectomy (RR=0.49; 95% CI:0.40-0.59). Similarly, the rate of parathyroidectomy reduced by half in the cinacalcet-treated

(7%) vs. placebo-treated (14%) patients in the EVOLVE trial (relative hazard, 0.44, 95% CI: 0.36-0.54) (Investigators *et al*, 2012). These RCT efficacy results support the potential effectiveness of cinacalcet in controlling SHPT in real-life clinical practice. In a real-life United States (US) observational study of approximately 14,550 patients undergoing dialysis with cinacalcet prescriptions, patients were more likely to achieve the KDOQI™ (National Kidney Foundation Kidney Disease Outcome Quality Initiative) guideline target (iPTH range 150-300 pg/mL) 12 months following cinacalcet initation compared to baseline (no cinacalcet prescription) (24% vs. 10%) (St Peter *et al*, 2009).

Despite the potential effectiveness of cinacalcet to manage SHPT, tolerance of cinacalcet may be limited. RCTs show a 7-fold increased risk of hypocalcemia (RR=6.98; 95% CI: 5.10-9.53) (Ballinger *et al*, 2014; Sekercioglu *et al*, 2016). Similar to RCTs, hypocalcemia is commonly observed in patients treated with cinacalcet in observational studies (Kilpatrick *et al*, 2013; St Peter *et al*, 2009). Although cinacalcet is effective in reducing calcium and PTH, the rate of hypocalcemia events increased by five-fold from baseline (2.3% to 10.1%). In another study of over 7600 patients initiating cinacalcet from a large dialysis provider in the US, low calcium levels at baseline have been found to be predictive of cinacalcet discontinuation, however, hypocalcemia prior to discontinuation of cinacalcet did not appear to be a factor in the decision to reinitiate use of cinacalcet (Kilpatrick *et al*, 2013). Its unclear how these hypocalcemia events may impact real-life cinacalcet utilization patterns and SHPT control.

In reponse to the development of cinacalcet-induced hypocalcemia (total calcium <8.4 mg/dl and >7.5 mg/dl), the Summary of Product Characteristics (SmPC) recommends clinicians to adjust calcium-containing phosphate binders, vitamin D sterols, dialysate calcium to raise serum calcium according their own clinical judgement (e.g. initiate, reduce or discontinue therapies); or if the patient has severe hypocalcemia (≤7.5 mg/dl), they are recommended to discontinue use until serum calcium levels are raised to levels of 8.0 mg/dl and symptoms have been resolved. Currently, there are limited data on the effectiveness of treatment interventions to manage cinacalcet-induced hypocalcemia and its impact on SHPT control. One observational study in the US found that 50% of patients who initiated cinacalcet experienced a hypocalcemia event (total calcium <8.4 mg/dL) (Brunelli *et al*, 2015). The proportion of patients who received at least one treatment intervention was directly related to increased severity of hypocalcemia: calcium <8.0-8.3 mg/dL (48.6%), 7.5-7.9 mg/dL (52.2%) and <7.5 (63.5%). The most common treatment intervention for low calcium was addition or up-titration of vitamin D

in about one-third of patients. Other management strategies included discontinuation or reduction in the dose of cinacalcet in 22% of patients and initiation or up-titration of a calcium-containing phostphate binder in 3% of patients; and there was also an increase in dialysate calcium concentration in 7% of patients. The majority of patients returned to normal calcium (total calcium ≥8.4 mg/dL) levels within 90 days following the hypocalcemia event irrespective of whether they continued or discontinued cinacalcet following the hypocalcemia event. However, the generalizability of these US treatment practices for management of hypocalcemia may be limited and cannot be extrapolated to management practices in a European hemodialysis population. It is recognized that patient characteristics and treatment with cinacalcet in Europe and the US differ hence treatment strategies and practices to address calcium reductions are also likely to differ.

7.2 Rationale

Hypocalcemia is a common adverse event in SHPT patients receiving cinacalcet as first-line treatment. Patients using cinacalcet are six times more likely to experience hypocalcemia (total calcium <8.4 mg/dl) compared to those who do not use it (Sekercioglu *et al*, 2016). The adverse effect can be symptomatic (i.e. include paresthesias, numbness, tingling, myalgias or cramping) or asymptomatic (Bover *et al*, 2016). Any side effects such as hypocalcemia could adversely affect a patient's level of medication compliance and/or adherence or changes in treatment strategies (e.g. discontinuation of cinacalcet). The SmPC recommends adjustments of standard therapies (calcium-containing phosphate binders, vitamin D sterols, dialysate calcium) at the clinician's discretion and to discontinue use of cinacalcet when the hypocalcemia event is severe (total calcium <7.5 mg/dl). Data from two clinical trials observed that <1% of asymptomatic patients with hypocalcemia were withdrawn from cinacalcet treatment, suggesting that it is tolerable and manageable in most cases (Block *et al*, 2008; Schaefer *et al*, 2008). In contrast, discontinuation was observed to be much higher (~17%) in a US retrospective observational study (unpublished data from (Brunelli *et al*, 2015)). The discrepancy in cinacalcet discontinuation rates between clinical trials (Block *et al*, 2008; Schaefer *et al*, 2008) and the observational study (Brunelli *et al*, 2015) raises the question as to what factors will drive changes in treatment strategies in real-life clinical practice. Dose reduction or cinacalcet discontinuation may negatively affect SHPT control.

To date, treatment strategies to manage cinacalcet-induced hypocalcemia has not been well-characterized in a European HD population. In addition, it is unclear whether

patients who recover from hypocalcemia will achieve adequate SHPT control. Therefore, we propose to characterize clinician treatment and management practices in the AROii European HD population. Data are needed to inform appropriate treatment guidance on the management of hypocalcemia among SHPT patients using cinacalcet.

7.3 Statistical Inference (Estimation or Hypothesis[es])

No formal hypothesis will be tested. Instead, point estimates and 95% confidence intervals will be derived to determine the rate of hypocalcemia following cinacalcet initiation, rate of cinacalcet discontinuation following hypocalcemia event and rate of cinacalcet re-initiation following discontinuation due to hypocalcemia. In addition, hazard ratios and 95% confidence intervals will be carried out for the predictor analyses.

8. Research Question and Objectives

8.1 Primary

Hypocalcemia HD patients with cinacalcet prescription (*Cohort 1)*

8.1.1 To determine the rate of cinacalcet discontinuation following hypocalcemia event

8.1.2 To describe prescribing patterns of any treatment intervention (e.g. vitamin D, phosphate binders and cardiovascular disease (CVD) medications) prior to cinacalcet initation, at time of cinacalcet initation and after hypocalcemia event

8.1.3 To describe time to any treatment intervention and treatment patterns (e.g. vitamin D, phosphate binders and cardiovascular disease (CVD) medications) prior to cinacalcet initation, at time of cinacalcet initation and after hypocalcemia event

8.1.4 To identify factors associated with changes to any treatment intervention following a hypocalcemic event

8.2 Secondary objectives

HD patients with cinacalcet prescription and serum calcium >8.4 mg/dL at baseline (*Cohort B)*

8.2.1 To determine the incidence of hypocalcemia in SHPT patients with CKD on HD following cinacalcet initiation

8.2.2 To determine the frequency of hypocalcemia events in cinacalcet patients

8.2.3 To determine time to first hypocalcemia event

8.2.4 To describe characteristics of cinacalcet patients who develop and who do not develop hypocalcemia

8.2.5 To identify factors associated with time to hypocalcemia event in cinacalcet patients

Hypocalcemia HD patients who discontinued cinacalcet prescription (*Cohort C*)

8.2.6 To determine time to cinacalcet re-initiation

8.2.7 To determine the rate of cinacalcet re-initiation among patients who discontinued cinacalcet following hypocalcemia event

8.2.8 To describe factors associated with time to cinacalcet re-initiation event in patients who discontinued cinacalcet following hypocalcemia event

8.2.9 To describe treatment patterns (e.g. vitamin D, phosphate binders and CVD medications) at time of hypocalcemia event, at time of discontinuation, and at time of cinacalcet re-initiation

8.2.10 To describe the trajectory of PTH, calcium and phosphate values following cinacalcet initiation, following hypocalcemia event, and following cinacalcet re-initiation

8.3 Exploratory

8.3.1 To explore the number of HD patients who initiate cinacalcet (i.e. first prescription) with hypocalcemia (*Cohort A)*

HD patients with cinacalcet prescription and serum calcium >8.4 mg/dL at baseline (*Cohort B)*

8.3.2 To explore the incidence of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels

8.3.3 To explore the frequency of hypocalcemia events among HD patients with cinacalcet prescription according to PTH levels

Hypocalcemia HD patients with cinacalcet prescription (*Cohort 1)*

8.3.4 To explore kinetics of hypocalcemia events in cinacalcet patients with more than one episoide of hypocalcemia

8.3.5 To explore the impact of hypocalcemia treatment on serum calcium level

8.3.6 To explore the impact of hypocalcemia cardiovascular events (CVE) or death

9. Research Methods

9.1 Study Design

A retrospective cohort of chronic kidney disease patients receiving hemodialysis in European Fresenius Medical Care (FMC) facilities will be carried out. The cohort design will allow us to utilize available data to study multiple outcomes (e.g. development of hypocalcemia and changes in treatment strategies, calcium recovery, and reinitiation of cinacalcet among hypocalcemia patients) and provide an opportunity to describe the management of hypocalcemia in the hemodialysis population.

9.2 Setting and Study Population

The source population for this study is the AROii cohort, which comprises of adult subjects presenting at one of 304 participating facilities in 14 European countries (*Czech Republic, France, Hungary, Ireland, Italy, Poland, Portugal, Romania, Russia, Serbia, Slovak Republic, Slovenia, Spain, and United Kingdom*) and Turkey enrolled between 01 January 2007 and 31 December 2009. Out of 10,600 patients enrolled, 1876 patients were still on follow-up by the end of 2014.

AROii, a closed-cohort by design, comprises of chronic (ten or more contiguous dialysis sessions) incident (<183 days since commencing renal replacement therapy (RRT)) haemodialysis patients with no history of renal transplantation or peritoneal dialysis. Data are captured electronically via the FMC European Clinical Database (EuCliD) and supplied on a quarterly basis. By Q4 2014, the AROii study population had accrued over 33,000) person-years of follow-up.

9.2.1.1 Study Period

The study period will be 01 January 2007 to 31 December 2014.

9.2.2 Subject/Patient/Healthcare Professional Eligibility

9.2.2.1 Inclusion/Exclusion Criteria

Inclusion criteria

HD patients in AROii cohort i) aged ≥18, ii) filled a cinacalcet prescription after 01 January 2007, iii) enrolled in AROii for at least 90 days prior to cinacalcet initiation and have at least 90 days follow-up following cinacalcet initiation (*Cohort A)*. HD patients who have serum Ca ≥8.4mg/dL at time of cinacalcet initation will be included in subsequent analyses (*Cohort B)*. A sub-set of HD patients who develop hypocalcemia (serum Ca <8.4 mg/dL) will define *Cohort 1* which will be used to evaluate the primary objectives. HD patients with hypocalcemia who discontinued cinacalcet will be included in further sub-analyses (*Cohort* C).

Exclusion criteria

Patients with i) parathyroidectomy up to and including the first 90 days of follow-up, ii) a prescription for cinacalcet is less than 15 days (2 weeks) with no further prescriptions

9.2.3 Baseline Period

Cohort 1: Hypocalcemia HD patients with cinacalcet prescription and serum calcium >8.4 mg/dL

The baseline period will be defined as period between cinacalcet initiation and development of hypocalcemia

Cohort B: HD patients with cinacalcet prescription

The baseline period will be defined as the 90 days prior to cinacalcet initiation.

Cohort C: Hypocalcemia HD patients who discontinued cinacalcet prescription

The baseline period will be defined as the period between development of hypocalcemia and ascribed date of discontinuation

9.2.4 Study Follow-up

In this retrospective AROii cohort study, follow-up commenced on the date they initiated a cinacalcet prescription.

- For Cohort 1 (hypocalcemia HD patients with Cinacalcet prescription), follow-up continued for 12 months following development of hypocalcemia.
- For Cohort B (HD patients with Cinacalcet prescription and serum calcium >8.4 mg/dL), continued for 12 months following cinacalcet initiation
- For Cohort C (hypocalcemia HD patients who discontinued cinacalcet prescription), follow-up continued for 12 months following discontinuation of cinacalcet.

9.2.4.1 Censoring

Patients will begin to accrue time at risk from the end of their relevant baseline period until they experienced hypocalcemia (*Cohort B*), discontinued cinacalcet (*Cohort 1*) or reinitiated cinacalcet (*Cohort C*). Patients will also be censored if they died, underwent parathyroidectomy or a renal transplant, or were lost to follow-up during the respective follow-up periods. Patients will be considered lost to follow-up if they left a dialysis facility for any reason and did not return to the same or another FMC facility within 45 days.

9.3 Variables

9.3.1 Exposure Assessment

9.3.1.1 Cinacalcet initiation

Cinacalcet initiation will be defined as the start date of patients' first prescription. If a patient's first prescription duration was less than 15 days (2 weeks) and the gap between the end date of the first prescription to the start date of the second prescription was greater than 15 days then the second prescription will be used to identify treatment initiation (same rules will apply for the second and subsequent prescription)

9.3.1.2 Development of hypocalcemia

Patients who developed hypocalcemia as defined by serum calcium <8.4 mg/dL.

9.3.1.3 Cinacalcet discontinuation

Cinacalcet discontinuation will be defined as occurring at the time point beyond which there were at least 90 consecutive days without a prescription of treatment (e.g. the gap between the end date of previous prescription and the start date of the following prescription is greater than 90 days) . A patient will be considered persistent until the time point at which discontinuation occurs. The choice for a 45-day window to define treatment discontinuation relies on the structure and content of ARO data: 45 days without data collection are defining loss-of-follow-up in ARO.

9.3.2 Outcome Assessment

Table 1 summarizes the outcomes to be assessed according to each objective.

Table 1. Outcomes for analysis

Objective	Definition
PRIMARY OBJECTIVES	
Hypocalcemia HD patients with cincalcet prescription	
- Rate of cincalcet discontinuation	- Number of patients who discontinue cinacalcet. Discontinuation is defined as the time point beyond which there were ≥90 consecutive prescription-free days.
-Cinacalcet prescribing pattern	- No change, dose reduced (relative to dose prior to hypocalcemia), discontinued, Dose increased (relative to dose prior to hypocalcemia)
- Prescribing pattern for any treatment and each of the following drugs: paricalcitol, older AVD, phosphate binder, dialysate calcium and CVD medications	- No change, dose reduced (relative to dose prior to hypocalcemia), discontinued, Dose increased (relative to dose prior to hypocalcemia)
SECONDARY OBJECTIVES	
HD patients with cincalcet prescription	
- Incidence of hypocalcemia	- First occurrence of a serum calcium (<8.4 vs. ≥8.4 mg/dL) - First occurrence of serum calcium defined by severity: mild (serum calcium ≥8.0 - < 8.4 mg/dL), moderate (serum calcium ≥ 7.5 - < 8.0 mg/dL), severe (serum calcium <7.5 mg/dL)
Hypocalcemia HD patients who discontinued cinacalcet prescription	
- Rate of cinaclcet re-initiation	- Number of patients who re-initiate cincalcet prescription following discontinuation (yes/no)
- Prescribing pattern for each of the following drugs: cinacalcet, paricalcitol, older AVD, phosphate binder, dialysate calcium and CVD medications	- No change, dose reduced (relative to dose prior to hypocalcemia), discontinued, Dose increased (relative to dose prior to hypocalcemia)
EXPLORATORY OBJECTIVES	
- Incidence of hypocalcemia by PTH	- No. of patients with hypocalcemia according to PTH levels: low (<10 ng/L or pg/mL), normal (10-65 ng/L or or pg/mL) or high (>65 ng/mL or pg/mL)
- Kinetics of hypocalcemia	- Progressors (subsequent events were more severe than initial events), remitters (subsequent events were less severe than initial events), fluctuators (events ranged in severity with no trend in one direction)
- Changes in calcium level	- Changes in mean or median calcium levels following changes in treatment treatment
- Cardiovascular event	- Any coronary artery event (CVE), cerebrovascular event (CVE), peripheral arterial event (PAE), congestive heart failure event (CHFE), or sudden cardiac event (SCE)

Definitions of specific outcomes are more detailed below.

9.3.2.1 Incidence of hypocalcemia

The cumulative incidence of first hypocalcemia event (# of new cases with serum calcium value <8.4 mg/dL) will be calculated overall and stratified by mutually exclusive groups of mild (≥8.0 - < 8.4 mg/dL), moderate (≥ 7.5 - < 8.0 mg/dL), and severe (< 7.5 mg/dL).

The time period will be monthly following cinacalcet initation. Patients are at risk for each specified time period if they have an active supply of cinacalcet and therefore at risk of hypocalcemia. Patients are not at-risk if they do not have an active supply of cinacalcet, died, had a parathyroidectomy or a renal transplant, or was lost to follow-up during the specified time period.

9.3.2.2 Frequency of hypocalcemia

The frequency of hypocalcemia events will be calculated as the total number of consecutive serum calcium values below 8.4 mg/dL during 12 months following cinacalcet initiation. Patients with less than 12 months follow-up will be excluded from this specific analysis.

9.3.2.3 Rate of cinacalcet discontinuation

Refer to definition defined in 9.3.1.3

9.3.2.4 Prescribing patterns of cinacalcet

Treatment patterns of cinacalcet at initiation, immediately prior to hypocalcemia event and after first occurrence of hypocalcemia (e.g. reduced dose or discontinuation of cinacalcet) during the 90 days (or up to 12 months) following hypocalcemia event.

9.3.2.5 Treatment patterns of active vitamin D, phosphate binders and CVD medications

Treatment patterns of active vitamin D, phosphate binders and CVD medications 90 days prior to cinacalcet initiation, immediately prior to cinacalcet initiation, and immediately after first occurrence of hypocalcemia during the 90 days (or up to 12 months) following hypocalcemia event

9.3.2.6 Cinaclacet re-initiation following cinacalcet discontinuation

Patients who discontinued cinacalcet are identified according to 9.3.1.3. Re-initation of cinacalcet during the 12 months following discontinuation of cinacalcet will be defined as patients who subsequently receive a new prescription. If the duration of a patient first prescription following discontinuation was less than 15 days (2 weeks) and the gap between the end date of that prescription to the start date of the subsequent prescription

was greater than 15 days then the subsequent prescription will be used to identify treatment re-initiation.

9.3.2.7 Kinetics of hypocalcemia events

For the exploratory objectives, the aim is to describe how many patients with more than one episode of hypocalcemia transitioned through the different disease severity stages. The aim is to assess whether hypocalcemia progresses slowly or whether patients tend to fall into the severe range immediately and then resolve or go back and forth.

Patients with more than one hypocalcemia event will be defined as one of the following:

- Progressors – Subsequent events were more severe than initial events
- Remitters - Subsequent events were less severe than initial events
- Fluctuators – events ranged in severity with no trend in one direction

9.3.2.8 Time to cardiovascular events or death

For exploratory objective of CVE and death events following hypocalcemia will be defined by ICD-10 codes in Appendix 1.

9.3.3 Covariate Assessment

Table 2 summarises the covariates to be evaluated. The most proximal values identified at the end of the baseline period will be used in the analysis. Time-varying covariates will be evaluated at consecutive 90 days intervals following the baseline period. Continuous (e.g. laboratory parameters, age, BMI, systolic blood pressure and etc) time varying covariates will be determined at the end of each interval (one value only). Categorical (e.g. concomitant medications, medical events etc) time-varying covariates will be determined by the occurrence of a prescription or event respectively within an interval. During intervals where an event of interest occurs, only data prior to the event will be used in the corresponding time-varying analysis (most recent value prior to the event for continuous covariates and vascular access). The presence (combined therapy) or absence of alternative therapeutic class (AVD for the cinacalcet-related analysis; cinacalcet for the AVD-related analysis) will be integrated as a covariate. For the exposure period pre-KDIGO covariate if the baseline period or the interval corresponding to the time-varying analysis included the date of the publication of the KDIGO guidelines, then the value of this covariate will be set to Yes.

Table 2: Baseline and time-varying covariates

Parameter type	Covariate	Categories	Variable type in time-varying analysis
Demographics	Age	<30, 30-49, 50-64, 65+	baseline
	Gender	Male/female	baseline
	Country		baseline
	Exposure period pre-KDIGO*	Yes/No	time-varying
	Mean time of follow-up following cincalcet initiation		
Clinical	Hospitalisation	Yes/No	time-varying
	BMI	<18.5, 18.5-<25, 25-<30, ≥30	baseline
	Pre-dialysis systolic blood pressure [mm Hg]	<120; 120-<130; 130-<140; 140-<160; 160+	time-varying
	Smoking status	Current, Former, Non-smoker	baseline
Medical history	CKD aetiology	Hypertension / vascular, Glomerulonephritis, Diabetes, Tubulo-interstitial, Polycystic Kidney Disease, Invalid/Missing, Unknown	baseline
Medical events	Diabetes	Yes/No	baseline
	Cancer	Yes/No	baseline
	CVD	Yes/No	time-varying
	Fracture	Yes/No	time-varying
Dialysis parameters	Vascular access	Catheter vascular access only, Non-catheter vascular access only, Missing	baseline
	Dialysis vintage	Continuous	time-varying
	Calcium dialysate concentration [mEq/L]	<2.5, ≥2.5	time-varying
	Net Ultrafiltration [L]	Quartiles	baseline
	Actual blood flow [mL/min] ¥	Quartiles	baseline
Laboratory parameters	Hemoglobin [g/dL]	<10, 10-<12, ≥12	baseline
	Ferritin [µg/L]	<500, ≥500	baseline
	CRP [mg/L] ¥	Quartiles	baseline
	Serum albumin [g/L] ¥	Quartiles	time-varying
	Creatinine [µmol/L] ¥	Quartiles	baseline
	Total calcium [mmol/L]	<2.10, 2.10-2.37, >2.37	time-varying
	Phosphate [mmol/L]	<1.13, 1.13-1.78, >1.78	time-varying
	Alkaline phosphatase	<44 IU/mL, 44-147 IU/mL (normal), >147	time-varying

	PTH [pg/mL]	<150, 150-<300, 300-<600, ≥600	time-varying
Medication use	Cinacalcet use[§]	Yes/No	time-varying
	Paricalcitol use[§]	Yes/No	time-varying
	Older AVD use[§]	None; Alfacalcidol; Calcitriol; both	time-varying
	Phosphate binder use[§]	None, Only calcium-based, Only non calcium-based, Any	time-varying
	Cardiovascular medication[§]	Yes/No	time-varying

*01 August 2009. If the baseline period or the interval corresponding to the time-varying analysis includes the publication of the KDIGO guidelines, then the value of the value of exposure period pre-KDIGO covariate will be set to Yes; [§] relevant ATC codes in Appendix B; [¥] The quartiles' cut-offs for these variables will be derived at baseline.

9.3.4 Validity and Reliability

Extensive procedures have been taken to ensure the analysis dataset contains prescriptions with correct ATC codes, dosage, start and end date, quantity, and route. The process and the QC procedure have validated more than 99.9% of all prescriptions Validation of other aspects of EuCliD database has been discussed elsewhere (Anker *et al*, 2016; de Francisco *et al*, 2016; Eckardt *et al*, 2015; Floege *et al*, 2015; Gillespie *et al*, 2015; Stenvinkel *et al*, 2016).

9.4 Data Sources

An extensive medical record for each haemodialysis patient, essential to their clinical care and encompassing general information, past medical history, physiological anamnesis, comorbidities, allergies, amputations, transfusion history, current medication, laboratory tests, vaccination status, residual renal function, dialysis access, surgery (including parathyroidectomy) and transplantation, is captured directly in the EuCliD system on adminission to an FMC facility. These data are reflected in AROii.

Demographic and clinical characteristics include age, gender, height, weight, mobility, marital status, education level, occupational status, smoking history, CKD etiology (coded according to ICD-10) and RRT initiation (country, center, modality and date). Detailed records on patient medical history were used to confirm history of cardiovascular disease, cerebrovascular disease, malignancy and diabetes. Information captured on HD therapy includes dialysis vintage, vascular access type and duration of placement, number of treatments per week, duration of treatment, actual blood flow, dialysis adequacy (Kt/V) as well as pre- and post-dialysis weight. Hospitalization data

including admission and discharge dates and ICD-10 codes, and death data, are routinely captured by all FMC sites.

Descriptive information on prescribed medications includes product (description and ATC code), start and end dates of prescription, dose and administration route. Laboratory data, including markers for anaemia, bone and mineral metabolism, inflammation and nutrition, reflects normal clinical care in terms of testing frequency. Specific to this study, calcium and phosphorous are typically tested on a monthly basis, whilst PTH is tested quarterly.

9.5 Study Size

Since this is a study with descriptive objectives, sample size calculations are based on the level of precision (i.e. half width) of the 95% confidence interval (CI) around the point estimate of the proportion discontinuiing cinacalcet (p) following development of hypocalcemia. Based on previous analysis by de Francisco et al (de Francisco *et al*, 2016), approximately 1245 HD patients in AROii were prescribed cinacalcet. Based on observational data (Brunelli *et al*, 2015), approximately 47% of patients on cinacalcet are expected to develop hypocalcaemia, so *Cohort 1* is expected to contain approximately 600 patients. The maximum half width of the 95% confidence interval for a proportion based on 600 patients is 0.040.

9.6 Data Analysis

9.6.1 Planned Analyses

9.6.1.1 Primary Analysis

Incidence of hypocalcemia will be examined during the first 12 months following cinacalcet initation overall and according to disease severity as defined by three hypocalcemia thresholds: < mild (≥8.0 - < 8.4 mg/dL), moderate (≥ 7.5 - < 8.0 mg/dL), and severe (< 7.5 mg/dL).

After identifying patients who develop hypocalcemia, sub-anlayses of hypocalcemia patients will be carried out to describe cinacalcet prescribing practices (i.e. reduced dose or discontinutation) and treatment patterns of other conventional therapies for SHPT during the 12 months following hypocalcemia event.

Among patients who discontinued cinacalcet, another sub-analysis will be carried out to determine rate of cinacalcet re-initiation and to identify factors associated with re-initation as well as describe treatment patterns prior to re-initiation during the 12 months following cinacalcet discontinuation.

9.6.2 Planned Method of Analysis

9.6.2.1 Descriptive Analysis

Baseline characteristics of patients who do vs. do not develop hypocalcemia until end of follow-up of 12 months will be summarized for patients who initiate cinacalcet using descriptive statistics. Summary statistics will be presented for the time-varying covariates of Table 2 for the 90 days prior to initiation, discontinuation and re-initiation for cinacalcet. Continuous variables will be described using the mean and standard deviation, median, 25th and 75th percentiles and minimum and maximum values. Ordinal or skewed variables will be described using a median and range or categorized into meaningful categories at which point categorical data anlaysis will be applied. Categorical data will be reported as counts and frequencies.

Summary statistics will also be presented for time to first hypocalcemia event, time to any treatment intervention following hypocalcemia event, and time to cinacalcet re-initiation. In addition, cinacaclet prescribing patterns at initiation, at time of hypocalcemia event and after hypocalcemia event and treatment patterns (e.g. vitamin D, phosphate binders and CVD medications) 90 days prior to cinacalcet initiation, at time of cinacalcet initiation, at time of hypocalcemia event, and time of cinacalcet re-initiation will be described. Number of HD patients who initiate cinacalcet with hypocalcemia (<8.4 ng/mL) amongst all cinacalcet users will also be counted.

Plot median and IQR of PTH, calcium and phosphate values every month or quarter during the 12 months following cinacalcet initiation, following hypocalcemia event and following cinacalcet re-initation. The resulting data will be arranged graphically, stratified by levels of control (Table 3) during the baseline period, to depic the longitudinal pattern of laboratory values following initation, discontinuation and re-initaition of treatment. Smoothing techniques may need to be employed to fit the patterns of the laboratory data trajectory.

Table 3. Levels of control

	Serum PTH (pg/mL)	Serum Calcium (mmol/L)	Serum Phosphate (mmol/L)
Low	<150	<2.10	<1.13
Medicum	Medium low 150<300	2.10-<2.37	1.13-<1.78
	Medium high 300-<600		
High	≥600	≥2.37	≥1.78

9.6.2.2 Analysis of the Primary, Secondary and Exploratory Endpoint(s)

9.6.2.3 Primary and secondary objective endpoints

Point estimates and 95% confidence intervals will be derived to determine the rate of hypocalcemia following cinacalcet initiation, rate of cinacalcet discontinuation following hypocalcemia event and rate of cinacalcet re-initiation following discontinuation due to hypocalcemia.

Time to first hypocalcemia event, time to any treatment intervention following hypocalcemia event, and time to cinacalcet re-initiation will be estimated using Kaplan-Meier methods according to baseline calcium levels (i.e. mild, moderate or severe) at baseline.

Cox regression models will be used to identify baseline covariates (Table 2) associated with time to first occurrence of hypocalcemia during the first 12 months of the study, time to any treatment intervention during the 12 months following hypocalcemia event and time to cinacalcet re-iniation during the 12 months following cinacalcet discontinuation. Subjects who do not experience hypocalcemia will be censored (according to 9.2.4.1 criteria). Univariate anlaysis will be performed. Variables significant at the 0.25 level in univariate anlaylsis will be entered into a multivariate analysis using a stepwise regression technique (significance levels of 0.10 for inclusion/exclusion). Hazard ratios and 95% confidence intervals will be presented.

9.6.2.4 Exploratory objective endpoints

Point estimates and 95% confidence intervals will be derived to determine the rate of hypocalcemia according to PTH levels during the 12 months following cinacalcet initiation.

To explore the kinetics of hypocalcemia, we will plot serum calcium level after first hypocalcemia event according to the treatment intervention employed to manage the hypocalcemia. These will be considered separately by treatment and in combination (i.e. any treatment). Average percentage change in serum calcium from the value at the time of hypocalcemia occurrence will also be summarized.

Point estimates and 95% confidence intervals will be derived to determine the rate of CV events during the 12 months following cinacalcet initiation.

9.6.2.5 Sensitivity Analysis

The analysis for primary endpoints will be repeated by:

- year of KDIGO guidelines introduction (year ≤2009 vs. >2009)
- definition of hypocalcemia based on calcium threshold (≤8.4 mg/dL) or ICD-10 code of hypocalcemia (E83.51)
- region (Eastern Europe, Western Europe, and Iberian Peninsula)

9.6.2.6 Stratified Analysis

- All primary and secondary analysis will be reported overall for all hypocalcemia patients and stratified by severity: mild (serum calcium ≥8.0 - < 8.4 mg/dL), moderate (serum calcium ≥ 7.5 - < 8.0 mg/dL), severe (serum calcium <7.5 mg/dL).
- For objective 8.2.4, summary statistics of cinacalcet patients who develop and do not develop hypocalcemia shall be stratified by sex and age.

9.6.3 Analysis of Safety Endpoint(s)/Outcome(s)

Safety data will not be collected or analyzed in this study.

9.7 Quality Control

AROii data, comprising detailed patient-level information on medical and drug history, and longitudinal records of biochemical measurements and medications, are provided by FMC to Amgen on a quarterly basis. Fresenius personnel process the data in an initial step (limiting data to the AROii cohort and encrypting patient-identifiable data) but otherwise the data provided are raw. Amgen programmers then undertake an extensive process of data cleaning and quality checking to eliminate double counts, wrongly coded patients, date mismatches and similar discrepancies.

In the final step of the data quality process described above, an ‘analysis database’ is created. Data are limited to adult chronic haemodialysis patients (defined as ten or more contiguous dialysis treatment sessions) presenting to FMC facilities between 2007 and 2009 and for whom laboratory data were available. Several distinct standardized datasets are created: 1) demographics; 2) laboratory values; 3) transfusion; 4) death; 5) hospitalizations; 6) co-morbidities; 7) medications.

A number of analyses and publications (Anker *et al*, 2016; de Francisco *et al*, 2016; Eckardt *et al*, 2015; Floege *et al*, 2015; Gillespie *et al*, 2015; Stenvinkel *et al*, 2016) have resulted from the standardized AROii dataset.

9.8 Limitations of the Research Methods

9.8.1 Internal Validity of Study Design

9.8.1.1 Measurement Error(s)/Misclassification(s)

In retrospective studies, persistence is often quantified as the number of days of medication available to the patient. This represents a simplified measure, since the definition does not access if the patient takes the medication (i.e. compliance/adherence). Nonetheless, in the absence of a valid measure and to maintain consistence and comparability between studies of persistence, the number of days the medication was prescribed to the patient during his/her follow-up period is used in this retrospective study.

9.8.1.2 Information Bias

Information bias is unlikely to affect the findings of this study unless investigators record exposures and/or outcomes more diligently for patients treated vs. untreated with cinacalcet. It is possible that hospitalized patients may artificially appear to be non-persistent (i.e. discontinued) as new prescriptions may not be issued until these patients return to FMC. If this is the case then both the time-varying and case cross-over analyses would identify hospitalization as a risk factor, although it should be acknowledged that it would be impossible to determine if this was true (a worsening clinical condition) or artificial effect.

9.8.1.3 Selection Bias

Patients included in the ARO study were selected at random, and therefore selection bias should not exist with regard to the overall patient population. The study will not, however represent the entire dialysis care population on cinacalcet with hypocalcemia in the participating countries, as it was chosen from a single network of providers belonging to Fresenius Medical Care. This limits the generalizability of the study findings but may still be relevant for other private providers in each of these countries. Specifically, patient exposure to these therapies of interest may not be balanced across countries.

9.8.1.4 Confounding

As with all observational studies, unmeasured confounding might occur if data, known to inform treatment decisions, is unavailable. Similarly, potential for residual confounding increases if measurement error exists for important confounding variables. However, the ARO data are derived from a clinically-validated database. It is imperative that these clinical data are complete and accurate as these are essential to inform nephrologists about their patients' care, particularly because this dialysis population requires intense

management of disease. These clinical safegurads will be reflected in the data available for analysis, reducing the potential for unmeasured confounding and residual confounding. If confounding is present, this should be considered non-differential with regard to the exposure measurements of interest.

9.8.2 External Validity of Study Design

Patients included in the study represent patients who receive dialysis from one network provider in Europe, Fresenius Medical Care. Selection bias and generalizability of the results are discussed in section 9.8.1.3. Briefly, the study population may not represent patients who receive dialysis from a different private provider nor represent patients who receive dialysis in the public sector where different treatment and management guidelines maybe used.

9.8.3 Analysis Limitations

A limitation of this study is that the EuCliD prescription module does not request clinicians to enter the exact reasons for initiating, discontinuing or reinitiating cinacalcet prescription or the decision-making process for how to manage hypocalcemia. The data limitation also applies to known side effects of cinacalcet, such as vomiting and nausea which are not accompanied by ICD-10 coding. Therefore, we are unable to assess clinican's perceptions in initiating, discontinuing or reinitiating cinacalcet treatment or the impact of vomitng or nausea alongside hypocalcemia on persistence. The study will rely on recorded information such as laboratory values and coded clinical events to characterize reasons for initiation, discontinuation and reinitiation of cinacalcet treatment following a hypocalcemia event.

Potential delayed use of cinacalcet may have occurred when KDIGO 2009 guidelines was introduced. For each of these cohorts, patients may initiate the treatment in various years between 2007 and 2014 and the lengths between initation of HD and cinacalcet may be very different between cohorts. Therefore, different prescribing and management patterns may have occurred in hypocalcemia patients who started cinacalcet pre-KDIGO (2007/8/9) and post-KDIGO (2010-2014). We will look at pre- and post-2009 KDIGO time periods to assess whether there are any differences in prescribing and management patterns.

There are also practical difficulties in analyzing physician prescribing patterns. The information of prescribing clinicians are not recorded in the database. In addition, amongst the 304 FMC facilities contributing to AROii, there are on average 3 to 4

physicans attending each facility and they share the responsibility of routine adaption of prescriptions. If information of prescribing physicians were available, we would have a similar ratio of physicians to patients which owoudl make it statistically impossible to study the prescribing patterns amongst physicians.

9.8.4 Limitations Due to Missing Data and/or Incomplete Data

Data completeness of variables will be assessed. Depending on the level of completeness, use of multiple imputation or another appropriate method will applied.

10. Protection of Human Subjects

10.1 Informed Consent

The study uses secondary data for analyses. There is no human or animal experimentation or which ethical approval is required.

10.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

All ethical and regulatory obligations concerning the use of patient data have been met at each participating ARO study site and all patient and facility information has been anonymized. Given the study uses existing data, there will be no risks posed to human life. Patient informed consent has been obtained whenever required by local law and regulations.

10.3 Patient Confidentiality

All patient identifiable information was removed from data before being supplied to FMC's central servers. Therefore, all data supplied to Amgen are fully anonymized.

11. Collection of Safety Information and Product Complaints

Reporting of individual adverse events is not applicable for secondary data collection studies.

12. Administrative and Legal Obligations

12.1 Protocol Amendments and Study Termination

Not applicable

13. Plans for Disseminating and Communicating Study Results

It is the intent that the results from this study be disseminated as published peer-reviewed scientific literature.

When available, the results of this tudy will be shared with the ARO Steering Committeee.

13.1 Publication Policy

Results generated from this analysis will be submitted to a relevant nephrology, epidemiology, or general medicine journal. Results will also be presented at a highly frequented professional conference.

Authorship of any publications resulting from this study will be determined on the basis of the International Committee of Medical Journal Editors (ICJME) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, which states:

- Authorship credit should be based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; (3) final approval of the version to be published and (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors should meet conditions 1, 2, and 3 and 4.
- When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship defined above.
- Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
- All persons designated as authors should qualify for authorship, and all those who qualify should be listed.
- Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

All publications (eg, manuscripts, abstracts, oral/slide presentations, book chapters) based on this study must be submitted to Amgen for corporate review.

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15. Appendices

Appendix A. List of Stand-alone Documents

None

Appendix 1. ICD-10 codes for cardiovascular events

Event	ICD-10 code/group
Coronary Artery Events (CAE)	I20-I25 Ischaemic heart diseases
Cerebrovascular Events (CVE)	F01 Vascular dementia; G45 Transient cerebral ischaemic attacks and related syndromes; G46 Vascular syndromes of brain in cerebrovascular diseases; I60 Subarachnoid haemorrhage; I61 Intracerebral haemorrhage; I62 Other nontraumatic intracranial haemorrhage; I63 Cerebral infarction; I64 Stroke, not specified as haemorrhage or infarction; I65 Occlusion and stenosis of precerebral arteries, not resulting in cerebral infarction; I66 Occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction; I67 Other cerebrovascular diseases; I68 Cerebrovascular disorders in diseases classified elsewhere; I69 Sequelae of cerebrovascular disease
Peripheral Arterial Events (PAE)	H34 Retinal vascular occlusions; I51.3 Intracardiac thrombosis, not elsewhere classified; I70 Atherosclerosis; I171 Aortic aneurysm and dissection; I73.1 Thromboangiitis obliterans [Buerger]; I73.9 Peripheral vascular disease, unspecified; I74 Arterial embolism and thrombosis; K55.0 Acute vascular disorders of intestine; K55.1 Chronic vascular disorders of intestine; N28.0 Ischaemia and infarction of kidney; R02 Gangrene, not elsewhere classified.
Congestive Heart Failure Events (CHFE)	I11.0 Hypertensive heart disease with (congestive) heart failure; I13.0 Hypertensive heart and renal disease with (congestive) heart failure; I13.2 Hypertensive heart and renal disease with both (congestive) heart failure and renal failure; I42 Cardiomyopathy; I43 Cardiomyopathy in diseases classified elsewhere; I50 Heart failure; I51.5 Myocardial degeneration; I51.7 Cardiomegaly
Sudden Cardiac Events (SCE)	I46 Cardiac arrest; I49.0 Ventricular fibrillation and flutter