WINE Study: Roxadustat Treatment of Anemia of Chronic Kidney Disease (CKD) in Real-world Clinical Practice - Observational Study Utilizing Routinely Collected Secondary Data from WiNe Registry in Germany

ISN/Protocol 1517-MA-3501

Version 1.0

28-Mar-2024

Sponsor:

Astellas Pharma Europe Ltd.

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SYNOPSIS

Title	WINE study: Roxadustat Treatment of Anemia of CKD in Real-world Clinical Practice – Observational Study Utilizing Routinely Collected Secondary Data from WiNe Registry in Germany
Study identifier/protocol number	ISN: 1517-MA-3501
Protocol version & date of last version of protocol	Version: 1.0 Date: 28-Mar-2024
Active substance	Roxadustat (ATC code: B03XA05); Erythropoiesis-stimulating agents (ESA) (ATC code: B03XA01, B03XA02, B03XA03)
Medicinal product	Roxadustat; ESA
Product reference	Roxadustat (EVRENZO - EMA/453588/2021)
Type of study	Secondary Use of Routinely Collected Data External Database Analysis study Post-Authorization Effectiveness Study (PAES) other
Procedure number	N/A
Joint PASS	(Select one below) ☐ Yes ☒ No
Research questions, objectives, and methodology	**Research Question:** This observational study will investigate patients with CKD who are prescribed roxadustat using routinely collected data, with a focus on characterizing patient demographics and comorbidities, evaluating clinical outcomes in patients treated with roxadustat for anemia management, and measuring mortality and morbidity rates. **Study Objectives:** *Primary objective* 1.1. To describe the characteristics and comorbidities at index of patients prescribed roxadustat overall and stratified by dialysis status [non-dialysis dependent (NDD) and dialysis dependent, (DD)] and prior erythropoietin stimulating agents (ESA) use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup) 1.2. To describe the clinical outcomes in patients treated with roxadustat for the management of anemia of CKD, overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup) *Secondary objectives* 2.1. To describe the mortality (among DD patients) and morbidity in patients with CKD prescribed roxadustat overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup) 2.2. To describe the characteristics and comorbidities at index of patients with CKD prescribed ESA and compare them with those prescribed roxadustat (stratified by dialysis status [NDD and

	DD] and prior ESA use (roxadustat ESA-naïve patients and new ESA patients; roxadustat patients converting from ESA and prevalent ESA users) within each NDD and DD subgroup
	
	Methods
	This observational study will utilize routinely collected patient data from the Wissenschaftliches Institut für Nephrologie (WiNe) registry reflective of real-world clinical scenarios. Detailed stratifications will be made based on patients' dialysis status (NDD and DD patients) and their prior ESA use, as follows:
	• For the roxadustat exposure group:
	  ○ NDD patients:
	  ▪ NDD ESA-naïve roxadustat user
	  ▪ NDD ESA conversion to roxadustat
	  ○ DD patients:
	  ▪ DD ESA-naïve roxadustat user
	  ▪ DD ESA conversion to roxadustat
	• For the ESA exposure group:
	  ○ NDD patients:
	  ▪ NDD incident/new ESA user
	  ▪ NDD prevalent ESA user
	  ○ DD patients:
	  ▪ DD incident/new ESA user
	  ▪ DD prevalent ESA user
	Descriptive statistics and survival analyses will underpin a data-driven approach to healthcare decision-making.

Country(-ies) of study	Germany
Description of data sources	The study will utilize the German WiNe registry, a comprehensive electronic medical record (EMR) database initiated in 2007. The WiNe registry contains routinely collected secondary care data from patients with CKD. With over 27,000 patients in total, it comprises all 5 CKD stages and covers about 13% of all DD patients in Germany. As of November 2023, there are 180 patients with CKD-related anemia who are prescribed roxadustat in the WiNe registry.
Description of target population	Adult patients with symptomatic anemia of CKD (all stages) requiring treatment with roxadustat or an ESA, irrespective of dialysis status.
Study dates	Start of data collection date: 18 August 2021 Completion of study finding date: 31 January 2025
Study lead or contact	*PPD* Medical Affairs Astellas Pharma Europe Ltd.

Marketing Authorization Holder(s)

Marketing authorization holder(s)	Astellas Pharma Europe Ltd. 300 Dashwood Lang Road Bourne Business Park Addlestone, Surrey KT15 2NX, UK
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1 RESPONSIBLE PARTIES

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2 LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

Abbreviations	Description of Abbreviations
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
CKD	Chronic kidney disease
CRF	Case report form
CRP	C-reactive protein
CV	Cardiovascular
DD	Dialysis dependent
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EMR	Electronic medical record
ESA	Erythropoiesis-stimulating agents
EPO	Erythropoietin
Hb	Hemoglobin
HD	Hemodialysis
HIF-PHI	Hypoxia-inducible factor prolyl hydroxylase inhibitors
ICD-10	International Classification of Diseases, 10th Revision
IQR	Interquartile range
IV	Intravenous
MACE	Major adverse cardiovascular events
NDD	Non-dialysis dependent
PAD	Peripheral artery disease
PAES	Post-Authorization Effectiveness Study
PTH	Parathyroid hormone
QoL	Quality of life
RAAS	Renin-angiotensin aldosterone system
RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SGLT2	Sodium-glucose cotransporter-2
SOP	Standard operating procedure
TIBC	Total iron-binding capacity
TSAT	Transferrin saturation
WiNe	Wissenschaftliches Institut für Nephrologie

3 AMENDMENTS AND UPDATES

None.

4 RATIONALE, BACKGROUND, AND RESEARCH QUESTION

4.1 Rationale (Why and How) - Purpose of the Rationale Section

Erythropoiesis-stimulating agents (ESAs) and intravenous (IV) iron have been the standard of care for anemia of chronic kidney disease (CKD) for over 30 years, whereas roxadustat (EVRENZO) was only approved by the European Medicines Agency (EMA) on 18 August 2021 for the treatment of anemia of CKD. Although the clinical trial program for roxadustat included nearly 10,000 patients and provided robust evidence on the efficacy and safety of roxadustat for both non–dialysis-dependent (NDD) and dialysis-dependent (DD) patients, the clinical outcomes and safety of roxadustat in routine clinical practice in Europe still needs investigation.

4.2 Background

Anemia, defined as hemoglobin (Hb) <12 g/dL in women and <13 g/dL in men, is a common complication of chronic kidney disease (CKD) affecting more than half of patients with stage 3b-5 disease under the care of a nephrologist and more than 9 out of 10 patients on dialysis[1]. Anemia adds to the symptom burden of CKD, causing or exacerbating fatigue, breathlessness, weakness, insomnia, headaches, and reduced mental acuity[2]. Such symptoms reduce patients' quality of life (QoL) and also increase the burden on healthcare systems[2-5].

Anemia in CKD has been shown consistently in observational studies to be associated with an increased risk of all-cause mortality and non-fatal cardiovascular (CV) events including heart failure and left ventricular failure. Such associations have been demonstrated for both NDD[6-8] and DD patients[9-11]. For example, in a large Danish retrospective study, patients with CKD with severe anemia (Hb <8 g/dL) were almost twice as likely to die or be hospitalized than patients with CKD without anemia[8]. Several studies have also suggested an association between anemia and progression to end-stage kidney disease[8-12]. In general, patients with the lowest Hb levels have the worst outcomes.[13]

The etiology of anemia of CKD is multifactorial. Progressive failure of the diseased kidney to produce the hormone erythropoietin (EPO) to meet oxygen demands and dysregulated iron metabolism play a predominant role. Such effects have been attributed to a deficit in the oxygen-sensing mechanism in the kidney in CKD, which under normal conditions would lead to coordinated erythropoiesis in response to cellular hypoxia.[2,14-16] Chronic inflammation associated with CKD is an important contributing factor to anemia; inflammation increases levels of the master iron regulator hepcidin, leading to sequestration of iron in the reticuloendothelial system and rendering iron unavailable to the bone marrow for erythropoiesis[14,17], resulting in functional iron deficiency; inflammation also impairs the response of hematopoietic stem cells to EPO, hindering the maturity of red blood cells (RBCs). Absolute and functional iron deficiency (e.g., iron stored not used), blood loss

associated with hemodialysis (HD), or gastrointestinal bleeding, nutritional deficiencies, and reduced RBC life span have also been identified as contributing factors.[18]

Current treatment of anemia of CKD involves iron supplementation (given orally or intravenously), injectable ESA, and, as a last resort, RBC transfusions[19]; RBC transfusions should generally be avoided, especially in patients who might be transplant candidates, to minimize the risk of human leukocyte antigen sensitization.[19]

Iron supplementation is indicated to correct iron deficiency, prevent its development in ESA-treated patients, raise Hb in the presence or absence of ESA treatment, and as a means to reduce ESA dose requirements in patients on ESA.

Although iron therapy is used universally in the treatment of anemia, it is not without drawbacks. Absorption of oral iron is limited, and gastrointestinal side effects, such as constipation, limit adherence. IV iron is more efficacious than oral iron and is the preferred iron treatment in dialysis patients. Treatment with IV iron can potentially cause severe adverse reactions,[2,19] including acute hypersensitivity reactions that, although rare, can be life-threatening.[20,21] IV iron injections also add to the healthcare burden, since iron should be given in an environment where resuscitation facilities are available, and caution should be exercised for every dose of IV iron that is given, even if previous administrations have been well tolerated.[22] This may be a burden for patients who are not yet on dialysis or are on home dialysis because, unlike those receiving HD, these patients receive nephrology care at outpatient clinics rather than in a hospital setting.

ESAs are indicated in patients with moderate to severe anemia and are most appropriate in patients who are iron replete and whose Hb levels have fallen below 10 g/dL.[19] Target Hb with ESA should be maintained in the range of 10-12 g/dL, since 4 prominent studies conducted 15-20 years ago in both dialysis or non-dialysis patients with CKD showed that the treatment strategy of aiming for full correction of anemia (Hb >13 g/dL) with ESA was associated with no benefit[23] or even excess CV risk.[24-26] The reasons for increased risk with ESA treatment when targeting normal Hb levels has not been fully understood, but the most plausible hypothesis is that high ESA doses (as opposed to higher Hb levels) given in an attempt to normalize Hb concentrations is a detrimental factor[27]; post hoc analyses of key studies show that patients who readily achieved higher Hb levels had better outcomes, whereas those receiving increasingly high doses of ESA in an attempt to reach target were most at risk of adverse outcomes.[28,29] In clinical practice, such patients are typically those with inflammation, in whom elevated hepcidin levels lead to iron-restricted erythropoiesis. Thus, administration of high doses of ESA in the setting of inflammation is generally regarded as problematic and potentially associated with excess CV risk.[30,31]

The 4 studies of full anemia correction with ESAs and the subsequent guideline updates have had a major impact on shaping anemia management over the last 2 decades. In general, there has been a shift in the pattern of anemia treatments in which ESA use and ESA doses have declined, and iron use has increased[32]; this has been reinforced by the results of the PIVOTAL trial showing beneficial effects of proactive high-dose iron therapy compared with low-dose reactive iron therapy in dialysis patients.[33] Despite widespread clinical use of ESA,

optimal Hb targets are not fully established, and the optimal balance of ESA and iron is still not known.[13,34] A key understanding is that erythropoiesis depends on both EPO and the timely availability of iron and that these entities must be managed in relation to each other to achieve the best outcomes for patients.

Improved understanding of the pathophysiology of anemia of CKD and oxygen-sensing mechanisms in the kidney have led to the development of a new class of drugs, the hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs).

These oral drugs mimic the effect of hypoxia and bring about a coordinated erythropoietic response by upregulation of the genes for EPO and iron absorption and mobilization.[35,36] The first drug in this new class available in Europe is roxadustat, which received a marketing authorization from the EMA in August 2021.[37] The global phase 3 program for roxadustat executed jointly by AstraZeneca, FibroGen, and Astellas involved more than 9,600 patients with anemia of CKD across 8 studies in nondialysis,[38-41] incident dialysis,[42] and stable dialysis.[43-45] Collectively, these studies indicated that roxadustat was superior to placebo in achieving and maintaining Hb to within the target range (10-12 g/dL) in NDD patients[38-40] and was comparable to treatment with ESA in NDD[41] and DD patients.[42-45] Importantly, although Hb levels were comparable in ESA- and roxadustat-treated patients, this result was achieved with less use of iron (oral and IV) in NDD patients[41] and less use of IV iron in dialysis patients[46]; this finding is in keeping with the mechanism of action of roxadustat, in which iron absorption and mobilization are enhanced. The mean dose of roxadustat to maintain Hb remained stable over time, but gradually increased in those receiving ESA. Fewer patients on dialysis required RBC transfusions on roxadustat than on ESA.[46] In terms of general safety, the main adverse reactions of note reported with roxadustat were sepsis, seizures, and vascular related events, including vascular access thrombosis, pulmonary embolism, and deep vein thrombosis. Given the history of ESAs, CV safety was a particularly important focus of the global clinical program. The composite endpoint of major adverse cardiovascular events (MACE; death from any cause, stroke, and myocardial infarction) and MACE+ (MACE plus hospitalization for angina or congestive heart failure) showed that overall, the CV safety profile of roxadustat was comparable to that of ESA.[46] There was a trend in favor of roxadustat in a study design setting where roxadustat was administered to correct Hb in patients not on dialysis (all of whom were ESA naïve) or to correct Hb in those initiating dialysis (most of whom were ESA naïve). In contrast, there was a trend in favor of ESA in settings where roxadustat was initiated in stable dialysis patients converted from ESA; these data derived in the conversion setting may reflect an inherent bias in the study design of converting patients stable on a treatment to a new treatment. The summary of product characteristics for Evrenzo[37] cautions against conversion of dialysis patients who are stable on ESA treatment to roxadustat unless there is a valid clinical reason. There was no evaluation of the comparative safety or efficacy of roxadustat versus ESA treatment in NDD patients converting from ESA.

The clinical trial program for roxadustat provides robust evidence of the clinical efficacy and safety of roxadustat in relation to placebo and standard of care. However, gaps in the evidence and unanswered clinical questions remain. Furthermore, the conditions of clinical

trials (randomization, specification of inclusion and exclusion criteria, and protocol-determined follow-up visits) mean that the results may not be fully generalizable to all patients in all circumstances. Hence, there is a need to generate real-world clinical evidence that better reflects the actual clinical environment in which roxadustat will be used in terms of patient demographics, comorbidities, adherence, and concurrent treatments. Such data will be a valuable complement to that gathered in clinical trials.

The ability to collect good-quality observational data on a new drug is dependent on the availability of an existing infrastructure, such as a registry, with sufficient scope (in terms of variables collected and relevant patient population) and size (geographical reach) to capture meaningful data in a reasonable length of time in a pragmatic way.

4.3 Research Question

The study aims to address the following research questions:

1. What are the characteristics and comorbidities of patients with CKD prescribed roxadustat, and how do these differ when patients are stratified by dialysis status (NDD and DD) and by prior ESA use (ESA-naïve and converting from ESA) ?
2. What are the clinical outcomes in patients treated with roxadustat for the management of anemia of CKD, both overall and when stratified by dialysis status (NDD and DD) and by prior ESA use (including ESA-naïve patients and those converting from ESA))?
3. What is the mortality (among DD patients) and morbidity rate among patients with CKD prescribed roxadustat, and how do these rates vary based on dialysis status and prior ESA use ?
4. How do the characteristics and comorbidities of patients with CKD prescribed ESA compare with those of patients prescribed roxadustat, especially when considering different ESA usage patterns (ESA-naïve patients treated with roxadustat, ESA patients converted to roxadustat, incident/new ESA users, and prevalent ESA users)?

5 OBJECTIVES, ENDPOINTS, AND ESTIMANDS

5.1 Study Objectives and Endpoints

There is no a priori hypothesis defined for this study. Table 1 describes the study objectives and endpoints.

Table 1. Study Objectives and Endpoints

Objectives	Endpoints
Primary	
1.1. To describe the characteristics and comorbidities at index of patients prescribed roxadustat overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup)	• Age at index date (defined as the date of the first recorded prescription of either roxadustat or ESA), sex, time since CKD diagnosis, anemia and time since anemia diagnosis, body mass index (BMI), primary cause of CKD, stage of CKD at diagnosis and at index, dialysis status/type of dialysis at index, roxadustat initiation dose at index, inflammatory status, Hb level, iron status (ferritin/serum iron/TSAT/TIBC), PTH levels, renal function (eGFR and albumin/creatinine ratio levels for NDD patients), and use of oral and IV iron treatment at baseline • Comorbidities: e.g., diabetes mellitus, hypertension, congestive heart failure, ischemic heart disease, cerebrovascular disease including stroke, peripheral vascular disease, pulmonary embolism, deep vein thrombosis, vascular access thrombosis, seizures, sepsis, history of cancer/malignancy, chronic inflammatory disease, history of transplants. [The details of reporting of comorbidities will be ascertained at analysis stage]. • Medications: RAASi and SGLT2i
1.2. To describe the clinical outcomes in patients treated with roxadustat for the management of anemia of CKD, overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup)	• Mean dose of roxadustat at initiation, mean dose of roxadustat over time and mean maintenance dose • Responsiveness to treatment: a. Time to meet Hb target threshold (See Section 6.4). b. Proportion of patients achieving Hb target threshold at specific intervals* (e.g., 1, 3, 6, and 12 months of treatment) c. Mean change of Hb over specific intervals* (e.g., 1, 3, 6, and 12 months of treatment) d. Mean change in eGFR and albumin/ creatinine ratio levels from baseline over specific intervals for NDD patients* (e.g., 1, 3, 6, and 12 months of treatment) e. Ferritin, TSAT, TIBC, serum iron levels at specific intervals* (e.g., 1, 3, 6, and 12 months of treatment) f. CRP levels at specific intervals g. Time to first IV iron treatment from index date h. IV iron dose and number of IV iron administrations i. Time to rescue therapy (ESA)* (e.g., 1, 3, 6, and 12 months of treatment) j. Mean PTH levels measured at specific intervals (e.g., 1, 2, 3, 6, and 12 months)

Objectives	Endpoints
Secondary	
2.1. To describe the mortality (among DD patients) and morbidity in patients with CKD prescribed roxadustat overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup)	• Mortality: all-cause† • Morbidity: hyperkalemia incidence stratified by dialysis status, rate of clinical events of special interest (stroke, pulmonary embolism, deep vein thrombosis, vascular access thrombosis, seizures, and sepsis)
2.2. To describe the characteristics and comorbidities at index of patients with CKD prescribed ESA and compare them with those prescribed roxadustat (stratified by dialysis status [NDD and DD] and prior ESA use (roxadustat ESA-naïve patients and new ESA patients; roxadustat patients converting from ESA and prevalent ESA users) within each NDD and DD subgroup	• Age at index date, sex, time since diagnosis, primary cause of CKD, stage of CKD at diagnosis and at index, dialysis status/type of dialysis at index, age at anemia of CKD diagnosis • Comorbidities (diabetes mellitus, hypertension, congestive heart failure, ischemic heart disease, cerebrovascular disease including stroke, peripheral vascular disease, pulmonary embolism, deep vein thrombosis, vascular access disease, seizures, sepsis, history of cancer/malignancy, chronic inflammatory disease, history of transplants) as per primary objective 1.1. • Medications: RAASi and SGLT2i

Abbreviations: BMI = body mass index; CKD = chronic kidney disease; CRP = C-reactive protein; DD = dialysis dependent; eGFR = estimated glomerular filtration rate; ESA = erythropoiesis-stimulating agents; Hb = hemoglobin; ICD-10 = International Classification of Diseases, 10th Revision; IV = intravenous; NDD = non-dialysis dependent; PTH = parathyroid hormone; RAAS = renin-angiotensin aldosterone system; SGLT2 = Sodium-glucose cotransporter-2 TIBC = total iron-binding capacity; TSAT = transferrin saturation

* Contingent upon data availability at specific timepoints

† Robust data available for DD patients; mortality for NDD is expected to be underreported

5.2 Estimands

Table 2. Estimands

Objective	Population	Endpoint	Intercurrent Events and Strategies	Population-level Summary
Primary Objective: 1.1. To describe the characteristics and comorbidities at index of patients prescribed roxadustat overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each	Patients with CKD diagnosed with anemia and prescribed roxadustat as part of their treatment regimen	Patient characteristics assessed during baseline period: age at index date, sex, time since diagnosis, BMI, primary cause of CKD, stage of CKD at diagnosis and at index, dialysis status/type of dialysis at index, roxadustat initiation dose at index, inflammatory status (CRP, hs-CRP) Hb level, iron status (ferritin/serum iron/TSAT/TIBC), PTH levels, renal function (eGFR and albumin/creatinine ratio levels for NDD patients), and use of oral and IV iron treatment during baseline	Not applicable as the endpoint is cross-sectional For laboratory measures (e.g., Hb level, iron status, renal function), if multiple values during baseline are recorded, the closest value to the index date will be reported	Descriptive statistics, such as mean, median, and distribution for continuous variables and number and proportions for categorical variables

Objective	Population	Endpoint	Intercurrent Events and Strategies	Population-level Summary
NDD and DD subgroup)		Comorbidities: e.g., diabetes mellitus; hypertension; congestive heart failure; ischemic heart disease; cerebrovascular disease, including stroke; peripheral vascular disease; pulmonary embolism; deep vein thrombosis; vascular access thrombosis; seizures; sepsis; history of cancer/malignancy; chronic inflammatory disease; history of renal transplant Medications: RAASi and SGLT2i		
1.2. To describe the clinical outcomes in patients treated with roxadustat for the management of anemia of CKD, overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup)	Patients with CKD diagnosed with anemia and prescribed roxadustat as part of their treatment regimen	The primary endpoint will be the mean change in Hb levels from baseline at specified intervals (e.g., 1, 3, 6, and 12 months from index) Secondary endpoints include the proportion of patients achieving Hb target at specific intervals; changes in ferritin, TSAT, TIBC, serum iron levels at specific intervals; CRP levels at specific intervals; time to first IV iron treatment; and dose and number of IV iron administrations	Initiation of rescue therapy with ESAs is expected to have direct effect on Hb Treatment discontinuation (including switch to ESA), initiation of ESA rescue therapy or loss to follow-up	Mean dose of roxadustat at initiation, mean dose of roxadustat over time and mean Maintenance dose Mean change in Hb from baseline at specific time intervals (e.g., 1, 3, 6, and 12 months from index) Time-to-event analysis using Kaplan-Meier methods to estimate time to achieving Hb target threshold (Hb within the range of ≥10 g/dL and ≤12 g/dL) during the follow-up period For continuous variables such as Hb, ferritin, TSAT, TIBC, PTH and serum iron levels, summary statistics will be provided at each specified time interval, without imputation for missing values Proportion of patients achieving Hb targets or requiring iron treatments during follow-up,

Objective	Population	Endpoint	Intercurrent Events and Strategies	Population-level Summary
				frequency, and percentage
Secondary Objective: 2.1. To describe the mortality (among DD patients) and morbidity in patients with CKD prescribed roxadustat overall and stratified by dialysis status (NDD and DD) and prior ESA use (ESA-naïve patients and those converting from ESA, within each NDD and DD subgroup)	Patients with CKD diagnosed with anemia and prescribed roxadustat as part of their treatment regimen	All-cause mortality Hyperkalemia incidence stratified by dialysis status and prior ESA use Rate of clinical events of special interest (stroke, pulmonary embolism, deep vein thrombosis, vascular access thrombosis, seizures, and sepsis)	Treatment discontinuation (including switch to ESA), initiation of ESA rescue therapy or loss to follow-up	Number and proportion of patients who died or developed hyperkalemia and each clinical event during follow-up
2.2. To describe the characteristics and comorbidities at index of patients with CKD prescribed ESA and compare them with those of patients prescribed roxadustat (stratified by dialysis status [NDD and DD] and prior ESA use (roxadustat ESA-naïve patients and new ESA patients; roxadustat patients converting from ESA and prevalent ESA users) within each NDD and DD subgroup	Patients with CKD diagnosed with anemia and prescribed roxadustat or ESA as part of their treatment regimen	Patient characteristics at baseline: age at index date, sex, time since diagnosis, BMI, primary cause of CKD, stage of CKD at diagnosis and at index, dialysis status/type of dialysis at index, treatments at index, inflammatory status, Hb level, iron status (ferritin/serum iron/TSAT/TIBC/PTH levels), renal function (eGFR and albumin/creatinine ratio levels for NDD patients), and use of oral and IV iron treatment at baseline Comorbidities: e.g., diabetes mellitus; hypertension; congestive heart failure; ischemic heart disease; cerebrovascular disease; including stroke; peripheral vascular disease; pulmonary embolism; deep vein thrombosis; vascular access thrombosis; seizures, sepsis; history of cancer/malignancy; chronic inflammatory disease; history of renal transplant Medications: RAASi and SGLT2i	Not applicable as the objective is cross-sectional	Descriptive statistics, such as mean, median, and distribution for continuous variables and proportions for categorical variables

Abbreviations: BMI = body mass index; CKD = chronic kidney disease; CRP = C-reactive protein; DD = dialysis dependent; eGFR = estimated glomerular filtration rate; ESA = erythropoiesis-stimulating agents; Hb = hemoglobin; hs-CRP = high-sensitivity C-reactive protein; IV = intravenous; NDD = non-dialysis dependent; PTH = parathyroid hormone; RAAS = renin-angiotensin aldosterone system; SGLT2 = Sodium-glucose cotransporter-2; TIBC = total iron-binding capacity; TSAT = transferrin saturation

6 RESEARCH METHODS

6.1 Study Design

This will be an observational cohort study using routinely collected secondary data of patients with anemia of CKD treated with roxadustat or ESA identified from the WiNe registry between August 2021 (EMA approval of roxadustat)[47] and 6 months before the end of available data, to allow all patients to have 6 months of follow-up time (identification period). Patients will be followed from the date of prescription to the end of the study period (latest available data; approximately 1 month before data request date), death, disenrollment from the registry, or loss to follow-up.

Study period: August 2021 to latest data available (not including lookback)

Identification period: August 2021 to 6 months before end of available data. The most recent data will be requested (current data available is through September 2023, expected to be through December 2023 at the time of data request).

Index date: Date of first roxadustat or ESA prescription during identification period

Baseline (lookback) period: All data available prior to/including index date (minimum 12-month baseline period available)

Follow-up period: The follow-up period will start on the day immediately after the index date and censor on the earliest of the following: end of available data, death, loss to follow-up from the WiNe registry.

Patients may leave the database without previous notice (e.g., moving away, seeking treatment from another site, or death). Usually, patients' follow-up visits happen every 3 months in the WiNe registry. Therefore, 9 months with no records will be interpreted as a loss to follow-up.

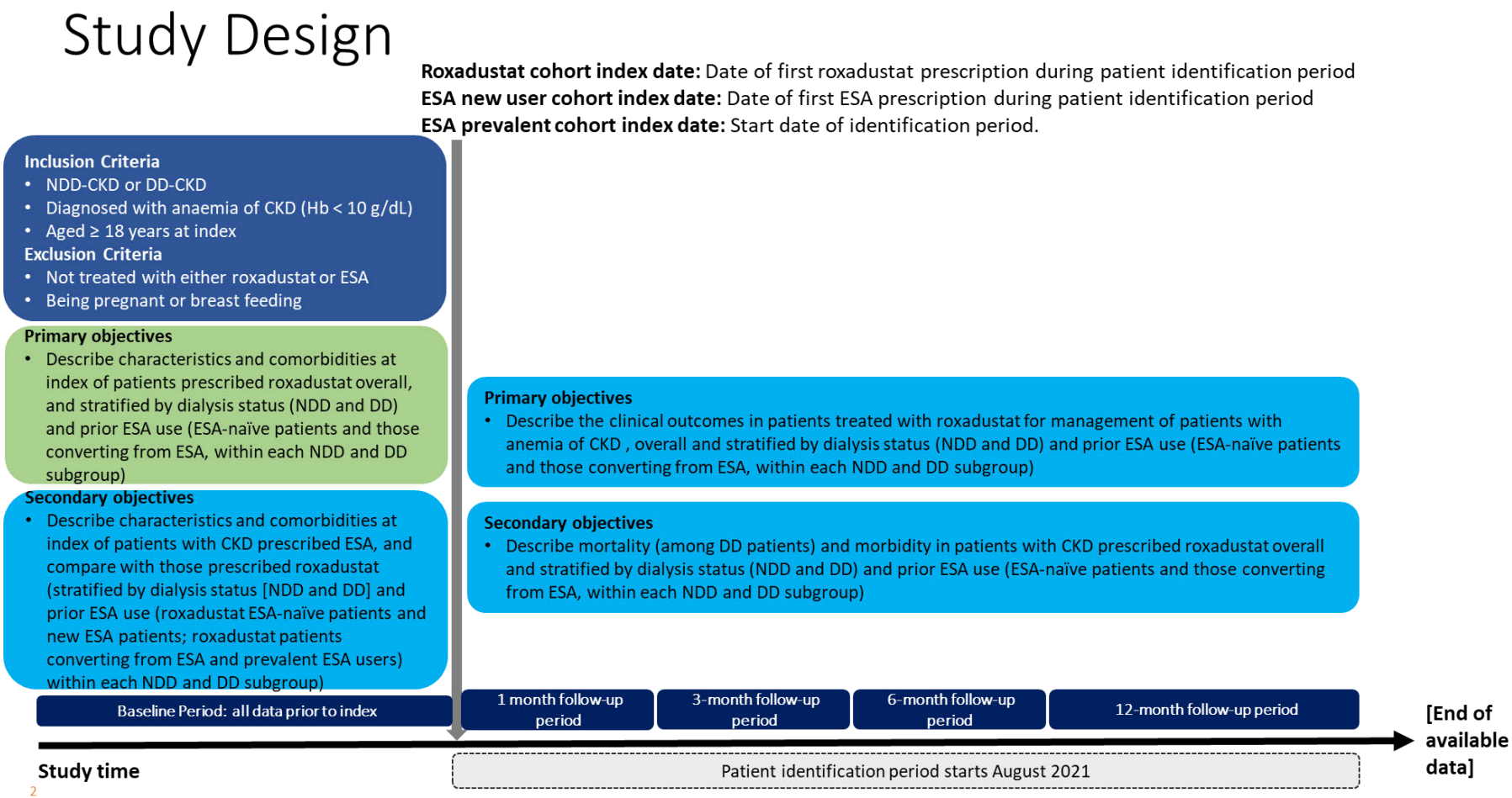
Discontinuation or initiation of rescue therapy with ESAs is expected to have a direct effect on Hb. Therefore, for the analyses on clinical outcomes and mortality in the roxadustat exposure group (primary objective 1.2 and secondary objective 2.1), the follow-up period will additionally be censored on the time of initiation of rescue therapy with ESA (including switching to ESA), discontinuation of roxadustat, and outcome of interest. Discontinuation of roxadustat will be defined as the stop date recorded for roxadustat in the WiNE registry. Initiation of rescue therapy with ESA will be determined by the record of ESA prescription that occurs after the commencement of roxadustat treatment, and the prescription date will be considered the initiation date. A switch to ESA will be defined as documented discontinuation of roxadustat followed by ESA prescription.

6.1.1 Study Schematic

Figure 1 illustrates the methodological framework of this study. The index date will serve as a pivotal reference point and is defined as the date of the first recorded prescription for either roxadustat or ESA from 18 August 2021 to 6 months before the latest available data (exact study period to be defined upon data receipt). This date marks the commencement of the study period for establishing patient eligibility and baseline characteristics.

Two distinct cohorts will be formed from the primary cohort of adult patients with anemia of CKD: the roxadustat exposure group and the ESA exposure group. The roxadustat exposure group will include patients who received at least one prescription for roxadustat during the identification period and will be stratified by dialysis status into NDD and DD and within those subgroups further stratified by ESA use DD-ESA-naïve, DD-ESA-converting, NDD-ESA naïve and NDD-ESA converting . Similarly, the ESA exposure group will comprise patients who received at least one ESA prescription and had no roxadustat prescriptions during the identification period and will be stratified by dialysis status and new or prevalent ESA use . The definitions of these subgroups and index dates are defined in the following section.

Figure 1. Study Schematic



Abbreviations: CKD = chronic kidney disease; DD = dialysis dependent; ESA = erythropoiesis-stimulating agents; NDD = non-dialysis dependent

6.2 Data Sources

The data source that will be used in this study is the German WiNe registry, an extensive electronic medical records database of patients with CKD attending outpatient nephrology clinics across Germany beginning in 2007. The registry contains over 27,000 patients with varying stages of CKD. Approximately 55% of patients in the registry have NDD CKD, and 45% have DD CKD. The database captures information on diagnoses, treatments (Anatomic Therapeutic Chemical [ATC] codes), and lab results. Patients are typically followed up every 3 months, and the registry database is updated every quarter. In compliance with the registry's guidelines and the ethical framework of our research, the WiNe Institute affirms that the patient data collected in this study will be entirely anonymous. Consequently, this precludes any attempts by the WiNe Institute to draw conclusions about or inquire into the specific medical history of any patient based on this anonymized data.

The majority of the variables of interest for this study are documented in the registry. Patient characteristics (including comorbidities), laboratory data, and medications are all reported. Notable limitations of the data include a lack of mortality data (available for DD patients only, with no cause of death), limited data on clinical events of special interest, and no information on RBC transfusions. No linkage to other sources of data is available. To identify clinical events of special interest, recorded diagnoses based on International Classification of Diseases, 10th Revision (ICD-10) codes will be used to pinpoint relevant conditions that occurred after the administration or prescription of the treatment. As of November 2023, there are 180 patients with CKD-related anemia who are prescribed roxadustat in the WiNe registry.

6.3 Study Population

6.3.1 Selection Criteria

Inclusion Criteria:

1. Patients to be included in this study must satisfy all of the following criteria:
2. Diagnosis of CKD (all stages)
3. Receiving a prescription for roxadustat or ESA during identification period
4. Age ≥18 years at time of roxadustat or ESA prescription

Exclusion Criteria:

1. Being pregnant or breastfeeding at time of roxadustat or ESA prescription

6.3.2 Patient Selection

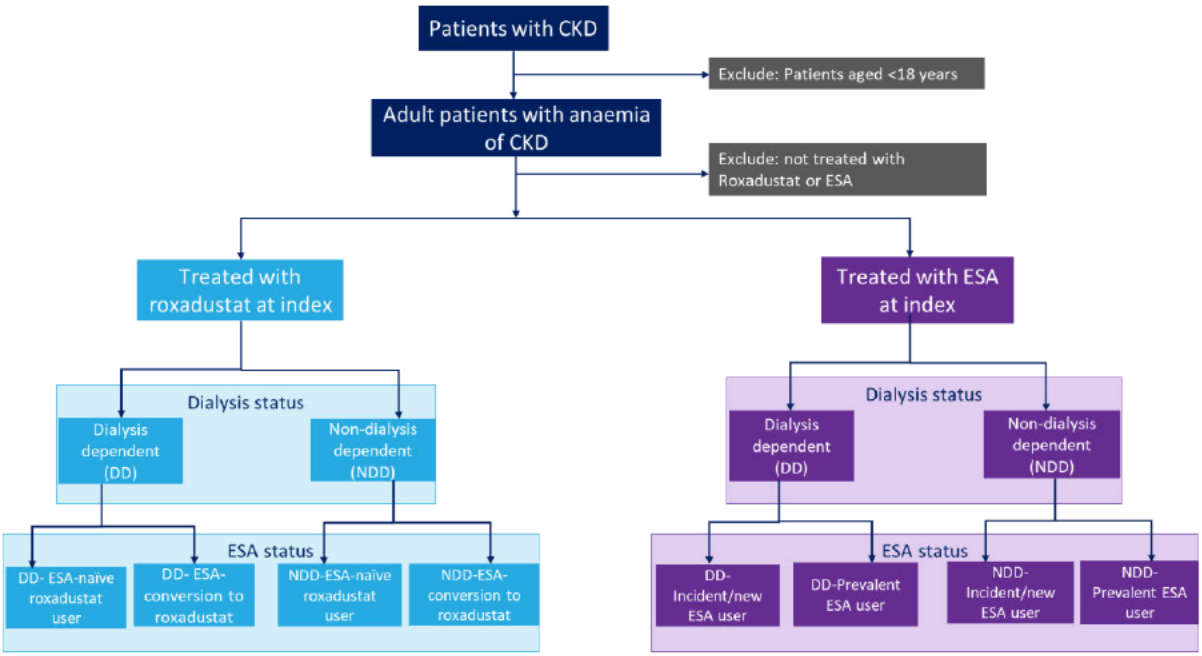
From the patients in the WiNe database who meet the eligibility criteria outlined in Section 6.3.1, participants will be selected for inclusion in the primary cohort of adult patients being treated for anemia of CKD.

From the primary cohort, patients will be stratified based on their prescription records during the identification period into 2 exposure groups:

- **Roxadustat exposure group**: Patients who have at least one prescription for roxadustat during the identification period. This group will also be further stratified as follows:
    - NDD patients: defined as patients with no history of dialysis at or before the first roxadustat prescription. Within the NDD subgroup, patients were further stratified by ESA status, as follows:
        - NDD ESA-naïve roxadustat user: defined as patients with no record of ESA prescription before the first roxadustat prescription.
        - NDD ESA conversion to roxadustat: defined as patients with at least one prescription for ESA before the first roxadustat prescription.
    - DD patients: defined as patients with a history of dialysis at or before the first roxadustat prescription. Within the DD subgroup, patients were also further stratified by ESA status, as follows:
        - DD ESA-naïve roxadustat user: defined as patients with no record of ESA prescription before the first roxadustat prescription
        - DD ESA conversion to roxadustat: defined as patients with at least one prescription for ESA before the first roxadustat prescription.
- **ESA exposure group**: Patients who have at least one prescription for ESA and no prescription of roxadustat during the identification period. The group will also be stratified as follows:
    - NDD patients: defined as patients with no history of dialysis at or before the first ESA prescription during the identification period. Within the NDD subgroup, patients were further stratified by ESA status, as follows:
        - NDD incident/new ESA user: defined as patients with no prescription for ESA before the first prescription of ESA during the identification period.
        - NDD prevalent ESA user: defined as patients with at least one prescription for ESA before the first prescription for ESA during the identification period.
    - DD patients: defined as patients with a history of dialysis (i.e., any record of dialysis) at or before the first ESA prescription during the identification period. Within the DD subgroup, patients were further stratified by ESA status, as follows:
        - DD incident/new ESA user: defined as patients with no prescription for ESA before the first prescription of ESA during the identification period.
        - DD prevalent ESA user: defined as patients with at least one prescription for ESA before the first prescription for ESA during the identification period.

No random selection will be applied in the cohort selection process, as the aim is to capture data reflective of real-world clinical practices from the WiNe registry.

Figure 2. Patient Selection



Abbreviations: CKD = chronic kidney disease; ESA = erythropoiesis-stimulating agents

Index dates:

- **Roxadustat users:** The index date is the date of the first roxadustat prescription recorded on or after 18 August 2021 (date of EMA approval of roxadustat).
- **ESA new users:** The index date is the date of the first ESA prescription recorded on or after 18 August 2021.
- **ESA prevalent users:** The index date is set as 18 August 2021. It is expected that patients who are prevalent users of ESAs will have a longer follow-up duration. We will evaluate the available follow-up time for these patients. Should there be a significant disparity in the follow-up duration, we may consider different methods to assign the index date in the prevalent ESA user group.

6.4 Variables

Table 3. Study Variables

Variable	Measurement	Definition	Period of Interest	Characterization
Exposure				
Exposure group	• Roxadustat group: at least one record of a roxadustat prescription during the identification period	ATC code: B03XA05 or name of drug, as recorded in the WiNe registry	Cohort identification period	Categorical: • Roxadustat • ESA

Variable	Measurement	Definition	Period of Interest	Characterization
	• ESA treatment group: a record of ESA prescription after 18 August 2021	ATC code: B03XA01, B03XA02, B03XA03, or name of drug as recorded in the WiNe registry		
Dose of exposure	Prescribed dose of first prescription for roxadustat or ESA during identification period (for the roxadustat and ESA exposure groups, respectively)	Prescribed dose at first prescription for roxadustat or ESA during identification period. This will be initiation dose for roxadustat patients.	Cohort identification period	Continuous
Stratifier				
Dialysis status	A stratifier based on whether patients had dialysis at index or during baseline, as recorded in the WiNe registry. • DD at baseline • NDD at baseline	Classification of patients as either DD or NDD at baseline based on dialysis status recorded in the WiNe registry	Baseline	Categorical: • DD • NDD
Dialysis type	Among DD patients, description of dialysis type (peritoneal dialysis or hemodialysis) at baseline		Baseline	Categorical: • Peritoneal dialysis • Hemodialysis
Prior ESA status for the roxadustat exposure group	The roxadustat exposure group will be categorized based on prior exposure to ESA into ESA-naïve or ESA-converting subgroups: • ESA-naïve: defined as no record of ESA prescription before first roxadustat prescription • ESA-converting:	ATC code: B03XA01, B03XA02, B03XA03, or name of drug as recorded in the WiNe registry	Baseline	Categorical: • ESA-naïve • ESA-converting

Variable	Measurement	Definition	Period of Interest	Characterization
	defined as at least one prescription for ESA before first roxadustat prescription			
ESA user type, for the ESA exposure group	The ESA exposure group will be split into 2 subgroups based on history of ESA prescription before 18 August 2021: • ESA new users: patients with no prior records of ESA prescription • ESA prevalent: patients with at least one prior record of ESA prescription	ATC code: B03XA01, B03XA02, B03XA03, or name of drug, as recorded in the WiNe registry	Baseline	Categorical: • ESA new users • ESA prevalent
Variables for demographic and clinical characteristics at baseline				
Age	Patient age at the time of first prescription (index date)	–	At index	Continuous Categorical: • <65 years • 65 – 74 years • ≥75 years
Sex	Biological sex as designated at birth	–	At index	Categorical: • Male • Female
BMI	BMI recorded closest to index date during baseline period	–	Baseline (closest value to index date in case of multiple records)	• Underweight: <18.5 • Healthy weight: 18.5 to <25 • Overweight: 25 to <30 • Obesity: ≥30
Primary cause of CKD	Underlying etiology of CKD recorded in WiNe registry	No coding. Primary cause of CKD as directly recorded in WiNe registry	Baseline	Categorical: • Diabetic nephropathy • Vascular nephropathy • Glomerular nephropathy • Interstitial nephropathy

Variable	Measurement	Definition	Period of Interest	Characterization
				• Cystic kidneys • Systemic diseases • Other nephropathies Note: specific categories will be confirmed after inspection of the relevant field in the WiNe database
Time since CKD diagnosis	Time from CKD diagnosis to the index date	–	Baseline	Continuous (days)
CKD stage	CKD stage closest to index date during baseline period recorded in WiNe registry	In the WiNe registry NDD CKD stages are classified based on KDIGO CKD stages.[48] DD are assigned to CKD5	Baseline	Categorical: • CKD-3 (including CKD-3a and CKD-3b) • CKD-4 • CKD-5
eGFR	eGFR recorded in WiNe registry, closest to index date during baseline period	In the WiNe registry eGFR is reported using the MDRD equation[49]	Baseline	Continuous and categories • ≥90 • 60–89 • 45–59 • 30–44 • 15–29 • <15
Albumin/ creatinine ratio	Albumin/ creatinine ratio measurement recorded closest to index date during baseline period	Albumin/ creatinine ratio as recorded in the WiNe registry	Baseline	Categories: • A2 (30–299 mg/g OR 3–30 mg/mmol) • A3 (≥300 mg/g OR >30 mg/mmol)
CRP	CRP measurement recorded closest to index date during baseline period	–	Baseline	Continuous
ESA dose at the time of switching to roxadustat	dose of ESA closest to index date during baseline period among patients converting from ESA	The last dose of ESA before initiation of roxadustat	Baseline	Continuous

Variable	Measurement	Definition	Period of Interest	Characterization
Hb level	Hb measurement recorded closest to index date during baseline period	–	Baseline	Continuous and categorical: • <10 g/dL • 10–12 g/dL • >12 g/dL
Ferritin	Ferritin measurement recorded closest to index date during baseline period		Baseline	Continuous
Serum iron	Serum iron measurement recorded closest to index date during baseline period		Baseline	Continuous
TSAT	TSAT iron measurement recorded closest to index date during baseline period		Baseline	Continuous
TIBC	TIBC measurement recorded closest to index date during baseline period		Baseline	Continuous
PTH	PTH measurement recorded closest to index date during baseline period		Baseline	Continuous
Oral iron treatment	At least one record of oral iron prescription 3 months prior or at index date	ATC: B03AB or name of drug with route of administration	Baseline	Categorical: • Yes • No
IV iron treatment	At least one record of oral iron prescription 3 months prior or at index date	ATC: B03AC or name of drug with route of administration	Baseline	Categorical: • Yes • No
Diabetes	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: E10-E14 or ATC code of antidiabetic medication: A10	Baseline	Categorical: • Yes • No
Hypertension	Registration of at least one ICD-10 code specified in definition column at any position	ICD-10: I10-I15	Baseline	Categorical: • Yes • No

Variable	Measurement	Definition	Period of Interest	Characterization
	during baseline period			
Heart failure	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: I110, I130, I132, I50	Baseline	Categorical: • Yes • No
Ischemic heart disease	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: I200, I21-I22, I201, I208, I209, I24, I25	Baseline	Categorical: • Yes • No
Stroke	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: I61, I63, I64	Baseline	Categorical: • Yes • No
PAD	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: I70, I72, I73	Baseline	Categorical: • Yes • No
Pulmonary embolism	Registration of at least one ICD-10 code during follow-up period	ICD-10: I802, I743, I829, I803, I828, I801, I800, I809, I808, I819, I822	Baseline	Categorical: • Yes • No
Deep vein thrombosis	Registration of at least one ICD-10 code during follow-up period	ICD-10: I80.1, I80.2, I80.3, I26	Baseline	Categorical: • Yes • No
Vascular access thrombosis	Registration of at least one ICD-10 code during follow-up period	ICD-10: T82. 868A	Baseline	Categorical: • Yes • No
Seizures	Registration of at least one ICD-10 code during follow-up period	ICD-10: G40 G41, R568	Baseline	Categorical: • Yes • No
Sepsis	Registration of at least one ICD-10 code during follow-up period	ICD-10: 038.xx, 790.7	Baseline	Categorical: • Yes • No

Variable	Measurement	Definition	Period of Interest	Characterization
Serum potassium	Potassium level recorded closest to index date during baseline period		Baseline	Continuous
Hyperkalemia at baseline	Potassium level of >5.5 mEq/L recorded closest to index the date during baseline period		Baseline	Categorical: • Yes • No
Cancer	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: C00-C97	Baseline	Categorical: • Yes • No
Chronic inflammatory disease	Registration of at least one ICD-10 code specified in definition column at any position during baseline period	ICD-10: • Psoriasis: L40.X except L405xx • Psoriatic arthritis: L405, M07.0-M07.3 • Rheumatoid arthritis, gout arthropathy: M05, M06, M10, M140, M315, M353 • Inflammatory bowel disease: M074, M075, M076, K50, K51 • Systemic lupus erythematosus: M32.9	Baseline	Categorical: • Yes • No
Renal transplant	Transplantation history as recorded in WiNe registry	–	Baseline	Categorical: • Yes • No
Medications				
RAAS inhibitors	At least one record of prescription 3 months prior or at index date	ATC code: C09A, C09B, C09C, C09D, C09X, or name of drug as recorded in the WiNe registry	Baseline	Categorical: • Yes • No

Variable	Measurement	Definition	Period of Interest	Characterization
SGLT2 inhibitors	At least one record of prescription 3 months prior or at index date	ATC code: A10BK01, A10BK02, A10BK03, A10BK04, A10BD15, A10BD19, A10BD20, A10BD21, A10BD23, A10BD24, or name of drug as recorded in the WiNe registry	Baseline	Categorical: • Yes • No
VARIABLES AT FOLLOW-UP				
Length of follow-up	Time in months	The duration of time from the index date to the end of follow-up period for each patient	From index date to the end of follow-up	Continuous
roxadustat dose over time	doses at monthly intervals from index date (e.g. 1, 2, 3, 4…months)	Dose over time refers to the quantification of roxadustat as recorded in WiNe registry at regular monthly intervals starting from the index date throughout the follow-up period.	Follow-up	Continuous
roxadustat maintenance dose	The first dose of roxadustat during follow-up that remains stable for three consecutive months	Stable roxadustat dose over 3 consecutive months	Follow-up	Continuous
Clinical outcomes				
Event: Hb target threshold achieved	Hb measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment)*	Whether the patient meets Hb target of ≥10 g/dL and ≤12 g/dL during the follow-up period	Follow-up	Categorical: • Yes • No
Time to Hb target	Date of first Hb measurement within range of ≥10 g/dL and ≤12 g/dL during the follow-up period	Time in days from index	Follow-up	Continuous (days)

Variable	Measurement	Definition	Period of Interest	Characterization
Hb	Hb measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment)*	Time points can be revised after data review	Follow-up	Continuous
Serum iron	Serum iron measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment)*	Time points can be revised after data review	Follow-up	Continuous
Ferritin	Ferritin measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment)*	Time points can be revised after data review	Follow-up	Continuous
TSAT	TSAT iron measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment)*	Time points can be revised after data review	Follow-up	Continuous
TIBC	TIBC measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment)*	Time points can be revised after data review	Follow-up	Continuous
CRP	CRP measurement recorded after index date at specific intervals (e.g., 1, 3, 6, and 12 months of treatment) *	Time points can be revised after data review	Follow-up	Continuous
Time to first IV iron treatment	Date of first IV iron prescription / administration after index date	Time from index date to the first IV iron prescription/ administration, as defined by ATC code B03AC or	Follow-up	Continuous

Variable	Measurement	Definition	Period of Interest	Characterization
		name of drug with route of administration		
IV iron treatments	Total count of IV iron prescriptions/ administrations after index date	Count of all longitudinal prescriptions/ administrations of IV iron, as defined by ATC code B03AC or name of drug with route of administration	Follow-up	Continuous
Dose of IV iron	Dose of each IV iron perception	Mean dose of IV iron (across all patients receiving IV iron)	Follow-up	Continuous (mean)
Time to rescue therapy with ESA	Date of first ESA prescription during follow-up among roxadustat patients	ATC: B03XA01, B03XA02, B03XA03, or name of drug, as recorded in the WiNe registry	Follow-up	Time to event (continuous)
Clinical events of interest				
Death	Death of DD patients recorded	Whether the DD patient died during follow-up	Follow-up	Categorical: • Yes • No
Time to All-cause death	Date of death during follow-up (for DD patients)	Time from index to death	Follow-up	Time to event
Hyperkalemia	First hyperkalemia measurement of >5.5 mEq/L during follow-up period	–	Follow-up	Categorical: • Yes • No
Stroke	Registration of at least one ICD-10 code during follow-up period	ICD-10: I63	Follow-up	Categorical: • Yes • No
Pulmonary embolism	Registration of at least one ICD-10 code during follow-up period	ICD-10: I802, I743, I829, I803, I828, I801, I800, I809, I808, I819, I822	Follow-up	Categorical: • Yes • No
Deep vein thrombosis	Registration of at least one ICD-10 code during follow-up period	ICD-10: I80.1, I80.2, I80.3, I26	Follow-up	Categorical: • Yes • No
Vascular access thrombosis	Registration of at least one ICD-10 code during follow-up period	ICD-10: T82. 868A	Follow-up	Categorical: • Yes • No

Variable	Measurement	Definition	Period of Interest	Characterization
Seizures	Registration of at least one ICD-10 code during follow-up period	ICD-10: G40 G41, R568	Follow-up	Categorical: • Yes • No
Sepsis	Registration of at least one ICD-10 code during follow-up period	ICD-10: 038.xx, 790.7	Follow-up	Categorical: • Yes • No

Abbreviations: ATC = Anatomical Therapeutic Chemical; BMI = body mass index; CKD = chronic kidney disease; CRP = C-reactive protein; DD = dialysis dependent; eGFR = estimated glomerular filtration rate; ESA = erythropoiesis-stimulating agents; Hb = hemoglobin; ICD-10 = International Classification of Diseases, 10th Revision; IV = intravenous; KDIGO = Kidney Disease: Improving Global Outcomes; MDRD = Modification of Diet in Renal Disease; NDD = non-dialysis dependent; PTH = parathyroid hormone; PAD = peripheral artery disease; RAAS = renin-angiotensin aldosterone system; SGLT2 = Sodium-glucose cotransporter-2; TIBC = total iron-binding capacity; TSAT = transferrin saturation; WiNe = Wissenschaftliches Institut für Nephrologie

*Specific time points, e.g., 1 month, 3 months, 6 months, and 12 months of treatment, to be determined and contingent upon data availability

6.5 Data Management

Data management will be in accordance with Evidera's standard operating procedures (SOPs) and will adhere to the minimum standards of Astellas. In particular, Evidera will collect deidentified electronic patient records from the WiNe registry in Germany. Routine procedures will include checking electronic files, maintaining security and data confidentiality, following analysis plans, and performing quality control checks of all programs.

The WiNe registry will maintain any patient-identifying information securely on site according to internal SOPs. Evidera will maintain the data cuts according to internal procedures.

An Evidera data analyst will write and review programs to implement the analyses outlined in Section 6.6. The project team will review all data outputs, including SAS® code as needed. Changes and corrections to programs stemming from the review will be made as appropriate. All programs will be saved, and the process documented. Security processes will be in place to ensure the safety of all systems and data.

6.6 Statistical Methods

6.6.1 Sample Size Justification

For the primary and secondary objectives, sample size will be determined from the WiNe registry. Data for all patients with CKD-related anemia who are being treated with roxadustat or ESA and satisfy the selection criteria specified in Sections 6.3.1 will be obtained from the WiNe registry between August 2021 and 6 months before the end of available data (identification period). As of November 2023, there are 180 patients with CKD-related anemia who are prescribed roxadustat in the WiNe registry.

6.6.2 Precision of the estimates

The group sample size considerations in the table below show the precision (smaller is better) achieved for binary outcomes such as the response rate.

	Precision Proportion (P)				
Sample Size	**P=10% or 90%**	**P=20% or 80%**	**P=30% or 70%**	**P=40% or 60%**	**P=50%**
25	12.2	15.1	16.9	17.9	18.2
50	8.5	10.9	12.3	13.1	13.4
75	6.9	9	10.2	10.8	11
100	6	7.8	8.8	9.4	9.6
125	5.3	7	7.9	8.5	8.6
150	4.8	6.4	7.3	7.7	7.9
200	4.2	5.5	6.3	6.7	6.9
250	3.7	4.9	5.6	6	6.2
300	3.4	4.5	5.2	5.5	5.6
375	3	4	4.6	4.9	5
500	2.6	3.5	4	4.3	4.4
625	2.4	3.1	3.6	3.8	3.9
750	2.2	2.9	3.3	3.5	3.6

The precision is calculated as the half width of the two-sided 95% confidence intervals using the Wilson (score) method for binomial proportions. The precision improves with higher sample sizes and more extreme proportions of outcome events (e.g., as the proportion gets closer to 0% or 100% from the 50% midpoint). For instance, the absolute precision assuming a sample size of 50 patients in the group would be 8.5%, 10.9%, and 12.3% when the response rate is 90%, 80%, and 70%, respectively. Assuming a total sample size of 200 patients would achieve an overall absolute precision ranging from 4.2% to 6.9% for an observed outcome proportion ranging from 90% to 50% (or alternatively from 10% to 50%).

6.6.3 Statistical Analysis

Data analysis will be performed by Evidera in accordance with Evidera’s SOP for statistics and clinical programming. All study specific processes and definitions will be documented.

Primary objective:

- The demographics and clinical characteristics of patients prescribed roxadustat, assessed during the baseline period, will be reported descriptively for the entire cohort and stratified by dialysis status (NDD and DD) and prior ESA use (within each NDD and DD subgroup), as follows:
    - NDD patients:
        - NDD ESA-naïve roxadustat user
        - NDD ESA conversion to roxadustat
    - DD patients:
        - DD ESA-naïve roxadustat user
        - DD ESA conversion to roxadustat

  Continuous variables will be summarized as mean standard deviations (SD), median, interquartile range, minimum, and maximum, as appropriate. Categorical variables will be presented as frequencies and percentages for the total patient sample and by subgroups.
- Length of follow-up will be captured by computing the duration each patient is followed in the study. This will be summarized using descriptive statistics [mean, SD, median, interquartile range (IQR) and min and max].
- Duration of exposure will be summarized using descriptive statistics (mean, SD, median, IQR and min and max).
- ESA dose at the time of conversion to roxadustat on index will be summarized using group-level statistic (mean, SD, median, IQR and min and max).
- For ‘roxadustat dose over time ', we will compute the frequency and dosage of roxadustat at monthly intervals from the index date. Group-level statistics will be used calculating the mean and median dosages for the study population over time. Appropriate graphical representations, such as box-whisker plot or similar, will be used to visualize the distribution and central tendencies of these variables.
- The roxadustat maintenance dose will be determined as the first dose of roxadustat during the follow-up period that remains stable over three consecutive months. This will involve analyzing the frequency and dosage of roxadustat at monthly intervals after the index date. The variable will be summarized using group-level statistic (mean, SD, median, IQR and min and max).
- Time to meet Hb target threshold (≥10 g/dL) will be estimated using Kaplan-Meier methods for the overall sample and stratified by dialysis status and prior ESA use. Only patients with a Hb value below the target threshold at index date will be included in the analysis. Patients will be followed from index until treatment discontinuation (including crossover to ESA), death, or end of study period (to be determined), whichever comes first.
- The proportion of patients achieving Hb targets at specific intervals (1, 3, 6, and 12 months since index) will be reported.
- Mean change in Hb and estimated glomerular filtration rate from baseline over specific intervals will be analyzed by linear mixed model for each of the exposure groups and

patient's baseline characteristics will be used as covariates in the model. To account for the repeated measures within individuals over time and potential within-patient correlation, a random intercept for each patient will be included.

- Other relevant markers (ferritin, transferrin saturation, total iron-binding capacity, serum iron levels, CRP) over follow-up will be summarized by mean (SD) at prespecified timepoints (currently assumed to be 1, 3, 6, and 12 months since index, but timepoints may be revised upon inspection of the data).
- Time to first IV iron treatment will be estimated using Kaplan-Meier methods. Details of the first IV iron administration (mean IV iron dose) and number of IV iron administrations during follow-up will be assessed by descriptive statistics of the incidence rate of IV iron administrations per patient/year.
- The proportion of patients in the roxadustat group who require therapy with ESA during the follow-up period will be reported.

Secondary objectives:

- Incidence rate of hyperkalemia, death, and clinical events will be reported during the entire follow-up period for the overall sample and stratified by a 2x2 matrix of subgroups (dialysis status and prior ESA use). Time to death for DD patients will be estimated using the Kaplan-Meier method for the overall sample and stratified by the dialysis status and prior ESA use. Using the on-treatment approach to estimate mortality, patients will be followed from index until treatment discontinuation (including crossover), outcome of interest (i.e. hyperkalemia, death and specific clinical events), or end of study period (to be determined), whichever comes first.

Handling of missing data

For variables related to disease conditions, medical procedures, and medication usage, the absence of a registered diagnosis, procedure code, or prescription or administration in the dataset will be interpreted as an indication that the patient does not have the respective condition, did not undergo the specified procedure, or is not using the mentioned medication. This approach assumes that if such events or conditions were present/did occur, they would have been recorded in the patient's medical records. Descriptive statistics will include a missing category where relevant.

6.7 Quality Control

SOPs and guidelines will be used to guide the conduct of the study. These procedures include internal quality audits, rules for secure and confidential data handling, methods to maintain and archive project documents, quality control procedures for programming, standards for writing analysis plans, and requirements for senior scientific review. Key programming modules written by a study analyst will be independently reviewed by a different analyst. Procedures will be consistent with the International Society for Pharmacoepidemiology Guidelines for Good Pharmacoepidemiology Practices.

All work will be subject to quality control and documentation procedures to make certain that the final report is accurate and thorough and that the analyses can be reproduced. All key

study documents, such as the analysis plan, abstraction forms, and study reports, will undergo quality control review, senior scientific review, and editorial review.

6.8 Strengths and Limitations of the Research Methods

- **Small sample size for roxadustat group**: A limitation is the expected small sample size for the roxadustat group. This may limit the robustness of our findings and affect the statistical power of the analyses, especially in subgroup evaluations.
- **Limited data on mortality and clinical events in NDD patients**: The absence of robust data concerning mortality and potential clinical events for NDD patients poses a challenge to the accurate assessment of these outcomes in this subgroup.
- **Assumption regarding RBC transfusion**: Our analysis will assume that patients have not received rescue therapy via RBC transfusion. The lack of information on RBC transfusions could potentially influence the interpretation of anemia management.
- **Stratified analysis limitations**: Stratified analyses based on dialysis status and prior ESA use are contingent on having a sufficient number of patients in each subgroup. The likelihood of smaller sample sizes in these subgroups may impact the reliability and applicability of our findings.
- **Reliance on registry coding accuracy**: The accuracy of the study data is dependent on the precision of diagnostic, procedure, and medication coding in the registry. Coding inconsistencies or errors could affect data reliability.
- **Routine clinical practice data**: The data will reflect routine clinical practice, rather than mandatory assessments at prespecified timepoints; therefore, time intervals of assessment might have to be revised upon inspection of the data.
- **Challenges with retrospective registry data**: Retrospective identification of patient cohorts from registry data inherently limits the ability to infer causal relationships. For example, clinical events are recoded as conditions developed by patients, and there is no direct association with any treatment received.
- **Missing data and generalizability issues**: Missing data, particularly for comorbidities and clinical events coded with the ICD-10, can result in a loss of power and sampling bias, potentially limiting the generalizability of study results.
- **Noninformative censoring assumption**: Our survival analyses assume noninformative censoring of time-to-event outcomes. Informative censoring, such as patients with more severe symptoms being lost to follow-up, could introduce bias.
- **Potential confounding in observational study**: As with all observational studies, there is a risk of unmeasured confounding. While we aim to address confounding through stratification, our ability to do so is limited to the variables recorded in the data and may be further constrained by the sample size.

7 PROTECTION OF PARTICIPANTS

This study will be conducted in compliance with national and EU requirements to ensure the participants rights in non-interventional studies.

7.1 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)/Competent Authorities (CA)/Regulatory Authority (RA)

Due to the study's retrospective nature, no IEC approval will be sought.

7.2 Ethical Conduct of the Study

This study will be conducted in compliance with all applicable requirements in Germany and EU for ensuring the rights of participants in non-interventional studies.

A SAP will be developed based on the final protocol. This document will be developed to address the structure and data content within the specific databases. The SAP will be finalized prior to data access. Documentation will clearly list the role(s) of each responsible party.

7.3 Participant Information and Consent

The data collection in this study is based on a pre-existing registry from representative patient populations and involves the secondary use of existing data, routinely collected in standard treatment and other processes. The Sponsor and/or any service provider working with the Sponsor for this study will receive only deidentified data and as a result the Sponsor does not receive access to directly identifying patient-level data at any time of the study.

7.4 Participant Confidentiality

During this study using secondary data, all applicable data protection and privacy regulations will be observed in collecting, forwarding, processing, and storing participant's data.

The Sponsor maintains confidentiality standards by ensuring that the Sponsor does not receive from those pre-existing registers and/or pre-existing data sources names or other directly identifying personal information of participants in any case report forms (CRFs) or other documents submitted to the Sponsor (unless this is required by law for reporting of AEs). This is achieved by using at inclusion codes or other random identification numbers to serve as the participant's identifiers in the study, as well in the study database retained by the Sponsor.

In some cases, a service provider may get access to study participants data but only within the controlled environment of the data source and/or the register concerned and always in conformity with all security and SOPs of the data owner. However, the Sponsor will not receive access to patient-level data. Only aggregated results will be presented to the Sponsor.

The Sponsor will use the data collected in order to run the study and to use and publish the results of the study. However, all study reports will contain aggregate data only and will not identify any individual participants.

8 MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

8.1 Secondary Data Collection

Reporting to Astellas Pharmacovigilance is not required for AEs which are observed in the secondary data source.

8.2 Notification of New Adverse Events (Serious and Nonserious) by Study Personnel to Sponsor

If during the conduct of the study, a new AE, special situation, or pregnancy is reported (e.g. study staff is made aware of a participant having an event on an Astellas product) the event should be sent to Astellas Pharmacovigilance. Reporting to Astellas Pharmacovigilance is not required for non-Astellas drugs unless the drug is a co-suspect drug for an Astellas drug AE or if it is related to the study objective.

Any AE associated with an Astellas product encountered in the data used for this study will be collected and listed in the final study report.

9 PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The study results will be disseminated and communicated in the following ways:

- A study report summarizing the study methods and results will be issued.
- The results will be considered for dissemination in the form of scientific publications (e.g., abstracts for scientific congress and/or manuscript for submission to a peer-reviewed journal). Publications will comply with internal Astellas standards and the International Committee of Medical Journal Editors guidelines.

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ANNEX 1 LIST OF STAND-ALONE DOCUMENTS

None

ANNEX 2 NON-INTERVENTIONAL STUDY CONTINUITY

Not applicable

ANNEX 3 ENCEPP CHECKLIST FOR STUDY PROTOCOLS

http://www.encepp.eu/standards_and_guidances/checkListProtocols.shtml

ANNEX 4 DEFINITIONS

DEFINITION OF ADVERSE EVENTS

An AE is defined as any untoward medical occurrence in a participant administered a study drug, and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product whether or not considered related to the medicinal (investigational) product.

In order to identify any events that may be associated with study assessments and could lead to a change in the conduct of the study, Astellas collects AEs even if the participant has not received study drug treatment.

An abnormality identified during a medical assessment is defined as an AE per the following criteria:

- Any abnormal laboratory test result (e.g., hematology, clinical chemistry, or urinalysis) or other safety assessment (e.g., ECGs, radiographic scans, vital signs measurements, physical examination), including those that worsen from baseline, that is considered to be clinically significant in the medical and scientific judgment of the investigator and not related to underlying disease, is to be reported as an AE/serious adverse event (SAE).
- Any clinically significant abnormal laboratory finding or other abnormal safety assessment which is associated with the underlying disease does not require reporting as an AE/SAE, unless judged by the investigator to be more severe than expected for the participant's condition.
- Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

DEFINITION OF SERIOUS ADVERSE EVENTS (SAES)

An AE is considered serious if, in the view of either the investigator or Sponsor, it:

- Results in death.
- Is life-threatening (an AE is considered "life-threatening" if, in the view of either the investigator or Sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death).
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital anomaly or birth defect of a child conceived during the exposure of one of the parents to the drug studied.

- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious).
- Is a medically important event or reaction.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in medically important events not on the Sponsor list, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These events, including those that may result in disability and/or incapacity, should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

The Sponsor has a list of events that they classify as “important medical events”. If an AE is reported that is considered to be an event per this classification as “important medical event”, additional information on the event may be requested.

11 SIGNATURES

INVESTIGATOR'S SIGNATURE

Not applicable

PROTOCOL APPROVED BY:

Protocol Approval Committee

Signature:

PPD

Date (DD Mmm YYYY)

Signature:

PPD

Date (DD Mmm YYYY)

Signature:

PPD

Date (DD Mmm YYYY)

Signature:

PPD

Date (DD Mmm YYYY)

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Date (DD Mmm YYYY)