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Official Title:	A non-blinded retrospective biomarker add-on study to **FIGARO-**DKD for **B**ioprofiling the phar**M**acodynamic response to finerenone in FIGARO-DKD subjects (**FIGARO-BM**)
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Title Page

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A non-blinded retrospective biomarker add-on study to **FIGARO-**DKD for **B**ioprofiling the phar**M**acodynamic response to finerenone in FIGARO-DKD subjects (**FIGARO-BM**)

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Acronym: FIGARO-BM

Sponsor Name: Bayer AG

Legal Registered Address:

Bayer AG, 51368 Leverkusen, Germany

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This is an electronically generated document that does not bear any sponsor signatures. The signature of the sponsor's medically responsible person is filed in the TMF and available on request.

Medical Monitor name and contact information will be provided separately.

Table of Contents

Table of Tables

1. Protocol Summary

1.1 Synopsis

Protocol Title:

A non-blinded retrospective biomarker add-on study to **FIGARO-**DKD for **B**ioprofiling the phar**M**acodynamic response to finerenone in FIGARO-DKD subjects (**FIGARO-BM**)

Brief Title: **FIGARO-BM**

Rationale:

A large body of evidence in animals demonstrates beneficial, anti-fibrotic and anti-inflammatory effects of finerenone treatment. In humans, persistent inflammation and fibrosis are characteristic features of chronic kidney disease and are independent predictors of disease progression. Yet, there is limited data in humans to support finerenone's mode-of-action (MoA) with regards to anti-fibrotic and anti-inflammatory activity. This analysis aims at contributing to a better understanding of the underlying mechanisms of drug efficacy and pharmacodynamics (PD) with particular focus on inflammation and fibrosis besides general bioprofiling and pathway analysis.

Objectives and Endpoints:

Objectives	Estimands/Endpoints
Primary	
• To investigate long-term effect of finerenone treatment, in addition to standard-of-care, on circulating blood biomarkers associated with fibrosis, congestion, inflammation and vascular function	• Change in plasma biomarker levels after 36 months (Visit 11) of treatment versus 4 months (Visit 3) of treatment in a set of 27 pre-defined biomarkers (provided in the SAP)

Overall Design:

FIGARO-BM (#21952) is a retrospective, non-interventional study to the multi-center, interventional Phase 3 trial FIGARO-DKD (#17530). Samples which had been collected in FIGARO-DKD will be analyzed by OLINK proteomics technology in FIGARO-BM. Samples for analysis in FIGARO-BM will be obtained from FIGARO-DKD trial sites with large enrollment numbers, from subjects who had been treated with finerenone or placebo for at least 24 months. Certain countries require a local protocol addendum to FIGARO-DKD (#17530) to conduct this planned biomarker analyses using OLINK technology, instead of a separate study protocol described in this document as FIGARO-BM (#21952). Biomarker data acquired under FIGARO-DKD #17530 will be pooled and reported together with biomarker data acquired under FIGARO-BM #21952.

Brief Summary:

Study #21952 in subjects with DKD will examine the mid- to long-term PD response to finerenone (vs placebo) after up to 36 months of treatment and will characterize relevant biological pathways which may be affected by treatment. The focus is on markers related to fibrosis and inflammation due to the mode-of-action of finerenone and preclinical and clinical evidence for finerenone and other MRAs.

Only existing stored biosample leftovers from FIGARO-DKD (#17530) will be used. No additional biological samples or data will be obtained from the subject, nor will any additional or new study intervention be introduced. No visit or patient contact other than for obtaining IC will be required.

Commercially available exploratory biomarker panels (OLINK Explore®) will be employed for analyses of the samples and all endpoints will be exploratory. OLINK Explore® couples antibody-array-based analyte-capturing with next generation sequencing (NGS) for analyte identification, thereby allowing simultaneous detection of hundreds of proteins and extensive bioprofiling of the response to finerenone.

Number of Participants for Reconsenting:

The targeted number of participants for final analysis is approximately 600 (or more) FIGARO-DKD patients (at a 1:1 randomization for finerenone versus placebo

Intervention Groups and Duration:

No new intervention will be administered in this retrospective biomarker study.

Data Monitoring/Other Committee:

Not applicable.

1.2 Schema

Not applicable.

1.3 Schedule of Activities (SoA)

- Obtain informed consent from former study participants of FIGARO-DKD to perform further analyses of existing biosamples (already collected in FIGARO-DKD).

2. Introduction

This protocol describes a biomarker add-on study to the Phase 3 trial FIGARO-DKD (#17530). While the original trial, FIGARO-DKD, investigated the efficacy and safety of finerenone (a next-generation, non-steroidal mineralocorticoid-receptor antagonist [MRA]) on the reduction of cardiovascular morbidity and mortality in subjects with type 2 diabetes mellitus and the clinical diagnosis of diabetic kidney disease in addition to standard of care, this study (#21952) will solely perform exploratory biomarker analyses. All analyses will be done on existing leftover samples from selected FIGARO-DKD patients. No additional biological samples or data will be collected, and no new or additional study intervention will be performed. The sponsor will obtain informed consent from former participants of FIGARO-DKD subjects to consent to this additional biomarker analysis on their sample leftovers. Biomarkers described in this protocol (analytes) will be analyzed by novel proteomics technology (OLINK Explore®) allowing quantitative measurements of a multitude of markers in a small sample volume.

2.1 Study Rationale

This study focuses on biomarkers of inflammation and fibrosis.

Persistent inflammation and fibrosis are characteristic features in patients with chronic kidney disease (CKD) and are independent predictors of disease progression. While a large body of evidence in animals demonstrates beneficial, anti-fibrotic and anti-inflammatory effects of finerenone treatment, there is limited data in humans to support the mode-of-action (MoA) with regards to anti-fibrotic and anti-inflammatory effects.

This analysis may contribute to a better understanding of the underlying mechanisms of drug efficacy and pharmacodynamics (PD) with regards to inflammation and fibrosis and will further knowledge of the biological pathways which underpin drug efficacy and PD. Due to the exploratory nature, this biomarker study is purely hypothesis-generating and will investigate the effect of treatment on biomarkers in the context of physiological and pathological pathway networks.

2.2 Background

2.2.1 Diabetic Kidney Disease (DKD)

Diabetic kidney disease (DKD) is a clinical syndrome affecting individuals with diabetes. It is defined by albuminuria on at least 2 occasions separated by 3 to 6 months and eGFR of less than 60 mL/min/1.73m^2 (1). DKD is the most frequent cause of end-stage renal disease (ESRD) in western countries (2). The risk of cardiovascular (CV) events and death increases in patients with DKD with decreasing glomerular filtration rate (GFR) and increasing albuminuria (3, 4). Patients with non–dialysis-dependent chronic kidney disease (NDD-CKD) are 10 times more likely to die of CV diseases (CVD) than the general population (5). As type 2 diabetes mellitus (T2DM) will rise rapidly throughout the world within the next years (6), and alongside DKD, there is an increasing need for new therapeutic agents that effectively target underlying disease mechanisms and slow or halt the progression of kidney disease, whilst also addressing the high CV morbidity and mortality in this population. Interventions to improve outcomes related to DKD focus on reducing risk, including counseling on lifestyle modifications (i.e. smoking cessation and dietary modifications to reduce proteinuria and aid in weight loss) and interventions aimed at glycemic control, dyslipidemia, and hypertension (7). Therefore, angiotensin-converting-enzyme inhibitors (ACEIs) and angiotensin-receptor

blockers (ARBs) are standard of care (SoC) therapy in patients with DKD and are often prescribed to patients with DKD at early stages (7). However, they slow but neither halt nor reverse kidney disease progression (8).

2.2.2 Mineralocorticoids Receptor Antagonists (MRAs) in Treatment of DKD and cardiovascular diseases

Long-term renin-angiotensin system (RAS) blockade with ACEIs and ARBs results in incomplete suppression of serum aldosterone levels. This is known as the '*aldosterone breakthrough*' phenomenon (9). It is associated with increased urinary albumin excretion and more rapid decline in estimated GFR (eGFR). This phenomenon is probably due to an increase in potassium and angiotensin serum levels in patients treated with ACEIs/ARBs, and it provides the scientific rationale for an additive benefit of mineralocorticoid-receptor (MR) blockade.

Inappropriate release of aldosterone contributes to organ damage found in chronic renal failure, heart failure (HF), myocardial infarction and hypertension. The MR is extensively expressed in the CV system including kidney mesangial cells, the heart, endothelial cells and vascular smooth muscle cells. The actual mechanisms behind the pathological effects of aldosterone in DKD have not yet been fully elucidated; however, aldosterone seems to be a key player in the progression of kidney disease. Chronically elevated aldosterone release participates in the generation of vascular, myocardial, and renal fibrosis, and eventually end-organ damage, dysfunction, and failure (10). The actual mechanisms behind the pathological effects of aldosterone in DKD have not yet been fully elucidated. Blockade of the action of aldosterone and potentially other MR ligands such as cortisol has been demonstrated to be of benefit in different forms of CVD (11). Steroidal MRAs currently on the market for the treatment of chronic heart failure (CHF) (spironolactone and eplerenone) have several disadvantages – particularly, an increased risk of hyperkalemia - which limit their use in clinical practice. None of the steroidal MRAs are currently approved for the treatment of patients with CKD as they have a high risk of hyperkalemia (12, 13). Finerenone is a novel, potent, and non-steroidal MRAs with superior selectivity vs. spironolactone in vitro and with improved potency and efficacy vs. eplerenone, which suggests an improved efficacy and safety profile and thus a potential treatment option for CKD in T2DM and HF (14).

2.2.3 Role of inflammation and fibrosis

By propagating inflammation and fibrosis, aldosterone plays a pivotal role in renal vascular damage. In hypertensive rat models, MRAs attenuated renal damage and supported the protective effect of aldosterone blockage in renal disease (15). Other preclinical data from ischemia models of AKI[1]-to-CKD progression in several species pointed towards a role of MRAs in polarizing macrophages to a less inflammatory phenotype. Along with these findings, kidney fibrosis was reduced (16). Finerenone was shown in animal models to act by targeting inflammatory and fibrotic pathways in the heart and kidney, reducing cardiac remodeling, and decreasing the release of inflammatory and profibrotic biomarkers (17).

2.3 Benefit/Risk Assessment

This study will only include patients who had participated in the FIGARO-DKD trial.

[1] AKI = Acute Kidney Injury

2.3.1 Risk Assessment

Participating in this study bears no foreseeable risk to the patient. There is no intervention, no blood nor data collected beyond what was already obtained during FIGARO-DKD.

2.3.2 Benefit Assessment

Patients with advanced kidney disease show high levels of fibrosis in the kidney. Therefore, data to substantiate anti-fibrotic activity of finerenone will open new treatment options for the patient beyond currently available treatment regimens which target other aspects of kidney pathophysiology.

This retrospective study offers the possibility to better characterize mid- to long-term PD effects of finerenone and to bioprofile the response to finerenone with particular focus on established aldosterone-driven, fibrosis- and inflammation-related pathways. The results of this study will be used to guide the decision on further development of finerenone by contributing to a better understanding of underlying mechanisms for better outcomes in future use of finerenone.

2.3.3 Overall Benefit: Risk Conclusion

The benefit-risk assessment is considered positive and justifies the conduct of this study within the selected cohort of patients with CKD and T2DM.

3. Objectives and Endpoints

The endpoints below all rely on data derived with an exploratory biomarker panel (OLINK technology) and hence, all analyses are considered exploratory in nature.

Objectives	Estimands/Endpoints
Primary	
• To investigate long-term effect of finerenone treatment, in addition to standard-of-care, on circulating blood biomarkers associated with fibrosis, congestion, inflammation and vascular function	• Change in plasma biomarker levels after 36 months (Visit 11) of treatment versus 4 months (Visit 3) of treatment in a set of 27 pre-defined biomarkers (provided in the SAP)
Other pre-specified	
• To characterize mid- to long-term PD effects of finerenone and profiling the response to finerenone (vs placebo) in patients with DKD to describe biological pathways	• Change in plasma biomarker levels after 12 months (Visit 5), 24 months (Visit 8) and 36 months (Visit 11), 48 months (Visit 14) [2] of treatment versus 4 months (Visit 3) of treatment
• To further investigate the study intervention and similar drugs (e.g. mode-of-action-related effects, safety) and to further investigate pathomechanisms deemed relevant to renal and cardiovascular diseases and associated health problems	• Change in various biomarkers (e.g. diagnostic, safety, pharmacodynamic, monitoring, prognostic or potentially predictive biomarkers)
• To assess relationship between pharmacokinetics of finerenone and PD effects	• Change in biomarker levels at 12 months (Visit 5), 24 months (Visit 8) and 36 months (Visit 11) of treatment compared to exposure

4. Study Design

4.1 Overall Design

FIGARO-BM (#21952) is a retrospective, non-interventional add-on study to the multi-center, interventional Phase 3 trial FIGARO-DKD (#17530). Blood plasma samples which were initially collected for pharmacokinetic (PK) analysis during the conduct of FIGARO-DKD study will be analyzed by OLINK proteomics technology (refer to Section 8.6 for details on biomarkers and intended technology) in FIGARO-BM.

This biomarker study will contact selected subjects from multiple centers in several countries who had participated in FIGARO-DKD and subjects will be re-consented for further biomarker analyses. Sites with a large number of subjects in FIGARO-DKD – preferentially with ~8 or more valid[3] subjects per site who were on treatment with finerenone (or placebo)

[2] Depending on availability of samples for visit

[3] Valid Subjects does not include subjects with known fatal outcome and/or subjects which otherwise do not meet enrolment criteria. Subjects with very high baseline risk (eGFR ≤25 mL/min/1.73m2) or low baseline risk (normal albuminuria and eGFR≥60 mL/min/1.73m2) will be excluded because the treatment effect on biomarkers is less likely to be observed in these subjects and would need a higher number of subjects per arm (see Section 9.2).

for at least 24 months - will be selected in order to investigate mid- to long-term treatment effects of finerenone in a cross-section of subjects from FIGARO-DKD. Countries will be selected based on above-average recruitment in FIGARO-DKD, short anticipated turn-around time to first patient first consent, and a sufficient number of sites and potentially available subjects interested in participation in this study.

Certain countries require a local protocol addendum to the parent study FIGARO-DKD #17530 to conduct this planned biomarker analyses using OLINK technology, instead of a separate study protocol described in this document as FIGARO-BM (#21952). Biomarker data acquired under FIGARO-DKD will be pooled and reported together with biomarker data acquired under FIGARO-BM.

The pooled data is intended to include approximately 600 (or more) FIGARO-DKD patients from 60 (or more) sites.

4.2 Scientific Rationale for Study Design

The overall rationale is described in detail in Section 2.1.

This study uses exploratory, commercially available biomarker panels (OLINK Explore®). Hence all endpoints are also exploratory in nature. OLINK Explore® is a novel technology which uses proteomics coupled to NGS[4]-detection methodologies (18). This is a very recent technology, which allows extensive bioprofiling of the PD response to finerenone. At the time FIGARO-DKD was set up, these technologies were not available. Since the introduction of OLINK Explore® to the market in mid-2020 only few groups have conducted similar analyses (e.g. (18-20)). The biomarker studies - which were carried out in ARTS (#14563 (22) and later, as sub-studies to FIGARO-DKD (#17530, report in preparation), FIDELIO-DKD (#16244 (21)) or) - suggest potential antifibrotic activity and warrant further investigation of circulating fibrosis markers.

The primary endpoint of FIGARO-BM focuses on fibrosis and inflammatory markers which have been reported in literature in the context of CVDs (see for example: (19)). FIGARO-BM will investigate first and foremost whether finerenone treatment (compared to placebo) affects markers of fibrosis and inflammation after mid- to long-term treatment. Impacting circulating levels of fibrosis-related biomarkers is generally assumed to be of long-term benefit to patients with CKD as kidney fibrosis is the common endpoint of CKD from various etiologies (16). Therefore, modulating fibrosis markers may reflect treatment efficacy and hence may translate into a clinically relevant benefit to the patient.

Taken together, multiple lines of evidence support that finerenone acts via inflammatory and fibrosis-related pathways. Yet, human data on fibrosis biomarker panels from randomized clinical trials (RCTs) with finerenone is missing to this point. While an individual biomarker of fibrosis or inflammation may not be informative to understand the intricate network of pathologic (or physiologic) processes and a potential interaction with the intervention, a network analysis of a large number of biomarkers may provide insights into linkages of

[4] Next generation sequencing (NGS) is used as a detection method in order to identify specific protein labels. There is no genetic information assessed, collected or analyzed. The current commercial version of this OLINK biomarker panel includes 1536 biomarkers but is expected to be extended to 3000 markers or more by the end of the year 2021.

pathways and how or if they are affected by treatment. In addition, biomarkers will be assessed longitudinally. Hence, this bioprofiling approach allows to analyze physiologically linked biomarkers over time which may more convincingly demonstrate remodeling events along the time axis than individual markers alone - without consideration of their context and time trajectory.

4.3 Limitations of the Study

Since PK blood samples were not collected at baseline in the main study, no changes from baseline will be analyzed. Hence, the focus of this study will be on mid- (i.e. ≥12 months of treatment) to long-term biomarker changes. Given that organ remodeling is a slow process over months to years, this limitation is considered acceptable. In addition, the study is a retrospective analysis of biomarkers from selected patients, sites and countries. It is likely that the focus on larger sites will lead to selection bias. In addition, patients with fatal outcome are no longer available for consenting, excluding risk analyses with regards to death. Yet, the emphasis is on understanding biological pathways and better describe the mode-of-action of finerenone in humans.

4.4 Justification for Dose

Not applicable.

4.5 End of Study Definition

The end of the study is defined as the date when the last subject provides consent into the study globally.

5. Study Population

This study will only include patients who were enrolled in the FIGARO-DKD study (see CSP FIGARO-DKD study #17530) and had received up to 20 mg finerenone or placebo for ≥24 months.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

- Signed informed consent to participate in FIGARO-BM[5]
- Randomized in the FIGARO-DKD trial
- For each participant, PK plasma samples from Visit 3 and at least 2 other Visits (Visit 5, Visit 8, Visit 11) must be available on storage from the main study FIGARO-DKD.[6,7]

[5] Capable of giving signed informed consent as described in Section 10.1.3 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.
[6] Participants with a complete set of PK blood samples up until 36 months of treatment (Visit 3, Visit 5, Visit 8, Visit 11) should be prioritized by sites over those which have incomplete sets of PK samples.
[7] Note that some subjects may have additional and/or unscheduled visits (PK samplings) which may be analyzed.

5.2 Exclusion Criteria

- Subjects which did not show overall compliance of 80 to 120% with study intervention in FIGARO-DKD
- Subjects which were not part of the full analysis set (FAS) of FIGARO-DKD.
- Subjects with known fatal outcome
- Subjects with baseline eGFR ≤25 mL/min/1.73m2
- Subjects with low baseline risk (normal albuminuria and eGFR≥60 mL/min/1.73m2)
- Sponsor request (after discussion with the investigator), for reasons such as a significant protocol deviation

5.3 Lifestyle Considerations

Not applicable.

5.4 Screen Failures

Not applicable.

5.5 Criteria for Temporarily Delaying

Not applicable.

6. Study Intervention(s) and Concomitant Therapy

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

6.1 Study Intervention(s) Administered

None.

6.2 Preparation/Handling/Storage/Accountability

Not applicable.

6.3 Measures to Minimize Bias: Randomization and Blinding

Not applicable.

6.4 Study Intervention Compliance

Not applicable.

6.5 Dose Modification

Not applicable.

6.6 Treatment of Overdose

Not applicable.

6.7 Concomitant Therapy

Not applicable.

7. Discontinuation of Study Intervention and Participant Discontinuation/Withdrawal

7.1 Discontinuation of Study Intervention

Not applicable.

7.2 Participant Discontinuation/Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request
- If the participant withdraws consent, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

7.3 Lost to Follow Up

Not applicable.

8. Study Assessments and Procedures

Protocol waivers or exemptions are not allowed. There are no study specific procedures for this biomarker study except for requesting the re-consent for use of existing samples. For details on biomarkers and sample analysis, refer to Section 8.6.

8.1 Efficacy Assessments

Not applicable.

8.2 Safety Assessments

Not applicable. There will be no study-specific safety assessments in this study. Safety of the participants was monitored within the FIGARO-DKD study.

8.3 Adverse Events (AEs), Serious Adverse Events (SAEs) and Other Safety Reporting

Not applicable.

8.4 Pharmacokinetics

Not applicable.

8.5 Genetics and/or Pharmacogenomics

Not applicable.

8.6 Biomarkers

8.6.1 Biomarker analysis

This study will analyze only blood-based biomarkers. Genetic biomarkers will not be measured.

The primary analyses will be carried out using the OLINK Explore® assay. The current version of the OLINK Explore® will provide biomarker data for 1536 biomarkers including hundreds of CV and inflammatory biomarkers[8]. "**Biomarkers of primary interest**" are summarized in the statistical analysis plan (SAP) and constitute the primary study endpoint. The full list of all biomarkers analyzed will be given in the study report.

The list of "biomarkers of primary interest" consists primarily of biomarkers of inflammation and fibrosis which have been published previously in the context of inflammation-related fibrosis and CKD. The list includes e.g. metallomatrix protein-2 (also known as CCL2), C-C motif chemokine 16 (CXCL16), connective tissue growth factor (CTGF, also known as CCN family member 2), or N-terminal pro-brain natriuretic peptide (NT-proBNP[9]).

Confirmatory analyses, e.g. to confirm biomarkers results from the primary analyses by using alternative methodologies - such as e.g. research-use immunoassays, routine clinical chemistry or immunoassay platforms (e.g. Roche Cobas, Abbott Architect, Siemens Centaur), high performance liquid chromatography (HPLC) or mass spectrometry, may be carried out.

Further biomarkers related to the mode of action or the safety of finerenone and similar drugs may be examined. The same applies to further biomarkers deemed relevant to renal and cardiovascular diseases and associated health problems. These investigations may include e.g. diagnostic, safety, PD, monitoring, or potentially predictive biomarkers.

8.6.2 Timing of sample collection

Not applicable[10].

8.6.3 Specimen type(s)

Leftover samples, originally collected for PK analysis (lithium heparin plasma) in FIGARO-DKD (#17530), will be used for this biomarker study. For details on sample collection refer to the study documentation of FIGARO-DKD (#17530 - e.g. sample handling sheets or lab manual).

8.6.4 Sample storage

Samples may be stored for a maximum of 15 years (or according to local regulations) following finalization of the study report for this study at a facility selected by the sponsor to enable further analyses.

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[8] This panel is subject to regular adaptations by the manufacturer to consider newly identified biomarkers. For this study, the latest available OLINK Explore Panel will be used. The composition of OLINK Explore® along with the UniProtID and gene names for each biomarker is published on www.olink.com. The complete protein list for the current Explore® can be found here: https://www.olink.com/content/uploads/2021/03/explore-1536-assay-list-20210227-web.xlsx (as of 31 May 2021 - Note: This link is not automatically updated.)

[9] NT-proBNP has also been analyzed in two other substudies of FIGARO-DKD using different analytical methodologies. Results for these substudies have been or, for still ongoing analyses under protocol #17530, will be reported under separated cover.

[10] There will be no new sample collection in the course of this biomarker study.

8.6.5 Analytical procedure and quality controls

Details of the analytical procedures and quality controls (if applicable) will be provided by the analytical laboratory (e.g. validation/analytical report) at the end of the study.

8.6.6 Data Pooling

Biomarker data acquired under FIGARO-DKD #17530 will be pooled and reported together with biomarker data acquired under FIGARO-BM #21952 (see also Section 4.1).

8.6.7 Reporting

Biomarker investigations as described herein (see Section 3) as "Primary" (see Section 3 or within the SAP) will be reported in a clinical study report. Other exploratory analyses may be reported separately (e.g. in a biomarker evaluation report).

8.7 Immunogenicity Assessments

Not applicable.

8.8 Health Economics or Medical Resource Utilization and Health Economics

Not applicable.

9. Statistical Considerations

9.1 Statistical Hypotheses

All analyses for this study are exploratory.

The **primary objective** (Section 3) is to analyze the change in plasma biomarker levels after 36 months (Visit 11) of treatment versus 4 months (Visit 3) of treatment in a set of 27 pre-defined biomarkers (provided in the SAP) in treatment group compared to placebo, based on the biomarker full analysis set (BFAS) as defined in Section 9.3. A two-sided moderated t-tests (23) between treatment and placebo group on the log-transformed ratios of biomarker levels from Visit 11 to Visit 3, based on the complete OLINK panel, will be used in the analysis. This analysis will be adjusted for relevant baseline covariates. P-values of the 27 biomarkers of primary interest will be adjusted for multiple testing according to Benjamini and Hochberg (24). A biomarker is considered to be affected by treatment, if the adjusted p-value is less or equal to 5%.

Summary statistics and line plots (mean with geometric standard deviation [gsd]- or standard deviation [sd] error bars over time) for the ratio to Visit 3 will be generated.

The remaining biomarkers will be analyzed according to SAP or under separate cover. Refer to SAP for further details.

9.2 Sample Size Determination

Table 9–1 summarizes the results of a power simulation based on the following assumptions:

- Beneficial effect of finerenone on **4** of the 27 biomarkers with a ratio of biomarker levels from Visit 11 to Visit 3 (month 4) under finerenone of **10%, 15%** or **20%** compared to placebo.
- Inter-subject variability of **25%** (geometric standard deviation of 1.25)

- Intra-subject variability from Visit 3 to Visit 11 of **20%** (gsd of 1.2)
- Assay-variability of **10%** (gsd of 1.1)

Table 9–1: Estimated power for the primary endpoint

Treatment effect size	Number of subjects per arm	Power to detect 4 biomarkers	Power to detect 3 biomarkers or more	Power to detect 2 biomarkers or more	Power to detect 1 biomarkers or more
10%	75	2.5%	5.4%	9.5%	16.8%
	100	4.5%	9.2%	15.2%	24.2%
	250	26.4%	42.0%	55.2%	67.4%
	300	**37.4%**	**55.4%**	**66.9%**	**77.4%**
	400	57.6%	74.4%	83.6%	90.3%
15%	75	13.4%	24.3%	34.9%	48.0%
	100	24.2%	39.3%	51.8%	63.8%
	250	86.1%	94.8%	97.7%	99.2%
	300	**94.2%**	**98.8%**	**99.5%**	**99.9%**
	400	99.4%	100.0%	100.0%	100.0%
20%	75	45.0%	62.4%	74.0%	83.4%
	100	67.3%	82.0%	89.5%	94.3%
	250	99.9%	100.0%	100.0%	100.0%
	300	**100.0%**	**100.0%**	**100.0%**	**100.0%**
	400	100.0%	100.0%	100.0%	100.0%

Power estimations are based on the methodology described in Section 9.4 and Monte-Carlo simulations with 10,000 iterations per scenario. Numbers in **bold** indicate the anticipated number of subjects per arm (total subjects = 600). Grey shading indicates a power of ≥80%

As shown in Table 9–1, the intended patient number of 600 with 300 subjects per arm (finerenone and placebo 1:1) would lead to a power of 94.2% to correctly detect all 4 biomarkers with beneficial effect at an estimated effect size of 15% between visit 3 (4 months) and visit 11 (36 months).

For description of the panels refer to Section 8.6. All simulations concerning sample size determination were conducted using R.

9.3 Analysis Set

Analysis sets are defined in Table 9–2:

Table 9–2: Analysis sets

Participant Analysis Set	Description
BFAS (Biomarker Full Analysis Set)	All participants with valid informed consent for this biomarker study and which meet study enrollment criteria as defined in Section 5.

9.4 Statistical Analyses

Selected demographics and disposition tables from FIGARO-DKD will be repeated for the biomarker subset. For details refer to the corresponding SAP.

For a detailed analysis plan and further exploratory analyses refer to SAP.

9.5 Interim Analysis

There will be no formal interim analysis. The study is unblinded.

10. Supporting Documentation and Operational Considerations

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants. Any substantial modification of the protocol will be submitted to the competent authorities as substantial amendments for approval, in accordance with ICH Good Clinical Practice and national and international regulations.
- Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
    - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines, the IRB/IEC, and all other applicable local regulations

10.1.2 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participants or their legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participants.

10.1.4 Data Protection

- Participants will be assigned the same unique identifier by the sponsor as in the FIGARO-DKD study. Any participant records, datasets or biological samples that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committees Structure

Due to the non-interventional nature, no committee structure has been established for this retrospective biomarker study. No additional subject data will be collected in this study.

10.1.6 Dissemination of Clinical Study Data

Results of clinical drug trials will be provided on EU Clinical Trials Register in line with the applicable laws and regulations. Result summaries of Bayer's sponsored clinical trials in drug development Phases 2, 3 and 4 and Phase 1studies in patients are provided in the Bayer Trial Finder application after marketing authorization approval in line with the position of the

global pharmaceutical industry associations laid down in the "Joint Position on the Disclosure of Clinical Trial Information via Clinical Trial Registries and Databases".

Bayer commits to sharing upon request from qualified scientific and medical researchers patient-level clinical trial data, study-level clinical trial data, and protocols from clinical trials in patients for medicines and indications approved in the United States and European Union on or after 01 JAN 2014 as necessary for conducting legitimate research.

All Bayer-sponsored clinical trials are considered for publication in the scientific literature irrespective of whether the results of the clinical trials are positive or negative.

10.1.7 Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data, biomarker data). The investigator is responsible for verifying that data entries are accurate and correct.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8 Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator’s site.
- Data reported in the CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the Source Data Location List (SDLL) or equivalent.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

- Study monitors will perform ongoing source data review to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- No source data verification will be performed

10.1.9 Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the first patient first consent.

Study/Site Termination

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further study intervention development

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
- Total number of participants included earlier than expected.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

10.1.10 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally

support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.2 Appendix 2: Abbreviations

ACEI(s)	angiotensin-converting-enzyme inhibitor(s)
AKI	acute kidney injury
ARB(s)	angiotensin-receptor blocker(s)
BFAS	biomarker full analysis set
BM	biomarker
CCL2	metallomatrix protein-2
CHF	chronic heart failure
CKD	chronic kidney disease
CRF	case report form
CSP	clinical study protocol
CTGF	connective tissue growth factor
CV	cardiovascular
CVD	cardiovascular disease
CXCL16	C-C motif chemokine 16
DKD	diabetic kidney disease
eGFR	estimated glomerular filtration rate
ESRD	end-stage renal disease
GCP	good clinical practice
GFR	glomerular filtration rate
gsd	geometric standard deviation
HF	heart failure
HPLC	high performance liquid chromatography
IC	informed consent
ICF	informed consent form

IEC	Independent Ethics Committee
IRB	Institutional Review Board
MoA	mode-of-action
MR	mineralocorticoid-receptor
MRA	mineralocorticoid-receptor antagonist
NDD-CKD	non–dialysis-dependent chronic kidney disease
NGS	next generation sequencing
NT-proBNP	N-terminal pro-brain natriuretic peptide
PD	pharmacodynamics
PK	pharmacokinetics
RAS	renin-angiotensin system
RCTs	randomized clinical trials
SAE	Serious adverse event
SAP	statistical analysis plan
sd	standard deviation
SoC	standard of care
T2DM	type 2 diabetes mellitus

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