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Title page

A non-blinded biomarker add-on study to FIGARO-DKD for Bioprofiling the pharMacodynamic response to finerenone in FIGARO-DKD subjects (FIGARO-BM)

Bayer study drug	BAY 94-8862 / Finerenone		
Study purpose:	Biomarker study		
Clinical study phase:	III	**Date:**	13 JUL 2022
Study No.:	21952	**Version:**	2.0
Author:	PPD		

This Statistical Analysis Plan is produced on a word-processing system and bears no signatures.

The approval of the Statistical Analysis Plan is documented in a separate Signature Document.

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Abbreviations

BFAS	Biomarker Full Analysis Set
gsd	Geometric standard deviation
LOD	Limit of Detection
mBFAS	Modified Biomarker Full Analysis Set
NPX	Normalized Protein expression
QC	Quality Check
SAP	Statistical Analysis Plan
sd	Standard deviation

1. Introduction

This statistical analysis plan 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. 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.

This SAP considers the main analysis of only 27 biomarkers as listed in section 10.2. Further analyses will be specified under separate cover in a biomarker analysis plan.

2. Study Objectives

Objectives and endpoints are listed in the table below:

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 (see section 10.2)
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) [1] of treatment versus 4 months (Visit 3) of treatment

[1] Depending on availability of samples for visit

• 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

3. Study Design

Data from subjects consented under FIGARO-DKD (addendum approach) will be analyzed 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. General Statistical Considerations

4.1 General Principles

The statistical evaluation will be performed by using the software package SAS release 9.4 or higher (SAS Institute Inc., Cary, NC, USA). The main efficacy analysis will be performed in R on a validated server.

4.2 Handling of Missing Data

Missing data will not be imputed, unless otherwise specified.

4.3 Interim Analyses and Data Monitoring

Not applicable.

4.4 Data Rules

The QC-flag, which is provided by OLINK, will be coded in a four-digit-code according to the table below:

	Value = 0	Value = 1	Description
1st digit	Data point passed OLINKs QC-metrics	Data point failed some of OLINKs QC-metrics	Pass/Fail of OLINKs predefined QC metrics
2nd digit	No outlier	Outlier	Outlier status, based on PCA
3rd digit	No outlier	Outlier	Outlier status, based on NPX median and range
4th digit	NPX > LOD	NPX < LOD	Above limit of detection or not

Data, which is QC-flagged by OLINKs predefined QC metrics (i.e. value = 1 in the first digit), will be set to missing for all analyses.

Visit 3 data will be considered as baseline measurements.

Demographic and baseline characteristics will be obtained from the FIGARO-DKD data.

NPX data is on $\log_2$-scale.

For further data rules, refer to the FIGARO-DKD SAP.

5. Analysis Sets

BFAS (Biomarker Full Analysis Set):

All participants with valid informed consent for this biomarker study and which meet study enrollment criteria as defined in the study protocol.

mBFAS (modified Biomarker Full Analysis Set):

All participants with valid informed consent for this biomarker study, which meet the following criteria:

- Study enrollment criteria as defined in the study protocol.
- Analyzed biomarker samples at Visit 3 (4 months) and Visit 11 (36 months). Biomarker samples that were shipped at ambient temperatures were not analyzed.
- On Treatment at Visit 3 (4 months) and Visit 11 (36 months) .

6. Statistical Methodology

6.1 Population characteristics

Population characteristics from FIGARO-DKD will be repeated for the modified Biomarker Full Analysis Set. Detailed information on the respective analyses are described in the FIGARO-DKD SAP.

6.1.1 Disposition

The number of subjects overall as well as by treatment group, region, country and study site will be repeated for the mBFAS.

6.1.2 Demography and baseline characteristics

The demographic and other baseline characteristics tables will be repeated for the mBFAS. For these tables the baseline definition of FIGARO-DKD will be used instead of Visit 3.

6.1.3 Medical history

The medical history tables will be repeated for the mBFAS.

6.1.4 Prior and Concomitant Medication

The prior and concomitant medication tables will be repeated for the mBFAS.

6.1.5 Treatment duration, extent of exposure and compliance

The treatment duration, extent of exposure and compliance tables will be repeated for the mBFAS.

6.2 Efficacy

All efficacy analyses will be performed on the 27 biomarkers listed in section 10.2. The remaining biomarkers analysed in the OLINK panel will only be included as prior information for the moderated t-test.

6.2.1 Main efficacy analysis

The primary efficacy variable is the change in plasma biomarker levels after 36 months (Visit 11) of treatment versus 4 months (Visit 3) of treatment of subjects on treatment at both visits. The NPX differences (corresponding to log-transformed ratio to baseline) of biomarker levels will be analysed for the set of 27 pre-defined biomarkers in treatment group compared to placebo, based on the modified Biomarker Full Analysis Set (mBFAS) as defined in section 5.

The hypotheses 'H_0^i: $\beta_i = 0$' (i=1,…,27) will be tested at a two-sided significance level of 5%, where β_i is the estimator for the difference in log-transformed ratios of biomarker levels of Visit 11 to Visit 3 between treatment and placebo group for the i-th of the 27 pre-specified biomarkers. The analysis will be adjusted for a selection of baseline characteristics and strafication factors. The selection of the adjustment covariates is related to the biomarker pre-processing of OLINK. The p-values obtained from the moderated t-statistics [1] testing the difference between treatment and placebo group on the log-transformed ratios of biomarker levels from Visit 11 to Visit 3, based on the OLINK Explore 1536 panel, will be adjusted for multiple testing according to Benjamini and Hochberg [2]. According to this method the false discovery rate can be controlled by ordering the 27 p-values p_i (i=1,…,27) by ascending value and comparing the i-th p-value with the adjusted significance level of $\frac{i}{27} \cdot 5\%$ respectively. Alternatively the i-th p-value p_i can be adjusted as $\min\left(\left(\min_{j \geq i} \frac{27}{j} \cdot p_i\right), 1\right)$, as described by Benjamini, Heller and Yekutieli [3].

The study will be considered as successful if at least one adjusted p-value is below the significance level of 5%.

Summary statistics and line plots (geometric mean with geometric standard deviation [gsd]-error bars over time) as well as boxplots for the ratio of Visit 11 to Visit 3 will be generated.

6.3 Pharmacokinetics/pharmacodynamics

Not applicable.

6.4 Safety

Not applicable.

7. Changes to the analyses specified in the study protocol

According to the protocol, the primary endpoint should be analyzed in the Biomarker Full Analysis Set (BFAS) including all subjects with signed informed consent. As the analysis is only feasible, if also a biomarker is measured, an new analysis set was defined including subjects with signed informed consent and a biomarker measurement. The primary analysis will be conducted in this modified Biomarker Full Analysis Set (mBFAS).

8. Document history and changes in the planned statistical analysis

SAP version 0.1 dated 08 DEC 2021 submitted for internal review

SAP version 0.2 dated 11 FEB 2022 submitted for internal review

Approved SAP version 1.0 dated 06 APR 2022

Approved SAP supplement version 2.0 dated 13 JUL 2022

- As delivery of second batch of biomarkers will take place earlier than expected, all biomarkers will be included in the primary analysis as described in the protocol, instead of only the first batch as described in SAP version 1.0.
- Added coding-table of OLINKs QC-flag, which was not available for SAP v. 1.0.
- Added statement on scale of NPX data.
- Added more detailed description of Benjamini-Hochberg procedure.

9. References

[1] G. Smyth, „Linear models and empirical Bayes methods for assessing differential expression in microarray experiments,“ in *Statistical Application in Genetics and Molecular Biology. vol 3*, Melbourne, 2004, pp. 1-26.

[2] H. Y. Benajmini Y, „Controlling the false discovery rate: a practical and powerful approach to multiple testing.,“ *Journal of the Royal Statistical Society Series B, 57,* pp. 289-300, 1995.

[3] Y. Benjamini, R. Heller and D. Yekutieli, "Selective inference in complex research," *Philosophical Transactions of the Royal Society,* vol. 367, pp. 1-17, 2009.

10. Appendix

10.1 Determination of sample size

Table 10-1 summarizes the results of a power simulation based on the following assumptions:

- Two sided test, significance level of 5%.
- 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% (geometric standard deviation of 1.2)
- Assay-variability of 10% (geometric standard deviation of 1.1)

Table 10-1 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). Greyshading indicates a power of ≥80%

As shown in Table 10-1, 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 the study protocol. All simulations concerning sample size determination were conducted using R.

10.2 List of Biomarkers

Uniprot	Protein name	Gene name
P07911	Uromodulin	UMOD
Q03167	Transforming growth factor beta receptor type 3	TGFBR3
O15467	C-C motif chemokine 16	CCL16
P02452	Collagen alpha-1	COL1A1
P04275	von Willebrand factor	VWF
P05121	Plasminogen activator inhibitor 1 // PAI-1	SERPINE1
P16581	E-selectin	SELE
Q9H2A7	C-X-C motif chemokine 16	CXCL16
Q9UBP4	Dickkopf-related protein 3	DKK3
P01033	Metalloproteinase inhibitor 1	TIMP1
Q16627	C-C motif chemokine 14	CCL14
P15144	Aminopeptidase N	ANPEP
Q99969	Retinoic acid receptor responder protein 2	RARRES2
P07585	Decorin	DCN
P09237	Matrilysin // MMP7	MMP7
Q03405	Urokinase plasminogen activator surface receptor	PLAUR
P29279	CCN family member 2 // CTGF	CCN2
Q13219	Pappalysin-1	PAPPA
P01137	Transforming growth factor beta-1 proprotein	TGFB1
Q13261	Interleukin-15 receptor subunit alpha	IL15RA
P35442	Thrombospondin-2	THBS2
P14780	Matrix metalloproteinase-9	MMP9
P19438	Tumor necrosis factor receptor superfamily member 1A	TNFRSF1A
P13500	C-C motif chemokine 2 // / MCP-1	CCL2
P12931	Proto-oncogene tyrosine-protein kinase Src	SRC
P02760	Protein AMBP	AMBP
O95388	WNT1-inducible-signaling pathway protein 1 //CCN4	WISP1