

Supplemental Material

Supplementary Methods 1.

Methodology of Sampling for Biobanking in EMPEROR-Reduced and EMPEROR-Preserved trials

Organizational Principles of Sampling for Biobanking in EMPEROR Trials

Participation in sampling for biobanking (including DNA) was voluntary and not a prerequisite for participation in the trial. Biobanking samples were taken only after separate informed consent form (ICF) was provided in accordance with local ethical and regulatory requirements.

Measures were in place to comply with the applicable rules for the collection, biobanking and future use of biological samples and clinical data, in particular

- Sample and data usage in accordance with the separate biobanking ICF.
- The sponsor internal facilities storing biological samples from clinical trial participants as well as the external banking facility are qualified for the storage of biological samples collected in clinical trials.
- An appropriate sample and data management system, including audit trail for clinical data and samples to identify and destroy such samples according to ICF in place
- A fit for the purpose documentation required (biomarker proposal, analysis plan and report) to ensure compliant usage
- A fit for purpose approach for assay/equipment validation required depending on the intended use of the biomarker data
- Possibility of samples and/or data transfer to third parties and other countries is specified in the biobanking ICF

The countries participating in biobanking in the EMPEROR trials

EMPEROR-Reduced	EMPEROR-Preserved
Argentina	Argentina
Australia	Australia
Canada	Canada
Czech Republic	Colombia
France	Czech Republic
Germany	Germany
Hungary	Hungary
India	India

Italy Japan Mexico Netherlands Poland Republic of Korea Spain United Kingdom United States	Italy Japan Mexico Netherlands Poland Republic of Korea Romania Singapore South Africa Spain United Kingdom United States
Countries not participating in biobanking	
Belgium Brazil China	Belgium Brazil China

Timing and Methodology of Sampling

Sampling for Biobanking in EMPEROR-Reduced and EMPEROR-preserved trials was performed at the following timepoints, specified by the clinical trial protocols:

(1) DNA: preferably at randomization; (2) plasma, serum and urine banking: randomization, week 12 and week 52.

DNA banking

Approximately 8.5 mL of blood were drawn into a PAXgene Blood DNA Tube

Plasma banking

Approximately 10 mL of blood were drawn into an EDTA blood collection tube

Serum banking

Approximately 8.5mL of blood were drawn into a serum separation tube.

Urine banking

Approximately 10 mL of urine (preferably morning mid-stream urine)

For all biosampling, detailed instructions on sampling, preparation, processing, shipment, and storage were provided in the laboratory manual. Plasma, serum, and urine samples have been being stored at an external biobanking facility contracted by the sponsor. Samples were stored at -80.

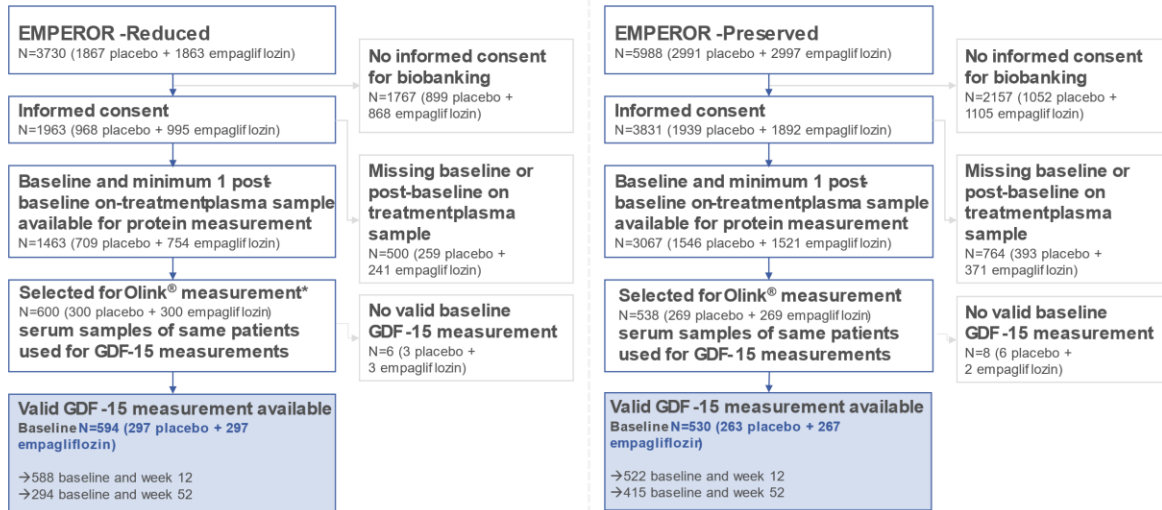
For the biomarkers measured by Elecsys®, Roche Diagnostics and Olink proteomics bioassays, there was one freeze/thaw cycle to produce aliquots from larger sample volumes, and another when samples were transferred to plates for analysis.

The analytes tested were stable under the storage and freeze/thaw cycle conditions (<10% influence)

DNA, extracted from the original whole blood sample, have been being stored with the sponsor.

Supplementary Methods 2. Samples selection and biomarkers evaluation in EMPEROR trials

Sampling for biobanking and GDF -15 measurement



*Random sampling stratified by treatment and availability of data, originally selected for OLINK measurement, same patients used for GDF -15 measurement.

Supplementary Table 3. Correlation analysis of GDF-15 serum concentrations with other circulating proteins at baseline (Spearman Rho \geq 0.5)

Protein	Spearman Rho
Insulin-like Growth Factor Binding Protein-7 [ng/mL]	0.64
Fatty Acid Binding Protein-3 [ng/mL]	0.63
Neurology: Tumor necrosis factor receptor superfamily member 10B	0.61
Oncology: Tumor necrosis factor receptor superfamily member 27	0.61
Oncology: WAP four-disulfide core domain protein 2	0.58
Cardiometabolic: Trefoil factor 3	0.57
Neurology: Layilin	0.57
Neurology: Tumor necrosis factor receptor superfamily member 1A	0.57
Neurology: Serine protease inhibitor Kazal-type 1	0.57
Neurology: Insulin-like growth factor-binding protein 4	0.56
Oncology: Ephrin type-A receptor 2	0.56
Inflammation: Tumor necrosis factor receptor superfamily member 11A	0.55
Neurology: Lysosome membrane protein 2	0.55
Neurology: Ephrin-A4	0.55
Neurology: Phosphoinositide-3-kinase-interacting protein 1	0.54
Oncology: CD302 antigen	0.54
Oncology: Tumor necrosis factor receptor superfamily member 12A	0.54
Inflammation: Collectin-12	0.54
Neurology: Ephrin-A1	0.53
Inflammation: Follistatin-related protein 3	0.53
Oncology: Neuroblastoma suppressor of tumorigenicity 1	0.53
Cardiometabolic: Nectin-2	0.53
Neurology: Tumor necrosis factor receptor superfamily member 1B	0.52
Inflammation: Tumor necrosis factor receptor superfamily member 14	0.52
Inflammation: Leukocyte-associated immunoglobulin-like receptor 1	0.52
Neurology: Tumor necrosis factor receptor superfamily member 19L	0.52
Neurology: V-set and immunoglobulin domain-containing protein 4	0.52
Oncology: CD27 antigen	0.51

Cardiometabolic: Collagen alpha-3	0.51
Oncology: Tumor necrosis factor receptor superfamily member 19	0.51
Cardiometabolic: Neural proliferation differentiation and control protein 1	0.51
Neurology: Serine/threonine-protein kinase receptor R3	0.51
Inflammation: Urokinase plasminogen activator surface receptor	0.51
Oncology: GDNF family receptor alpha-1	0.51
Inflammation: Cytoskeleton-associated protein 4	0.51
Cardiometabolic: Protein FAM3C	0.51
Neurology: UL16-binding protein 2	0.51
Cardiometabolic: Actin, aortic smooth muscle	0.5
Oncology: CCN family member 4	0.5
Oncology: TGF-beta receptor type-2	0.5

Supplementary Table 4. Effect of Empagliflozin on GDF-15 level changes over time by metformin use at baseline

Visit	Placebo Geometric mean (coef. variation)	Empagliflozin Geometric mean (coef. variation)	Empa. vs Pbo. Ratio of Relative Change (95%CI) P-value*
No metformin use at baseline			
N	404	439	
Baseline	2276 (68)	2436(73)	-
Week 12	2397 (73)	2650 (74)	1.05 (0.99, 1.11) P=0.10
Week 52	2407 (73)	2815 (69)	1.12 (1.04, 1.20) P=0.003
Metformin use at baseline			
N	151	124	
Baseline	3261(68)	2995 (64)	-
Week 12	3464 (76)	3140 (58)	0.96 (0.87, 1.06) P=0.39
Week 52	3406 (66)	3084 (65)	0.98 (0.86, 1.10) P=0.68

* MMRM Model includes age, baseline values of eGFR and LVEF, study, sex, region, diabetes status, visit by treatment by baseline use of Metformin interaction and baseline GDF-15 by visit interaction as fixed effects. Interaction P-value for treatment-by-metformin use at baseline, for week 12 =0.12; for week 52 =0.06.