Online supplement

Title:

Protocol of the *Berlin Long-term Observation of Vascular Events (BeLOVE)* - a prospective cohort study with deep phenotyping and long term follow up of cardiovascular high-risk patients

Joachim E. Weber[1,2,3,6 (ORCID: 0000-0002-1666-6021) *]; Michael Ahmadi[1,2,3(0000-0001-5792-4704) *]; Leif-Hendrik Boldt[5,6,26 (ORCID: 0000-0002-3320-0880)]; Kai-Uwe Eckardt[1,4 (0000-0003-3823-0920)]; Frank Edelmann[1,5,6 (0000-0003-4401-5936)]; Holger Gerhardt[1,6,9 (0000-0002-3030-0384)]; Ulrike Grittner [1,10 (0000-0003-2595-0224)]; Kathrin Haubold[1(0000-0001-5108-5356)]; Norbert Hübner[1,6,11,12 (0000-0002-1218-6223)]; Jil Kollmus-Heege[1,10 (0000-0003-3183-4932)]; Ulf Landmesser[1,6,13 (0000-0002-0214-3203)]; David Leistner[13 (0000-0002-4351-420X)]; Knut Mai[6,14,15,24 (0000-0003-0126-3155)]; Dominik N. Müller[1,6,9, 12 16 (0000-0003-3650-5644)]; Christian H Nolte[1,2,3,6 (0000-0001-5577-1775)]; Burkert Pieske[1,5,6 (0000-0002-6466-5306);] Sophie K. Piper[1,10,25 (0000-0002-0147-8992)]; Simrit Rattan[1,10 (0009-0006-3684-0660)]; Geraldine Rauch[10 (0000-0002-2451-1660)]; Sein Schmidt[1,2,3 (0000-0002-6698-5717)]; Kai M. Schmidt-Ott[1,4,11,27 (0000-0002-7700-7142)]; Katharina Schönrath[1(0000-0002-7973-7536)]; Jeanette Schulz-Menger[6,11 (0000-0003-3100-1092)]; Oliver Schweizerhof[1,10(0000-0002-8954-4818)]; Bob Siegerink[1,17(0000-0002-8454-9142)]; Joachim Spranger[1,6,14,15,24 (0000-0002-8900-4467)]; Ramachandran S. Vasan[1,19,20 (ORCID: 0000-0001-7357-5970)], Martin Witzenrath[18,21 (0000-0002-9787-5633)]; Matthias Endres[2,3,6,7,8(0000-0001-6520-3720)]; Tobias Pischon[1,6,12, 22,23 (ORCID: 0000-0003-1568-767X)]

1 *Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany*

2 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Center for Stroke Research Berlin, Berlin, Germany,*

3 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Neurology, Berlin, Germany,*

4 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Nephrology and Medical Intensive Care, Berlin, Germany*

5 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Internal Medicine and Cardiology, Berlin, Germany,*

6 *German Centre for Cardiovascular Research (DZHK), partner site Berlin, Berlin, Germany*

7 *German Center for Neurodegenerative Diseases (DZNE), partner site Berlin, Berlin, Germany*

8 *ExellenceCluster NeuroCure, Berlin, Germany*

9 *Max Delbruck Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany*

10 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany, Institute of Biometry and Clinical Epidemiology*

11 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin,ECRC, Department of Cardiology, Berlin, Germany*

12 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany*

13 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Cardiology, Berlin, Germany,*

14 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Endocrinology & Metabolism, Berlin, Germany,*

15 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Charité-Center for Cardiovascular Research (CCR), Berlin, Germany*

16 *Experimental and Clinical Research Center (ECRC), a cooperation of Charité - Universitätsmedizin Berlin and Max Delbruck Center for Molecular Medicine (MDC), Berlin, Germany*

17 *Leiden University Medical Center, Department of Clinical Epidemiology, Leiden, Netherlands*

18 *Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Division of Pulmonary Inflammation, and Department of Infectious Diseases and Respiratory Medicine, Charitéplatz 1, 10117 Berlin, Germany.*

19 *Sections of Preventive Medicine and Epidemiology, and Cardiovascular Medicine, Department of Medicine , Boston University School of Medicine, Boston, Massachusetts, USA;*

20 *Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts, USA, USA*

21 *German Center for Lung Research (DZL)*

22 *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Molecular Epidemiology Research Group, Berlin, Germany*

23 *Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Biobank Technology Platform, Berlin, Germany*

24 *German Center for Diabetes Research, München-Neuherberg, Germany*

25 *Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Medical Informatics*

26 *Deutsches Herzzentrum der Charité, Department of Cardiology, Angiology and Intensive Care Medicine, Augustenburger Platz 1, 13353 Berlin, Germany*

27 *Hannover Medical School, Department of Nephrology and Hypertension, Hannover, Germany*

* equal contributions

Corresponding authors:

Prof. Dr. med. Tobias Pischon, Deputy Spokesperson of the BeLOVE study

Group Leader, Molecular Epidemiology Research group Max-Delbrück-Center for Moecular Medicine (MDC)

Robert-Rössle-Straße 10

13125 Berlin, Germany

Tel.: +49 30 94064563

tobias.pischon@mdc-berlin.de

Dr. med. Joachim E. Weber, Scientific Officer of the BeLOVE study,

Berlin Institute of Health (BIH)

Anna-Louisa-Karsch-Str. 2, 10178 Berlin,

Tel. +49 30 450543524

Fax +49 30 4505437524

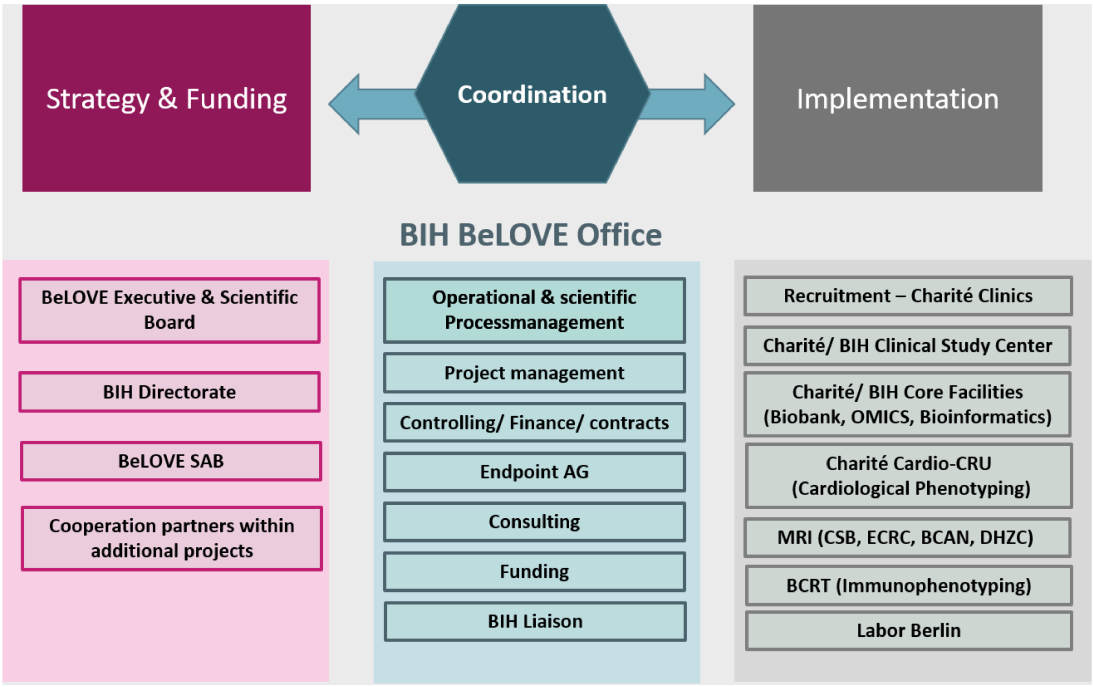
joachim.weber@bih-charite.de

Sponsor:

Charité – Universitätsmedizin Berlin,

Charitéplatz 1, 10117 Berlin, Germany

Structures for study planning and execution



Supp. figure 1: Institutions and structures involved in the execution of BeLOVE: SAB = Scientific Advisory Board; Cardio-CRU = Cardiological Clinical Research Unit; MRI = Magnetic Resonance Imaging; ECRC = Experimental and clinical Research Center; BCAN = Berlin Center for Advanced Neuroimaging (Charité); DHZC = German Heart Center Charité; BCRT = BIH Center for Regenerative Therapies

The study is funded by the *Berlin Institute of Health (BIH)*. The study concept, governance and financial plan was evaluated and approved by the international BeLOVE Scientific Advisory Board (SAB). The operative and scientific strategy is determined by the BeLOVE Executive and Scientific Board which is a an assembly of principal investigators of the *Charité – Universitätsmedizin Berlin* from the fields of cardiology, neurology, nephrology, endocrinology, pneumology, infectiology, biostatistics, of the Charité and of the *Max-Delbrück Center for Molecular Medicine Berlin (MDC)* from the fields of epidemiology, basic cardiovascular and metabolism research. The study is open to multiple externally funded subproject initiatives with different partners within the BIH, Charité and MDC as well as from other cohorts (e.g. the German National Cohort, NAKO).

The BeLOVE Office is the central project managing and controlling facility that is coordinating the execution of all strategic decision as well as cooperations, endpoint adjudication and finances/funding.

The study is executed by the following institutions:

- Recruitment is carried out by professional clinical trial teams of the Charité´s departments for cardiology, neurology and endocrinology
- Patient visits including all phenotyping is performed by the studys own BeLOVE Trial Unit (BTU) at different Campuses of the Charité in Berlin.
- Specialized personal of the BTU is also performing all telephone visits and research for relevant new clinical events (endpoints)
- Echocardiography is carried out under supervision of the Charité department for cardiology
- MRI is performed and analyzed by the department for neuroradiology of the Center for Stroke Research Berlin (CBS), the Berlin Center for Advanced Neuroimaging (BCAN), the Experimental and Clinical Research Center (ECRC) of the MDC and Charité Berlin and the German Heart Center Charité (DHZC). Further, data from metabolic MRI is analyzed by the department of radiology at Universität Tuebingen and pulmonary and kidney MRI at the department of radiology of the Klinikum Rechts der Isar at the Technische Universität Munich (TUM)
- Biobanking is performed by the BIH Biobank Core Facility.
- Blood samples are analyzed for routine measures by Labor Berlin
- Induced pluripotent stem cells are programmed at the MDC
- Immunophenotyping is performed by the BIH Center for Regenerative Therapies (BCRT)
- Proteomics analyses are performed by the Core Facility Proteomics (BIH)

- Metabolomics analyses are performed by the Core Facility Metabolomics (BIH)
- Microbiome analyses are performed at the MDC.
- Genomics analyses are performed at the MDC
- Integration of OMICs data is performed by the Core Unit Bioinformatics (CUBI) (BIH)

Inclusion criteria

Specific inclusion criteria: acute cardiovascular event (CVE) group

Supp. table 1: Detailed specific inclusion and exclusion criteria for the acute CVE group

Specific inclusion criteria: acute CVE events	
Hospitalization for acute heart failure (AHF)	• AHF ≥ NYHA II *OR* clinical deterioration of chronic heart failure* **AND** • escalation of preexisting loop diuretic therapy *OR* new prescription of loop diuretic therapy **exception*: if the main primary diagnosis is not AHF but acute coronary syndrome, acute stroke or acute kidney injury, patients shall *not* be included in the study
Hospitalization for acute coronary syndrome (ACS)	characteristics of cardiac chest pain OR angina equivalents AND electrocardiographic manifestations of STE-ACS (ST-segment elevation in ≥ two contiguous leads with the cut-points: ≥ 2.5mm in men < 40 years; ≥ 2.0 mm in men ≥40 years; ≥ 1.5mm in women regardless of age in the leads V2/V3 OR ST-segment elevation ≥ 1.0 mm in the other leads OR new-onset of LBBB/RBBB OR ST-segment changes in the presence of a preknown BBB OR ST-segment depression in ≥ 8 leads in the presence of ST-segment elevation in aVR/V1 OR the presence of pathological Q-waves) OR electrocardiographic manifestations NSTE-ACS (Horizontal ST-segment depression of ≥ 0.5mm OR T-wave inversions of ≥ 1mm in at least 2 contiguous leads) OR laboratory evidence of myocardial necrosis (detection of elevation of hs-TnI or hs-TnT greater than the 99th percentile of UR OR normal hs-Tn baseline values at (hospital) admission with a dynamic change in hs-Tn values within 1 hour, confirmed through a TnI-TnT specific assay)
Hospitalization for acute cerebrovascular disorders	

a. TIA	an acute-onset neurological deficit with clinical restitution within 24 hours without evidence for acute ischemia or hemorrhage on imaging AND initial neurological deficit was verified by a neurologist OR ABCD2-Score ≥ 3 OR main hospital diagnosis is amaurosis fugax
b. Ischemic stroke	• an acute-onset neurological deficit lasting more or less than 24hrs with a fresh ischemic lesion on neuroimaging (MRI or CT) ***OR*** for patients that did *not* receive cerebral MR imaging during the acute phase*: acute-onset typical cerebrovascular clinical syndrome without a definite fresh ischemic lesion on CT-imaging but with symptom duration > 24hrs ***OR*** acute monocular vision impairment and evidence for retinal central artery occlusion * pts. with a symptom duration > 24h, who received cerebral MRI-imaging including diffusion- and T2*-weighted sequences during the acute phase that did not show acute diffusion-restriction or fresh intracerebral hemorrhage *cannot* be included
c. non-traumatic intracerebral hemorrhage	• an acute-onset neurological deficit ***AND*** • evidence for fresh intracerebral hemorrhage on neuroimaging (MRI or CT)

Specific Inclusion and exclusion criteria: chronic very high CV-risk group

The very high risk criteria are in accordance with the recommendations of the ESC/EAS-guidelines for the management of lipid disorders[1] and cardiovascular disease prevention.[2]

Supp. table 2: Detailed specific inclusion and exclusion criteria for the chronic CV-risk group

Specific inclusion criteria: chronic CV-risk group	
At least 1 out of the following 8 conditions	*definitions*
1.) CV events ≥12 months ago	• non-traumatic hemorrhagic stroke, ischemic stroke (including central retinal artery occlusion) or TIA ≥ 12 months ago OR • a history of acute coronary syndrome or myocardial infarction ≥ 12 months ago
2.) Atherosclerosis	• Coronary artery calcium score >100 on coronary CT *OR* • Significant atherosclerosis on coronary angiography *OR* • Coronary artery revascularization (PCI, stenting, cardiac bypass surgery) • *OR* Carotid artery stenosis ≥ 50% *OR* • History of carotid artery revascularization (TEA or stenting) *OR*

	• Significant peripheral artery disease (≥ 50% stenosis, PCI, stenting, bypass surgery or amputation))
3. Severe chronic kidney injury	• GFR < 30 ml/min per 1,73 m2 *OR* • GFR 30-44 ml/min pro 1,73 m2 AND Albumin Creatinin-Ratio (ACR) > 30mg/g
4. Diabetes mellitus type 2 AND arterial hypertension AND hypercholesterolemia	• diabetes mellitus type 2: pathological findings in oral glucose tolerance test *OR* documented HbA1c ≥ 6.5 % *OR* intake of any antidiabetic medication *OR* fasting blood glucose ≥ 126 mg/dl) *AND* • arterial hypertension ≥ grade 1: blood pressure of ≥ 140/90 mmHg *OR* intake of anti-hypertensive drugs *AND* • hypercholesterinemia: LDL-cholesterol > 130mg/dl *OR* intake of a lipid-lowering medication that was initiated to treat dyslipoproteinemia
5. Diabetes mellitus type 2 AND at least moderate diabetic kidney injury	• Diabetes mellitus type 2 (see 4.) *AND* • estimated glomerular filtration rate (eGFR) < 60 ml per minute per 1.73 m2 (according to the creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation) *OR* requirement of renal replacement therapy *OR* urinary albumin concentration >20mg/l *OR* 24hour albumin excretion > 30mg/24hours *OR* urinary albumin to creatinine ratio > 30mg/g
6. Diabetes mellitus type 2 AND diabetic retinopathy	• Diabetes mellitus type 2 (see 4.) *AND* • documented funduscopic lesions (e.g., micoraneurysms, intraretinal hemorrhage, diabetic maculopathy), former laser therapy, former injection therapy, former vitrectomia, former intervention because of neovascular glaucoma (Nationale Versorgungsleitinie diabetische Netzhautkomplikationen, 2015)
7. Diabetes mellitus type 2 AND diabetic neuropathy	• Diabetes mellitus type 2 (see 4.) *AND* • neuropathy defined as ≥ 2 of the following: decreased/ absent ankle jerk reflexes and/or decreased distal sensory perception (touch/pressure, vibration (dorsal hallux: < 30yrs: < 6/8; >30yrs: <5/8; medial malleolus: <40 yrs.: <6/8; > 40 yrs: <5/8), pain, temperature) and/or neuropathic symptoms (MNSI, NDS, NSS) *OR* neuropathy as evident by neurophysiological examination (neurography +/- EMG) *OR* small-fiber neuropathy as evident by skin biopsy)
8. patients without any history of CVE, atherosclerosis Diabetes or familial hypercholesterinemia but with very high cardiovascular risk	• < 50 years: CV-risk SCORE2 ≥ 7,5%. • 50-60 years: CV-risk SCORE2 ≥ 10% • ≥70 years: CV-riksk SCORE2-OD ≥ 15% (SCORE2/SCORE2-OD was calculated for a European moderate risk-region using the ESC-CVD-Risk-Calculation-App

according to SCORE2/ SCORE2-OD	(https://www.escardio.org/Education/ESC-Prevention-of-CVD-Programme/Risk-assessment/esc-cvd-risk-calculation-app)
Specific exclusion criteria: chronic CV-risk group	
a < 12 months history of one of the following acute events (as defined by the inclusion criteria for the acute trigger event group)	acute coronary syndrome or myocardial infarction hospitalization for acute heart failure ischemic or hemorrhagic stroke (including retinal central retinal artery occlusion) hospitalization for transitory ischemic attack (TIA) (including Amaurosis fugax)

Research visits

Blood and urine samples: parameters analysed immediately during study visits

Supp. table 3: Blood and urine analysis performed immediately at the day of the inclusion visit and the day of deep phenotyping respectively

Blood sample	
Hematology	• hemoglobin, hematocrit • blood cell count and differential white blood cell count • fibrinogen
Clinical chemistry/biochemistry	• electrolytes (sodium, potassium, chloride, calcium, inorganic phosphate, magnesium) • creatinine, urea, estimated glomerular filtration rate (eGFR), cystatin c • total cholesterol (TCHOL), high density lipoprotein (HDL) cholesterol, non-HDL cholesterol, low density lipoprotein (LDL) cholesterol, lipoprotein a (LP-A), triglycerides • lipase • uric acid • glycated hemoglobin (HbA1c) • liver transaminases (ALT, AST, GGT), alkaline phosphatase (aP), total bilirubin, lactate dehydrogenase (LDH) • total iron binding capacity (TIBC), transferrin, ferritin • total protein, albumin • partial thromboplastin time (aPTT), thromboplastin time (TP, quick), international normalized ratio (INR) • creatin kinase (CK), CK muscle/brain (CK-MB), high sensitive troponin (hsTroponin) • MR pro atrial natriuretic peptide (MR-proANP), NT pro brain natriuretic peptide (NT-proBNP), copeptin • glucose, proinsulin, insulin, c-peptide, homeostasis model assessment (HOMA-IR) • 25-OH-vitamin D • thyroid stimulating hormone (TSH), fT3, fT4
Immunology	• interleukin 6 (IL-6)

	• high sensitive procalcitonin (PCT) • high sensitive c-reactive protein (hsCRP)
Urine	
	• albumin, creatinine, albumin/creatinine ratio

Deep phenotyping visits: Standard plus program

Supp. table 4: Overview of the (optional) standard plus deep phenotyping, that may be joined by all participants of standard deep phenotyping

Disease overarching measures	
Cardiovascular function	24h ECG and 24h blood pressure
Glucose metabolism	cutaneous Advanced Glycation End product (AGE) accumulation
Disease specific measures [study arm]	
Carotid ultrasound [ACS, AHF, Reference]	Intima Media Thickness (IMT); Plaque qualitatively
Electroencephalography (EEG) [Stroke]	3 min neuronal resting state EEG
Physical activity extended [Stroke, ACS, AHF]	[Stroke]: 9-Hole-Peg-Test, 2min finger tapping test [ACS, AHF]: 6-minute-walk test
Somatosensory function testing [Stroke, chronic risk]	cold/warmth detection thresholds (QST), vibration threshold, touch perception, achilles tendon reflexes, sural neurography (point of care)
Magnet Resonance Imaging (MRI)	
cranial MRI	neuroimaging
cardial MRI	cardial muscle and valve imaging
pulmonary MRI	
kidney MRI	
metabolic MRI	liver fat, intraabdominal fat, abdominal subcutaneous fat

Biosample processing

One part of the blood- and urine sample that is collected from the patients is send to our local laboratory (Labor Berlin) for immediate analysis of routine clinical parameters, while the other part is prepared at the BeLOVE Trial Unit`s (BTU) own preanalytical lab for biobanking. .The following probes are processed and aliquoted before transfer to the biobank (all tubes are labelled with 2D barcodes):

- Serum: standing for 30-35 min at room temperature (RT), centrifugation at 2500g for 10 min (RT), pooled, aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen).
- EDTA (whole blood): aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- EDTA (buffy coat): direct centrifugation at 2500g for 10 min (RT), aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- EDTA (plasma): direct centrifugation at 2500g for 10 min (RT), aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- EDTA (aprotinin-plasma): direct centrifugation at 2500g for 10 min (RT), aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- EDTA (citrate-fluoride-plasma): direct centrifugation at 2500g for 10 min (RT), aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- Heparine (PBMC): isolation, aliquoting in 1.9 ml tubes (from 20ml starting material), storage in the liquid phase of nitrogen
- Heparine (plasma): direct centrifugation at 2500g for 10 min (RT), aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- Citrate (plasma): direct centrifugation at 2500g for 15 min (RT), aliquoting in 0.5 ml tubes (storage at -80°C) and 0.25 ml tubes (storage in the liquid phase of nitrogen)
- Cellular Preparation Tubes (PBMC): isolation, aliquoting in 1.9 ml tubes (from 20ml starting material), storage in the liquid phase of nitrogen
- Tempus: upright for 2h (RT), for 24h at -20°C, then storage at -80°CUrine: centrifugation at 2500g for 10 min (RT), pooled, aliquoting in 2.0 ml tubes with a 2D barcode, storage at -20°C/-80°Cand in liquid nitrogen.

- Stool/feces: storage in OMNIgeneGut tubes at 0°C
- Peripheral blood mononuclear cells (PBMC): isolated and cryconserved in liquid nitrogen

Collection of clinical data from medical records concerning history and the index event

Supp. table 5 Data concerning medical history and the treatment of the index event collected from medical records from the HIS

Data concerning medical history	
Cardiovascular	• Acute/ chronic heart failure; Cardiomyopathies, Cardiac contractility management • Acute myocardial infarction; Coronary artery disease, interventional therapy • Arterial hypertension; Artrial fibrillation; Cardial pacemaker • Endocarditis; Myocarditis • Aortic aneurysm, interventional therapy, Persistant foramen ovale, interventional closure
Cerebrovascular/ neurological	• Acute stroke; TIA; Intracerebral hemorrhage; Carotid artery stenosis, interventional therapy; Cerebral aneurysm, interventional therapy • Epilepsy; Parkinson´s disease; Polyneuropathy
Peripheral vascular	• Peripheral artery disease, interventional therapy • Pulmonary artery embolism
Metabolic	• Diabetes • Hyperlipidemia, Hypercholesterolemia, Hypertriglyceridemia • Hyperuricemia, gout • Thyroid disorders
Psychiatric	• Anxiety disorder; Dementia; Depression
Behavioral	• Smoking; Alcohol addiction
Other	• Acute/ chronic kidney injury • Cancer, type of cancer, active vs. in remission • CIBD • Chronic viral infections, type of infection • Collagenosis, type of collagenosis • MGUS • Nephritis • Polymyalgia rheumatic • Psoriasis • Retinal disease • Sacrcoidosis • Thrombophilia, type of thrombophila • Vasculitis, type of vasculitis
Data concerning the index event	

All arms	• Acute infection • any of the diseases described under medical history, if they were newly diagnosed during the index event treatment • Echocardiography (if performed) • ECG
AHF arm	• clinical symptoms; prior therapy; NT-pro-BNP; HFpEF; ECG
ACS arm	• type of ACS; symptoms; mode of admission; door-to-groin puncture time; coronary interventional therapy; blood pressure, heart rate on admission; non-interventional therapy
Stroke arm	• type of stroke; symptom duration; type of imaging and result of imaging • for TIA and ischemic stroke: $ABCD^2$ Score • for ischemic stroke: NIHSS and mRS on admission and discharge, clinical symptoms, stroke localization (vascular territory, anatomical), reperfusion therapy (if performed, type), blood pressure and sugar on admission, intervention for carotid artery stenosis, stroke etiology (TOAST)
Abbrevations: CIBD, Chronic Inflammatory Bowel Disease; HFpEF, Heart Failure with preserved Ejection Fraction; MGUS, Monoclonal Gammopathy of Unknown Significance; NIHSS, National institute of Health Stroke severity Scale; mRS, modified Rankin Scale; TOAST, Trial of ORG 10172 classification of stroke etiology	

Outcome measures: Definition

Supp. table 6: Main event- and value-based outcome measures of BeLOVE. Event-based outcomes are specific clinical events occurring during observation specified by clear endpoint definitions (for the process of endpoint adjudicaton, all endponits are defined in more detail in a comprehensive endpoint repository). Value-based outcomes on the other hand are those that are directly reported by with no direct interpretation processing by the investigations

Event-based outcomes (clinical endpoints)	
Primary endpoint **=** *the first major adverse cardiovascular event (MACE), that is a composite of:*	*specification*
○ non-fatal myocardial infarction (MI) or	acute MI type 1 or MI type 4b or MI type 4a or MI type (each STEMI or NSTEMI) or MI Type 2
○ non-fatal stroke or	ischemic stroke (documented diagnosis of acute ischemic stroke and typical clinical symptoms presenting for ≥ 24 hrs or typical clinical symptoms presenting for < 24 hrs with evidence for acute ischemia on CT- or MR imaging) or non-traumatic intracerebral hemorrhage (acute clinical symptoms and imaging evidence for a brain parenchyma hemorrhage)
○ hospitalization for heart failure or	clinical manifestation heart failure (at least one sign: dyspnea, orthopnea, paroxysmal nocturnal dyspnea, edema, pulmonary basilar crackles, jugular venous distension requiring at least 12 hrs of hospitalization, third heart sound or gallop rhythm, radiological evidence of worsening heart failure) AND heart failure is requiring hospitalization AND additional/increased therapy (at least one: initiation of oral diuretic or IV diuretic or inotrope therapy or vasodilator therapy or uptitration of oral or intravenous therapy if already under therapy or initiation of mechanical or surgical intervention or the use of ultrafiltration, hemofiltration, or dialysis that is specifically directed at the treatment of alteration of biomarkers subsequent to heart failure
○ cardiovascular death	fatal myocardial infarction (death within 14 days after MI without evidence for another cause of death) or sudden cardiac death (witnessed vs. unwitnessed) or fatal new or worsening

	heart failure (including fatal cardiogenic shock) or fatal cerebrovascular disease (death within 30 days following a documented ischemic stroke, non-traumatic intracerebral hemorrhage, non-traumatic SAH cerebral vein thrombosis without without evidence for another cause of death) or fatal cardiac arrhythmia or fatal cardiac valve disease or fatal coronary catheterization or fatal coronary or carotid or cerebral artery intervention or fatal aortic aneurysm or fatal mesenterial infarction or fatal ischemia of the extremities or fatal pulmonary artery embolism
Secondary endpoints *	specification
cardiovascular	
▪ recurrent myocardial infarction (MI)	any MI occurring in a participant for whom a prior MI was the composite primary endpoint
▪ MI following stroke or acute heart failure (AHF)	any MI occurring in a participant for whom a prior stroke or acute heart failure that was the composite primary endpoint
▪ unstable angina pectoris	hospitalization for unstable angina that does not meet criteria of STEMI or NSTEMI
▪ coronary artery revascularization	urgent vs. elective endovascular revascularization or bypass surgery
▪ hospitalization for suspected MI	any hospitalization that ruled out MI
▪ any hospitalization for diagnostic cardiac catheterization	cardiac catheterization without revascularization
▪ recurrent AHF	any AHF occurring in a participant for whom a prior AHF episode was the composite primary endpoint
▪ AHF following MI or stroke	any AHF occurring in a participant for whom a stroke or a MI was the composite primary endpoint
▪ terminal heart failure therapeutic intervention	ventricular assist device implantation, heart transplantation
▪ severe cardial arrhythmia	hospitalization for ventricular tachycardia or bradycardia; cardial pacemaker implantation; hospitalization for or diagnosis of atrial fibrillation
▪ heart valve surgery	open heart surgery; TAVI
cerebrovascular	
▪ recurrent stroke	any stroke occurring in a participant for whom a prior stroke that was the composite primary endpoint
▪ stroke following MI or AHF	any stroke occurring in a participant for whom a prior AHF episode or MI that was the composite primary endpoint
▪ acute revascularization of acute ischemic stroke	therapeutic thrombolysis; endovascular thrombectomy
▪ transient ischemic attack (TIA)	typical transient acute cerebral symptoms with no correlate in cerebral MRI imaging
▪ carotid artery revascularization	endarterectomy; stenting, extra-intracranial bypass surgery
▪ persistent foramen ovale (PFO) intervention	PFO closure for secondary stroke prevention
▪ subarachnoid hemorrhage (SAH)	any non-traumatic SAH
▪ cerebral aneurysm intervention	surgical or endovascular treatment of a cerebral aneurysm
▪ cerebral vein thrombosis	cortical, cerebral sinus or internal cerebral vein thrombosis
peripheral vascular	
▪ amputation for peripheral artery disease (PAD)	therapeutic amputation of a lower or upper extremity due to severe PAD
▪ PAD revascularization	surgical or endovascular revascularization of any artery except for the coronaries, carotids, intracerebral arteries or aorta
▪ aortic aneurysm intervention	resection and interposition of the thoracic or abdominal aorta
▪ peripheral vein disease	deep vein thrombosis, pulmonary artery embolism
diabetic end-organ damage/ complications	
▪ diabetic microangiopathy	new diagnosis of diabetic retinopathy, neuropathy, or nephropathy
▪ diabetic foot syndrome	due to diabetic neuropathy and/or PAD, with or without amputation
▪ hospitalization for hypo- or hyperglycemia	with or without ketoacidosis or hyperosmolar coma
renal	
▪ major adverse kidney events (MAKE)	end stage renal disease (transplantation or dialysis) or renal death
▪ acute kidney injury	stage AKIN I-III with or without acute dialysis
death	
▪ all-cause mortality	any vascular or non-vascular death or death by an unknown cause
▪ death caused by infection	in immunocompromised vs. in non-immunocompromised patients

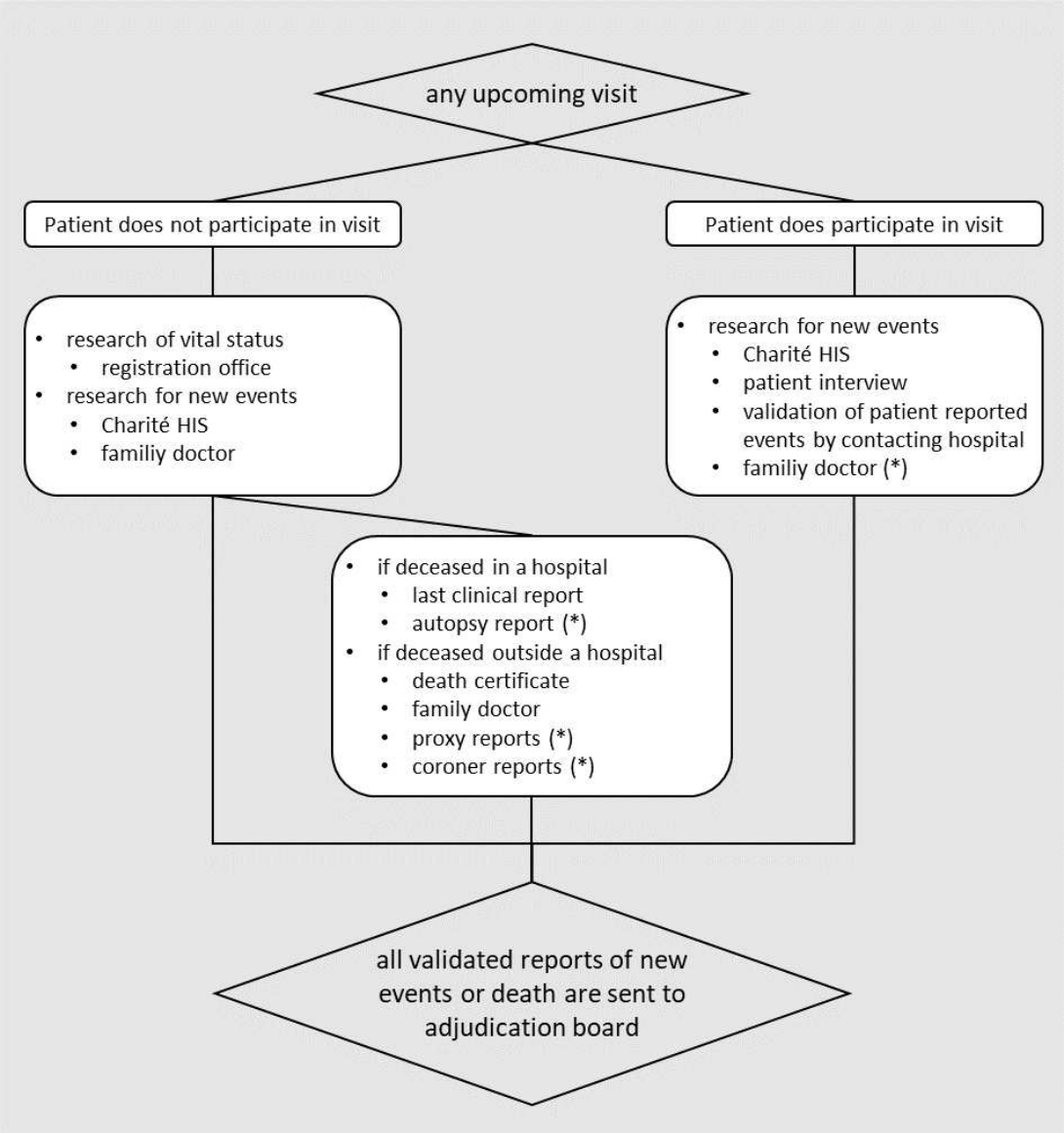
▪ death caused by terminal kidney disease	death directly attributable to kidney failure without sufficient renal replacement therapy
▪ death caused by cancer	death in the context of terminal cancer or cancer therapy complications
▪ death caused by COVID-19	any death that occurs during hospitalization for COVID-19
▪ death caused by other reasons	e.g., death by suicide, death following trauma
▪ unknown cause of death	this category can only be adjudicated in exceptional cases
other hospitalizations	
▪ hospitalization for or with neuro-psychiatric reasons	schizophrenia, depression, bipolar disorder, anxiety disorder, delirium, dementia
▪ hospitalization for epilepsy	first epileptic seizures, epilepsy, status epilepticus
▪ hospitalization for endocrinological/ metabolic reasons	hypothyroidism, hyperthyroidism, hyponatremia, non-alcoholic fatty liver disease
▪ hospitalization or new diagnosis of cancer	any kind of cancer
▪ any other hospitalization	any hospitalization that does not meet criteria of a more specific endpoint
COVID-19 related outcomes	
▪ in-hospital treatment for COVID-19	with or without ICU admission, with or without mechanical ventilation or ECMO
medical consequences of BeLOVE incidental findings	medical treatment related to urgent incidental findings from MRI, ECG, 24hr-ECG, 24hr blood pressure, echocardiography, optical fundus examination, blood samples
* secondary endpoints are defined and coded in much more detail in a comprehensive inventory	
Value-based outcomes (quality of life) §	
o health related quality of life	PROMIS Profile® EQ5d5l SF 36 **
o domain specific quality of life**	depression: PROMIS; PHQ-8 anxiety, physical function, pain, fatigue, sleep, social roles: PROMIS
o disease-specific quality of life	heart failure: KCCQ, MLHFQ angina: Seattle Angina Questionnaire diabetes: ADDQoL stroke: SSQoL
§ value-based outcomes are also measured during ~1hr basic deep phenotyping ** these measures are not performed during telephone visits	
Abbrevations: TAVI = transcatheter aortic valve implantation; STEMI= ST-segment elevation myocardial infarction; NSTEMI= non--segment elevation myocardial infarction; ECMO= extracorporeal membrane oxygenation; ICU = intensive care unit; PROMIS= patient reported outcome measure instrument system; EQ5d5l = five-level scale of the EuroQoL group; SF36= short form 36; PHQ 8= depression related part of the Patient Health Questionnaire sparing suicidality; KCCQ = Kansas City Cardiomyopathy questionnaire; MLHFQ= Minnesota Living with Heart Failure Questionnaire; ADDQoL= Audit of Diabetes Dependent Quality of Life; SSQoL= Stroke Specific Quality of Life Scale	

Endpoint assessment and adjudication procedures

Assessment of relevant new clinical events during follow up

Assessment of new clinical events is performed repeatedly (after ~30, ~60 and ~90 days and biannually) in every participant by a specialized team of BTU staff. All available patients are interviewed (see clinical events interview table 3 in the main paper) for new clinical events (see sup. table 7) at deep phenotyping and all telephone visits. Self-reported patient information on clinical events by participants or proxies will only be considered as an endpoint if it can be validated by medical documents from hospitals of family doctors. Systematic research of the Charité hospital information system (HIS) is carried out irrespective of declarations made

during the participant interview every 6 months. For participants that are unavailable for questioning, the assessment is expanded by inquiries of citizen registration offices and family doctors. In case of death any available information concerning the cause of death including the latest medical reports, death certificates and information by family doctors and proxies are obtained (see suppl. figure 2 for an overview of the research methods). Further, repeated queries of diagnostic data from health insurance companies shall be implemented as soon as possible as an additional measure.

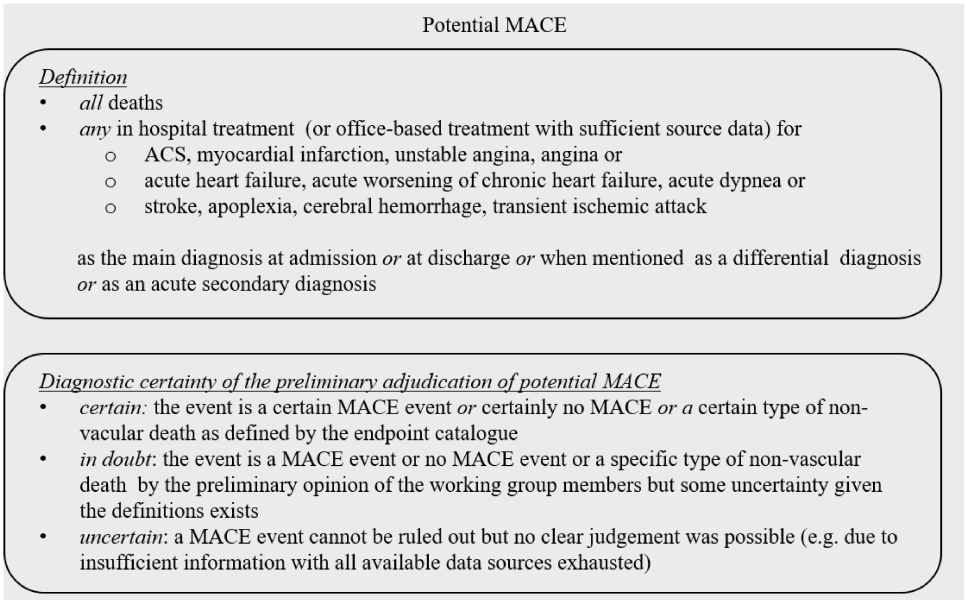


Supp. Figure 2: Endpoint assessment is performed at every upcoming deep phenotyping or telephone visit. The hospital information system (HIS) of the Charité is researched for any new treatments or events in any case. The use of all other research methods are depending on the availability of the participant. In unavailable participants the family doctor is contacted for additional information on any new clinical events (e.g., that were not treated within the Charité). Since registration of all citizens is mandatory in Germany the current address and information about the vital status, that is information about death and date of death can usually obtained by the citizen registration office databank. If a participant deceased in a hospital, all relevant last medical reports and if available, any autopsy report will be obtained. In cases of death outside of a hospital death certificates and any other available information by the family doctor, by proxies or autopsy reports will be obtained. All available participants will be interviewed concerning clinical events. Importantly, every self-reported event will be validated by documents from HIS, other hospitals or the family doctor and reported events that could not be validated will not be considered as endpoints. Finally, the whole process of endpoint research is documented in the study databank and all medical documents obtained are forwarded to the endpoint adjudication board. (*) means, that this source data is considered whenever it is available.

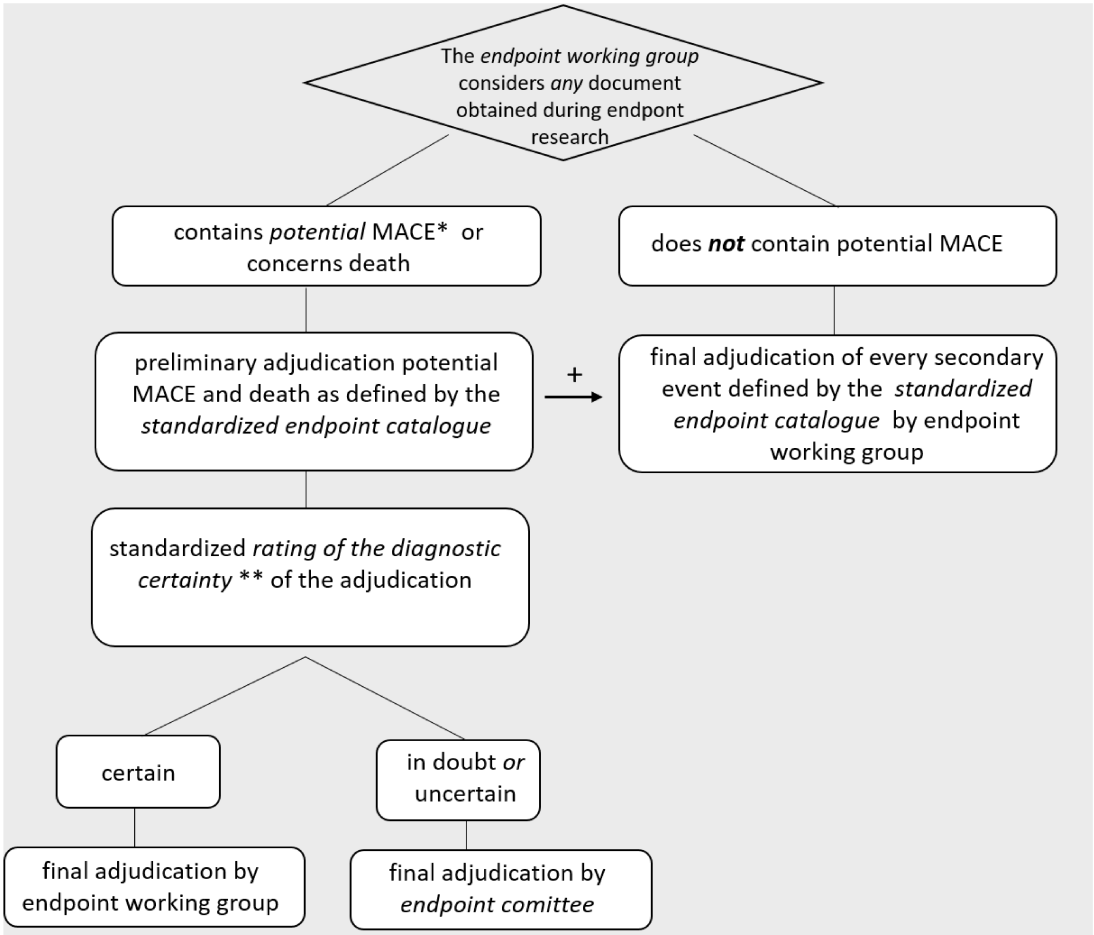
Endpoint adjudication procedures

Endpoint adjudication is primarily and constantly carried out by a group of physicians that is reviewing all medical information on clinical events retrieved during the follow up. All endpoints of interest are predefined and coded in an extensive inventory containing standardized definitions which is providing the tool for a standardized adjudication procedure (see supp. table 7 for an overview). Every medical document is reviewed for any of the endpoint-defining events as defined in the inventory, therefore multiple secondary endpoints may be extracted from a single document. Further, data on the patient history in every document is compared to already existing endpoint data in the study database. Additional medical source will be obtained for all potential endpoint data that is missing in our database.

For every potential MACE (see supp. figure 3) or in any case of death that cannot be determined with absolute certainty the final adjudication is carried out by a superior endpoint committee consisting of the clinical PIs of the study (see supp. figure 4). Only events that manifested clinically are considered as MACE while events that are evident by paraclinical exams only (e.g., signs of incidental stroke or myocardial infarction on neuroimaging) are not.



Supp. figure 3 Definition of potential MACE events and categories to be used to rate the diagnostic certainty of preliminary adjudication by the endpoint working group. For all events categorized as in doubt or uncertain final adjudication is carried out by the internal endpoint committee.



Supp. figure 4 Endpoint adjudication is primarily carried out by the l endpoint working group of study physicians who examine every document obtained during endpoint research for contained endpoints. Endpoints are defined in a comprehensive catalogue defining multiple standardized endpoints categorized in 9 chapters (cardiovascular events, cerebrovascular events, peripheral vascular events, diabetes complications, renal events, other clinical events (including any kind of hospitalization not defined elsewhere, medical treatments as a consequence of BeLOVE findings management, COVID-19 associated events and events occurring during the acute phase of the index event). The definition* of potential MACE and the categories for rating the certainty of their adjudication are described in supp. figure 4.

Methods used to improve retention

Participants receive several calls and letters to remind them of their appointments. Different protocols with shorter vs. longer examination times are available for the on-site visits to accommodate both physically impaired participants and those who are physically more resilient and highly motivated (see figure 2). Communication of individual study results (which is embedded in incidental findings management, see below) is conducted in a highly standardized

process, that is transparently communicated to the participants during informed consent. Furthermore, participants are informed about the overall study progress by newsletters.

Data management, Quality assurance (QA) and Quality control (QC)

Data management is conducted by BeLOVE´s own data management team in close cooperation with the clinical study center (CSC) of the Charité – Universitaetsmedizin Berlin. BeLOVE collects and manages study data using the secure, open-source web-based software platform REDCap hosted at Charité – Universitaetsmedizin Berlin [3, 4]. Manually captured data (e.g., self-administered questionnaires, interview results, and results of bedside examinations) are collected using a web-based central electronic case report forms (eCRF) on a tablet. Data from medical devices are captured automatically to the Health Data Platform (HDP) of the Charité, which includes an archive for raw data as well as a structured repository for metadata. Similarly, measurements performed on biosamples that are not stored in the biobank is processed using a central laboratory information management system (LabVantage). The repository for all laboratory data, including metadata, is centrally managed. This management includes central execution of data validation procedures as well as data query management. The independent third trust party of the Charité, which is separated from the main study database, is keeping a master participant index and is managing pseudonymization and a central electronic informed consent management.

Our QA and QC concept was developed and will constantly be updated, in close cooperation with the central structures for internal and external quality management at the Clinical Study Center (CSC) of the Charité – Universitaetsmedizin Berlin. Our concept is in line with principles and guidelines for Good Clinical Practice (ICH-GCP), Good Laboratory Practices (GLP) and Good Epidemiological Practice (GEP).[5] Standard operating procedures for all elements of data collection as well as a delegation log of responsibilities have been implemented to standardize our efforts. This includes also the periodic calibration of data capturing devices

to reduce measurement errors and batch effects. More importantly, the training and certification of all personnel involved in collection of data and biosamples, as well as the continuous testing of our data collection procedures will help to ensure high-quality data collection throughout the study period. This is supported by data monitoring in the responsibility of the CTO ensuring that rights and well-being of participants are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study complies with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirements.

Research questions (examples) addressed in BeLOVE

Supp. table 7: Examples for research questions/ hypotheses to be investigated by specific phenotyping methods

Research question/Hypotheses	Method	main outcomes
Myocardial structure as determined by cardiac MRI predicts major adverse cardiovascular events	cardiac MRT	MACE
Stroke induced functional and structural alterations in the central autonomic network predict long-term cardiac alterations	cerebral MRI, cardiac MRI, Echocardiography, clinical parameters	myocardial morphology and cardiac function, Diagnosis of Heart Failure
Alterations in myocardial morphology and cardiac function predict cognitive decline and cerebrovascular events	cerebral MRI, cardiac MRI, Echocardiography, clinical parameters	Cognitive decline, cerebrovascular events
Cognitive decline after ischemic stroke is determined by multiple factors such as genetic, inflammatory, metabolic, structural, psychosocial, and lifestyle predispositions.	cerebral MRI, EEG, Cognitive measures (MOCA; CANTAB), bio sampling, PROMs	Cognitive decline, cerebrovascular induced brain lesions
Fasting, feeding, resting, and physical activity induce different dynamics of metabolic biomarker profiles predicts future cardiovascular events	Nutritional and physical (spiroergometry) challenge, bio sampling,	MACE, secondary cardiovascular events
Glucose variation as measured by continuous glucose monitoring improves prediction of recurrent cardiovascular events and health outcome	Continouus glucose monitoring, bio sampling	MACE, secondary cardiovascular events
Patterns of physical activity, sedentary behavior, diet, and psychosocial stress predict cardiovascular outcomes	Physical Activity, Food diaries, Eating questionnaires, PROMs, metabolomics	MACE, secondary cardiovascular events
Advanced assessment of diabetic microvascular complications is able to identify biomarkers for adverse macrovascular outcomes	Somatosensory phenotyping, Ophthalmologic phenotyping, bio sampling	Diabetic neuropathic and retinopathic patterns, MACE
Genetic and epigenetic variability are associated risk factors for cardiovascular events	Genomics	MACE
Alterations of microbiome-driven immunological and metabolic homeostasis predict cardiovascular risk	Stool sampling, bio sampling, immunophenotyping	MACE, secondary cardiovascular events

Addressing potential Sources of bias

There are methodological challenges in BeLOVE as in any other clinical observation cohorts. Patients consenting to participate in the study represents a selection of patients who would be recruitable. To estimate this selection bias, we compare patient characteristics of participants to aggregated data (sex, age, comorbidity) of all other patients treated at Charité and main hospital diagnosis of CVD. In addition, the number of patients who are able to participate in face-to-face visits may decrease over time due to worsened disease status or other issues. We will address this potential attrition bias with a comprehensive concept of active and passive patient follow-up, such as telephone interviews and the use of hospital registry data. Additionally, reasons for drop out will be documented if participants are contacted and withdraw from the study. Other types of bias such as collider stratification bias or reverse causation need to be considered in analyses of the BeLOVE study. Thus, conditioning on disease groups may open up a backdoor path, and thus violate the conditional exchangeability assumption. Such backdoor paths can be identified using Directed Acyclic Graphs (DAGs). In BeLOVE, we will therefore ensure that specific research questions will be put into the framework of sound DAG theory. Therefore, data collection regarding pre-existing risk factors is just as important as the data collection on current potential risk factors.

Reverse causation is another concern for potential bias when studying patients with pre-existing diseases. This type of potential bias does not uniformly apply to the study design per se but is dependent on the underlying research questions. In recent years, situations have been identified in which reverse causation has been an issue, and several approaches have been suggested to identify reverse causation bias, such as serial tracking of data, stratified analysis, and instrumental variable analysis.[6] Because of the intensive monitoring of the patients in the BeLOVE cohort and the close integration with the Charité electronic health record system, serial tracking will be an efficient way to be included into the analysis. In addition, given the deep phenotyping in BeLOVE and the assessment of many potential risk factors we will be able to conduct pre-specified stratified analyses (e.g., by age or follow-up time) to assess the possibility of reverse causation.

Sample size justification and detailed power statement

While many different types of analyses and models will be applied in BeLOVE, time-to-event analyses will be a major focus. The Cox proportional hazards regression model is the basic approach for modeling time to event data. Other models for recurrent or competing events that will also be applied are all extensions of this well-known Cox-model. Therefore, we based our power considerations on the Cox model approach.

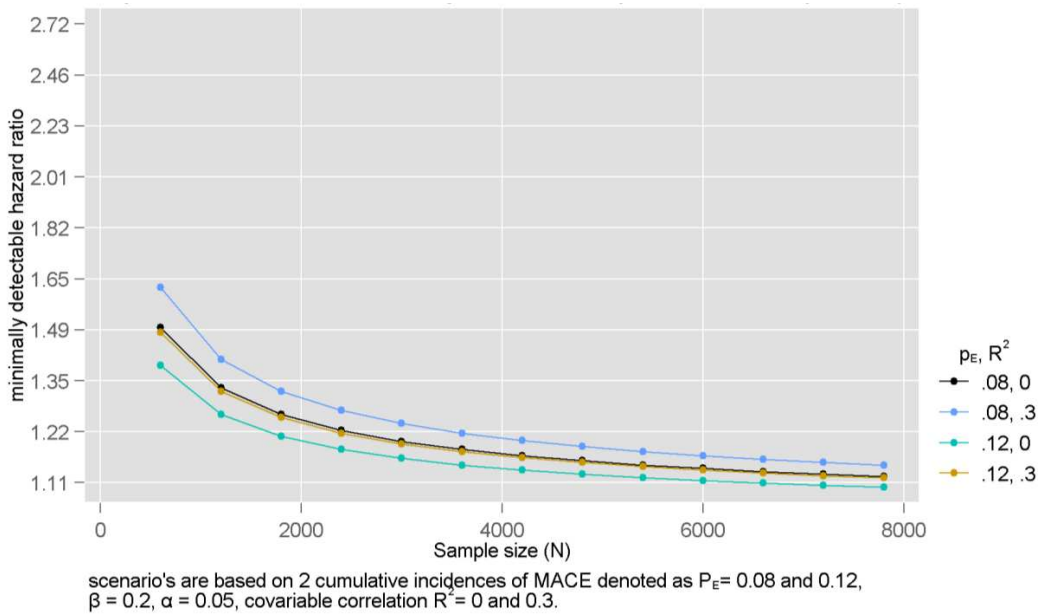
The sample size calculation of a Cox model is influenced by several factors, for which we have made the following assumptions:

- The anticipated power value (set as 80%).
- The number or percentage of observed events at the investigated follow-up time point. For BeLOVE, it is assumed that within the 1-year follow-up 8-12% of the patients experience a new cardiovascular event of any type.[7-9] For the individual disease entities, the event rates might differ. Note that the BeLOVE study is planned with total follow-up time of 10 years. With an increasing follow-up period, the number of expected events will increase and as a consequence, the power will increase as well. Therefore, the considerations made in here for the one-year follow-up define a conservative scenario and even better power values can be expected for longer follow-up times.
- The type, the distribution and the number of independent variables included in the model. For the sake of simplicity, we assumed two types: continuous and binary. For continuous we have modelled the exposure per standard deviation increase (standardized effect) and for binary variables we assumed equal group sizes (50% prevalence of the exposure of interest).
- The anticipated effect of the risk factor, expressed for the Cox model as the hazard ratio (HR) or the logarithm of the HR (i.e., beta-coefficient).

- The anticipated degree of correlation among risk factors of interest and all other independent variables in the model, which is given as pseudo-R^2, which lays within [0;1]. Values close to 0 indicate that the risk factor of interest is independent of all other covariates. As there are always multiple factors associated with the final outcome, a correlation among independent variables of 0.3 seems often more reasonable.

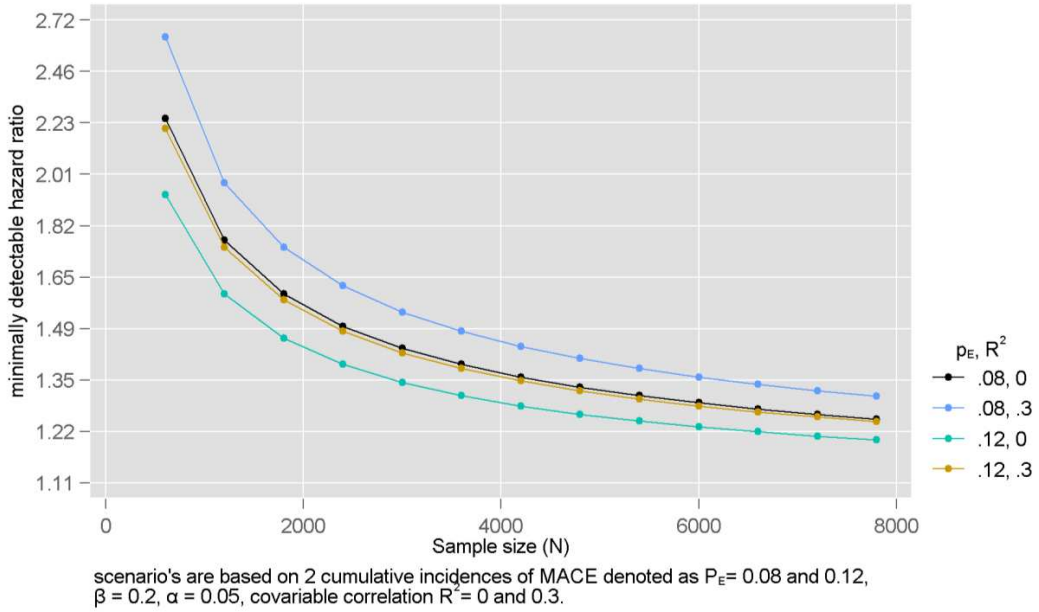
Based on these parameters and assumptions, we constructed two figures that provide an overview of the precision and minimally detectable effects for continuous exposures modeled per standard deviation increase (suppl. figure 5) and binary exposures (suppl. figure 6), respectively that can be expected. Specifically, the figures investigate a range of sample sizes from 1200 to 7800. The graphs were obtained by the "power" package from STATA 14.0 with the following details:

1. power cox, sd(1.0) n(600 1200:8000) r2(0 0.3) failprob(0.08 .12) effect(hratio) power(0.8) direction(upper)
2. power cox, sd(0.5) n(600 1200:8000) r2(0 0.3) failprob(0.08 .12) effect(hratio) power(0.8) direction(upper)



scenario's are based on 2 cumulative incidences of MACE denoted as P_E= 0.08 and 0.12, β = 0.2, α = 0.05, covariable correlation R^2= 0 and 0.3.

Supp. figure 5: BeLOVE Power Scenarios: minimally detectable effect after one year of follow-up: **continuous exposure**, per SD



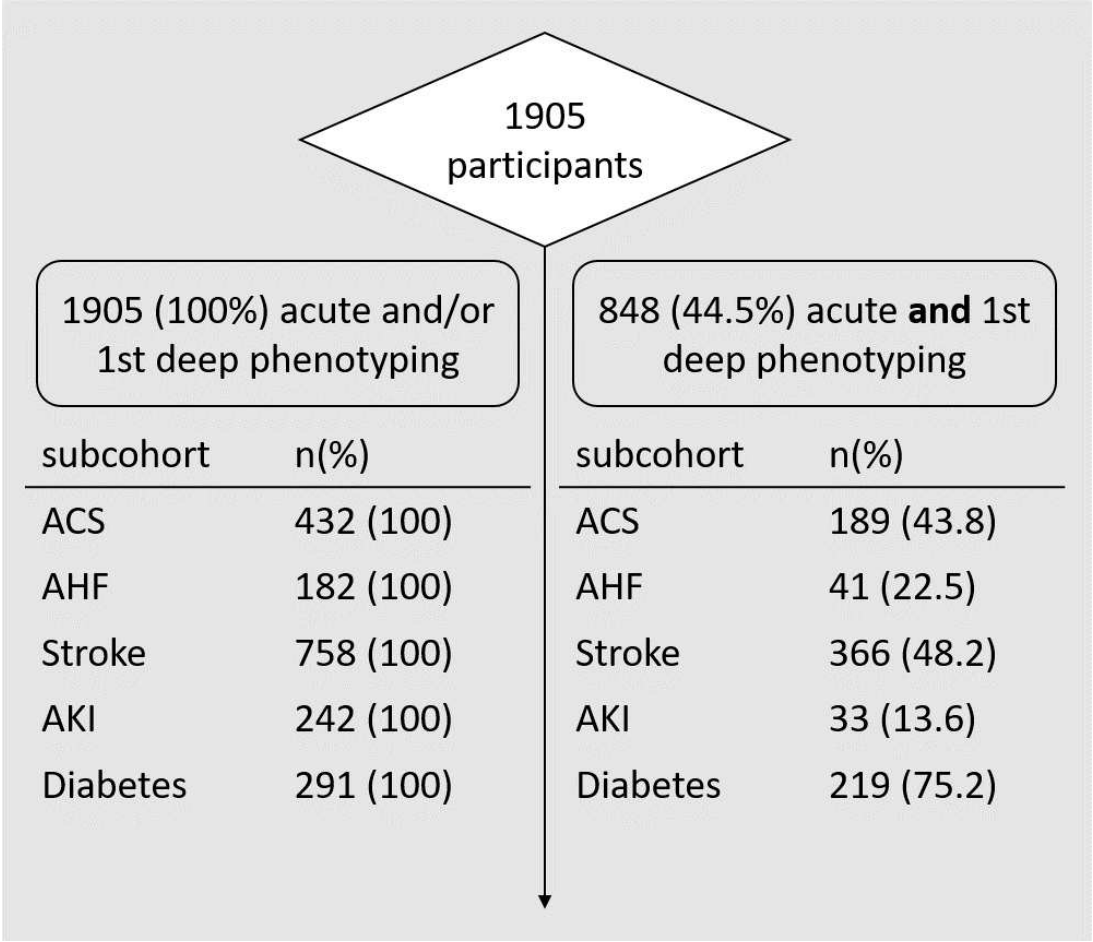
Supp. figure 6: BeLOVE Power Scenarios: minimally detectable effect after one year of follow-up: **binary exposure** (50%)

As an exemplary interpretation, for a two-sided significance level of 5%, a power of 80%, and no correlation between the predictors and an annual event proportion of 8%, the minimal detectable effect in 7000 participants is HR 1.13 (black line), which is lowered to HR 1.10 when a 12% annual incidence of outcome is assumed (green line). This shows that with all patients in a combined analysis, BeLOVE has sufficient statistical power to pick up small effect sizes. Our calculations also include more conservative scenarios, e.g. if the study population at 1 year is reduced due to loss-to-follow-up, or in case of subgroup analyses in patients with one specific disease. Moreover, there will be the need to adjust for other covariates as for the predictor of interest and these set of predictors will usually be correlated. The figure shows these different scenarios, by varying the sample size as well as plotting separate lines for a single independent variable (R^2=0) as well as for several correlated independent variables, for example, adjusted for age, sex, and other traditional cardiovascular risk factors (R^2 =0.3). It can be seen that if the risk factor of interest is correlated to other independent variables (blue and yellow lines), the required sample sizes are larger than for uncorrelated independent variables (black and green

lines). The flattening of the lines with increasing sample sizes indicate that the added precision obtained from increasing sample size reduces with sample sizes above N=2000.

A similar picture, but with higher minimally detectable hazard ratios, is obtained when looking at the minimally detectable differences for binary exposures with a prevalence of 50%.

Recruitment, acute and 1st deep phenotyping visits performed during first study phase (implementation)



Supp. Figure 7: Patients recruited during first study phase between July 18, 2017 and December 31, 2020. Originally, 2248 participants were recruited and signed the informed consent. Of those, 343 were excluded for screening failure or dropped out of the study (by withdrawal, death etc.) before any baseline data could be obtained. Therefore 1905 participants were available for phenotyping and follow up. Acute phenotyping was performed at max. 7 days after the acute event or study inclusion in the diabetes arm. Deep phenotyping was performed after ~90 days, 2 years and 4 years. Please note, that in the initial study phase deep phenotyping was offered every second year. Only participants that joined the 1st deep phenotyping could participate in the later deep phenotyping visits. Follow up by telephone is continued for all participants that did not end study participation. * 848 (96.6%) participants of the 1st deep phenotyping also joined acute phenotyping before; figure is based on data export from 15 May 2023

Baseline characteristics of patients recruited in the first study phase (implementation)

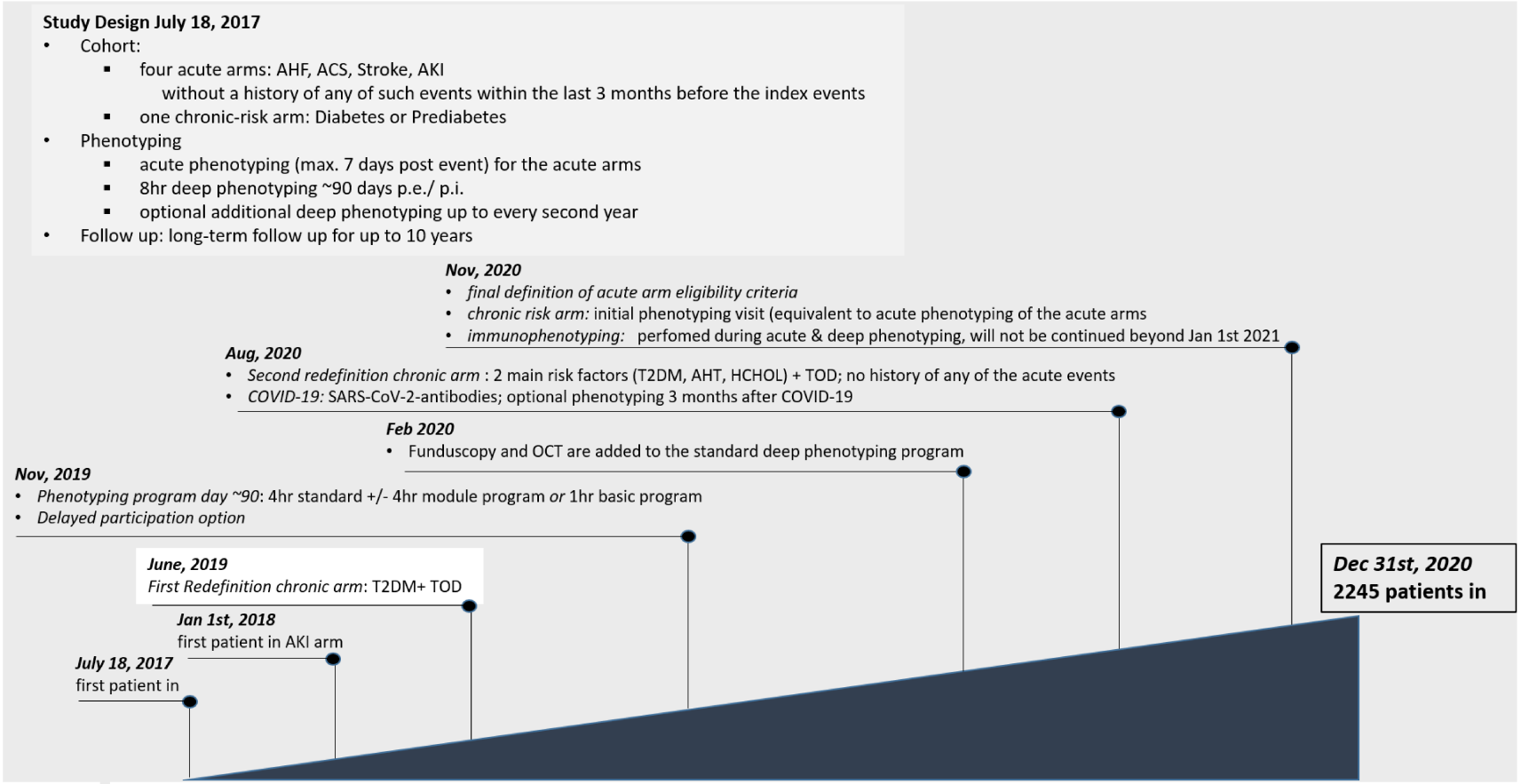
Supp. table8: Baseline characteristics at enrollment of patients recruited during the first study phase. Data is based on self-reported history which was largely validated by reviewing medical records. Data is related to the time before event or recruitment in the diabetes group. Except for ischemic stroke/TIA, myocardial infarction, acute heart failure and peripheral artery disease primary diagnosis of the condition during the treatment of the index event was also considered. Available number of data for every parameter are presented in [ ]. Table is based on data export from 15 May 2023.

	total cohort	**subcohorts**				
		ACS	*AHF*	*Stroke*	*AKI*	*Diabetes*
total partcipants n	1905	432	182	758	242	291
age, mean (SD) [n total]	66.0 (13.0)[1905]	65.0 (12.0) [432]	71.0 (10.9)[181]	67.5 (12.7)[768]	63.0 (15.9)[242]	63.1 (12.0)[291]
sex, female, n (%) [n total]	680 (35.7) [1905]	121 (28.0) [432]	61 (33.5) [181]	282 (37.2) [768]	103 (42.6) [242]	113 (38.8) [291]
hypercholesterolemia, n (%) [n total]	1646 (91.5) [1799]	418 (98.4) [425]	143 (83.1) [172]	694 (93.7) [741]	136 (70.5) [193]	255 (95.1) [255]
arterial hypertension, n (%) [n total]	1627 (88.2) [1845]	425 (98.4) [432]	169 (93.4) [181]	625 (85.0) [735]	157 (71.0) [221]	251 (90.9) [276]
diabetes mellitus, n (%) [n total]	801 (42.7) [1892]	148 (34.3) [431]	98 (54.1) [181]	171 (22.6) [757]	93 (40.1) [232]	291 (100) [291]
coronary artery disease, n (%) [n total]	749 (40.3) [1859]	398 (93.4) [426]	107 (59.1) [181]	107 (14.2) [752]	58 (26.1) [222]	79 (28.4) [278]
atrial fibrillation, n (%)[n total]	467 (25.1) [1857]	74 (17.4) [426]	122 (67.4) [181]	164 (21.8) [752]	59 (26.5) [223]	48 (17.5) [275]
current smoking, n (%)[n total]	410 (22.9) [1792]	132 (32.2) [410]	26 (15.7) [166]	155 (21.6) [717]	49 (22.3) [220]	48 (17.2) [279]
former smoking n (%)	532 (29.6)	111 (27.1)	62 (37.3)	208 (29.0)	76 (34.5)	75 (26.8)
Hx of chronic heart failure, n (%) [n total]	345 (19.5) [1770]	72 (17.8) [404]	155 (88.1) [176]	46 (6.7) [691]	37 (16.8) [220]	35 (12.5) [279]
Hx of ischemic stroke or TIA, n (%)[n total]	271 (14.1) [1866]	30 (7.0) [426]	26 (14.4) [181]	187 (24.9) [752]	14 (6.3) [223]	14 (5.0) [279]
Hx of myocardial infarction, n (%)[n total]	241 (13.0) [1860]	86 (20.2) [426]	44 (24.3) [181]	63 (8.4) [752]	24 (10.8) [222]	24 (8.6) [279]
Hx of carotid artery stenosis, n (%)[n total]	133 (7.4) [1793]	18 (4.4) [407]	1 (0.6) [162]	89 (12.3) [725]	7 (3.2) [221]	18 (6.5) [278]
Hx of peripheral artery disease, n (%)[n total]	129 (6.9) [1858]	29 (6.8) [426]	23 (12.7) [181]	42 (5.6) [752]	19 (8.6) [221]	16 (5.8) [278]
Hx of acute heart failure, n (%)[n total]	113 (6.1) [1854]	7 (1.6) [426]	83 (45.9) [181]	11 (1.5) [752]	7 (3.2) [218]	5 (1.8) [277]

Study design adjustments

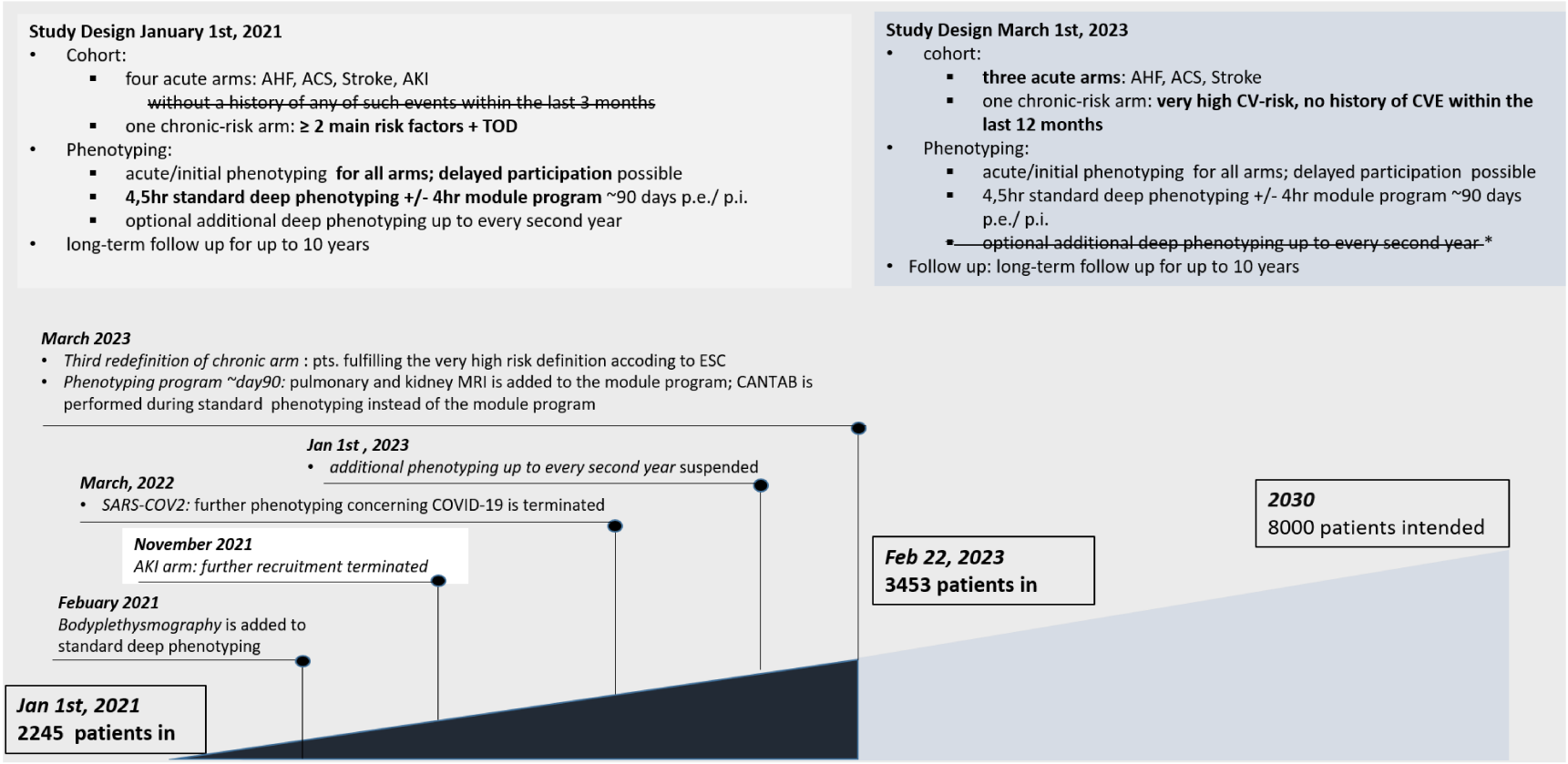
Several adjustments of the study design were necessary First, while we intended to include high-risk patients in our study, we observed a misbalance among study arms in a way that in some arms patients were too sick to attend study visits. Therefore, we adapted the inclusion and exclusion criteria particularly of the AHF-arm to facilitate participation of less severely affected patients and in the chronic high risk-arm to achieve a more balanced design. Further, the original design of BeLOVE included an additional fifth arm of patients with recent acute kidney injury (AKI); however, this arm was terminated because health-impairment in this population was too severe to participate in the study, and no option for adaption of the inclusion or exclusion criteria was deemed feasible. In consequence, the total sample size aimed for was reduced from 10,000 to 8,000 patients. Second, it was originally planned to have deep-phenotyping visits every two years for each patient in addition to the 90-day visit. Because of budget restrictions these visits have been dropped from the main protocol. Third, the study program was tightened to reduce the burden of clinical examinations for the participants and to optimize adherence rates. A detailed timeline and description of all relevant modifications can be found in supp. figures 8 and 9 and supp. table 9.

Timeline of study design adjustments – part 1



Supp. figure 8: Timeline of study design adjustments between July 18 2017 and December 31st, 2021. Timeline is continued in supp. figure 10. Please see supp. Table 10 for more details on the adjustments. AHF, acute heart failure NYHA ≥ IIIb; ACS, acute coronary syndrome; Stroke,ischemic stroke,TIA,, non-.traumatic intracerebral hemorrhage and cerebral vein thrombosis; AKI, acute kidney injury ≥ AKIN II; p.e., post event date; p.i. , post inclusion date; TOD; target organ damage

Timeline of study design adjustments – part 2



Supp. figure 9: Timeline of study design adjustments between Jan 1 2021 and March 1st 2023. Please see supp. Table 10 for more details on the background and implications of the major adjustments. AHF, acute heart failure NYHA ≥ II; ACS, acute coronary syndrome; Stroke included ischemic stroke,TIA, and non-.traumatic intracerebral hemorrhage; AKI, acute kidney injury ≥ AKIN II; p.e., post event date; p.i. , post inclusion date; CANTAB, Cambridge Neuropsychological Test Automated Battery; * phenotyping in 2 year intervals may be continued for pat. subsets if additional funding is available

Major adjustments of the study design during study implementation

Supp. table 9: Major adjustments of the study protocol during the initial implementation and current second phase of the study

Topic/ Study procedure	**Previous status**	**Experience**	**Major adjustment**
inclusion criteria acute kidney injury (AKI)	a subcohort of patients with AKI KDIGO stage AKIN ≥ 2 as an additional CV high risk population was recruited since January 2018	recruitment and retention of this subcohort was critical due to severe overall health impairment which was also expressed by a high mortality- (~25%) and withdrawal (~17%) rate	• recruitment was terminated in November 2021 • a total 252 patients were recruited from January 2018 until November 2021 • currently 140 participants remain in the study for whom a telephone follow up is continued • the subcohort will be described in more detail in forthcoming publications
inclusion criteria, chronic CV risk arm	initial definition was pts. with diabetes mellitus type 2 or prediabetes with target organ damage	• Recruitment: diabetes: n= 219; prediabetes n=2 • *1st redefinition June 18, 2019*: recruitment of prediabetes was terminated; diabetes typ2 (T2DM) + microangiopathy (nephropathy, retinopathy, or neuropathy) or + macroangiopathy (cerebrovascular disease, CAD, or PAD) was defiend as elegible; n=90 pts were recruited until Aug 17, 2020 • there were concomittant concerns about the limitation of the chronic risk group to diabetics only • *2nd redefinition Aug 17, 2020*: due to concomittant concerns about the limitation of the chronic risk group to diabetes only; patients with at least to major risk factors (T2DM, arterial hypertension, or hypercholesterinemia) and target organ damage (atherosclerosis, chronic kidney injury, hypertensive heart disease, diabetic or hypertensive retinopathy, diabetic nephropathy, or diabetic neuropathy); pts. with *any* history of acute stroke, TIA, AHF, ACS or AKI were excluded from recruitment • n= 99 patients recruited under this definition; > 70% were diabetics and recruitment of all candidates was heavily impeded by the exclusion of previous acute events	• *3rd redefinition Febuary 2023*: according to the CV very high risk definitions proposed by the ESC (2029, 2021) which includes patients with previous acute CV events, patients with severe chronic kidney injury, atherosclerosis, T2DM+ arterial hypertension+ hypercholesterolemia, diabetic microangioiopathy or very high risk SCRORE2/SCORE2-OD • patients with pervious CV events or AHF are only excluded if such events occurred within the last 12 months • the adjustment does not affect the majority of participants who have been recruited previously, as they will remain eligible under the new criteria
inclusion criteria, stroke arm	cerebral venous thrombosis (CVT) was defined as a possible inclusion criterion	no patients with CVT could be recruited during the first study phase	• CVT was discarded as an inclusion criterion • central retinal artery occlusion was defined as a ischemic stroke equivalent

Topic/ Study procedure	Previous status	Experience	Major adjustment
			• the adjustment does not affect participants who have been recruited previously, as they will remain eligible under the new criteria
inclusion criteria, acute heart failure arm	AHF was defined as dyspnea ≥ NYHA IIIb and NTproBNP ≥ 300 pg/nl or MRproANP ≥ 120 pmol/l	recruitment and retention were found to be compromised by the severity of the overall health impairment of these patients	• AHF ≥ NYHA II was defined to be sufficient for study inclusion • specific cut of values for biomarkers were no longer mandatory • the adjustment does not affect participants who have been recruited previously, as they will remain eligible under the new criteria
exclusion criteria general	patients with an acute event, that did already experience a previous event within the last 3 months were excluded from the study	this practice did significantly limit the number of eligible patients, in particular in the AHF arm	• criterion was discarded since Jan 1st, 2021 • change does not affect participants who have been recruited previously, as they will remain eligible under the new criteria. • the adjustment does not affect participants who have been recruited previously, as they will remain eligible under the new criteria
follow up phenotyping schedule	additional and repeated deep phenotyping visits with a program equivalent to the ~day 90 visit u to every 2 years was offered to the first ~3000 participants	current funding does not allow to continue additional deep phenotyping beyond day 90	• deep phenotyping visits beyond day 90 have not been performed since January 1st, 2023 • number and rates of pts. that up till then participated in deep phenotyping after 2,4 and 6 years are shown in supp. figure 8 • results of the phenotyping performed will be analyzed for publication • additional phenotyping may be continued for patient subsets currently recruited if additional funding is available in the future
follow up phenotyping schedule	deep phenotyping was an 8hr program splitted between two days without other options	8hr phenotyping was not feasible for many old and/or very illl participants and the lack of other options impaired recruitment and retention	• participants can chose between an 4hr standard program or 4hr standard + 4 hr module program • a ~1hr basic program is available for participants that would otherwise not participate in deep phenotyping at all for health-related and other reasons
follow up discaredd methods	• blood samples obtained during acute and deep phenotyping were used for immunophenotyping	• immunophenotyping and hair sample analysis cannot be continued due to funding restrictions • COVID-19-related measures were stopped due to the declining incidences and increasing reates of seroconversion	• Methods and associated research questions will be described in detail in forthcoming publications. Available sample sizes are: Immunophenotyping was performed from blood samples of ~1000 participants

Topic/ Study procedure	Previous status	Experience	Major adjustment
	• besides saliva, hair samples were obtained during day ~90 deep phenotyping • to investigate the impact of the pandemic on the CV high-risk cohort, additional phenotyping (3 months post infection) and measurement of SARS-CoV2-antibodies was offered to the participants		during acute and of ~750 participants of ~day 90 deep phenotyping • hair samples were obtained from 339 participants • additional phenotyping after COVID-19-infection was performed in 34 participants; data will be shared with the German National Pandemic Cohort Network (NAPKON)between August 2020 and March 2022 SARS-CoV-2 antibodies were obtained at least once 1362 participants in
follow up new methods	several measures of deep phenotyping were not yet available by the start of the first study phase	the following method were implemented during the course of the study (see timeline supp. figure 9) : standard deep phenotyping: optical funduscopy, optical coherence tomography (OCT), bodyplethysmography, Montreal Cognitive Assessment (MoCA); module program: 24hr - ECG	all methodes are well established and measures are continued during phneotyping

Supplemental References

1. Mach, F., et al., *2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk.* Eur Heart J, 2020. **41**(1): p. 111-188.
2. Visseren, F.L.J., et al., *2021 ESC Guidelines on cardiovascular disease prevention in clinical practice.* Eur J Prev Cardiol, 2022. **29**(1): p. 5-115.
3. Harris PA, et al. *Research electronic data capture (REDCap) – A metadata-driven methodology and workflow process for providing translational research informatics support*, J Biomed Inform. 2009. **42**(2):377-81
4. Harris PA, et al. *The REDCap consortium: Building an international community of software partners*, J Biomed Inform. 2019 [doi: 10.1016/j.jbi.2019.103208]
5. Hoffmann, W., et al., *Guidelines and recommendations for ensuring Good Epidemiological Practice (GEP): a guideline developed by the German Society for Epidemiology.* Eur J Epidemiol, 2019. **34**(3): p. 301-317.
6. Sattar, N. and D. Preiss, *Reverse Causality in Cardiovascular Epidemiological Research: More Common Than Imagined?* Circulation, 2017. **135**(24): p. 2369-2372.
7. Amarenco, P. and T.o. Steering Committee Investigators of the, *Risk of Stroke after Transient Ischemic Attack or Minor Stroke.* N Engl J Med, 2016. **375**(4): p. 387.
8. Jernberg, T., et al., *Cardiovascular risk in post-myocardial infarction patients: nationwide real world data demonstrate the importance of a long-term perspective.* Eur Heart J, 2015. **36**(19): p. 1163-70.
9. Maggioni, A.P., *Epidemiology of Heart Failure in Europe.* Heart Fail Clin, 2015. **11**(4): p. 625-35.