

Protocol

Protocol for: Zimmermann WH, Ensminger S, Kutschka I, et al. Stem-cell–derived biologic ventricular assist tissue in heart failure. *N Engl J Med* 2026;394:1991-2001. DOI: 10.1056/NEJMoa2513525

This trial protocol has been provided by the authors to give readers additional information about the work.

This supplement contains the following items:

1. Original protocol (Version 1.0, pages 2-82), final protocol (Version 7.0, pages 83-212), and a summary of changes (pages 213-222) for BioVAT-HF-DZHK20.
2. The statistical analysis plan (Version 1.0, pages 223-242) for BioVAT-HF-DZHK20



Clinical Trial Protocol

Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure

BioVAT-HF

Engineered Human Myocardium (EHM) in patients with terminal heart failure

EudraCT No.	2019-000885-39
UMG Registration No.	02289
ClinicalTrials.gov ID	NCT04396899
Protocol Version	V 1.0
Therapeutic area	Terminal heart failure
Revision chronology, if applicable	n.a.
Development Phase	Phase I/II
Sponsor	University Medical Center Göttingen represented by the Head of the Clinical Trials Unit Von-Bar-Str. 37075 Göttingen, GERMANY
Coordinating Investigator	Prof. Wolfram-Hubertus Zimmermann
"Leiter der Klinischen Prüfung/LKP" (in accordance with German Drug Law)	University Medical Center Göttingen Robert-Koch-Str. 40 37075 Göttingen

This Clinical Trial Protocol contains confidential information. Circulation of this material to individuals who are not involved in the carrying out of the study or any kind of publication requires the approval of the sponsor. These limitations similarly relate to all confidential information and data which will be obtained in the future.

Approval of the Clinical Trial Protocol

Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure

EudraCT No.: 2019-000885-39

Protocol Version No: V 1.0

R. Bredenkamp

Sponsor Representative

Date

Signature

Prof. Dr. W. H. Zimmermann

Coordinating Investigator

Date

Signature

"Leiter der Klinischen Prüfung/LKP"
(in accordance with German Drug
Law)

Prof. Dr. Tim Friede

Biostatistician

Date

Signature

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R. Bredenkamp

Sponsor Representative

28/5/20

Date



Signature

Prof. Dr. W. H. Zimmermann

Coordinating Investigator

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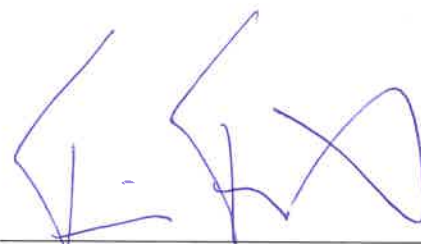
Prof. Dr. Tim Friede

28 MAY 2020

Date

Biostatistician

Signature



Investigator Statement

Protocol Short Title: BioVAT-HF
EudraCT No.: 2019-000885-39
Protocol Version No: V 1.0
Trial Site:

I confirm that I have read the Clinical Trial Protocol (CTP) and hereby commit to adhering to all actions and terms as specified in the relevant sections of the clinical, ethical and general paragraphs.

I confirm that I and my colleagues will comply with the local legislation (in Germany, the German Drug Law with the appropriate amendments). I further confirm that the clinical trial will be carried out in compliance with the Declaration of Helsinki and ICH-GCP guidelines.

I acknowledge that all confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

Under my supervision I put copies of this CTP and possible updates as well as access to all information regarding the carrying out of this clinical trial at the disposal of my colleagues; in particular I will promptly forward all information from the Sponsor in relation to pharmaceutical safety (SUSARs, SmPC and IB updates, if applicable) to my colleagues.

I confirm that I and my colleagues were informed by a responsible scientist about the results and expected risks of the pharmacological and toxicological examination associated with the clinical trial.

I will discuss this CTP in detail with my colleagues and ensure that they are comprehensively informed about the trial compound/preparation and the execution of the trial.

I confirm that I will be responsible for supervising any individual or party to whom I delegate study tasks conducted at the trial site.

Furthermore I commit myself not to commence patient enrolment prior to approval of the competent authorities (CA) and acceptance by the responsible Independent Ethics Committee (IEC).

_____	_____	_____
Date	Name (in CAPITALS)	Signature of Investigator

_____	_____	_____
Date	Name (in CAPITALS)	Signature of Deputy

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List of Abbreviations

[The following list includes abbreviations which have already been used in this document, please complete/adapt it.]

6MWT	6-Minute-Walk-Test
AE	Adverse Event
AMG	Medicinal Products Act / German Drug Law (<i>Arzneimittelgesetz</i>)
BID	twice a day
BioVAT	Biological Ventricular Assist Tissue
BP	Blood Pressure
CA	Competent Authority
CEC	Clinical endpoint adjudication committee
CONSORT	Consolidated Standards Of Reporting Trials
CRA	Clinical Research Associate (on-site monitor)
CRF	Case Report Form
CRP	C-reactive protein
CRT-D	Cardiac Resynchronization Therapy-Defibrillator
CSR	Clinical Study Report
CT	Computerised Tomography
CTCAE	Common Terminology Criteria for Adverse Events
cTnT	Cardiac troponin T
CTP	Clinical Trial Protocol
CTU	Clinical Trials Unit
DAMAST	SAS®-based data management system
DM	Data management
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DRKS	German Clinical Trials Register (<i>Deutsches Register Klinischer Studien</i>)
DSUR	Development Safety Update Report
DZHK	German Center for Cardiovascular Research (<i>Deutsches Zentrum für Herzkreislaufforschung</i>)
ECG	Electrocardiogram
EDC	Electronic Data Capture
EF	Ejection Fraction
EHM	Engineered Human Myocardium
EMA	European Medicines Agency
EOT	End Of Treatment
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	Full Analysis Set
FDG-PET	Fluorodeoxyglucose-Positron Emission Tomography
FU	Follow Up
GCP-V	Ordinance on the implementation of Good Clinical Practice in the conduct of clinical trials on medicinal products for use in humans (<i>GCP-Verordnung</i>)
GFR	Glomerular Filtration Rate
HF	Heart Failure
HFrEF	Heart Failure with reduced Ejection Fraction
HIV	Human Immunodeficiency Virus
HTLV1	Human T-Lymphotropic Virus 1
i.v.	Intravenous(ly)

IB	Investigator's Brochure
ICD	Implantable Cardioverter Defibrillator
ICF	Informed Consent Form
ICH-GCP	ICH Topic E6: Guideline for Good Clinical Practice (GCP)
IEC	Independent Ethics Committee
IL-6	Interleukin-6
IMP	Investigational Medicinal Product /study medication
ISF	Investigator Site File
ITT	Intention To Treat
LV	Left Ventricle
MED	Minimally Effective Dose
MFD	Maximal Feasible Dose
MH	Medical History
MLHFQ	Minnesota Living with Heart Failure Questionnaire
MRI	Magnetic Resonance Imaging
MTD	Maximal Tolerable Dose
NCT No	National Clinical Trial (NCT) number in ClinicalTrials.gov registry
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
NYHA	New York Heart Association
p.o.	Per os
PC	Project Coordination
PHI	Protected Health Information
PI	Principal Investigator
PP	Per-Protocol
PR	Pulse Rate
PV	Pharmacovigilance
QD	Once a Day
QOL	Quality of Life Questionnaire
RBC	Red Blood Cell Count
RSI	Reference Safety Information (current SmPC or/and current IB)
RV	Right Ventricle
s.c.	Subcutaneous(ly)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SDV	Source Data Verification
SF-36	Short Form-36
SMD	Safe Maximal Dose
SmPC	Summary of Product Characteristics (<i>Fachinformation</i>)
SOP	Standard Operating Procedure
SSC	Scientific Steering Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAPSE	Tricuspid Annular Plane Systolic Excursion
TDM	Therapeutic drug monitoring
TMF	Trial Master File
UAR	Unexpected Adverse Reaction
VO2max	Maximum rate of oxygen consumption

WBC	White Blood Cell Count
WHO	World Health Organization

Synopsis

TITLE OF TRIAL	Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure
SHORT TITLE	BioVAT-HF
EUDRACT NO	2019-000885-39
UMG REGISTRATION NUMBER	02289
HEALTH CONDITION STUDIED	Terminal heart failure
PHASE	Phase I/II
OBJECTIVE(S)	<p>Primary objective:</p> <ul style="list-style-type: none"> to assess safety and efficacy of Engineered Human Myocardium (EHM) in patients with terminal heart failure (HFrEF EF $\leq 35\%$) with or without RV dysfunction (TAPSE < 16 mm) <p>Secondary objective:</p> <ul style="list-style-type: none"> to assess effects of EHM-grafts on disease-specific events and symptoms

TREATMENT(S)	<p><u>Experimental intervention/Index test:</u> Implantation of EHM on dysfunctional left or right ventricular myocardium in patients with HFrEF (EF \leq35%).</p> <p>Phase I: Dose Finding Cohort to determine the Minimally Effective Dose and Optimally Effective Dose Range, and if possible the Safe Maximal Dose of EHM.</p> <p>Phase II: Refinement Cohort to specify the most optimal EHM target heart wall, i.e., the left ventricle (LV) or the right ventricle (RV).</p> <p>Phase III: Expansion Cohort to collect proof-of-concept data as to efficacy of EHM mediated remuscularization/augmentation of the LV or RV.</p> <p>Epicardial implantation will be via a minimal invasive left lateral thoracotomy performed as standalone procedure in case of LV targeting and concomitant to a scheduled open chest LV surgery if the RV is targeted. This strategy will reduce confounding effects as to the interpretation of EHM efficacy data.</p> <p><u>Duration of intervention per patient:</u></p> <ul style="list-style-type: none"> • Baseline visit and start of immune suppression (calcineurin inhibitor and corticosteroid) 7\pm3 days before EHM implantation • Implantation of EHM: 1 h according to experience from preclinical studies and similar surgical procedures (i.e., epicardial pacemaker lead placement) • 12 months follow-up <p>Note: After the final study visit, patients will be further monitored by their treating physician. Immune suppression (calcineurin inhibition) will be continued until end-of-life if evidence for efficacy without safety concerns can be obtained within the 12 month study period (corticoids will be weaned-off after 3-6 months according to guidelines for immune suppression in organ heart transplantation). The treating physician is requested to report clinically relevant observations to the principal investigator. After 12 month follow-up, study patients will be enrolled in a separate registry study (BioVAT-registry set up by the Study Center) until end of life for the documentation of long-term outcome.</p>
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	<p><u>Follow-up per patient:</u></p> <ul style="list-style-type: none"> • 17 segment high-resolution echocardiography and/or MRI (if possible) to study global and regional heart/graft function (before EHM implantation as well as 2 weeks, 1 month, 2 months, 6 months, and 12 months after surgery) • Biomarkers: CK, CK-MB, cTnT, CRP, IL-6, and NT-proBNP; in addition, experimental assessment of graft derived DNA for the monitoring of graft retention/rejection (before EHM implantation as well as 2 weeks, 1, 3, 6, and 12 months after surgery) • Telemetric monitoring via Implantable Cardioverter Defibrillator (ICD)- or Cardiac Resynchronization Therapy-Defibrillator (CRT-D)-devices with event recorder for the whole duration of the study. • Pathology to obtain data on graft survival, integration, and maturation upon heart transplantation or death (according to patient consent). • If clinically indicated: FDG-PET (Fluorodeoxyglucose-Positron-Emission Tomography) at 12 months to assess heart and EHM graft vitality. • If clinically indicated: Monitoring of pulmonary artery pressure with a CardioMEMS HF Device (St. Jude Medical) or a similar device. <p><u>Accompanying measures:</u></p> <ul style="list-style-type: none"> • Therapeutic drug monitoring (TDM) to verify effective trough levels of accompanying immune suppressive drugs (calcineurin inhibitors) according to the proceeding in orthotopic heart transplantation (ISHLT Guidelines; Costanzo et al. 2010), i.e., Tacrolimus: 10-15 ng/ml at the time of implantation maintained for 2 month followed by a reduction to 8-12 ng/ml until 6 months and finally a reduction to 5-10 ng/ml in stable patients or Cyclosporine A: 275-375 ng/ml at the time of implantation maintained for 6 weeks followed by a reduction to 200-350 ng/ml until week 12 and then followed by a reduction to 150-300 ng/ml until 6 months and then followed by a further reduction to maintenance levels at 150-250 ng/ml. • Biomarker analysis to monitor rejection: CK/CK-MB, cTnT, circulating cell-free allograft DNA (experimental method; liquid biopsy) • Monitoring of specific allograft immune responses: donor specific antibodies (DSA)
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INCLUSION CRITERIA	<ol style="list-style-type: none"> 1. HFrEF (EF \leq 35%) as assessed by high-resolution echocardiography or MRI 2. No realistic chance or not eligible for heart transplantation 3. At least one hypo- or dyskinetic segment to demark the implant target area 4. Stable disease condition allowing for an elective left-lateral mini-thoracotomy (for LV applications) or open-chest surgery (for RV applications) for a clinically indicated intervention on the LV (e.g., coronary bypass surgery, valve repair, mechanical circulatory support device implantation) with concomitant RV dysfunction, diagnosed using the Tricuspid Annular Plane Systolic Excursion (TAPSE) index <16 mm (Rudski et al. 2010). 5. 18-80 years of age 6. Previous implantation of an ICD or CRT-D with event recorder 7. New York Heart Association (NYHA) Class III or IV under optimal medical therapy 8. Willingness and ability to give written informed consent 9. Female subjects of childbearing potential must agree to use acceptable method(s) of contraception for the full study duration.
EXCLUSION CRITERIA	<ol style="list-style-type: none"> 1. Contraindication to immunosuppressive drugs (e.g. known history of unresolved cancer, hepatitis B/C, HIV, HTLV1) 2. Hypertrophic cardiomyopathy (HCM) 3. Terminal kidney failure (stage 4; GFR <30 ml/min) 4. Terminal liver failure (Child-Pugh stage C; score >10) 5. Alloimmunisation against EHM implant cells 6. Autoimmune disease 7. History of stroke 8. Reduced life expectancy in the short term due to non-cardiac disease 9. Simultaneous participation in another interventional trial 10. Pregnant or breastfeeding females 11. Known or suspected alcohol and/or drug abuse

ENDPOINTS	<p><u>Primary efficacy endpoint:</u> Evidence for anatomical and functional muscular augmentation of target myocardium determined as enhanced target heart wall thickness (HWT) and thickening fraction (HWTF)</p> <p><u>Key secondary endpoint:</u></p> <ul style="list-style-type: none"> • Recurrent HF hospitalizations <p><u>Further secondary endpoints:</u></p> <ul style="list-style-type: none"> • Identification of a Minimal Effective Dose (Dose Finding Cohort) • Identification of the Optimal Effective Dose Range (Dose Finding Cohort) • Ejection Fraction • Functional status in patients as determined by six-minute walk test (6MWT), hand-grip strength, and cardiopulmonary stress testing (VO₂max) • Patient reported outcomes assessed by NYHA classification, quality of life score (MLHFQ, KCCQ, EQ-5D), and physical score (SF-36) • All-cause and cardiovascular mortality
	<ul style="list-style-type: none"> • <u>Assessment of safety:</u> • Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke and cardiovascular death) • Frequency and severity of arrhythmic events • Incidence of immune rejection (allograft DNA, CK/CK-MB, cTnT) • Incidence of mechanical perturbation of ventricular function by EHM graft • Identification of a Safe Maximal Dose (SMD) or Maximum Tolerated Dose (MTD) in Dose Finding Cohort
TRIAL DESIGN	Combined, open-label, 3-stage, phase I/II safety and efficacy study

STATISTICAL ANALYSIS	<p><u>Primary Endpoints:</u> Primary efficacy analyses are based on the changes in HWT/HWTF between baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. To test for a time effect a generalized linear mixed model will be employed for each of the two primary endpoints. In case of detecting a time effect this is followed by Dunnett-type pairwise comparisons to baseline.</p> <p><u>Secondary endpoints:</u> Secondary endpoint analyses will be similar as the analyses of the primary endpoint and comprise of Gaussian longitudinal models evaluating changes over time from baseline prior to EHM implantation. For recurrent event data such as HF hospitalizations appropriate regression models such as the negative binomial regression model or the semiparametric LWYY model will be used.</p> <p><u>Safety:</u> The maximal feasible dose (MFD; 20 g EHM comprised of 800 million cells) was chosen conservatively based on preclinical experience in Rhesus macaque and allometric scaling considerations. The probability of dose-limiting toxicity will be modelled by a Bayesian two-parameter logistic regression model. Safety events will be summarized as rates with 95% confidence intervals. Survival will be displayed as Kaplan-Meier curve and analyzed using a Cox proportional hazards model exploring the prognostic quality of the biomarkers assessed at baseline.</p>	
	<p><u>Effect size assumed for estimation of sample size:</u> A sample size of 30 patients (including 5 + 25 from the Refinement and Expansion Cohorts) yields a power of 80% (90%) in a pre-post comparison of means at a two-sided significance level of 10% given a standardized mean difference (Cohen's d) of 0.47 (0.55).</p>	
SAMPLE SIZE	<p><u>Phase I:</u></p> <p><u>Phase II:</u></p> <p><u>Phase III:</u></p> <p>To be assessed for eligibility:</p> <p>To be allocated to trial:</p> <p>To be analysed:</p>	<p>n = 18 (max.), in dose cohorts of 2-4 patients</p> <p>n=10 (5 with LV and 5 with RV EHM placement)</p> <p>n=25 (additional patients with LV or RV EHM placement)</p> <p>n = 65</p> <p>n = 53</p> <p>n = 53</p>
TRIAL DURATION	<p>Time for preparation of the trial :</p> <p>Recruitment period (part I to part III):</p> <p>First patient in to last patient out:</p>	<p>6 months</p> <p>30 months</p> <p>42 months</p>

	Post processing after LPO:	6 Months
	Duration of the entire trial:	54 months
	Duration of surgical intervention per patient:	1 hour (according to experience from preclinical studies and similar surgical procedures)
	Follow up duration per patient:	12 months
PLANNED DATES	Enrolment of first patient, first patient in (FPI)	4st quarter 2020
	Enrolment of last patient, last patient in (LPI)	2rd quarter 2023
	End of trial defined as last patient last visit (LPLV)	2rd quarter 2024
	Final statistical analysis	4st quarter 2024
	Planned interim analysis	Interim analysis will be performed after end of study phase I and phase II.
PARTICIPATING SITES	3 sites (Göttingen, Lübeck, Bad Oeynhausen) are planned in Germany.	
FUNDER(S)	The trial is funded by the DZHK (<i>Deutsches Zentrum für Herz-Kreislauf-Forschung e.V.</i>)	

Table 1 Visit schedule and assessments – Flowchart

Visits	Title in eCRF	Study registration (Visit 1)	Baseline (Visit 2)	Start of Immun- suppression (Visit 3)	Implantation (Visit 4)	Follow Up 1 (Visit 5)	Follow Up 2 (Visit 6)	Follow Up 3 (Visit 7)	Follow Up 4 (Visit 8)	Follow Up 5 (Visit 9)
	Time Section	Month -4 to -1	Day -10 to -5	Day -10 to -4	Day 0	2 weeks (± 2 days)	1 months (± 7 days)	3 months (± 7 days)	6 months (± 7 days)	12 months (± 7 days)
Informed Consent		x								
Inclusion / Exclusion Criteria		x	x ¹							
Medical History			x							
DZHK basic data set			x ²		x ³	x ³	x ³	x ³	x ³	x ³
Concomitant heart failure medications			x		x	x	x	x	x	x
Patient registration eCRF		x								
Adverse events documentation					x	x	x	x	x	x
Laboratory assessments										
CBC with differentials and platelet count			x		x	x	x	x	x	x
CRP, IL6			x		x	x	x	x	x	x
Liver panel			x		x	x	x	x	x	x
Albumin			x		x	x	x	x	x	x
Serum Creatinine			x		x	x	x	x	x	x
PTT or PT/INR as applicable			x		x	x	x	x	x	x
Plasma free haemoglobin; Iron			x		x	x	x	x	x	x
Troponins, CK, CK-MB			x		x	x	x	x	x	x
NT-proBNP			x		x	x	x	x	x	x
Pregnancy Test (for females of childbearing potential)			x							
Blood draw for allograft DNA assessment			x			x	x	x	x	x
HLA/KIR-Typing			x							
Donor Specific Antigens			x				x	x	x	x

Further assessments / Scores										
12-lead ECG			x		x	x	x	x	x	x
Transthoracic Echocardiography (TTE)			x		x	x	x	x	x	x
Cardiac-MRI ⁴			x			x	x	x	x	Cardiac-MRI ⁴
Cardiopulmonary exercise testing			x			x	x	x	x	Cardiopulmonary exercise testing
Hand grip strength			x			x	x	x	x	Hand grip strength
6 minute walking test			x			x	x	x	x	6 minute walking test
EuroSCORE II and/or Logistic EuroSCORE I ⁵			(x)			(x)	(x)	(x)	(x)	EuroSCORE II and/or Logistic EuroSCORE I ⁵
Positron-Emission Tomography (FDG-PET) ⁶			(x)							Positron-Emission Tomography (FDG-PET) ⁶
Quality of life questionnaires (patient reported outcome)										
MLHFQ			x			x	x	x	x	MLHFQ
SF-36			x			x	x	x	x	SF-36
KCCQ			x			x	x	x	x	KCCQ
EQ-5D			x			x	x	x	x	EQ-5D
Treatment / therapy										
Surgical Procedure					x					Surgical Procedure
ICD/CRTD-event recorder readout			x		x	x	x	x	x	ICD/CRTD-event recorder readout
TDM Calcineurin Inhibitors				x	x	x	x	x	x	TDM Calcineurin Inhibitors

DZHK-Biobanking Data Set ⁷			x							DZHK-Biobanking Data Set ⁷
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¹check if In- / Exclusion criteria are still valid
²according to DZHK-SOP-K-01 "Basisdatensatz – Anamnese / Klinische Diagnosen / Körperliche Untersuchung"
³a reduced data set of the DZHK-SOP-K-01 "Basisdatensatz – Anamnese / Klinische Diagnosen / Körperliche Untersuchung" needs to be collected as defined in chapter 7.6.2
⁴Patients with devices may or due to other circumstances might not be eligible to MRI investigations. For these patients see further instructions in chapter 7.8.8
⁵only if site uses it as a standard risk calculator
⁶only if done according to standard of care
⁷Collection of biological samples according to patient consent

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1 Background and rationale

Heart failure is the most common cause of death. The underlying disease mechanism is a loss of heart muscle cells, which correlates with reduced heart function. Approximately 2 million patients in Germany, 6.5 million patients in Europe, and 23 million patients globally are diagnosed with heart failure. Due to its positive correlation with life expectancy and the dramatic demographic changes of our aging population, prevalence of heart failure will further increase.

Incidence of heart failure is reported to be at ~300,000 new cases/year in Germany. Mortality is despite advances in pharmacological and interventional heart failure therapy 20% within 1 and 50% within 5 years upon the initial diagnosis (Lopez Sendon and Montoro, 2015). Heart transplantation is the only curative option with excellent outcome (80% 5-year survival rates; ISHLT-Registry data). Shortage of donor organs results in ~300 orthotopic heart transplantations (OHTs) per year in Germany. There is an estimated need for ~8.000 OHT per year in Germany

Heart failure patients upon listing for heart transplantation present a 12 month survival of 80% and a 24 month survival of 70% (Hsich et al., 2016). Once under inotrope support in advanced heart failure, average life expectancy is 1.1 years if eligible and 9.4 months if ineligible for OHT (Long et al., 2014).

In addition to reduced life expectancy, quality of life in patients living with advanced heart failure is greatly reduced and was found to be comparable to the quality of life in patients with acute aphasic stroke (Franzen-Dahlin et al., 2010; Nieminen et al., 2015).

The socioeconomic burden of heart failure is anticipated to increase markedly; e.g., direct and indirect cost associated with heart failure were \$28 billion in the US in 2010 with an anticipated increase to \$78 billion by 2030 (Lopez Sendon and Montoro, 2015). Ischemic heart disease is the main cause of heart failure and the leading contributor to disability-adjusted life years (DALYs; (Murray et al., 2012)).

Because of the enormous medical unmet need for new treatment options various technical and regenerative therapeutic approaches, like, e.g., artificial hearts, xenotransplantation of hearts, implantation of a mechanical pump device (left ventricular assist device, LVAD), and more recently cell- and tissue-based regenerative therapies have emerged. Cell- and tissue-based repair approaches aim at the reconstitution of heart muscle *in situ* and therefore at long lasting therapeutic improvement of heart failure by *bona fide* remuscularization. Based on constantly rising numbers of patients suffering from heart failure and the limited availability of donor hearts or therapeutic alternatives, the development of regenerative, cell-/tissue-based therapeutic approaches is warranted.

The BioVAT-HF trial will test the hypothesis that cardiomyocyte implantation via engineered human myocardium (EHM), the proposed investigational medicinal product (IMP; designated "Biological Ventricular Assist Tissue" or BioVAT), results in sustainable remuscularization and biological enhancement of myocardial performance in the failing heart. EHM are constructed from defined mixtures of induced pluripotent stem cell (iPSC)-derived cardiomyocytes and stromal cells in a bovine collagen type I hydrogel. Comprehensive preclinical testing confirmed the rationale for the clinical translation of the myocardial remuscularization strategy by EHM implantation. The patient target population for EHM therapy is patients suffering from advanced heart failure with reduced ejection fraction (HFrEF; EF: ≤35%) and no realistic option for heart transplantation.

1.1 Scientific background

Despite encouraging results from recent studies investigating the implantation of embryonic stem cell-derived cardiac progenitor cells (Menasche et al., 2018) and exosome therapeutics (Liu et al., 2018b), there is a common understanding that direct integration of cardiomyocyte implants would be the most promising option if successfully translated into the treatment of patients with endstage heart failure. As to cardiomyocyte delivery and retention, tissue engineering-based cardiomyocyte delivery has a clear advantage over direct intramyocardial injection (Nguyen et al., 2016; Riegler et al., 2015). In addition, there is comprehensive preclinical evidence for safety and efficacy of tissue engineered heart repair with EHM and EHM precursors obtained by the applicant's lab in 3 preclinical animal species (rat, mouse, and Rhesus macaque):

- 1) Rat model with uncompromised heart function (Zimmermann et al., 2002)
- 2) Rat model of chronic (severe) heart failure after permanent LAD occlusion (Zimmermann et al., 2006)
- 3) Mouse model of acute myocardial infarction by permanent LAD occlusion (Didie et al., 2013)
- 4) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Riegler et al., 2015)
- 5) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Qin et al., 2016)
- 6) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Tiburcy et al., 2017)
- 7) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (extension of Tiburcy et al., 2017 with focus on EHM patch retention; data presented in IMPD)
- 8) Rhesus macaque model with uncompromised heart function (data presented in IMPD)

Additional pilot studies were performed in pig models, but found to be of no predictive value because of limited xenograft retention, despite administration of comprehensive immune suppression regimens.

The preclinical *in vivo* studies 1-8 collectively provide the rationale for the BioVAT-HF trial. Study 1 demonstrated feasibility and safety of Engineered Rat Myocardium (ERM) allograft implantation under immune suppression in a healthy rat model. Study 2 demonstrated electromechanical integration, safety and efficacy of ERM allografts in a rat model of chronic heart failure. Safety and efficacy was further confirmed in a mouse model of subacute myocardial infarction and the application of pluripotent stem cell-derived Engineered Mouse Myocardium (EMM) allografts (Study 3). Studies 2 and 3 employed MRI and echocardiography to document efficacy of ERM and EMM allograft-based heart repair by detection of an enhancement of thickness and contractility of the target heart wall, in line with the proposed mode of action. Studies 4-7 established and validated GMP-compatible EHM as well as EHM xenograft retention upon implantation in immunocompromised nude rats. Long term engraftment for more than 6 months without unwanted effects was demonstrated in study 4. In contrast to rodent allografts (Zimmermann et al., 2006), reliable evidence for electromechanical integration of human xenografts could not be obtained, which is in-line with data from other groups using guinea pig (Weinberger et al., 2016) or rat (Gerbin et al., 2015). In the rat xenograft study (study 4), enhanced left ventricular ejection fraction (+5%) was observed to be independent of the implantation of contractile or non-contractile EHM. These findings confirmed earlier findings (studies 2 and 3) in that mechanical stabilization of the ventricular wall or indirect (paracrine) effects of the epicardial patch on the underlying myocardial milieu may be of therapeutic value, which was however inferior to the therapeutic effects observed in rodent models of heart failure and contractile ERM as well as EMM allograft implantation (studies 2 and 3). In a pivotal Rhesus macaque allograft study

(study 8), Engineered Non-Human Primate Myocardium (ENHPM) allografts thickened the target heart wall by ~1 and ~5 mm in a dose dependent manner (1x and 5x EHM assemblies, respectively). The augmentation of the target heart wall was sustained for the whole study duration (up to 6 months) with no evidence for arrhythmia, tumor formation, perturbation of heart performance, and immune suppression related side effects.

Detailed *post mortem* analyses in the 14 Rhesus macaques implanted with 1x (n=7) or 5x (n=7) ENHPM grafts revealed terminally differentiated chondrocytes and osteocytes in 5 of the 14 ENHPM implanted Rhesus macaques (1 of 7 with 1x ENHPM and 4 of 7 with 5x ENHPM). Impurities in the Rhesus macaque iPSC-cardiomyocytes differentiations were identified by bulk and single cell RNA-sequencing as cause for these observations. Comparative RNA sequencing analyses to the human iPSC-derived cardiomyocytes populations did not reveal evidence for similar impurities in the GMP process. Importantly, osteochondral differentiations were never observed in preclinical *in vitro* and *in vivo* studies with EHM (Riegler et al. 2015; Qin et al. 2016; Tiburcy et al. 2017), including a combined GLP toxicity, tumorigenicity, biodistribution study (refer for details to IMPD). Adaptations of the NHP-iPSC-differentiation process provided cardiomyocyte populations with markedly reduced transcriptional heterogeneity and similar cardiomyocytes purity as set as release criterion for the human GMP process (>90%). ENHPM allografts constructed from accordingly optimized Rhesus iPSC-differentiations are studied in an extension of the NHP Feasibility and Safety study (study 8) with 4 additional Rhesus macaques implanted with 2x EHM [n=3] and 5x EHM [n=1]) and un-eventful follow-up. Preclinical *in vivo* studies will be continuously conducted in parallel to clinical testing, following a bed-to-bench-to-bed (reverse translation) strategy.

We consider the 5x EHM assembly, confirmed to be safely applicable as epicardial allograft to augment the target heart wall according to the proposed mode of action in Rhesus macaques, as minimal effective dose (MED) for the BioVAT-HF trial and anticipate a similar thickening of the target heart wall as observed in the Rhesus macaque study (~5 mm); given that the human heart wall has an average thickness of 6-10 mm (Kawel et al., 2012), we anticipate clinical impact with the predetermined MED. Considering the differences in heart and body size in Rhesus macaque and human (10-fold) and applying allometric scaling a 5x EHM assembly in Rhesus macaque resembles a 50x EHM assembly in human. With an anticipated maximal feasible dose (MFD) of 20x EHM and no observed safety issues in the Rhesus macaque study or any of the preclinical studies testing EHM xenografts under non-GLP and GLP conditions, we do not anticipate graft related safety concerns in the BioVAT-HF trial.

1.2 Overview of investigational medicinal product(s) (IMP(s))

The designated IMP is Engineered Human Myocardium (EHM) for applications as Biological Ventricular Assist Tissue (BioVAT). EHM are constructed from induced pluripotent stem cell (iPSC)-derived cardiomyocytes and stromal cells mixed at a defined ratio and suspended in a bovine collagen type I hydrogel. EHM are produced under GMP-conditions at the University Medical Center Göttingen (UMG) under the auspices of the Institute of Transfusion Medicine. An application for manufacturing authorization has been submitted to the local competent authority (LCA; Gewerbeaufsichtsamt Braunschweig) in October 2019. The manufacturing process was reviewed with no critical concerns by the LCA and representatives of the Paul-Ehrlich-Institute in January 2020. Critiques will be fully addressed before final approval of the clinical trial application.

Two studies delivering pluripotent stem cell-derivatives to the heart of patients with heart failure have been registered at ClinicalTrials.gov, so far:

(1) the ESCORT trial (NCT02057900) tested the delivery of 5-10 million embryonic stem cell (ESC)-derived cardiac progenitor cells (identified by the expression of the SSEA1 surface marker) immobilized in a fibrin patch to the heart (n=6 patients). The study is completed and the data have been published (Menasche et al., 2018);

(2) the HEAL-CHF trial (NCT03763136) is presently recruiting patients for an intramyocardial injection of 100 million iPSC-derived cardiomyocytes in patients with heart failure (total of 5 patients planned; no data reported).

A third study (UMIN000032989) on the delivery of iPSC-derived cardiomyocytes via a cell sheet approach has been announced (Cyranoski, 2018) with start of recruitment in 2019 (personal information provided by Dr. Sawa). In this trial cell sheets (0.1 x 40 mm) comprising 100 million iPSC-derived cardiomyocytes are delivered epicardially (n=3 patients). An extension of this trial (n=10 patients) is registered as jRCT2053190081.

The epicardial implantation of EHM, IMP of the BioVAT-HF trial, has not been attempted clinically. For further details on IMP(s) please refer to section 9.

1.3 Trial purpose and rationale

To counter the irreversible and progressive loss of cardiomyocytes and effectively remuscularize the failing heart, cardiomyocyte therapeutics are being developed (refer to section 1.1. and 1.2). Human cardiomyocytes can be derived from embryonic (ESC) and induced pluripotent (iPSC) stem cells using scalable processes with reproducible quality (Chen et al., 2015). The suggested clinical route of administration is either by direct intramyocardial injection (Chong et al., 2014; Liu et al., 2018a; Romagnuolo et al., 2019; Shiba et al., 2016; Zhao et al., 2018) or by epicardial implantation of tissue engineered heart muscle (Didie et al., 2013; Riegler et al., 2015; Tiburcy et al., 2017; Weinberger et al., 2016; Zimmermann et al., 2006). A prerequisite for therapeutically effective remuscularization of the human heart is cardiomyocyte survival and sustainable retention after implantation. Long term retention of intramyocardially injected cells, including cardiomyocytes, is negligible (<1%; (Nguyen et al., 2016)). Substantially more cardiomyocytes are retained after delivery via an epicardial patch approach ((Riegler et al., 2015); refer also to own data from additional rat and NHP model data in IMPD). Approximately 0.8-1 billion cardiomyocytes (i.e., 25% of the left ventricle) are lost in patients with advanced stage heart failure (Gepstein, 2002) Bergmann et al. 2015). Replacing the lost cardiomyocytes and thereby restoring heart function is the primary goal of cardiomyocyte therapeutics.

For the epicardial delivery, we propose a tissue engineered product, namely EHM, for epicardial application as BioVAT. The EHM-IMP is composed of defined mixtures of iPSC-derived cardiomyocytes and stromal cells seeded in a collagen type I hydrogel, cast into custom-made molds to obtain a desired geometry, and conditioned by exposure to mechanical load to simulate the hemodynamic loading conditions during a natural contraction-relaxation cycle of the heart (Tiburcy et al., 2017) (refer to IMPD for details).

The proposed primary mode of action (MoA) is functional remuscularization of the targeted hypokinetic heart wall (Fujita and Zimmermann, 2017a), resulting in (1) enhanced target heart wall thickness and (2) enhanced target heart wall thickening fraction.

The primary target patient population comprises subjects with advanced heart failure with a reduced ejection fraction (HFrEF; EF ≤ 35%) despite optimal medical therapy (OMT) and segmental hypo- or dyskinesia of the left ventricular (LV) free wall. This patient population is

according to guideline recommendations protected from sudden cardiac death by implanted cardioverter/defibrillator (ICD) devices. Patients may be listed for heart transplantation, but are unlikely to receive a heart transplant due to for example age (>65 of age), blood group, and co-morbidities or other exclusion criteria. By targeting the hypokinetic heart wall with contractile EHM, we aim at a functional remuscularization, which will, if successful, improve local and global heart function and patient outcome (mortality and quality of life).

Preclinical allograft (Didie et al., 2013; Zimmermann et al., 2002; Zimmermann et al., 2006) and xenograft (Qin et al., 2016; Riegler et al., 2015; Tiburcy et al., 2017) studies in rodents and in Rhesus macaque (unpublished data in IMPD) have demonstrated the feasibility of EHM implantation onto the beating heart via a left-lateral mini-thoracotomy, using a similar route of administration as used for surgical Transapical Aortic Valve Implantation (TAVI), Minimally Invasive Direct Coronary Artery Bypass (MIDCAB) surgery, or surgical placement of epicardial pacemaker leads. This will expose the target patient population to a minimal surgical risk with no anticipated mortality (own experience and experience from others (Puglisi et al., 2004)). The Rhesus macaque allo- and autograft study further underscored that this route of administration and the implantation of clinically relevant doses of a surrogate therapeutic candidate is safe without unwanted side effects (no deaths, no tumor, no arrhythmia, no LV perturbation, no immune suppression related complications).

Patients will receive immune suppression, using a similar protocol as found effective in the Rhesus macaque study and commonly used in clinical organ transplantation, i.e., (1) calcineurin inhibitors (e.g., tacrolimus) and (2) methylprednisolone, to prevent BioVAT allograft rejection. Surveillance of graft retention will be by echocardiography and MRI as well as biomarkers, including allograft-derived cell-free circulating DNA, which we aim to establish as an “online monitor” for graft retention/rejection (Beck et al., 2015). Patients will be on the BioVAT-HF study protocol for 12 months with frequent clinical investigations (biomarkers, MRI/echocardiography, quality of life assessments; refer to Visit schedule below). After the 12-month study period, patients will be included in a registry off-study until death. In line with our preclinical data (6 months data in the Rhesus macaque model), we anticipate that remuscularization of the failing heart will be sustained and as such improve heart failure symptoms, quality of life, and ultimately mortality in patients with heart failure.

1.4 Rational for choice of control interventions/comparators

This is a first-in-patient study to obtain safety and efficacy data from patients with advanced heart failure. The lowest EHM dose was chosen as a minimally effective dose (MED) based on the observed effect in the pivotal preclinical non-human primate study (unpublished; refer to IMPD). Data from the BioVAT-HF trial will be compared to the data from patients under standard of care. Given the intention to treat design of the BioVAT-HF first-in-patient trial it is not acceptable to include a placebo control intervention.

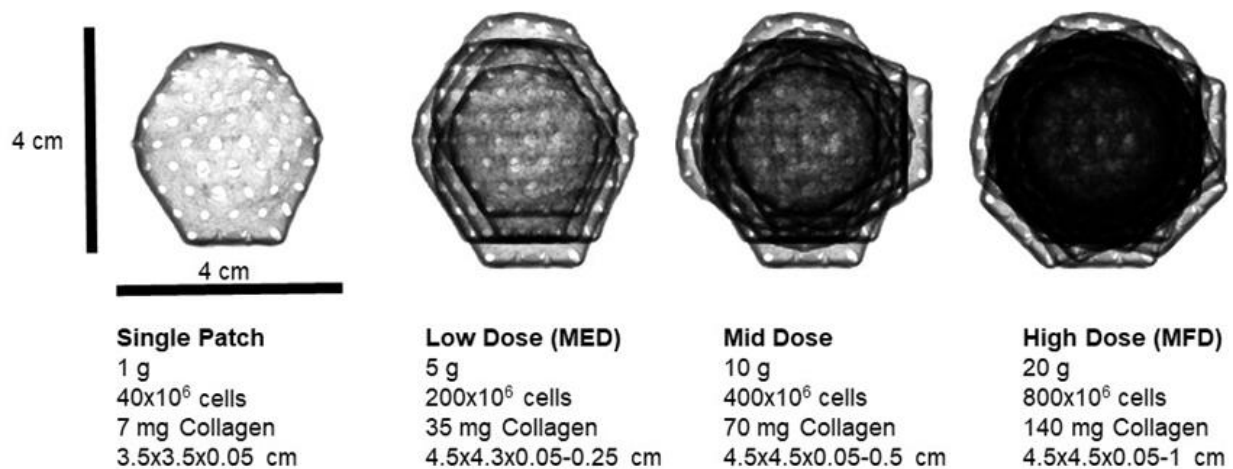
1.5 Rational for dose selection

We propose a dose finding study with the highest dose tested preclinically in large animal models (Rhesus macaque; 5x EHM assemblies) as a starting dose. The starting dose (minimal effective dose [MED]) is selected because of an anticipated and clearly discernible effect on target heart wall thickness (we anticipate an increase in heart wall thickness by 5 mm according to our observations in the Rhesus macaque study). EHM patches will be sutured to cover hypo- or

dyskinetic myocardium with suture points in the surrounding healthy myocardium, similarly as performed in previous studies (Didie et al., 2013; Riegler et al., 2015; Tiburcy et al., 2017; Zimmermann et al., 2002) and further refined in the completed Rhesus macaque study (refer to IMPD for a detailed description of the implantation procedure). To prevent potential epicardial bleeding events, EHM implants are stabilized with a 4.8x4.8 cm fibrinogen (5.5 mg cm²) and thrombin (2 I.U./cm²) coated equine collagen membrane (TachoSil®; BLA125351, EU/1/04/277/001-005; Takeda).

EHM-patch assemblies will be prepared by an overlapping sandwich format strategy. The Figure below depicts an individual EHM patch, a schematic of an assembly of 5 EHM (low dose), and the strategy to stack 2 (mid dose) and 4 (high dose) layers of 5-EHM assemblies (Fig. 1):

Figure 1 EHM dosing



The BioVAT stacking strategy can be adapted according to individual clinical needs. For the dose escalation part of the BioVAT-HF trial multiples of 5x EHM comprising 200 (5x EHM), 400 (10x EHM), 600 (15x EHM), or 800 (20x EHM) million cells are considered; this may be further adapted as to recommendations by the dose determining committee (DDC). With the highest dose we aim to match the loss of cardiomyocytes as it typically occurs in advanced heart failure (Fujita and Zimmermann, 2017a; Gepstein, 2002).

Patients will be under standard heart failure medication. Rejection of EHM allografts will be prevented by clinically established (in OHT) immune suppression (Costanzo et al., 2010), including calcineurin inhibition (Tacrolimus: 5-15 ng/ml or Cyclosporine A: 150-375 ng/ml) and corticosteroids (5-10 mg/day; 0.15 mg/kg bodyweight * day) starting 7±3 days before EHM implantation. Adaptations of immune suppression may be acceptable according to clinical recommendations. Specific criteria are defined for stopping immunosuppression and thus initiating graft rejection, in case of immune suppression-related complications or lack of a defined treatment outcome. In the ESCORT trial (Menasche et al., 2018), with a weaning of immune suppression 1-2 months after cell implantation, and in our own studies in Rhesus macaque after withdrawal of immune suppression in a study subcohort (refer to IMPD) no evidence for rejection related events were observed.

1.6 Risk-benefit assessment

Administration of immune suppression will start 7 ± 3 days before EHM implantation. This allows for an individual dose adjustment of the calcineurin inhibitors to steady state levels and the identification of potential side effects (e.g., liver damage [increase in ALT/AST], kidney damage [increase in creatinine]) prior to EHM implantation. Calcineurin inhibition will be continuously applied throughout the study and beyond the 12 month study period if clinical follow-up suggests efficacy of EHM implantation without safety concerns. Corticoids (preferably methylprednisolone) will be maintained at low dose to avoid Cushing symptoms and weaned after 3-6 months in line with the proceeding in heart transplant patients.

The surgical procedure related risk of a EHM implantation via a minimal left lateral thoracotomy is best compared to the low risk associated with the surgical fixation of epicardial pacemaker leads (experience of the participating surgical teams and for example (Puglisi et al., 2004)). Typical surgical complications include bleeding, adhesions, and impaired wound healing.

The immune suppression protocol and surgical procedure were simulated in a pivotal preclinical Rhesus macaque allo- and autograft study at dose levels simulating the starting dose for the BioVAT-HF trial (5x EHM assemblies). Moreover, the 5x EHM assembly in Rhesus macaque represents a 50x EHM assembly dose in human according to allometric scaling (body and heart weight in Rhesus macaque is approximately 1/10 of the body and heart weights in human subjects). No critical unwanted effects (no deaths, no tumor, no arrhythmia, no perturbation of heart function, no immune suppression related complications) were observed at the tested doses, establishing a safety margin of 2.5 compared to the maximal feasible dose (MFD; 20x EHM). For details refer to IMPD.

Patients will remain hospitalized for 2 weeks after EHM implantation and will be closely monitored for side effects, in particular for arrhythmia. Arrhythmia (ventricular tachycardia) represent the main complications in preclinical studies, testing iPSC-derived cardiomyocytes injections (Chong et al., 2014; Liu et al., 2018b; Romagnuolo et al., 2019; Shiba et al., 2016). Conversely, arrhythmia were not observed in preclinical studies of tissue engineered heart repair, which is most likely explained by the implantation of a preformed functional syncytium in tissue engineering approaches, rather than individual mostly spontaneous active cardiomyocytes. The observation of ectopy rather than re-entry in macaque models is in agreement with this assumption (Liu et al., 2018b). Patients will be under OMT, which includes ICD/CRT-D for protection from sudden cardiac death. Controlled rejection of BioVAT implants by withdrawal of immune suppression will serve as a rescue measure if intolerable side effects occur.

Patients will remain under standard of care, which may include inotrope treatment, mechanical circulatory support, and OHT as clinically indicated.

A DSMB, comprised of external independent experts with expertise in cell-based heart repair studies will be charged with the oversight of the clinical trial: Prof. P. Menasche, Paris (Cardiothoracic Surgeon), Prof. S. Janssens, Leuven (Cardiologist), Prof. S. Zohar, Paris (Statistician) have agreed to be members of the BioVAT-HF DSMB. The DSMB may recommend that the sponsor suspends enrolment, amends the study or discontinues the study at any time. Clinical investigators will report all events to the principle investigator (PI). PI will report all events to the sponsor and the DSMB. DSMB will review events and recommend continuation, modification, or discontinuation of the study. Discontinuation will be by stopping the immune suppression and thus initiating graft rejection. Discontinuation will be according to pre-specified stopping criteria such as (1) immune suppression related complications (e.g., sepsis, kidney failure, liver failure), (2) lack of EHM patch retention (e.g., no evidence for enhanced thickness of

the target hypokinetic heart segment), (3) graft related perturbations of heart function (e.g., sustained arrhythmia, decrease of EF, end-organ failure due to low-output syndrome), or (4) exaggerated disease progression (e.g., increased frequency in recurrent hospitalizations due to worsening of heart failure, need for sustained inotrope support).

Preclinical studies have provided no palpable evidence for toxicity or tumorigenicity of EHM implantation (refer to IMPD). Potential risks associated with the nature of the IMP and companion treatment (immune suppression), include arrhythmia (as a result of faulty cardiomyocyte integration), tumor formation (as a result of pluripotent stem cell impurities), perturbation of heart function (as a result of mechanical compression or constriction of the heart), and immune suppression related side effects.

2 Objectives and endpoints

Table 2 Objectives and related endpoints

	Objective	Endpoint
Primary	To assess safety and efficacy of engineered human myocardium (EHM) in patients with terminal heart failure (HFREF EF ≤ 35%).	Evidence for anatomical and functional muscular augmentation of target myocardium determined as enhanced target heart wall thickness and thickening fraction
Secondary	To assess effects of EHM-grafts on disease-specific events and symptoms	<p><u>Key secondary endpoint:</u> Frequency of recurrent hospitalizations for worsening of heart failure</p> <p><u>Further secondary endpoints:</u></p> <ul style="list-style-type: none"> • Identification of a Minimal Effective Dose (Dose Finding Cohort) • Identification of the Optimal Effective Dose Range (Dose Finding Cohort) • Change of EF • Change in functional status in patients by six-minute walk test (6MWT), hand-grip strength, and cardiopulmonary stress testing (VO₂max) • Change in patient reported outcomes assessed by NYHA classification, quality of life score (MLHFQ, KCCQ, EQ-5D), and physical score (SF-36) • All-cause and cardiovascular mortality
Safety		<ul style="list-style-type: none"> • Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death) • Frequency and severity of arrhythmic events

	Objective	Endpoint
		<ul style="list-style-type: none"> • Incidence of immune rejection (DNA, CK/CK-MB, cTnT, circulating cell-free allograft DNA) • Incidence of mechanical perturbation of ventricular function by EHM graft • Identification of a Safe Maximal Dose (SMD) or Maximum Tolerated Dose (MTD) in Dose Finding Cohort

2.1 Evaluation of primary / secondary objectives and endpoints

Safety and efficacy of EHM implantation will be key outcome measures. These will inform the strategy to advance EHM-based heart repair from early clinical testing towards routine clinical application. The dose finding cohort will define the Optimal Effective Dose Range. Based on our preclinical work and allometric scaling efforts we anticipate that EHM size, rather than toxicity, will be dose-limiting in the selected patient cohort. Accordingly, we consider 20 EHM the Maximum Feasible Dose (MFD). In a pivotal preclinical study in a non-human primate model, 1x to 5x EHM assemblies, representing clinical doses of 10x to 50x EHM according to allometric scaling were tested and found to not be associated with dose limiting toxicity. These observation provide a comfortable (2.5) safety margin. After determination of the optimal effective dose range (identified by an increase in target heart wall thickness and systolic thickening fraction), we aim at testing LV and RV support in a refinement cohort to address the unmet need for functional augmentation by muscularization in both ventricles. The expansion cohort, targeting the LV or RV according to DSMB recommendations, will serve to consolidate the safety and efficacy data.

Outcome measures will be determined by MRI or echocardiography as well as recording of heart failure symptoms. Safety outcome will be supported by ECG and if clinically indicated pulmonary artery pressure telemetry recordings and biomarkers (CK/CK-MB, BNP). Enhanced heart wall thickness with enhanced thickening fraction as primary efficacy endpoint was chosen to best determine retention and function of EHM-grafts, which will be key for therapeutic impact. Heart wall augmentation by EHM implants has been used and validated in preclinical studies (Didie et al., 2013; Zimmermann et al., 2006); refer to IMPD for data from the pivotal non-human primate study); we further anticipate that enhanced thickness of the ventricular wall will reduce heart wall stress according to the LaPlace law and by implantation of contractile EHM result in biological ventricular assistance best assessed by an enhancement of wall thickness and systolic thickening fraction. As key secondary endpoint, recurrent hospitalization due to worsening of heart failure was chosen because it may best represent the trajectory of disease progression and quality of life in the proposed patient population.

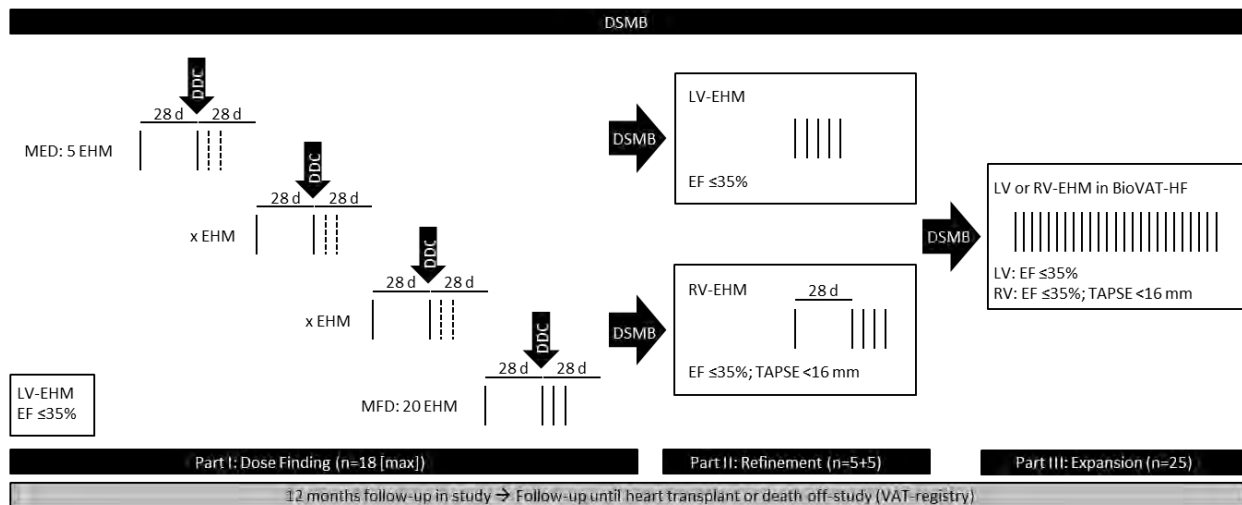
3 Clinical trial plan

3.1 Trial design

This is a combined, open-label, 3-stage, phase I/II safety and efficacy study investigating induced pluripotent stem cell-derived engineered human myocardium (EHM) as biological ventricular

assist tissue (BioVAT) in patients with terminal heart failure. 53 patients will be recruited over 30 months. The study consists of three consecutive phases (Fig. 2).

Figure 2 Trial design



3.2 Treatment phases

All patients in this trial receive induced pluripotent stem cell (iPSC)-derived EHM as BioVAT.

Within **phase I** a dose finding regimen to determine the Optimally Effective Dose Range and if possible the Safe Maximal Dose (SMD) of EHM will be applied (Fig. 2). A minimum of 8 and a maximum of 18 patients will be included with 4 patients treated with the identified MFD. 5x EHM assemblies will be applied as starting dose. 5x EHM assemblies resulted in an augmentation of the target heart wall by ~5 mm in the pivotal macaque study and thus is considered a minimally effective dose (MED) in the BioVAT-HF study. Dose escalation may be recommended after review of the clinical data obtained during the first 4 weeks after treatment of the first two patients in each dosing cohort by the Dose Determining Committee (DDC). Multiples of 5x EHM assemblies, i.e. 5, 10, 15, 20x EHM assemblies are considered as dosing intervals (refer to IMPD and page 26 for details). Up to 4 patients may be included in each dose cohort. The earliest time point for inclusion of a second patient in each dose cohort is 4 weeks after treatment of the first patient in the respective dose cohort.

During **phase II** the most optimal EHM target heart wall, i.e., the left ventricle (LV) or the right ventricle (RV) will be specified (Fig. 2). In this phase ten patients will receive EHM-implantation on LV (n = 5) or RV (n = 5). RV treatment addresses the unmet need for RV support in patients referred to a surgical intervention on the LV (i.e., CABG, LVAD, valve reconstruction) with concurrent RV failure.

After evaluation of phase II further patients (n = 25) will be included to collect proof-of-concept data as to efficacy of EHM mediated assistance/augmentation of the LV or RV (target heart wall determined in phase II) within the final **phase III** (Fig. 2).

3.3 Treatment duration

With respect to experience from preclinical studies and similar surgical procedures the implantation of EHM-patches will take approximately 1 h. Immunosuppressive treatment will be started 7 ± 3 days prior to the scheduled EHM implantation and continued for 12 months unless unwanted effects require withdrawal of immune suppression for controlled EHM rejection. Immune suppression will be continued off-study if patients present evidence for efficacy (i.e., enhanced heart wall thickness, enhanced heart wall thickening fraction, improved symptoms) without palpable safety concerns. Patients will be included in a registry off-study until death (BioVAT-HF Registry). Data from routine heart failure management will be recorded.

3.4 Number of patients

Up to 53 patients are planned to be enrolled in the trial (Phase I: max. 18, Phase II: 10, Phase III: 25).

3.5 Participating sites

The three participating sites are highly specialized centers for heart failure therapy:

University Medical Center Göttingen:

PI: Prof. Dr. I Kutschka (Cardiothoracic [CT] Surgery)

Deputy: Prof. Dr. G. Hasenfuß (Cardiology)

University of Schleswig-Holstein, Campus Lübeck:

PI: Prof. Dr. S. Ensminger (CT Surgery)

Deputy: Prof. Dr. I. Eitel (Cardiology)

Heart Center Bad Oeynhausen:

PI: Prof. Dr. J. Gummert (CT Surgery)

Deputy: Prof. Dr. V. Rudolph (Cardiology)

3.6 Recruitment rate

The participating centers run active heart failure programs, including specific programs for OHT and/or LVAD implantation. Other physicians may refer patients to the study via consultation with the primary study center in Göttingen. The participating centers have agreed to recruit 12 patients/year to the BioVAT-HF trial.

3.7 Trial timetable

Planned dates:

Enrolment of first patient, first patient in (FPI)	4th quarter 2020
Enrolment of last patient, last patient in (LPI)	2nd quarter 2023
End of trial for last patient, last patient last visit (LPLV)	2nd quarter 2024
Final statistical analysis	3rd quarter 2024
Planned interim analysis	After end of phase I and phase II and after half of the patients in Part II+III with either LV or RV administration of EHM (i.e., in total 15 patients) have completed the 12 month follow-up.

4 Trial population and selection criteria

4.1 Target population

Patients will only be allowed to enter the trial if they provide written informed consent to their participation (following full explanation of the trial) (see section 5.1).

4.1.1 Health condition studied

Patients with terminal heart failure will be enrolled into this trial; eligible patients must present with reduced ejection fraction ($EF \leq 35\%$) and overt heart failure symptoms (NYHA III-IV), be on OMT with no realistic chance for heart transplantation or be ineligible for heart transplantation. A hypo- or dyskinetic free heart wall must be identified as target for EHM implantation.

4.1.2 Gender distribution

There will be no selection of patients according to gender. No evidence for differences in outcome are anticipated for male and female subjects. In agreement with other clinical trials in patients with endstage heart failure, we anticipate an overrepresentation of male subjects.

4.2 Inclusion criteria

Patients eligible for this trial must meet all of the following criteria:

1. HFrEF ($EF \leq 35\%$) as assessed by echocardiography or MRI
2. No realistic chance or not eligible for heart transplantation
3. At least one hypo- or dyskinetic segment to demark the implant target area
4. Stable disease condition allowing for an elective left-lateral mini-thoracotomy (for LV applications) or open-chest surgery (for RV applications) for a clinically indicated intervention on the LV (e.g., coronary bypass surgery, valve repair) with concomitant RV dysfunction, diagnosed using the Tricuspid Annular Plane Systolic Excursion (TAPSE) index <16 mm (Rudski et al. 2010).

5. 18-80 years of age
6. Previous implantation of an ICD or CRT-D with event recorder
7. New York Heart Association (NYHA) Class III or IV under optimal therapy
8. Willingness and ability to give written informed consent

4.3 Exclusion criteria

Patients eligible for this trial must not meet any of the following criteria:

1. Contraindication to immunosuppressive drugs (e.g. history of unresolved cancer, infections like hepatitis B/C, HIV, HTLV1)
2. Hypertrophic cardiomyopathy (HCM)
3. Terminal kidney failure (stage 4; GFR <30 ml/min)
4. Terminal liver failure (Child-Pugh stage C; score >10)
5. Alloimmunization against EHM implant cells
6. Autoimmune disease
7. History of stroke
8. Reduced life expectancy in the short term due to non-cardiac disease
9. Simultaneous participation in another interventional trial
10. Pregnant or breastfeeding females
11. Known or suspected alcohol and/or drug abuse

5 Enrolment and patient registration

5.1 Patient eligibility

The investigator will inform the patient about the trial and ask the patient for his/her written consent. It is imperative that written consent is obtained prior to any trial-specific procedures. The investigator will then record the details of these trial patients on the following trial-specific lists:

- Subject identification log: A confidential log of the names of all trial patients with the identification code **Fehler! Textmarke nicht definiert..** However, Sponsor representatives, clinical research associates (CRAs), auditors and representatives of competent authorities (CA) must be allowed to inspect the list on request. However, Sponsor representatives, clinical research associates (CRAs), auditors and representatives of competent authorities (CA) must be allowed to inspect the list on request.

Afterwards the investigator will check the in- / exclusion criteria. Only if the patient has signed the informed consent and meets all of the inclusion criteria and none of the exclusion criteria the patient is eligible for the study.

Furthermore the investigator should ask the patient to provide biological samples for the DZHK-Biobank project. A separate consent form needs to be signed for drawing biological samples for this project. This consent-process is independent from the BioVAT-HF-study and patients can participate in the BioVAT-HF without consent for the DZHK-Biobanking project

5.2 Patient registration

Patient registration will be done within the eCRF(secutrial). After registration an individual Patient Identification number will be assigned to each patient

6 Treatment plan and procedure

6.1 Dosing regimen and IMP administration

Immune suppression (calcineurin inhibitor combined with a corticoid) will be started 7 ± 3 days prior to the scheduled EHM implantation. Dosing of the calcineurin inhibitor and corticoid will be daily *per os* according to standard protocols in patients with heart transplantation, i.e. Tacrolimus (5-15 ng/ml) or Ciclosporine A (150-375 ng/ml) and Methylprednisolone (5-10 mg/day; ~ 0.15 mg/kg bodyweight * day). Trough levels for the administered calcineurin inhibitor will be assessed before EHM implantation and dose adjusted if needed.

Adaptations of immune suppression, for example administration of other calcineurin inhibitors or other immune suppressants in clinical use in organ transplantation (e.g. MMF), may be acceptable if clinically indicated and recommended by the treating physician.

EHM will be delivered individually from the manufacturing site (University Medical Center Göttingen) to the point-of-care and assembled to 5x, 10x, 15x, or 20x EHM assemblies at the point-of-care (refer to IMPD). EHM assemblies will be sutured to a TachoSil® membrane (refer to IMPD) and then sutured to the target heart wall via a left lateral thoracotomy.

Table 3 Dosing schedule

Study phase	ATMP form and route of administration	Dose	Regimen
Part I: Dose finding cohort	Implantation of EHM assemblies to the LV composed of increasing EHM layers	5x EHM 10x EHM 20x EHM Dosing intervals will be recommended by the DDC and may include an intermediate dose of 15x EHM	<u>Minimum (8 patients):</u> Patient I: 5x EHM Patient II: 5x EHM Patient III: 10x EHM Patient IV: 10x EHM Patient V: 20x EHM Patient VI: 20x EHM Patient VII: 20x EHM Patient VIII: 20x EHM <u>Maximum (18 patients):</u> Patients I-IV: 5x EHM Patients V-VIII: 10x EHM Patients IX-XII: 15x EHM Patients XIII-XVI: 20x EHM

Study phase	ATMP form and route of administration	Dose	Regimen
Part II: Refinement cohort	Implantation of EHM patches to LV and RV	Dose for LV as defined in Part 1 Dose for RV suggested by DDC after DSMB review (approx.. 50% of optimal dose determined in Part I)	Patients will be included in LV and RV cohorts according to eligibility for LV or RV augmentation.
Part III: Expansion cohort	Implantation of EHM patches to LV or RV according feasibility, safety and efficacy considerations obtained from Part II.	Dose according to Part II	Extension from Part II

6.2 Dose modification and dose delay / or dose reduction

There will be no modifications of the IMP dose after EHM implantation. Immune suppression may be adapted according to clinical routine proceedings in case of evidence for immune suppression related toxicity (liver, kidney) or rejection. Withdrawal of immune suppression may be recommended in case of intolerable unwanted effects. Immune suppression dose reduction or withdrawal must be recorded on the appropriate eCRF page.

6.3 Concomitant treatment/medication

6.3.1 Permitted prior/concomitant treatment/medication

Treatment with transfusions (red blood cells and platelets) and supportive care are permitted after the initiation of study treatment.

The patient must notify the investigational site of any new medication he/she starts taking after the start of the trial medication. All medications (other than IMP) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts treatment with IMP must be listed in the eCRF.

6.3.2 Guidelines for rescue medications and/or non-drug therapies or supportive care

Patients should receive treatment/medication appropriate to their clinical condition in an emergency. The following alternative treatments could be given in an emergency:

Withdrawal of immune suppression to initiate controlled rejection of EHM implants in case of intolerable side effects.

6.3.3 Permitted concomitant therapy requiring caution and/or action

Calcineurin inhibitors (tacrolimus and cyclosporine) are substrates of CYP3A4. Pharmacokinetic interactions with other CYP3A4 substrates, inhibitors and activators (such as for example HMG-CoA reductase inhibitors) must be considered according to standard clinical practice.

6.3.4 Prohibited concomitant therapy

CYP3A4 accelerating drugs (e.g., St. John's Wort) must not be administered. Patients must be informed about the potential need for dose modification if CYP3A4 inhibitors (e.g., macrolide antibiotics, HMG-CoA reductase inhibitors) are concurrently administered. Control of trough levels of calcineurin inhibitors must be performed according to routine practice and dosing adjusted accordingly.

6.4 Unblinding of treatment assignment

Not applicable, trial is unblinded.

6.5 Treatment and Follow-up after end of the trial

Upon completion of the BioVAT-HF trial with 12 months follow-up, study subjects will be included in a registry off-study until death. Immune suppressive therapy will be continued according to the German treatment guidelines in patients with organ transplantation to prevent rejection of EHM.

7 Visit schedule and assessments

7.1 Flow and visit schedule

A detailed Flowchart is provided in Table 1 Visit schedule and assessments – Flowchart

The schedule of assessment lists all of the assessments and indicates with an “x” when they have to be performed. All data obtained from these assessments must be available in the patient's source documentation.

7.2 Visit and assessment windows

During the course of the trial, visits and test procedures should occur on schedule whenever possible; visits that occur within a certain timeframe from the scheduled date will not constitute a protocol deviation. These timeframes are defined in Table 1 and the following descriptions.

7.3 Study registration (Visit 1, Month -4 to -1)

The investigator is obliged to give the patient thorough information about the trial and the trial related assessments, and the patient should be given ample time to consider his or her participation. The investigator must not start any trial-specific procedure before Informed Consent Form (ICF) is signed and dated by both the patient (and impartial witness, if applicable) and the investigator. The investigator must keep the original signed ICF (a signed copy is given to the patient), (see section 15.3). Study registration visit is performed 4 to 1 month before Implantation. Patients considered eligible by the investigator will be included and registered to the trial (see section 5.2).

Following data will be collected during the registration visit (please refer to section 7.8 for a precise definition of assessments):

- Informed consent
- Verification of inclusion / exclusion criteria

7.4 Baseline (Visit 2, Day -10 to -5)

The data that will be collected at baseline include the following (please refer to section 7.8 for a precise definition of assessments):

- Verification of inclusion / exclusion criteria
- Medical history
- DZHK basic data set
- Concomitant heart failure medications

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, IL6
- Liver panel
- Albumin
- Serum Creatinine
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- Troponins (high-sensitive troponin T), CK, CK-MB
- NT-proBNP
- Pregnancy Test (for females of childbearing potential)
- Blood draw for allograft DNA assessment
- HLA/KIR-Typing
- Donor Specific Antigens

Further assessments / Scores

- 12-lead ECG
- Transthoracic Echocardiography (TTE)
- Cardiac-MRI (if possible)
- Cardiopulmonary exercise testing
- Hand grip strength
- 6 minute walking test

- EuroSCORE II; Logisitec EuroSCORE I, if site uses it as a standard risk calculator (not mandatory)
- Positron-Emission Tomography (FDG-PET) (not mandatory, only if used as standard of care)

Quality of life questionnaires (patient reported outcome)

- MLHFQ
- SF-36
- KCCQ
- EQ-5D

Treatment / therapy:

- ICD/CRTD-event recorder readout
- DZHK-Biobanking Data Set (not mandatory, only if separate Consent available)

7.5 Start of Immunosuppression (Visit 3, Day -10 to -4)

Immune suppression (calcineurin inhibitor combined with a corticoid) will be started 7 ± 3 days prior to the scheduled EHM implantation. Dosing of the calcineurin inhibitor and corticoid will be daily *per os* according to standard protocols in patients with heart transplantation, i.e. Tacrolimus (5-15 ng/ml) or Ciclosporine A (150-375 ng/ml) and Methylprednisolone (5-10 mg/day; 0.15 mg/kg bodyweight * day).

Further data to be collected:

- Adverse events
- TDM Calcineurin Inhibitors 2-3 days after start of immune suppression

7.6 Implantation (Visit 4; Day 0)

On this day, EHM patch implantation will be performed. **Before** surgical procedures following data has to be collected:

- Reduced DZHK basic data set according to chapter 7.8.2
- Concomitant heart failure medications
- Adverse events

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, IL6
- Liver panel
- Albumin
- Serum Creatinine
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- Troponins (high-sensitive troponin T), CK, CK-MB
- NT-proBNP
- Blood draw for allograft DNA assessment

Further assessments / Scores

- 12-lead ECG
- Transthoracic Echocardiography (TTE)

Treatment / therapy:

- ICD/CRTD-event recorder readout
- TDM Calcineurin Inhibitors (see chapter 7.5)

7.7 Follow up

Following implantation of EHM as BioVAT (Visit 4), the patient will remain hospitalized for 2 weeks to monitor potential adverse events and adjust the calcineurin inhibitor dose according to TDM data obtained every day after EHM implantation. After release from the hospital, patients are scheduled for follow-up visits 1, 3, 6 and 12 months after BioVAT implantation. For details see sections below.

7.7.1 Assessments at Visit 5 (Week 2 ± 2 days)

- Reduced DZHK basic data set according to chapter 7.8.2
- Concomitant heart failure medications
- Adverse events

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, IL6
- Liver panel
- Albumin
- Serum Creatinine
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- Troponins (high-sensitive troponin T), CK, CK-MB
- NT-proBNP
- Blood draw for allograft DNA assessment

Further assessments / Scores

- 12-lead ECG
- Transthoracic Echocardiography (TTE)
- Cardiac MRI (if possible)
- Cardiopulmonary exercise testing
- Hand grip strength
- 6 minute walking test
- EuroSCORE II; Logisitc EuroSCORE I, if site uses it as a standard risk calculator (not mandatory)

Quality of life questionnaires (patient reported outcome)

- MLHFQ
- SF-36
- KCCQ
- EQ-5D

Treatment / therapy:

- ICD/CRTD-event recorder readout
- TDM Calcineurin Inhibitors (see chapter 7.5)

7.7.2 Assessments at Visit 6 (Month 1 ± 7 days), Visit 7 (Month 3 ± 7 days), and Visit 8 (Month 6 ± 7 days)

- Reduced DZHK basic data set according to chapter 7.8.2
- Concomitant heart failure medications
- Adverse events

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, IL6
- Liver panel
- Albumin
- Serum Creatinine
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- Troponins (high-sensitive troponin T), CK, CK-MB
- NT-proBNP
- Blood draw for allograft DNA assessment
- Donor Specific Antigens

Further assessments / Scores

- 12-lead ECG
- Transthoracic Echocardiography (TTE)
- Cardiac MRI (if possible)
- Cardiopulmonary exercise testing
- Hand grip strength
- 6 minute walking test
- EuroSCORE II; Logisitec EuroSCORE I, if site uses it as a standard risk calculator (not mandatory)

Quality of life questionnaires (patient reported outcome)

- MLHFQ
- SF-36
- KCCQ
- EQ-5D

Treatment / therapy:

- ICD/CRTD-event recorder readout
- TDM Calcineurin Inhibitors (see chapter 7.5)

7.7.3 Assessments at Visit 9 (Month 12 ± 7 days)

Assessments will be done according to visits 6 - 8. Optional, a Positron-Emission Tomography (FDG-PET) can be performed, if it is done according to standard of care.

7.8 Assessments and specifications

7.8.1 Medical history

Stage D heart failure patients (Yancy et al., 2013) with a documented history of LVEF ≤35% are eligible to enrol in the BioVAT-HF trial. LVEF will be re-evaluated by echocardiography (ECHO; refer to description in 7.8.7) and/or magnetic resonance imaging (MRI; refer to description in 7.8.8). Upon the baseline visit medical history is gathered according to DZHK-SOPs:

DZHK-SOP-K-01 „Basisdatensatz Anamnese/Klinische Diagnosen/Körperliche Untersuchung“
DZHK-SOP-K-02 „Anamnese/Klinische Diagnosen“

Data will be reported according to the eCRF modules in DZHK-SOP-K-01 and DZHK-SOP-K-02.

7.8.2 DZHK basic data set

For the DZHK basic data set data collection is performed according to DZHK-SOP_K_01 „Basisdatensatz – Anamnese/Klinische Diagnosen/Körperliche Untersuchung“. For baseline visit all mentioned examinations have to be performed. For all other visits a reduced data set should be collected. Items not necessary are marked in the distributed DZHK-SOP_K_01 „Basisdatensatz – Anamnese/Klinische Diagnosen/Körperliche Untersuchung“.

7.8.3 Concomitant heart failure medications

Patients will be under optimal medical therapy, according to the most recent guidelines (i.e., at present the 2016 ESC Guidelines).

7.8.4 Pregnancy Test (for females of childbearing potential)

Recommendation by the Clinical Trial facilitation Group as to contraception and pregnancy testing will be followed (www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf).

Women of childbearing potential will be subjected to urinary pregnancy testing at baseline to confirm eligibility in the trial (see Table 1). During the study period women of childbearing potential must adhere to appropriate contraceptive measures.

The trial treatment cannot be withdrawn after administration. The concomitant immune suppression can be terminated, which will result in IMP rejection.

In case of pregnancy, the pregnancy will be followed up by the sponsor to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence/absence of any birth defects, congenital abnormalities or maternal and new-born complications.

7.8.5 Laboratory assessments

Collection of blood and urine will be according to DZHK-SOP-B01 for obtaining:

(A) a DZHK Basic Set

(B) a DZHK Study Set

From the DZHK Study Set following blood tests will be performed:

Blood tests:

- CBC with differentials and platelet count
- CRP, IL6
- Liver panel
- Albumin
- Serum Creatinine
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- Troponins (high-sensitive troponin T), CK, CK-MB
- NT-proBNP
- Blood draw for allograft DNA assessment
- HLA/KIR typing
- Donor specific antigens (DSA)

Urinalysis:

- Pregnancy test

Blood and urinalysis from the DZHK Study Set will be performed by the accredited central laboratories of the participating study sites. For circulating cell-free allograft DNA assessment, blood will be sent to the Liquid Biopsy Center in Göttingen (www.liquidbiopsy.center). Details on all laboratory procedures, collections, shipment of samples and reporting of results, alerting of extreme values and notable values to the principle investigator will be provided to investigators in the laboratory manual. The central laboratories of the study sites will provide the sponsor with a copy of the laboratory certification and tabulation of reference ranges for the individually measured parameters.

Details on collection and handling of laboratory samples are provided in the laboratory manual/instruction.

7.8.6 ECG

A resting 12-lead surface electrocardiogram (ECG) will be performed at all visits according to DZHK-SOP-K-03. Each ECG tracing will be kept in the source documents at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K03.

7.8.7 Echocardiography

Transthoracic echocardiography will be performed at all visits according to DZHK-SOP-K-08. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K08.

7.8.8 Cardiac-MRI

Cardiac MRI will be performed at the baseline (Visit 1) and follow-up visits (Visits 3-7) if possible according to DZHK-SOP-K-06. Patients with devices may not be eligible to MRI investigations. In these patients ECHO data will be used to investigate the primary (target heart wall thickness and thickening fraction) and secondary (LVEF) endpoints. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K06.

7.8.9 FDG-PET (optional; not mandatory)

Data on heart and EHM glucose metabolism will be used as additional measures for EHM graft survival and integration if patients are subjected to FDG-PET analyses before or during the trial period. FDG-PET studies will not be performed as part of the BioVAT-HF trial, but may be clinically indicated. The participating centers will share the obtained FDG-PET data with the principle investigator for ancillary analyses.

7.8.10 Cardiopulmonary exercise testing

Spiroergometry will be performed at the baseline (Visit 1) and follow-up visits (Visits 3-7) according to DZHK-SOP-K-07 if possible, i.e., if patients can be subjected to the spiroergometry. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K07. VO2 max will be reported as secondary endpoint.

7.8.11 EuroSCORE II; Logistic EuroSCORE I (only if site uses it as a standard risk calculator, not mandatory)

The surgical risk will be estimated at the baseline visit (Visit 1) using the EuroSCOREII risk calculator and/or logistic EuroScoreI if implemented as a standard at the participating study sites (<http://euroscore.org/calc.html>).

7.8.12 Hand grip strength

Hand grip strength is measured with a hand dynamometer. Maximum hand grip strength correlates well with total muscle mass. Loss in muscle mass or cachexia are associated with poor prognosis. Hand grip strength measurements will be done according to a SOP by the Nutritional Assessment Platform.

7.8.13 6 minute walking test (6MWT)

The 6MWT will be performed at the baseline (Visit 1) and follow-up visits (Visits 3-7) according to DZHK-SOP-K-04 if possible, i.e., if patients can be subjected to the 6MWT. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K04. VO2 max will be reported as secondary endpoint.

7.8.14 Assessment of quality of life

The Quality of life of the patients will be evaluated using the following Quality of Life Questionnaire:

MLHFQ (Minnesota LIVING WITH HEART FAILURE® Questionnaire)

The MLHFQ is used to determine whether a treatment for heart failure is effective for improving quality of life by reducing the adverse impact of heart failure. The standardized questionnaire is comprised of 21 questions as to physical, emotional and socioeconomic ways heart failure can adversely affect a patient's life. After a standardized instruction patients patient mark a 0 (zero) to 5 scale to indicate how much each item has prevented the patient from living as he or she wanted to live during the past 4 weeks. Patients will be provided with a paper questionnaire at the Baseline (Visit 1) and follow-up visits 2-5 (Visits 4-7).

SF-36 (short form 36)

The SF-36 questionnaire is a comprehensive measure of general health status. It includes one multi-item scale that assesses eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. Patients will be asked to fill in the questionnaire at Baseline (Visit 1) and follow-up visits 2-5 (Visits 4-7).

KCCQ (Kansas City Cardiomyopathy Questionnaire)

The KCCQ is a 23-item instrument that quantifies physical function, symptoms (frequency, severity and recent change), social function, self-efficacy and knowledge, and QoL in patients with heart failure. Patients will be asked to fill in the questionnaire at Baseline (Visit 1) and follow-up visits 2-5 (Visits 4-7).

EQ-5D

EQ-5D questionnaire measures health-related quality of life in cost-effectiveness analysis. Patients will be asked to fill in the questionnaire at Baseline (Visit 1) and follow-up visits 2-5 (Visits 4-7).

7.8.15 ICD/CRTD-event recorder readout

ECG with potential arrhythmia events will be monitored by ICD or CRT devices with in-build event recorders. Data will be documented at visit 2 and 4-9.

7.9 Additional biological specimen collection

7.9.1 Biological specimen collection for translational program

Biological samples will only be collected during the course of the trial if the patient consents to the DZHK-Biobanking project. Collection of biological samples is not mandatory for participation in the BioVAT-HF-study.

Detailed instructions on sample collection, processing, handling and shipment are provided in the DZHK-SOP-B-02 "Biomaterial Proccessing Basic Set". Following sample are included in this SOP:

- Serum
- EDTA-Plasma
- Citrate-Plasma
- Urine
- Buffy Coat

8 Discontinuation criteria

8.1 Premature termination of one of the treatment arms or the entire trial

The sponsor/coordinating investigator is under obligation to monitor the progress of the clinical trial with regard to safety-relevant developments and, if necessary, initiate the premature termination of a treatment arm or the entire clinical trial. The sponsor/coordinating investigator will be supported in this responsibility by the DSMB, if necessary.

A treatment arm or the entire clinical trial must be terminated prematurely if:

- the benefit-to-risk ratio for the patients changes markedly,
- the sponsor/coordinating investigator OR the DSMB considers that the termination of the trial is necessary,
- indications arise that the trial patients' safety is no longer guaranteed,
- the question(s) addressed in the trial can be clearly answered on the basis of an interim analysis,
- the questions(s) addressed in the trial can be clearly answered on the basis of results of another trial on the same subjects,
- an insufficient recruitment rate makes a successful conclusion of the clinical trial unrealisable/no longer feasible.

If the clinical trial is prematurely terminated or suspended for any reason, the investigator should promptly inform the trial patients and ensure appropriate therapy and follow-up for the patients. Where required by the applicable regulatory requirements, the CA(s) and the IEC(s) will also be informed (this is usually done by the sponsor).

8.2 Premature termination of the trial at one of the trial sites

Both the investigator and the sponsor have the right to terminate the trial at one of the sites.

The clinical trial can be terminated prematurely at his site by the investigator if, for instance unforeseeable circumstances have arisen at the trial site which preclude the continuation of the clinical trial, the investigator considers that the resources for continuation are no longer available, the investigator considers that the continuation of the trial is no longer ethically or medically justifiable.

The sponsor/coordinating investigator can initiate the exclusion of a site from further participation if, for instance, patient recruitment is inadequate, serious problems arise with regard to the quality of the collected data which cannot be resolved.

Premature termination at one of the trial sites does not automatically mean a termination of the trial for already enrolled trial patients. A separate decision on further treatment must be made for each patient, depending on the overall situation. Adequate further treatment and follow-up of already enrolled trial patients must be ensured. The documentation of already enrolled trial

patients will be reviewed for completeness and plausibility. Queries may be raised for further clarification before the site is closed. These queries must be answered properly by the site. The CA(s) and IEC(s) must be duly notified of the site's closure, including reasons, within the specified period. The trial site concerned will be closed in stages by the CRA when a decision has been made on the further treatment of the patients concerned.

8.3 Discontinuation of trial treatment or trial participation for individual patients

It has to be distinguished if trial treatment of a patient has been stopped prematurely (by withdrawal of immune suppression or surgical removal of the EHM implant) or if the trial participation of a patient was stopped prematurely.

In the case trial treatment of a patient has been stopped prematurely, further follow-up visits and the assessment of the trial endpoints are essential to enable an analysis of the full analysis set according to the intention-to-treat principle. Further visits, follow-up and documentation should always be striven for/ensured in this case. This includes the follow-up of AEs, the time of termination, the results available at that time and, if known, the documentation of the termination of treatment on the eCRF and in the medical record, giving reasons, a final examination and documentation according to the protocol (if possible).

In the case trial participation of a patient was stopped prematurely, the conduct of further follow-up visits is no longer possible. The documentation should be completed as far as possible under these circumstances, e.g. a final examination and documentation according to the protocol (if possible), a documentation of the premature trial termination on the CRF and in the medical record, giving reasons, appropriate further treatment and follow-up outside the trial should be ensured; inform general practitioner of the termination, if necessary (provided that the patient agrees). In studies that assess the survival status, an attempt should at least be made to assess the patient's survival status by telephone follow-up (unless informed consent for documentation has been withdrawn).

8.3.1 Premature discontinuation of trial treatment

After implantation the EHM patch can be removed by a discontinuation of immunosuppressive treatment or by surgical excision in case of unwanted effects.

8.3.2 Premature termination of trial participation

The trial patient can withdraw his/her consent at any time, without having to give reasons, and have his/her entire trial participation terminated prematurely. However, the prerequisite for this is that the patient actively terminates trial participation by withdrawing his/her consent for the follow-up and documentation.

The responsible investigator may only withdraw a patient from participation in the trial for the following reasons:

- Extreme circumstances arise which make any trial-relevant follow-up impossible

9 Investigational medicinal product (IMP)

9.1 Engineered Human Myocardium (EHM) background information

Engineered Human Myocardium (EHM) in a clinically translatable format was introduced in 2017 (Tiburcy et al., 2017). The production protocol was optimized for GMP production and application as BioVAT. The EHM protocol originates from ~25 years of preclinical development (Eschenhagen et al., 1997; Didie et al., 2013; Fujita and Zimmermann, 2017a, b, 2018; Riegler et al., 2015; Tiburcy et al., 2017; Zimmermann et al., 2000; Zimmermann et al., 2006).

9.1.1 Preclinical data

Feasibility, safety, and efficacy of tissue engineered heart repair was thoroughly investigated in 3 preclinical animal species (rat, mouse, and Rhesus macaque):

- 1) Rat model with uncompromised heart function (Zimmermann et al., 2002)
- 2) Rat model of chronic (severe) heart failure after permanent LAD occlusion (Zimmermann et al., 2006)
- 3) Mouse model of acute myocardial infarction by permanent LAD occlusion (Didie et al., 2013)
- 4) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Riegler et al., 2015)
- 5) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Qin et al., 2016)
- 6) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Tiburcy et al., 2017)
- 7) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (extension of Tiburcy et al., 2017 with focus on EHM patch retention; data presented in IMPD)
- 8) Rhesus macaque model with uncompromised heart function (data presented in IMPD)

Additional pilot studies were performed in pig models, but found to be of no predictive value because of limited xenograft retention, despite administration of comprehensive immune suppression regimens.

These studies collectively provide the rationale for the BioVAT-HF trial. Study 1 demonstrated feasibility and safety of Engineered Rat Myocardium (ERM) implantation under immune suppression in a healthy rat model. Study 2 demonstrated electromechanical integration, safety and efficacy of ERM allografts in a rat model of chronic heart failure. Safety and efficacy was further confirmed in a mouse model of subacute myocardial infarction and the application pluripotent stem cell-derived Engineered Mouse Myocardium (EMM) allografts (Study 3). Studies 2 and 3 employed MRI and echocardiography to document efficacy of ERM and EMM allograft-based heart repair by detection of an enhancement of thickness and contractility of the target heart wall, in line with the proposed mode of action. Studies 4-7 established and validated a human GMP-compatible EHM as well as retention upon implantation. In a pivotal Rhesus macaque allograft study (study 8), a Engineered Non-Human Primate Myocardium (ENHPM) graft dose dependent increase in target heart wall thickness by approximately 1 and 5 mm was observed as a result of the implantation of 1x and 5x ENHPM assemblies, respectively, in line with the observations from allograft studies 2 and 3. The augmentation of the target heart wall was sustained for the whole study duration (investigated for up to 6 months in the 5x ENHPM group) with no evidence for unwanted effects (no arrhythmia, no tumor, no perturbation of heart performance, no immune suppression related side effects). The observed osteochondral

differentiations in 5 of 14 ENHPM treated Rhesus macaque were identified as non-proliferative with no unwanted effects on heart function. The observation of osteochondral differentiations was identified as a NHP model specific finding, with no evidence for the occurrence of osteochondral differentiations obtained from any of the EHM implantation studies (studies 4-7), including a dedicated GLP toxicity, tumorigenicity and biodistribution study.

Collectively, the preclinical data provided strong evidence for the safety and efficacy of the EHM-based heart remuscularization strategy and the rationale for the proposed BioVAT-HF early clinical trial.

9.1.2 Pharmacokinetics

Not applicable.

9.1.3 Pharmacodynamics

We consider the 5x EHM assembly as minimal effective dose (MED) for the BioVAT-HF trial and anticipate a similar thickening of the target heart wall as observed in the Rhesus macaque study. Considering the differences in heart and body size in Rhesus macaque and human (10-fold) and applying allometric scaling a 5x EHM assembly in Rhesus macaque resembles a 50x EHM assembly in human. With an anticipated maximal feasible dose (MFD) of 20x EHM and no observed safety concerns in the Rhesus macaque study, we do not anticipate graft related safety concerns in the BioVAT-HF trial.

9.1.4 Adverse reactions

Arrhythmia (in case of irregular electromechanical integration), tumor formation (in case of the contamination with pluripotent stem cells), perturbation of heart performance (in case of compression or stiffening of the target heart wall), and immune suppression related side effects may be anticipated. Preclinical studies did not provide evidence for adverse reactions.

9.2 IMP(s) pharmaceutical characteristics

The IMP(s) used in this trial are characterised as follows, according to the applicable IB:

Proprietary name:	Engineered Human Myocardium (EHM)
Name of substance:	Human heart muscle comprised of iPSC-derived cardiomyocytes and stromal cells supported to self-organize into heart muscle with structural, functional, and molecular properties of juvenile myocardium by a bovine collagen type I hydrogel environment
Manufacturer:	University Medical Center Göttingen
Approved indications:	IMP not yet approved
Dosage form:	Refer to Figure 1
Strength:	Not applicable
Total daily dose:	Not applicable

For further characteristics, see current version of the corresponding IB.

9.3 Packaging and labelling

EHM is packaged as single patch mounted on a holder in a screw container filled with 10 ml transport medium. EHM will be delivered directly to the point-of-care at the required quantity, i.e., 5x, 10x, 15x, or 20x EHM patches.

Medication labels will be in German and comply with GMP Annex 13 and legal requirements in Germany.

9.4 Supply and ordering

EHM will be ordered from the University Medical Center Göttingen. EHM will be ordered 1 - 4 months prior to implantation by E-mail to the following address:

BioVAT-HF.Bestellung@med.uni-goettingen.de

9.5 Receipt and storage

EHMs will be delivered as individual patches in a sterile screw-cap container to the point-of-care. After receipt, EHM will be left in the unopened screw-cap containers at room temperature until implantation on the day of delivery.

Delivery of the EHM patch to the point-of-care is further described in a separate protocol.

Upon opening the screw container at the point-of-care under aseptic conditions, EHM mounted on stretch devices with transport medium will be transferred into a container (e.g., a kidney dish). EHM will then be released from the stretch devices using a custom-made fork and transferred into a second container (e.g., a kidney dish) filled with Ringer Acetate solution (200 ml) for a first washing step using a custom-made shovel. After 10 min EHM will be transferred into a third container (e.g., a kidney dish) filled with Ringer Acetate solution (200 ml) for a second washing step using a custom-made shovel and subsequent assembly into the desired EHM dose (5x, 10x, 15x, or 20x assemblies) in a custom-made assembly device. EHM should be implanted within 30-60 min after opening of the screw container.

9.6 Preparation of EHM patch

EHMs will be delivered as individual patches in sterile screw-cap containers to the point-of-care. Upon opening the screw-cap container at the point-of-care under aseptic conditions, EHM mounted on stretch devices with transport medium will be transferred into a container (e.g., a kidney dish). EHM will then be released from the stretch devices using a custom-made fork and transferred into a second container (e.g., a kidney dish) filled with Ringer Acetate solution (200 ml) for a first washing step using a custom-made shovel. After 10 min EHM will be transferred into a third container (e.g., a kidney dish) filled with Ringer Acetate solution (200 ml) for a second washing step using a custom-made shovel and subsequent assembly into the desired EHM doses (5x, 10x, 15x, or 20x assemblies) in custom-made assembly devices. Assembled EHM will be sutured onto a TachoSil™ membrane and subsequently transferred onto the heart. EHM should be implanted within 30-60 min after opening of the screw container.

Preparation of EHM patch is further described in a separate protocol.

9.7 EHM Implantation

EHM assemblies will be sutured to the target heart wall via a left lateral thoracotomy. The surgical access route and fixation procedure is similar to the proceeding in epicardial pacemaker lead implantations.

EHM implantation is further described in a specific protocol depicting the surgical procedures.

9.8 Return and destruction

Unused IMP can be destroyed at the study site.

9.9 Drug compliance and accountability

The investigator or designee should maintain records of the delivery of the IMP, the use in individual trial patients, and the disposal of unused IMP(s). The investigator should ensure that the IMP is only used according to this protocol.

- The investigator bears the responsibility for the proper storage in an appropriate place to which unauthorised persons have no access.
- The investigator may only use the IMP for implantation in patients who have been enrolled in the study. Usage of the IMP for implantation in patients outside of this clinical trial is not permitted.
- The investigator or designee should explain the IMP implantation to each trial patient and check at regular intervals whether the IMP has been rejected or not.
- The investigator should take notice of the IMP Handling Manual, if applicable.

9.10 Treatment adherence

Not applicable as EHM patch is surgically implanted and cannot be removed.

10 Safety monitoring and reporting

10.1 Adverse Events (AEs)

10.1.1 Definition of AEs

An adverse event (AE) is any untoward medical occurrence in a patient administered any dose of a pharmaceutical product and which does not necessarily have to have a causal relationship with the use of the product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not related to the product.

- In order to monitor the conditions of the patients from the time the patients receive the first dose of IMP the investigator is requested to report any untoward clinical event on the AE-page

of the eCRF. Any untoward medical occurrence, which occurs after the period of patient follow-up defined in the protocol, is not considered an AE.

- Irrespective of any causal relationship, all AEs spontaneously reported by the patient or observed by the investigator will be continuously documented in the medical record and on the designated case report form (AE eCRF page).
- All AEs must be described by diagnosis or, if an underlying diagnosis is not known, by symptoms or medically significant laboratory or instrumental abnormalities. The AEs will be documented as shown in section 10.1.2. Please note that medical or surgical procedures (e.g., tooth extraction, transfusion, surgery) performed are not AEs *per se*; the medical condition that leads to the procedure is an AE;
- Symptoms, medically significant laboratory, or instrumental (e.g. electrocardiographic) abnormalities of a pre-existing disease are not to be considered an AE. Occurrences of new symptoms or laboratory or instrumental abnormalities, as well as worsening of pre-existing ones, are considered AEs.
- All AEs, no matter how intense, are to be followed up by the investigator in accordance with ICH-GCP until resolved or judged no longer clinically relevant, or in case of a chronic condition, until it is fully characterised.
- Overdose without clinical sequelae is not to be considered an AE. For the purposes of this study, an overdose is defined as a single dose of IMP that exceeds the prescribed dose for each age range.

10.1.2 Documentation of AEs

Adverse events have to be documented in the eCRF starting from EHM implantation until the last study visit (patient's individual study end date).

- Characterization of the event (diagnosis; if not available, symptoms)
- Onset/end date
- Severity according to the current version of CTCAE
- Relationship to the IMP (related/not related)

Note: According to the CIOMS VI Working group the causal relationship between the investigational product and the adverse event should be characterized as “related” or “not related” (the various gradients of relatedness offer little or no advantages in data analysis or regulatory reporting).

The expression “related” means, that there is evidence or argument to suggest a reasonable causal relationship between the event and the administration of the study drug, e.g. close temporal connection, exclusion of other causes.

The assessment “not related” is appropriate, if the SAE is clearly or most likely explained by other causes even if a potential relationship between study drug and the SAE cannot be completely excluded.

- Serious / non-serious
- Action taken with IMP
- Outcome

10.2 Serious Adverse Events (SAEs)

10.2.1 Definition of SAEs

A Serious Adverse Event (SAE) is any untoward medical occurrence that results in any of the following outcomes:

- Death,
- Life-threatening situation (patient is at immediate risk of death),
- Inpatient hospitalization or prolongation of existing hospitalization (excluding those for study therapy and/or assessments, placement of an indwelling catheter, social/convenience admissions, respite care, elective or pre-planned treatment/surgery)
- Persistent or significant disability/incapacity,
- Congenital anomaly/birth defect,
- Other, medically important condition: conditions which, in the investigator's opinion, may not be immediately life-threatening or result in hospitalization, but may jeopardize the patient's safety or may require intervention to prevent one of the other outcomes listed in the definition above, may also be considered serious. Examples of such conditions include: allergic bronchospasm requiring treatment in an emergency room or at home, unexpected convulsions (i.e. convulsions which cannot be explained by the underlying illness) that do not result in hospitalization, development of IMP dependency or drug abuse, suspected transmission of infectious agents by medicinal product, etc.

Clarification of SAEs:

- NOTE: The term “life-threatening” in the definition of “serious” refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe,

10.2.2 Documentation of SAEs

All SAEs (with the exception of the special situation described below) that starting from EHM implantation until the patient's last study visit (patient's individual study end date) will be documented in the eCRF and on the provided SAE reporting form.

The SAE reporting form will be processed as described in the section below.

10.2.3 Investigator reporting requirements

10.2.3.1 Reporting policy

The following events must be reported by investigator to the sponsor:

- SAEs

The events above must be reported by fax to the following address within 24 hours after knowledge by the investigator:

Pharmacovigilance
Clinical Trials Unit
Medical Center - University of Freiburg

Elsaesser Str. 2, 79110 Freiburg
SAE Fax No.
+49 761 270-74 390

If only limited data are initially available, a follow-up report is required. If new information including outcome becomes available or e.g. relationship to IMP is reconsidered, a SAE follow-up report should be sent within 24 hours using the same procedure as for transmitting the initial SAE report (details will be provided in SAE reporting manual).

10.2.3.2 Specific protocol exceptions to expedited SAE reporting

As this trial involves patients suffering from severe heart failure (NYHA Class III or IV) associated with significant mortality/morbidity, and as the frequency of recurrent hospitalizations for worsening of heart failure is one of the secondary endpoints (i.e. anticipated clinical outcomes) thoroughly collected on the specific eCRF pages and taking into consideration recommendations of the CIOMS working group VI concerning management of safety information from clinical trials, the following events have not to be notified to the sponsor as SAEs:

- worsening of heart failure, except if the investigator evaluates worsening of heart failure as being related to the IMP, this case has to be reported to the Sponsor as a SAE;

10.2.3.3 Reporting of patient death

Please note that “death” is usually an SAE outcome and not an SAE *per se*. Only in cases where the clinical circumstances before the death are unknown (i.e. patient died without a determinable cause of death), then the diagnosis “death” itself should be reported as an SAE. In case of fatal outcome of an already-registered SAE, a follow-up notification must be done.

According to section 12, subsection 6 GCP-V, in case of patient’s death the investigator must submit on demand all information to the competent IEC, the other IEC(s) involved, the CA and the sponsor, that is required for the fulfilment of their duties (note that personal data must be transmitted using the trial-specific patient identification number, i.e. in pseudonymised form).

10.2.4 Sponsor reporting requirements

The sponsor’s reporting requirements are divided into expedited reporting and reporting that must be performed on request or annually.

10.2.4.1 Definition of SUSARs

The sponsor’s expedited reporting requirements are particularly relevant to suspected unexpected serious adverse reactions (SUSARs). The definition is a combination of the definitions of serious adverse reaction (for seriousness criteria see section 10.2) and unexpected adverse reaction (adverse reaction: the nature or severity of which is not consistent with the applicable RSI. As this is the first in man clinical study, all SAEs judged by the investigator to be related to the IMP will be classified as a SUSAR.

10.2.4.2 SUSAR/ circumstance requiring a review of the benefit/risk evaluation

The sponsor’s expedited reporting requirements comprise the following:

- All SUSARs must be reported within 15 days after knowledge (section 13, subsection 2 GCP-V),
- All SUSARs that are life-threatening or result in death must be reported within 7 days after knowledge (section 13, subsection 3 GCP-V),
- All circumstances requiring a review of the benefit/risk evaluation of the IMP must be reported within 15 days after knowledge (e.g. expected serious adverse reaction with unexpected outcome, increased incidence of expected serious adverse reactions, SUSARs after the end of the patient's participation in the clinical trial, events in connection with the trial conduct or the development of the IMP which may affect the safety of the trial patients) (section 13, subsection 4 GCP-V).

10.2.4.3 Development Safety Update Report (DSUR)

In addition to the expedited reporting, the sponsor shall submit an annual report once a year or on request throughout the clinical trial period, according to section 13, subsection 6 GCP-V and ICH guideline E2F. The aim of the DSUR is to concisely describe all new safety information relevant for one or several clinical trial(s), to assess the safety conditions of subjects included in the concerned trial(s) and to evaluate whether the benefit / risk ratio is still favourable.

10.2.5 Pregnancies

Any pregnancy (female trial participant or female partner of male trial participant) that occurs during trial participation must be reported. To ensure patient safety each pregnancy must be reported to Pharmacovigilance CTU on the pregnancy reporting form within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence/absence of any birth defects, congenital abnormalities or maternal and new-born complications.

11 Data handling and data management

11.1 Data confidentiality

Information about trial patients will be kept confidential and managed under the applicable laws and regulations. Those regulations require a signed patient authorization informing the patient of the following:

- what protected health information (PHI) will be collected from patients in this trial;
- who will have access to that information and why;
- who will use or disclose that information;
- the rights of a research patient to revoke their authorization for use of their PHI.

In the event that a patient revokes authorisation to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For patients that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the patient is alive) at the end of their scheduled trial phase.

The data collection system for this trial uses built-in security features to encrypt all data for transmission in both directions, preventing unauthorised access to confidential participant

information. Access to the system will be controlled by individually assigned user identification codes and passwords, made available only to authorised personnel who have completed prerequisite training.

11.2 Documentation of trial data

11.2.1 Documentation in medical records

The investigator will record the participation in the trial, the frequency of the trial visits, the relevant medical data, the concomitant treatment and the occurrence of adverse events in the medical record of each trial patient.

11.2.2 Documentation in eCRF

An electronic data capture (EDC) system will be used in this trial (called e-CRF). All data collected during the trial will be entered on the trial-specific e-forms by the responsible investigator, or designated person, as timely as possible. Data entry and data corrections on e-forms are automatically tracked in the audit trail created by the EDC system.

Data corrections in the e-CRF due to queries are performed by the responsible investigator, or designated person, as timely as possible.

11.3 Data management

Data handling

The Department of Medical Informatics at the University Medical Centre Göttingen manages the medical data for the DZHK (Data Handling). For this purpose, the secuTrial® system is being provided in which electronic case report forms (eCRF) are being modeled. The DZHK aims to standardise data collect on amongst its clinical studies in order to enable secondary use of the data across studies. Therefore, Standard Operating Procedures (SOPs) as well as data capture modules for common cardiological assessment procedures are available. The DZHK item catalogue comprises all currently standardised data capture modules.

secuTrial

Data capture using secuTrial software is achieved by entering the items queried during collection using the eCRFs created and provided by the Data Handling (DH) unit. With secuTrial it is possible to conduct multicentre clinical studies and post-marketing studies. secuTrial allows direct, decentralised electronic capture of study data (remote data entry) in a central database. Operation of secuTrial is completely browser-based, which means it is not necessary to install software for either management or data capture. Authorised users can define the studio setup, manage participants, export data and enter patient data from any internet-enabled PC. Within the application there is both a separate test area (setup) and a productive area. This means that prior to the start of the actual study or in the case of changes implemented after the go-live, any function can be tested before it is unlocked for users. The tested study setup can go live after it has successfully completed testing. The changes are subject to constant version control. secuTrial

complies with all regulatory standards (CRF, GCP) and is certified for all FDA-compliant functions, such as audit trail, a roles and rights concept and electronic signature.

Data Collection

Although the data collection process runs parallel to the data capture process in most cases, this is not absolutely necessary from a technological point of view. The collected data can initially be stored intermediately on printed questionnaires. The collected data is captured in the eCRFs provided and is usually captured by a study nurse or study doctor. The medical data (MDAT) and identifiable information (IDAT) are separated before data collection. This is achieved by incorporating an IDAT input mask provided by the Independent Trusted Third Party (TTP) before the MDAT is actually entered. The IDAT is entered directly on the TTP servers. This tunnelling ensures that the IDAT is at no time known to the secuTrial system.

secuTrial also implements query management within studies, registers and cohorts. Queries are designed to help monitors and other authorized users to investigate unclear entries. Study doctors and study nurses can read, review, answer (if necessary) and close queries. This process can also only be accessed by users who are set up in the system.

Data Management

In addition to providing suitable capture tools, another core task of the Central Data Management unit is the long-term storage and management of the collected data. A non-bypassable audit trail is created for the corresponding dataset as early as the data capture stage. This audit trail saves which person (login information in secuTrial) with which role (derived from the login information) conducted which operation at which time, on which date and on which dataset. This allows consistent version control and traceability for all amendments. The aforementioned query management is also recorded in its entirety in the audit trail. The audit trail offers an overview of all changes made to the data and saved in the up-to-date form. It can be accessed after the form has been saved for the first time. Entering and saving comments, conducting and answering queries, conducting (Source Data Verification) SDV, reviews and form-locking actions, and ending data capture are all storage processes for the respective form. For this reason, all these actions are illustrated in the storage history in the upper part of the audit trail. Every storage operation documents the current project version so that changes to the project setup can be traced here as well. It also displays whether an e-signature was used for saving and if it is still valid.

Details on data management (e.g., software, procedures, responsibilities) will be described in a data management plan prior to the trial. During the trial, the performance of data management and any deviations from the data management plan will be documented in a data management report. Technical specifications of the trial data base and all data checks will be documented in a data validation plan.

The trial data base has been fully validated before any data entry will be performed. Data entry personnel will not be given access to the trial data base until they have been trained. An audit trail provide a data history which data were entered, changed or deleted, by whom and when.

11.4 Data coding

Concomitant treatments or procedures entered into the database will be coded using the WHO Drug Reference List.

Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

12 Quality assurance

The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted, data are generated, documented, and reported in compliance with the protocol, ICH-GCP, and the applicable regulatory requirement(s).

12.1 Monitoring procedure

Monitoring is performed by the CRAs of the CTU, University Medical Center Göttingen. Risk-based monitoring will be done according to ICH-GCP E6 and standard operating procedures (SOP) to verify that patients' rights and wellbeing are protected, reported trial data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with the currently approved protocol/amendment, with ICH-GCP and with the applicable regulatory requirements to ensure safety and integrity of clinical trial data.

The investigator will accept monitoring visits before, during and after the clinical trial. Prior to the trial, a site initiation visit at each site is conducted in order to train and introduce the investigators and their staff to the trial protocol, essential documents, handling of IMP and related trial specific procedures, ICH-GCP and national/local regulatory requirements.

During the trial, the CRA will visit the site regularly to monitor recruitment rate and quality of data. During these on-site visits, the CRA verifies that the trial is conducted according to the trial protocol, trial specific procedures, ICH-GCP and national/local regulatory requirements. The presence of signed informed consents, eligibility of patients, primary endpoint, handling of IMP and documentation/reporting of safety data (e.g., AE/SAE) will be verified by the CRA. The CRA performs also source data verification and drug accountability to ensure that the clinical trial data which are recorded in the source data and CRFs are complete and accurate. Extent of source data verification and monitor visit frequency will be adapted for individual sites in case of lack of data quality or a high number of protocol violations. All trial specific monitoring procedures, monitoring visit frequency and extent of SDV will be predefined in a trial specific monitoring manual. The investigator must maintain source documents for each patient in the trial, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments (see section 7). All information recorded on CRFs must be traceable to source documents in the patient's file as defined in section 11.2. The investigator must also keep the original signed informed consent form (a signed copy is given to the patient).

The investigator must give the CRA access to all relevant source documents to confirm their consistency with the CRF entries.

12.2 Source data verification (SDV)

Source data as defined by ICH-GCP include original documents, data, and records such as hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or

evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial.

12.3 Auditing procedures and inspections

According to the ICH-GCP guidelines, audits may be performed as a quality measure. Audits may be conducted by the sponsor or an independent external party, inspections by CA(s).

The investigator needs to inform the CTU immediately of an inspection requested by a regulatory authority. The investigator is responsible for providing / giving access to source data/documents to auditors/inspectors.

13 Biostatistical planning and analysis

Before the final analysis (see section 13.65) a detailed statistical analysis plan (SAP) will be prepared. This will be completed before data base lock, at the latest. If the SAP contains any changes to the analyses outlined in the trial protocol, they will be marked as such, and reasons for amendments will be given.

All statistical programming for analysis will be performed with the Statistical Analysis System (SAS) or the statistical software package R.

13.1 Trial design

For details on trial design see section 3.1 of the protocol.

13.2 Objectives and endpoints

For details on endpoints see section 2 of the protocol.

13.3 Sample size calculation

The required number of patients to be enrolled results as a total of the numbers needed for the three parts of the trial. A maximum of $n=18$ patients in dose finding cohorts of $n=2-4$ patients is planned for the first part, the Dose Finding stage. This number of patients is not uncommon for phase I dose-escalation studies and can be justified through simulation studies. In the second part, the Refinement stage, $n=10$ patients in two cohorts ($n=5$ per LV and RV cohort) are needed to provide additional confidence to the DSMB as to the expansion for LV or RV augmentation in the third part which will focus on the most feasible, safe, and effective approach. In the third part, the Expansion stage, sample size planning is based on the primary endpoint heart wall thickness and systolic thickening fraction. In a pre-post comparison of means a sample size of 30 patients yields a power of 80% (90%) to detect a standardized mean difference (Cohen's d) of 0.47 (0.55) at a two-sided significance level of 10% given α . As this is an early study to evaluate trends the slightly larger than usual significance level is justified (Kianifard and Islam, 2011). Using the 5

patients from the Refinement stage, this means, an additional number of n=25 patients are needed for the third part. Summing up a total n=53 patients have to be enrolled in the entire study. Calculations were done using the statistical software nQuery.

13.4 Definition of populations included in the analyses

Primary analysis will be performed on all patients with complete observations at baseline and complete or imputed observation at month 12. This will be referred to as the intention-to-treat population (ITT). If not mentioned otherwise, all analyses are based on the ITT population. Imputation will be performed using predictive mean matching.

Sensitivity analysis will be performed on all patients with complete observations at baseline and month 12. This will be referred to as the per-protocol (PP) population.

A CONSORT (consolidation standards of reporting trials) flow chart will be provided to report disposition of patients. Safety of patients and interim analyses will be supervised by the DSMB.

13.5 Methods of analysis

13.5.1 Patient demographics/other baseline characteristics

Demographic and other baseline data (including disease characteristics) will be summarised descriptively using the FAS.

Continuous data will be summarised by arithmetic mean, standard deviation, minimum, 25% quantile, median, 75% quantile, maximum, and the number of complete and missing observations. If appropriate, continuous variables will also be presented in categories.

Categorical data will be summarised by the total number of patients in each category and the number of missing values. Relative frequencies are displayed as valid % (number of patients divided by the number of patients with non-missing values).

13.5.2 Trial medication

Numbers of patients in the dose cohorts of the Dose Finding stage of the trial with corresponding dose levels will be reported.

13.5.3 Concomitant medication

The concomitant medications will be summarised by ATC level 1/3/5. In each table, patients will be counted once, if they took at least one medication from the respective ATC level. The number of patients and the percentage of the total number of patients in the respective population will be given.

13.5.4 Primary endpoint

There are two primary endpoints: Target heart wall thickness (HWT) and heart wall thickening fraction (HWTF) as determined by echocardiography and/or cardiac MRI (AHA17-segment model

and comparison of EHM target vs. contralateral wall thickness). Primary efficacy analyses are based on the changes in HWT/HWTF between baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. To test for a time effect a generalized linear mixed model will be employed for each of the two primary endpoints. In case of detecting a time effect this is followed by Dunnett-type pairwise comparisons to baseline. Due to the explorative character of the efficacy analysis testing will be performed at a 10% two-sided significance level. Mean differences will be reported along with 90% confidence intervals.

13.5.5 Secondary endpoints for efficacy

Key secondary endpoints will be tested at a two-sided 10% significance level as well due to the explorative character of the trial. Further secondary endpoints will be tested at a two-sided 5% significance level. Type I error rate control for subsequent additional analyses, e.g. pairwise comparisons, will be ensured using appropriate closed testing procedures, e.g. Bonferroni-Holms.

Recurrent hospitalizations for heart failure

Another key secondary endpoint is the frequency of recurrent hospitalizations for worsening of heart failure. For the analysis a negative binomial regression model will be fitted. The annual event rate will be reported with a 90% confidence interval.

Ejection fraction

Change in ejection fraction over time will be tested via a generalized linear mixed model using EF measurements at baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. If there is a significant time effect this may be followed by Dunnett-type pairwise comparisons to baseline. Mean differences will be reported along with 90% confidence intervals.

Functional status

The functional status is measured by three endpoints

- Six-minute walk test (6MWT)
- Hand-grip strength
- Cardiopulmonary stress testing

Changes over time will each be analysed with a generalized linear mixed model comparing measurements at baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. Subsequent Dunnett-type pairwise comparisons to baseline may be performed in case of significant ANOVA results. Effects will be reported with 90% confidence intervals.

Patient reported outcomes

Change in patient reported outcomes is assessed by

- Quality of life score (MLHFQ)
- Physical score (SF-36)

- NYHA classification

MLHFQ and SF-36 changes over time will each be analysed with a generalized linear mixed model comparing measurements at baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. Subsequent Dunnett-type pairwise comparisons to baseline may be performed in case of significant ANOVA results. Mean differences will be reported along with 90% confidence intervals.

NYHA classification change over time will be analysed giving proportions of patients with NYHA Class I/II at time points baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. Proportions are accompanied by 90% confidence intervals.

Mortality

Both all-cause mortality and cardiovascular mortality will be displayed showing Kaplan-Meier curves with either pointwise 90% confidence intervals or 90% confidence bands and analysed using a Cox proportional hazards model exploring the prognostic quality of the biomarkers assessed at baseline. The baseline mortality in the target patient population is 20% within 12 months (Hsich et al., 2016).

Minimal Effective Dose and Optimal Effective Dose Range

During the Dose Finding stage of the trial determining, identification of the minimal effective dose and the optimal effective dose range is based on heart wall thickness and thickening fraction measurements and agreed upon with the DDC and DSMB.

13.5.6 Safety parameters

Safety is assessed by frequencies of adverse events including:

- major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke and cardiovascular death – further defined by a clinical endpoint adjudication committee - CEC)
- Frequency and severity of arrhythmic events
- Incidence of immune rejection (allograft DNA, CK/CK-MB, cTnT)
- Incidence of mechanical perturbation of ventricular function by EHM graft

All safety parameters will be listed and displayed in summary tables. Adverse events (AEs) are displayed in summary tables as follows:

The total number of AEs, the minimum, maximum and mean number of AEs per patient, the total number of follow-up days (number of days in the observation period), the number of AEs per FU-day (total number of AEs divided by the total by the number of follow-up days), and the number of patients who had at least one AE.

The incidence of AEs defined by preferred term (PT) according to MedDRA will be calculated as the number of patients who experienced at least one AE with the respective PT in percentage of the total number of patients in the safety population. In the incidence tables the PTs will be grouped by system organ class (SOC) according to MedDRA. Additionally, the incidence of AEs defined by SOC will be calculated as the number of patients who experienced at least one AE in the respective SOC as percentage of the total number of patients in the safety population.

Incidences of AEs will be calculated with 95%-confidence intervals.

Laboratory data will be presented in the measured units (or in SI units, being converted from the original units, if necessary). Values outside the investigator's reference range will be flagged as above or below the reference range in the listings. Shift tables for all parameters will also be generated.

13.6 Interim analyses

A formal interim analysis is planned for the time point when half of the patients in Part 2+3 with either LV or RV administration of EHM (i.e., in total 15 patients) have completed the 12 month follow-up. The interim analysis and the interim report will describe patient recruitment, treatment compliance as well as safety, tolerability, and efficacy for the patients in this period. An adjustment of the type I error will be performed using a type I error-spending function.

The results of the interim analysis will be reported only to the independent data safety monitoring board (DSMB), see section 14. The DSMB will give advice to the Coordinating Investigator concerning further conduct of the trial.

Naturally, each transfer from one study phase to another is accompanied by an interim evaluation.

14 Scientific steering and data monitoring committees

All applicable DZHK-SOPs will be used in the proposed study. Data management and biosampling will be according to DZHK rules. Data will be monitored by Dose Determining (DDC) and Data Safety Monitoring Board (DSMB) comprised of members with appropriate scientific and medical expertise to monitor the study. The DDC and DSMB will be charged with ensuring the safety of the subjects. The DDC is charged primarily with determining the appropriate dose escalation steps according to hands-on experience gained within the study. The DSMB is composed of independent experts and charged with the whole study oversight. The DSMB may recommend that the Sponsor suspends enrolment, amends the study, or discontinues the study at any time.

14.1 Dose Determining Committee (DDC)

A trial related dose determining committee (DDC) will be appointed by the sponsor prior to the start of the trial comprising of 3-5 investigators participating in the trial and sponsor representatives from the clinical trial team and representatives from the CTU including the responsible biostatistician.

The DDC will be involved in the development of the protocol and will ensure transparent management of the trial according to the protocol through recommending and approving modifications as circumstances require. The DDC will review protocol amendments as appropriate.

14.2 Data Safety Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) will be established. The members of the DSMB are given in section "Responsibilities". The function of the DSMB is to monitor the course of the trial and if necessary to give a recommendation to the sponsor, coordinating investigator

and the DDC for discontinuation, modification or continuation of the trial. The underlying principles for the DSMB are ethical and safety aspects for the patients. It is the task of the DSMB to examine, whether the conduct of the trial is still ethically justifiable, whether security of the patients is ensured, and whether the process of the trial is acceptable. For this the DSMB has to be informed about the adherence to the protocol, the patient recruitment, and the observed adverse events. The DSMB will receive the corresponding reports twice a year and at the time of the planned interim analyses. The composition and responsibilities of the DSMB, the structure and procedures of its meetings, and its relationship to other key trial team members (DDC), will be laid down in a DSMB charter.

15 Ethical and legal principles

15.1 Regulatory and ethical compliance

This clinical trial was designed, shall be implemented and reported in accordance with the ICH-GCP, with applicable local regulations (including European Directive 2001/20/EC), and with the ethical principles laid down in the Declaration of Helsinki.

Before initiating the clinical trial, the sponsor/coordinating investigator should submit the CTP and any required application(s) to the appropriate competent authority for review, acceptance, and/or permission, as required by the applicable regulatory requirements.

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Independent Ethics Committee (IEC) before trial start. A signed and dated statement that the protocol and informed consent have been approved by the IEC must be available prior to initiation of the trial.

15.2 Responsibilities of the investigator

Before the start of the trial, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the trial in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to sponsor CRAs, auditors, sponsor Clinical Quality Assurance representatives, designated agents of sponsor, IECs and CA(s) as required.

15.3 Informed consent procedures

Before enrolment in the clinical trial, the patient will be informed that participation in the clinical trial is voluntary and that he/she may withdraw from the clinical trial at any time without having to give reasons and without penalty or loss of benefits to which the patient is otherwise entitled.

The treating physician will provide the patient with information about the treatment method and the possible risks involved. At the same time, the nature, significance, implications, expected benefits and potential risks of the clinical trial and alternative treatment will be explained to the patient. During the informed consent discussion, the patient, if applicable, will also be informed about the insurance cover that exists and the insured's obligations. The patient will be given ample time and opportunity to obtain answers to any open questions. All questions relating to the clinical trial should be answered to the satisfaction of the patient and/or his/her legal representative. In

addition, the patient will be given a patient information sheet which contains all the important information in writing.

The patient's written consent must be obtained before any trial-specific tests/treatments.

For this purpose, the written consent form will be personally dated and signed by the trial patient and the investigator conducting the informed consent discussion.

By signing the consent form, the patient agrees to voluntarily participate in the clinical trial and declares his/her intention to comply with the requirements of the clinical trial and the investigator's instructions during the clinical trial. By signing the form, the patient also declares that he/she agrees to the recording of personal data, particularly medical data, for the trial, to their storage and codified ("pseudonymised") transmission to the sponsor, CA(s), and further agrees that authorised representatives of the sponsor, who are bound to confidentiality, national or foreign CA(s) may inspect his/her personal data, particularly medical data, which are held by the investigator.

After signing, the patient will be given one copy of the signed and dated written consent form and any other written information to be provided to the patients.

In the case of substantial amendments, the patient must be informed with an appropriate revised patient information/consent form. Changed trial procedures can only be carried out if they have been approved by the CA and the leading IEC, and if the patient has been appropriately informed and has given his/her written consent.

Fertile men and women of child bearing potential should be informed that taking the IMP may involve unknown risks to the foetus if pregnancy were to occur during the trial and agree that in order to participate in the trial they must adhere to the contraception requirement for the duration of the trial. The patients have to agree to data collection related to pregnancy and its outcome. If there is any question that the patient will not reliably comply, they should not be entered in the trial.

Separately, the patients will be informed about the DZHK project regarding Data & Biospecimen collection. Consent to DZHK Biospecimen collection is not mandatory for participation in the BioVAT-HF-study

15.4 Patient insurance

Subject insurance (minimum: € 500,000 per subject) according to applicable law has been taken out with

Newline Europe Versicherung AG
Schanzenstraße 28a
51063 Köln

for all subjects participating in the clinical trial.

The investigator, or an individual who is designated by the investigator, will inform the subject of the existence of the insurance, including the obligations arising from it. The trial subjects must be afforded access to insurance documents and provided with a copy of the general conditions of insurance on request.

15.5 Confidentiality of trial documents and patient records

The investigator must ensure anonymity of the patients; patients must not be identified by names in any documents submitted to sponsor. Signed informed consent forms and patient enrolment log must be kept strictly confidential to enable patient identification at the site.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded identification number (see section 5.1) only to maintain participant confidentiality.

15.6 Financial disclosure

Financial disclosures should be provided by trial personnel who is directly involved in the treatment or evaluation of patients at the site - prior to trial start.

16 Trial documents and archiving

16.1 Trial documents/investigator site file

The investigator will be given an investigator site file containing all the necessary essential trial documents for the initiation of the trial at his/her site. The essential documents include a list on which the investigator will enter all appropriately qualified persons to whom he/she has delegated important trial-related tasks.

The investigator, or an individual who is designated by the investigator, will be responsible for the maintenance and completeness of the trial documents during the clinical trial. At the request of the CRA, auditor, IEC or CA(s), the investigator shall make available all the requested trial-related records for direct access. Essential documents must not be removed permanently.

16.2 Archiving

After completion of the clinical trial, the essential trial documents - as defined by ICH-GCP E6 section 8 - will be retained at the trial site for a sufficient period so that they will be available for audits and inspections by the CA(s).

The investigator will be responsible for the storage. The following retention periods will apply after the completion/termination of the clinical trial:

- The above-mentioned essential documents must be retained for at least 10 years (section 13, subsection 10 GCP-V).
- The medical records and other source documents must be retained for the longest possible period allowed by the hospital, the institution or the private practice.

The investigator/the institution should take measures to prevent accidental or premature destruction of these documents. The sponsor will notify the investigator in writing when the trial-related essential documents are no longer required.

16.3 Access to trial data

The trial investigators and all authors of the main publications of the trial result have access to the full trial dataset in order to ensure that the validity of the results can be verified.

17 Protocol adherence and amendments

17.1 Protocol adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact sponsor or its agents, if any, monitoring the trial to request approval of a protocol deviation, as no authorised deviations are permitted. If the investigator feels a protocol deviation would improve the conduct of the trial this must be considered a protocol amendment, and unless such an amendment is agreed upon by sponsor and approved by the IEC it cannot be implemented.

17.2 Amendments to the protocol

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by sponsor, CA where required, and the IEC.

Only changes of the protocol that are required for patient safety may be implemented prior to IEC approval.

Regardless of the need for approval of formal protocol amendments, the investigator is expected to take immediate action required for the safety of any patient included in this trial, even if this action represents a deviation from the protocol. In such cases, the sponsor has to be notified as soon as possible of this action; the IEC should be informed correspondingly.

Information regarding important protocol modifications will be provided in due time to further relevant parties (e.g. investigators, trial participants, trial registries, journals).

17.3 Protocol deviations

Details will be described in the Monitoring Manual.

18 Administrative Agreements

18.1 Financing of the trial and role of funders

The clinical trial will be financed/financially supported by Deutsches Zentrum für Herz-Kreislauf-Forschung e.V (DZHK). Costs for the IMP production for Parts II and III will be covered by Repairon GmbH.

18.2 Trial agreement- investigator compensation

According to ICH-GCP 4.9.6, a trial agreement on the conduct of the clinical trial and the compensation for conducting the trial will be signed between the sponsor of the clinical trial and the investigators including their heads of administration.

18.3 Reimbursement of trial patients

There is no payment planned for patients.

18.4 Trial reports

After completion of the analysis by the responsible biostatistician, the coordinating investigator will prepare and sign the final integrated medical and statistical report, a synopsis of the results and publication(s) containing the results of the study jointly with the biostatistician.

Except when required by law, no one will disclose a result of the clinical trial to third parties unless all parties involved have first agreed on the results of the analysis and their interpretation.

The final trial report will be written and signed in co-operation between the coordinating investigator and the CTU of the University Medical Center Göttingen.

18.5 Clinical trials registry

The sponsor ensures that the key design elements of this protocol will be posted in publicly accessible clinical trials registries: clinicaltrials.gov.

18.6 Publication of trial protocol and results

The sponsor assures that the key design elements of this protocol will be posted in a publicly accessible clinical trials registry (see section 18.5). In addition, upon trial completion the results of this trial will be submitted for publication and posted in a publicly accessible database of clinical trial results irrespective of the results of the trial.

Reporting guidelines will be taken into account (see www.equator-network.org), the CONSORT statement will be adhered to in the preparation of papers on the results of randomised studies.

Each publication of trial results will be in mutual agreement between the principal investigator and the other investigators involved. All data collected in connection with the clinical trial will be treated in confidence by the coordinating investigator and all others involved in the trial, until publication. Interim data and final results may only be published (orally or in writing) with the agreement of the coordinating investigator.

18.7 Authorship in publications of trial protocol and results

Authorship criteria defined by the International Committee of Medical Journal Editors will be adhered to. Investigators with a substantive contributions to the design, conduct, interpretation, and reporting of a clinical trial are recognized through the granting of authorship on the trial report. Individual contributions will be acknowledged in publications according to the publisher's

regulations. The coordinating investigator will be responsible to mediate in case of disputes as to authorship.

19 Appendices

Appendix 1 New York Heart Association “Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels”

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

19.1 Relevant Guidelines and Laws

[List and reference to websites; delete not applicable links]

Declaration of Helsinki	http://www.wma.net/en/30publications/10policies/b3/
ICH E6 - GCP Guideline	http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/good-clinical-practice.html
ICH E8 – General considerations for clinical trials	http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/general-considerations-for-clinical-trials.html
ICH E2F - DSUR	http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2F/Step4/E2F_Step_4.pdf
EMA Guidelines	http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/landing/human_medicines_regulatory.jsp&mid=WC0b01ac058001ff89
AMG/GCP-V	http://www.gesetze-im-internet.de
Recommendations related to contraception and pregnancy testing in clinical trials- Heads of Medicines Agencies (HMA)	http://www.hma.eu/fileadmin/dateien/Human_Medicines/01_-_About_HMA/Working_Groups/CTFG/2014_09_HMA_CTF_G_Contraception.pdf
2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure	https://leitlinien.dgk.org/2016/2016-esc-guidelines-for-the-diagnosis-and-treatment-of-acute-and-chronic-heart-failure/

19.2 Summary of translational project

The combined Phase I/II (open label, non-randomized) BioVAT-HF Early Clinical Study is designed to obtain first clinical data on feasibility, safety and efficacy of epicardial EHM implantation in patients with end-stage heart failure and reduced ejection fraction (HFrEF; EF $\leq 35\%$). It will comprise of three parts: (I) a dose finding cohort (n=18 patients) starting with the anticipated minimally effective dose (i.e., EHM-assemblies constructed from 200 million induced pluripotent stem cell-derived cardiomyocytes and stroma cells [iPSC-CM/StC]) up to an anticipated maximal feasible dose (MFD; i.e., EHM-assemblies constructed from 800 million iPSC-CM/StC); (II) a refinement cohort to test for optimal applications on the left (n=5) and right (n=5) ventricles; (III) an expansion cohort (n=25) to obtain clinical proof-of-concept for the most suitable indication and dosing according to the data obtained in I and II. Route of administration will be via minimal left-lateral thoracotomy as a standalone intervention in LV applications and concomitant to an open-chest LV intervention in RV applications.

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Clinical Trial Protocol

Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure

BioVAT-HF

Engineered Human Myocardium (EHM) in patients with terminal heart failure

EU CT No.	2024-515708-38-01
FOMA-ID	02289
ClinicalTrials.gov ID	NCT04396899
Protocol Version	V 7.0, 20.11.2024
Therapeutic area	Terminal heart failure
Revision chronology, if applicable	Version 6.0 Version 5.2 Version 5.1 Version 5.0 Version 4.2 Version 3.0 Version 2.0
Development Phase	Phase I/II
Sponsor	University Medical Center Göttingen represented by the Head of the Clinical Trials Unit Von-Bar-Str. 37075 Göttingen, GERMANY
Coordinating Scientist	Prof. Dr. Wolfram-Hubertus Zimmermann University Medical Center Göttingen Robert-Koch-Str. 40 37075 Göttingen

This Clinical Trial Protocol contains confidential information. Circulation of this material to individuals who are not involved in the carrying out of the study or any kind of publication requires the approval of the sponsor. These limitations similarly relate to all confidential information and data which will be obtained in the future.

Approval of the Clinical Trial Protocol

Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure

EU CT No.: 2024-515708-38-01

Protocol Version No: V 7.0, 20.11.2024

R. Tostmann

Sponsor Representative

22.11.24

Date



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Prof. Dr. Wolfram-Hubertus Zimmermann

Coordinating Scientist

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Biostatistician

Date

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Approval of the Clinical Trial Protocol

Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure

EU CT No.: 2024-515708-38-01

Protocol Version No: V 7.0, 20.11.2024

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21. Nov 24

Date

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Approval of the Clinical Trial Protocol

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Date

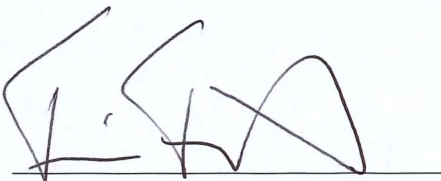
Signature

Prof. Dr. Tim Friede

Biostatistician

21 Nov 2024

Date



Signature

Investigator Statement

Protocol Short Title: BioVAT-HF

EU CT No.: 2024-515708-38-01

Protocol Version No: V 7.0, 20.11.2024

Trial Site:

I confirm that I have read the Clinical Trial Protocol (CTP) and hereby commit to adhering to all actions and terms as specified in the relevant sections of the clinical, ethical and general paragraphs.

I confirm that I and my colleagues will comply with the local legislation (in Germany, the German Drug Law with the appropriate amendments). I further confirm that the clinical trial will be carried out in compliance with the Declaration of Helsinki and ICH-GCP guidelines.

I acknowledge that all confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

Under my supervision I put copies of this CTP and possible updates as well as access to all information regarding the carrying out of this clinical trial at the disposal of my colleagues; in particular I will promptly forward all information from the Sponsor in relation to pharmaceutical safety (SUSARs, SmPC and IB updates, if applicable) to my colleagues.

I confirm that I and my colleagues were informed by a responsible scientist about the results and expected risks of the pharmacological and toxicological examination associated with the clinical trial.

I will discuss this CTP in detail with my colleagues and ensure that they are comprehensively informed about the trial compound/preparation and the execution of the trial.

I confirm that I will be responsible for supervising any individual or party to whom I delegate study tasks conducted at the trial site.

Furthermore I commit myself not to commence patient enrolment prior to approval of the competent authorities (CA) and acceptance by the responsible Independent Ethics Committee (IEC).

Date

Name (in CAPITALS)

Signature of Investigator

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List of Abbreviations

[The following list includes abbreviations which have already been used in this document, please complete/adapt it.]

6MWT	6-Minute-Walk-Test
AE	Adverse Event
AMG	Medicinal Products Act / German Drug Law (<i>Arzneimittelgesetz</i>)
ATMP	Advanced Therapy Medicinal Product
BID	twice a day
BioVAT	Biological Ventricular Assist Tissue
BP	Blood Pressure
CA	Competent Authority
CABG	Coronary Artery Bypass Grafting
CBC	Complete Blood Count
CEC	Clinical endpoint adjudication committee
CK	Creatine Kinase
CK-MB	Creatine Kinase-MB
CONSORT	Consolidated Standards Of Reporting Trials
CRA	Clinical Research Associate (on-site monitor)
CRF	Case Report Form
CRP	C-reactive protein
CRT-D	Cardiac Resynchronization Therapy-Defibrillator
CSR	Clinical Study Report
CT	Computerised Tomography
CTCAE	Common Terminology Criteria for Adverse Events
cTnT	Cardiac troponin T
CTP	Clinical Trial Protocol
CTU	Clinical Trials Unit (ZKS, Zentrum Klinische Studien)
DAMAST	SAS®-based data management system
DDC	Dose Determining Committee
DM	Data management
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DRKS	German Clinical Trials Register (<i>Deutsches Register Klinischer Studien</i>)
DSA	Donor Specific Antibodies
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
DZHK	German Center for Cardiovascular Research (<i>Deutsches Zentrum für Herzkreislaufforschung</i>)
ECG	Electrocardiogram
EDC	Electronic Data Capture
EF	Ejection Fraction
EHM	Engineered Human Myocardium
EMA	European Medicines Agency
EMM	Engineered Mouse Myocardium
EOT	End of Treatment
ERM	Engineered Rat Myocardium
EQ-5D	EuroQol Five Dimension Scale

FAS	Full Analysis Set
FU	Follow Up
GFR	Glomerular Filtration Rate
HF	Heart Failure
HFrEF	Heart Failure with reduced Ejection Fraction
HIV	Human Immunodeficiency Virus
HTLV1	Human T-Lymphotropic Virus 1
HWT	Heart Wall Thickness
HWTF	Heart Wall Thickening Fraction
i.v.	Intravenous(ly)
IB	Investigator's Brochure
ICD	Implantable Cardioverter Defibrillator
ICF	Informed Consent Form
ICH-GCP	ICH Topic E6: Guideline for Good Clinical Practice (GCP)
IEC	Independent Ethics Committee
IL-6	Interleukin-6
IMP	Investigational Medicinal Product /study medication
INR	International Normalized Ratio
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File
ISHLT	International Society for Heart and Lung Transplantation
ITT	Intention To Treat
KCCQ	Kansas City Cardiomyopathy Questionnaire
LPO	Last Patient Out
LV	Left Ventricle
LVAD	Left Ventricular Assist Device
MACE	Major Adverse Cardiac Events
MED	Minimally Effective Dose
MFD	Maximal Feasible Dose
MH	Medical History
MRI	Magnetic Resonance Imaging
MTD	Maximal Tolerable Dose
NCT No	National Clinical Trial (NCT) number in ClinicalTrials.gov registry
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
NYHA	New York Heart Association
OHT	Orthotopic Heart Transplantations
p.o.	Per os
PC	Project Coordination
PHI	Protected Health Information
PI	Principal Investigator
PP	Per-Protocol
PR	Pulse Rate
PT	Prothrombin Time
PPT	Partial Thromboplastin Time
PV	Pharmacovigilance
QD	Once a Day
QOL	Quality of Life Questionnaire
RBC	Red Blood Cell Count

RSI	Reference Safety Information (current SmPC or/and current IB)
RV	Right Ventricle
s.c.	Subcutaneous(ly)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SAS	Statistical Analysis System
SDV	Source Data Verification
SMD	Safe Maximal Dose
SmPC	Summary of Product Characteristics (<i>Fachinformation</i>)
SOP	Standard Operating Procedure
SSC	Scientific Steering Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAPSE	Tricuspid Annular Plane Systolic Excursion
TDM	Therapeutic drug monitoring
TMF	Trial Master File
UAR	Unexpected Adverse Reaction
VO2max	Maximum rate of oxygen consumption
WBC	White Blood Cell Count
WHO	World Health Organization

Synopsis

TITLE OF TRIAL	Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure
SHORT TITLE	BioVAT-HF
EU CT NO	2024-515708-38-01
FOMA-ID	02289
HEALTH CONDITION STUDIED	Terminal heart failure
PHASE	Phase I/II
OBJECTIVE(S)	<p>Primary objective:</p> <ul style="list-style-type: none">to assess safety and efficacy of Engineered Human Myocardium (EHM) in patients with terminal heart failure (HFrEF EF ≤35%) with or without RV dysfunction (TAPSE <16 mm) <p>Secondary objective:</p> <ul style="list-style-type: none">to assess effects of EHM-grafts on disease-specific events and symptoms

<p>TREATMENT(S)</p>	<p><u>Experimental intervention/Index test:</u> Implantation of EHM on dysfunctional left or right ventricular myocardium in patients with HFrEF (EF ≤35%).</p> <p>Part A: Dose Finding Cohort to determine the Minimally Effective Dose and Optimally Effective Dose Range, and if possible the Safe Maximal Dose of EHM.</p> <p>Part B: Refinement/Expansion Cohort to specify the most optimal EHM target heart wall, i.e. the left ventricle (LV) or the right ventricle (RV), and to collect proof-of-concept data as to efficacy of EHM mediated augmentation of the LV or RV by remuscularization.</p> <p>Epicardial implantation will be via a minimal invasive left lateral thoracotomy performed as standalone procedure in case of LV targeting and concomitant to a scheduled open chest LV surgery if the RV is targeted. This strategy will reduce confounding effects as to the interpretation of EHM efficacy data.</p> <p><u>Duration of intervention per patient:</u></p> <ul style="list-style-type: none"> • Start of immune suppression 7±3 days before EHM implantation until the end of the study (daily intake of a calcineurin inhibitor and a corticosteroid for 12 months after EHM implantation) • Implantation of EHM: 90 minutes according to experience from preclinical studies and similar surgical procedures (i.e., epicardial pacemaker lead placement) <p>Note: Immune suppression will be according to revised ISHLT-guidelines (Velleca et al., 2023) with a preference for a combined use of Calcineurin inhibitors (CNI) and mTOR inhibitors with Methylprednisolone:</p> <p>Calcineurin inhibitors (CNIs), preferably Tacrolimus, will be adjusted to trough levels of 8-10 ng/ml for 3-6 months (Baran et al. 2011) and then (1) continued at a reduced dose to maintain trough levels of 5-8 ng/ml (in case of no drug related side effects), (2) reduced to 3-8 ng/ml in combination with a mTOR-inhibitor (in case of drug related side effects) or (3) discontinued (CNI-free protocol; in case of intolerable CNI-related side effects).</p> <p>mTOR-inhibitors, preferably Everolimus, in combination with CNI are adjusted to trough concentrations of 3-8 ng/mL. In case of CNI-free protocol Everolimus maintenance trough levels of 6-10 ng/ml are targeted.</p> <p>Proliferation signal inhibitors (PSIs), preferably mycophenolat mofetil (MMF), may be added according to ISHLT-guidelines (Velleca et al., 2023) and the reported proceeding in the TICTAC trial for 2 weeks post operatively (NCT00299221; Baran et al. 2011) at the discretion of the treating physician.</p> <p>Methylprednisolone (0.15 mg/kg bodyweight) administered daily for 3-6 months, preferably 6 months.</p>
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	<p>Perioperative antibiotics will be selected based upon their activity against skin flora, including <i>Staphylococcus</i> species, according to standard protocols in cardiothoracic surgery at the respective transplant centers.</p> <p>Pneumocystis jiroveci (PJP) prophylaxis will be initiated in the early postoperative phase by daily administration of trimethoprim/sulfamethoxazole (80 mg TMP/160 mg SMZ per day) and continued for 6-12 months. In the setting of a sulfa allergy or glucose-6-phosphate dehydrogenase deficiency, alternative regimens can be used, including: (1) aerosolized pentamidine (AP) isethionate (300 mg every 3–4 weeks); (2) dapsone (diaminodiphenylsulfone) with or without TMP or pyrimethamine (50–100 mg/day). Pyrimethamine may be administered weekly (25 or 50 mg) to supplement dapsone (50-100 mg/day). (3) Atovaquone (1,500 mg PO QD). (4) Clindamycin and pyrimethamine.</p> <p>After the final study visit (Visit 10), patients will be further monitored by their treating physician. Immune suppression by calcineurin inhibition and/or mTOR inhibition will be prescribed to prevent allograft rejection with a recommendation for continuation until end-of-life if the benefit-risk assessment remains positive at the 12-month study endpoint and beyond.</p> <p>The treating physician is requested to report clinically relevant observations to the principal investigator. After 12-month follow-up, study patients will be asked for informed consent to be enrolled in the VAT-registry until end-of-life for the documentation of long-term outcome.</p>
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	<p><u>Follow-up per patient:</u></p> <ul style="list-style-type: none"> Echocardiography and cardiac CT to study global and regional heart/graft function; cardiac MRI can be performed instead of cardiac CT if sufficient image quality can be anticipated/confirmed at the baseline investigation: Echo: before EHM implantation (baseline) as well as 2 weeks, 1 month, 3 months, 6 months, and 12 months after surgery CT: before EHM implantation (baseline) as well as 3 months and 12 months after surgery MRI (only if high image quality can be anticipated/confirmed at the baseline investigation): before EHM implantation (baseline) as well as 1 month, 6 months, and 12 months after surgery Biomarkers: CK and CK-MB in case of elevated CK, hs-cTnT, NT-proBNP, CRP, creatinine, cystatin C, blood urea nitrogen (BUN) Assessment of graft derived cell-free DNA for the monitoring of graft retention/rejection (before EHM implantation as well as 2 weeks, 1, 3, 6, and 12 months after surgery) Telemetric monitoring via Implantable Cardioverter Defibrillator (ICD)- or Cardiac Resynchronization Therapy-Defibrillator (CRT-D)-devices with event recorder for the whole duration of the study. Pathology to obtain data on graft survival, integration, and maturation upon heart transplantation or death (according to patient consent). Monitoring of pulmonary artery pressure with a CardioMEMS HF Device (St. Jude Medical) or a similar device, if device has been implanted electively.
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	<p><u>Accompanying measures:</u></p> <ul style="list-style-type: none"> Therapeutic drug monitoring (TDM) to verify effective trough levels of accompanying immune suppressive drugs according to the proceeding in orthotopic heart transplantation (ISHLT Guidelines; Velleca et al. 2023), i.e.: <u>for Tacrolimus:</u> 8-10 ng/ml for 3-6 months (Baran et al. 2011) and then (1) continued to maintain trough levels of 5-8 ng/ml (in case of no drug related side effects), (2) reduced to 3-8 ng/ml if combined with a mTOR-inhibitor (in case of drug related side effects) or (3) discontinued (CNI-free protocol; in case of intolerable CNI-related side effects). <u>for Everolimus:</u> 3-8 ng/ml if combined with a CNI or 6-10 ng/ml if administered in a CNI-free protocol. Cylcosporine may be applied instead of Tacrolimus, Sirolimus may be applied instead of Everolimus, Mycophenolat Mefetil (MFF) may be applied as a proliferation signal inhibitor (PSI) at the discretion of the treating physician and in accordance with guideline recommendations or clinical best practice in heart transplantation. TDM will be performed to monitor trough levels. Biomarker analysis to monitor rejection: CK and CK-MB in case of elevated CK, hs-cTnT, cell-free allograft-derived DNA (experimental method; substudy) Monitoring of specific allograft immune responses: donor specific antibodies (DSA)
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INCLUSION CRITERIA	<ol style="list-style-type: none"> 1. Symptomatic heart failure (NYHA II-IV) with reduced ejection fraction (HFrEF with LVEF $\leq 35\%$) as assessed by echocardiography. 2. Patients on guideline-directed medical therapy 3. NT-proBNP >300 pg/mL for patients in sinus rhythm or >900 pg/mL if in atrial fibrillation 4. History of previous heart failure hospitalization in the past 12 months 5. At least one hypo- or dyskinetic segment or dilated heart chamber to demark the implant target area. 6. (A) Stable disease condition allowing for an elective left-lateral mini-thoracotomy (for LV applications) or (B) open-chest surgery (for RV applications) for a clinically indicated intervention on the LV (e.g., coronary bypass surgery, valve repair, mechanical circulatory support device implantation) with concomitant RV dysfunction, diagnosed using the Tricuspid Annular Plane Systolic Excursion (TAPSE) index <16 mm (Rudski et al. 2010). 7. 18-80 years of age 8. Willingness and ability to give written informed consent 9. Female subjects of childbearing potential must agree to use acceptable method(s) of contraception for the full study duration.
EXCLUSION CRITERIA	<ol style="list-style-type: none"> 1. Contraindication to immunosuppressive drugs (e.g. known history of unresolved cancer, hepatitis B/C, HIV, HTLV1) 2. Contraindication to TachoSil® (e.g. hypersensitivity to human fibrinogen, human thrombin, horse collagen, human albumin, Riboflavin, Natriumchloride, Natriumcitrate, L-Arginin-Hydrochloride) 3. Hypertrophic cardiomyopathy (HCM) 4. Terminal kidney failure (stage 4; GFR <30 ml/min) at the time of enrolment 5. Terminal liver failure (Child-Pugh stage C; score >10) at the time of enrolment 6. History of disabling stroke 7. Reduced life expectancy in the short term due to non-cardiac disease 8. Any condition that excludes adherence to study protocol (in particular lack of adherence to prescribed medication) 9. Simultaneous participation in another interventional trial 10. Pregnant or breastfeeding females 11. Known or suspected alcohol and/or drug abuse

SAFETY ENDPOINTS	<p><u>Assessment of safety:</u></p> <p><u>Primary Safety Endpoint</u></p> <ul style="list-style-type: none"> Part A (Dose Escalation steps): Adverse events related to the procedure, including in particular arrhythmic events and worsening of disease progression within 28 days (based on a comparison of data obtained during visit 2 and visit 7) Part B: Adverse events related to the procedure, including in particular arrhythmic events and worsening of disease progression within the whole study duration <p><u>Secondary Safety Endpoints:</u></p> <ul style="list-style-type: none"> Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke and cardiovascular death) Frequency and severity of arrhythmic events Incidence of immune rejection (allograft DNA, CK/CK-MB, hs-cTnT, DSA) Incidence of mechanical perturbation of ventricular function by EHM graft
EFFICACY ENDPOINTS	<p><u>Primary efficacy endpoints:</u></p> <ul style="list-style-type: none"> Structural endpoint: Change from baseline in EHM target heart wall thickness (TWTh) measured by echocardiography or cardiac CT or cardiac MRI over 12 months Functional endpoint: Change from baseline in left/right ventricular ejection fraction (LV-EF/RV-EF) measured by echocardiography or cardiac CT or cardiac MRI in the LV/RV cohorts over 12 months Patient reported outcome/quality of life: Change from baseline in Kansas City Cardiomyopathy Questionnaire-23 Overall Summary Score (KCCQ-23 OSS) over 12 months <p><u>Key secondary endpoint:</u></p> <ul style="list-style-type: none"> Recurrent HF hospitalizations <p><u>Further secondary endpoints:</u></p> <ul style="list-style-type: none"> Time to mechanical circulatory assist device implantation. Time to heart transplantation. Functional status in patients as determined by cardiopulmonary stress testing (VO₂max), six-minute walk test (6MWT) distance, and hand-grip strength measurements Patient reported outcomes assessed by NYHA classification, quality of life score (EQ-5D-5L), and study adherence motivation (HADS, MoCA, IPQ-8, TEX-Q, medication adherence) All-cause and cardiovascular mortality
TRIAL DESIGN	Combined, open-label, phase I/II safety and efficacy study

STATISTICAL ANALYSIS	<p><u>Primary Endpoints:</u> Primary efficacy analyses are based on the changes from baseline (visit 2) in structural outcome (TWTh), functional outcome (LV/RV-EF), and/or patient-reported outcome (KCCQ-OSS) at 1 month (visit 7), 3 months (visit 8), 6 months (visit 9) and 12 months (visit 10) after implantation. To test for a time-effect a linear mixed model will be employed for each of the three primary endpoints. For all three primary endpoints the hypothesis of a positive trend over 12 months will be tested. All three primary endpoints will be assessed independently. Type-1 error will be controlled using the Hochberg procedure.</p> <p><u>Secondary endpoints:</u> Secondary endpoint analyses will be similar as the analyses of the primary endpoint and comprise of Gaussian longitudinal models evaluating changes over time from baseline prior to EHM implantation. For recurrent event data such as HF hospitalizations, appropriate regression models such as the negative binomial regression model or the semiparametric LWYY model will be used. Time to event outcomes such as the time to mechanical assist device implantation or the time to heart transplantation will be displayed using Kaplan-Meier curves. If sufficient number of events occurred, Cox regression analyses would be applied.</p> <p><u>Safety:</u> The maximal feasible dose (MFD; 20 g EHM comprised of 800 million cells) was chosen conservatively based on preclinical experience in Rhesus macaque and allometric scaling considerations. The probability of dose-limiting toxicity will be modelled by a Bayesian two-parameter logistic regression model. Safety events will be summarized as rates with 95% confidence intervals. Survival will be displayed as Kaplan-Meier curve and analyzed using a Cox proportional hazards model exploring the prognostic quality of the biomarkers assessed at baseline.</p>	
	<p><u>Effect size assumed for estimation of sample size:</u> A sample size of 30 patients (in Part B) yields a power of 80% (90%) in a pre-post comparison of means at a two-sided significance level of 10% given a standardized mean difference (Cohen's d) of 0.47 (0.55).</p>	
SAMPLE SIZE	<p><u>Part A:</u></p>	<p>n = 18 (max.), in dose cohorts of minimally 2 patients</p>
	<p><u>Part B</u></p>	<p>n = 35 (min. 5 with LV and min. 5 with RV EHM placement; max. 30 per LV or RV indication)</p>
	<p>To be assessed for eligibility:</p>	<p>n = 65</p>
	<p>To be allocated to trial:</p>	<p>n = 53</p>
	<p>To be analysed:</p>	<p>n = 53</p>

TRIAL DURATION	Time for preparation of the trial:	6 months
	Recruitment period (part A to part B):	60 months
	First patient in to last patient out (LPO):	72 months
	Post processing after LPO:	6 months
	Duration of the entire trial:	84 months
	Duration of surgical intervention per patient:	120 minutes
	Follow up duration per patient:	12 months
PLANNED DATES	Enrolment of first patient, first patient in (FPI)	1st quarter 2021
	Enrolment of last patient, last patient in (LPI)	4 th quarter 2026
	End of trial defined as last patient last visit (LPLV)	4 th quarter 2027
	Final statistical analysis	2 nd quarter 2028
	Planned interim analysis	Interim analysis will be performed after end of study part A and after 15 of the patients treated with the SMD either with LV or RV EHM implantation have completed at least 3 months follow-up.
PARTICIPATING SITES	Up to 8 sites are planned in Germany. 1 site outside Germany is planned.	
FUNDER(S)	The trial is funded by the DZHK (<i>Deutsches Zentrum für Herz-Kreislauf-Forschung e.V.</i>) and Repairon GmbH.	

Table 1 Visit schedule and assessments – Flowchart

Visits	Title in eCRF	Baseline		Start of Immuno-suppression (Visit 3)	Hospital admission (Visit 4)	Implantation (Visit 5)	Follow Up 1 (Visit 6)	Follow Up 2 (Visit 7)	Follow Up 3 (Visit 8)	Follow Up 4 (Visit 9)	Follow Up 5 (Visit 10)
		Baseline 1 (Visit 1)	Baseline 2 (Visit 2)								
	Time Section	min. 24 h before Baseline 2	Month -3 to week - 2	Day -10 to -4	Day -6 to -1	Day 0	Before release from hospital	1 month (± 7 days)	3 months (± 7 days)	6 months (± 7 days)	12 months (± 7 days)
Informed Consent		x									
Inclusion / Exclusion Criteria	4.2 / 4.3		x								
Medical History	7.9.12		x								
DZHK basic data set	7.9.3		x ¹		x ²		x ²	x ²	x ²	x ²	x ²
Concomitant heart failure medications	7.9.4		x		x		x	x	x	x	x
Patient registration eCRF	5.2		x								
Adverse events documentation	10				x	x	x	x	x	x	x
CBC with differentials and platelet count	7.9.6		x		x		x	x	x	x	x
CRP, Procalcitonine, IL6	7.9.6		x		x		x	x	x	x	x
Liver panel, albumin	7.9.6		x		x		x	x	x	x	x
Creatinine, cystatin C, BUN	7.9.6		x		x		x	x	x	x	x
PTT or PT/INR as applicable	7.9.6		x		x		x	x	x	x	x
Plasma free haemoglobin; Iron	7.9.6		x		x		x	x	x	x	x
hsTnT, CK/CK-MB	7.9.6		x		x		x	x	x	x	x
NT-proBNP	7.9.6		x		x		x	x	x	x	x
HBsAg, Anti-HCV, Anti-HIV, Anti-HTLV-1	7.9.6		x								

Pregnancy Test (for females of childbearing potential)	7.9.6		x								
Blood draw for cell-free allograft DNA assessment ³	7.10.2/ Appendix 19.3		x				x	x	x	x	x
HLA/KIR-Typing	7.9.6		x								
Donor Specific Antibodies	7.9.6		x					x	x	x	x
12-lead ECG	7.9.7		x		x		x	x	x	x	x
Echocardiography	7.9.8		x		x		x	x	x	x	x
Cardiac-CT ⁴	7.9.9		x						x		x
Cardiac-MRI ⁵	7.9.10		x						x	x	x
Chest x-rays	7.9.11				(x)	x ⁶					
6MWT	7.9.12		x					x	x	x	x
Cardiopulmonary exercise testing	7.9.13		x					x	x	x	x
Hand grip strength	7.9.14		x					x	x	x	x
EuroSCORE II	7.9.15		x								
INTERMACS	7.9.16		x								x
KCCQ-23	7.9.177		x						x		x
EQ-5D-5L	7.9.177		x						x		x
HADS	7.9.177		x						x		x
MoCA	7.9.177		x						x		x
Medication adherence	7.9.177		x						x		x
B-IPQ	7.9.177		x								
TEX-Q	7.9.17		x								
Surgical Procedure	9.7					x					
ICD/CRTD-event recorder readout	7.9.18		x		x		x	x	x	x	x
TDM Calcineurin Inhibitors	6.1 / 6.2			x	x	x	x	x	x	x	x
DZHK-Biobanking Data Set ⁷	7.9.6		x				x	x	x	x	x

¹according to DZHK-SOP-K-01 "Basisdatensatz – Anamnese / Klinische Diagnosen / Körperliche Untersuchung"

²a reduced data set of the DZHK-SOP-K-01 "Basisdatensatz – Anamnese / Klinische Diagnosen / Körperliche Untersuchung" needs to be collected as defined in chapter 7.6.2

³only if patients consent

⁴performed if cardiac MRI is viewed insufficient to assess heart/implant structure and function – this is generally the case if patients are implanted with a device such as ICD, CRT-D, mitra-Clip, stents

⁵performed if patients are eligible to cardiac MRI investigations and high image quality can be anticipated

⁶Two chest x-rays necessary: ~2h after Implantation and ~2h after chest drainage removal (see also chapters 7.9.10 and 9.7)

⁷Collection of biological samples according to patient consent

(x): only if indicated

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1 Background and rationale

Heart failure is the most common cause of death. The underlying disease mechanism is a loss of heart muscle cells, which correlates with reduced heart function. Approximately 2.5 million patients in Germany, 10 million patients in Europe, and 64 million patients globally are diagnosed with heart failure. Due to its positive correlation with age and the dramatic demographic changes of our aging population, prevalence of heart failure will further increase and is thought today to affect 2-4% of the global adult population (Savarese et al. 2023, Shahim et al., 2023).

Incidence of heart failure is reported to be at ~300,000 new cases/year in Germany. Mortality is despite advances in pharmacological and interventional heart failure therapy 20% within 1 and 50% within 5 years upon the initial diagnosis (Lopez Sendon and Montoro, 2015). Advanced heart failure is characterized by repeated hospitalization for heart failure decompensation and increasing mortality with every hospitalization for heart failure (HFH; Setoguchi et al. 2007, Butler et al. 2019).

Heart transplantation is the only curative option with excellent outcome (80% 5-year survival rates; ISHLT-Registry data). Shortage of donor organs results in ~300 orthotopic heart transplantations (OHTs) per year in Germany. There is an estimated need for ~8.000 OHT per year in Germany.

Heart failure patients upon listing for heart transplantation present a 12 month survival of 80% and a 24 month survival of 70% (Hsieh et al., 2016). Once under inotrope support in advanced heart failure, average life expectancy is 1.1 years if eligible and 9.4 months if ineligible for OHT (Long et al., 2014).

In addition to reduced life expectancy, quality of life in patients living with advanced heart failure is greatly reduced and was found to be comparable to the quality of life in patients with acute aphasic stroke (Franzen-Dahlin et al., 2010; Nieminen et al., 2015).

The socioeconomic burden of heart failure is anticipated to increase markedly; e.g., direct and indirect cost associated with heart failure were \$28 billion in the US in 2010 with an anticipated increase to \$78 billion by 2030 (Lopez Sendon and Montoro, 2015). Ischemic heart disease is the main cause of heart failure and the leading contributor to disability-adjusted life years (DALYs; (Murray et al., 2012)).

Because of the enormous medical unmet need for new treatment options various technical and regenerative therapeutic approaches, like, e.g., artificial hearts, xenotransplantation of hearts, implantation of a mechanical pump device (left ventricular assist device, LVAD), and more recently cell- and tissue-based regenerative therapies have emerged. Cell- and tissue-based repair approaches aim at the reconstitution of heart muscle *in situ* and therefore at long lasting therapeutic improvement of heart failure by *bona fide* remuscularization. Based on constantly rising numbers of patients suffering from heart failure and the limited availability of donor hearts or therapeutic alternatives, the development of regenerative, cell-/tissue-based therapeutic approaches is warranted.

The BioVAT-HF trial will test the hypothesis that cardiomyocyte implantation via engineered human myocardium (EHM), the proposed investigational medicinal product (IMP; designated "Biological Ventricular Assist Tissue" or BioVAT), results in sustainable remuscularization and biological enhancement of myocardial performance in the failing heart. EHM are constructed from defined mixtures of induced pluripotent stem cell (iPSC)-derived cardiomyocytes and stromal cells in a bovine collagen type I hydrogel. Comprehensive preclinical testing confirmed the rationale for the clinical translation of the myocardial remuscularization strategy by EHM implantation. Data from Part A (Dose-Finding) of BioVAT-HF confirm that EHM can result in cardiac remuscularization

in patients with advanced heart failure (Jebran et al. 2024 [in press]). The patient target population for EHM therapy is patients suffering from advanced heart failure with reduced ejection fraction (HFrEF; EF: $\leq 35\%$) with a history of hospitalization for heart failure and classified in Class II-IV according to the New York Heart Association (or Stage D according to the American Heart Association) despite optimized guideline-directed medical therapy.

1.1 Scientific background

Despite encouraging results from recent studies investigating the implantation of embryonic stem cell-derived cardiac progenitor cells (Menasche et al., 2018) and exosome therapeutics (Liu et al., 2018b), there is a common understanding that direct integration of cardiomyocyte implants would be the most promising option if successfully translated into the treatment of patients with endstage heart failure. As to cardiomyocyte delivery and retention, tissue engineering-based cardiomyocyte delivery has a clear advantage over direct intramyocardial injection (Nguyen et al., 2016; Riegler et al., 2015). In addition, there is comprehensive preclinical evidence for safety and efficacy of tissue engineered heart repair with EHM and EHM precursors obtained by the applicant's lab in 3 preclinical animal species (rat, mouse, and Rhesus macaque):

- 1) Rat model with uncompromised heart function (Zimmermann et al., 2002)
- 2) Rat model of chronic (severe) heart failure after permanent LAD occlusion (Zimmermann et al., 2006)
- 3) Mouse model of acute myocardial infarction by permanent LAD occlusion (Didie et al., 2013)
- 4) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Riegler et al., 2015)
- 5) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Qin et al., 2016)
- 6) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Tiburcy et al., 2017)
- 7) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (extension of Tiburcy et al., 2017 with focus on EHM patch retention; data presented in IMPD)
- 8) Rhesus macaque model with uncompromised heart function (Jebran et al. 2024 [in press]; data presented in IMPD)
- 9) Rhesus macaque model with post-myocardial infarction chronic heart failure (Jebran et al. 2024 [in press]; data presented in IMPD)

Additional pilot studies were performed in pig models, but found to be of no predictive value because of limited xenograft retention, despite administration of comprehensive immune suppression regimens.

The preclinical *in vivo* studies 1-9 collectively provide the rationale for the BioVAT-HF trial. Study 1 demonstrated feasibility and safety of Engineered Rat Myocardium (ERM) allograft implantation under immune suppression in a healthy rat model. Study 2 demonstrated electromechanical integration, safety and efficacy of ERM allografts in a rat model of chronic heart failure. Safety and efficacy were further confirmed in a mouse model of subacute myocardial infarction and the application of pluripotent stem cell-derived Engineered Mouse Myocardium (EMM) allografts (Study 3). Studies 2 and 3 employed MRI and echocardiography to document efficacy of ERM and EMM allograft-based heart repair by detection of an enhancement of thickness and contractility of the target heart wall, in line with the proposed mode of action. Studies 4-7 established and validated GMP-compatible EHM as well as EHM xenograft retention upon implantation in immunocompromised nude rats. Long term engraftment for more than 6 months

without unwanted effects was demonstrated in study 4. In contrast to rodent allografts (Zimmermann et al., 2006), reliable evidence for electromechanical integration of human xenografts could not be obtained, which is in-line with data from other groups using guinea pig (Weinberger et al., 2016) or rat (Gerbin et al., 2015). In the rat xenograft study (study 4), enhanced left ventricular ejection fraction (+5%) was observed to be independent of the implantation of contractile or non-contractile EHM. These findings confirmed earlier findings (studies 2 and 3) in that mechanical stabilization of the ventricular wall or indirect (paracrine) effects of the epicardial patch on the underlying myocardial milieu may be of therapeutic value, which was however inferior to the therapeutic effects observed in rodent models of heart failure and contractile ERM as well as EMM allograft implantation (studies 2 and 3). In a pivotal Rhesus macaque allograft study (study 8; Jebran et al. 2024 [in press]), Engineered Non-Human Primate Myocardium (ENHPM) allografts thickened the target heart wall by ~1 and ~5 mm in a dose dependent manner (1x [n=7] and 5x [n=7] ENHPM assemblies, respectively). The augmentation of the target heart wall was sustained for the whole study duration (up to 6 months) with no evidence for arrhythmia, tumor formation, perturbation of heart performance, and immune suppression related side effects. In an extension of the pivotal Rhesus macaque study (study 9; Jebran et al. 2024 [in press]), ENHPM were implanted in Rhesus macaques ~6 months after myocardial infarction by ischemia/reperfusion injury, inflicted by a period of 3 h percutaneous balloon occlusion of the mid-left anterior descending artery (LAD). ENHPM (2x ENHPM, n=3 and 5x ENHPM, n=4) were sutured onto the epicardium to cover the visible infarct scar in a similar manner as anticipated for the BioVAT-HF study. Rhesus macaques with myocardial infarction and no EHM graft without (n=3) and with (n=4) immune suppression served as controls. Similar as in study 8 (ENHPM in healthy Rhesus macaques), a sustained augmentation of the target heart wall was observed for the whole study duration (6 months) with no evidence for arrhythmia, tumor formation, perturbation of heart performance, and immune suppression related side effects. One animal in the 5x ENHPM group died in the post-anaesthesia recovery phase due to low cardiac output syndrome, which was unrelated to the EHM implant. Whereas ENHPM target heart wall contractility was unaffected in control animals at ~5%, enhanced target heart wall thickening fraction to at least 20% (as a measure for synchronized contractility of the treated heart wall) was observed in 2 macaques treated with 2x ENHPM and in 2 macaques treated with 5x ENHPM. In 3 of the 4 animals with an ENHPM-enhanced target heart wall contractility, enhancement of left ventricular ejection fraction by ≥5% was observed suggesting a contribution of the ENHPM patch not only to local, but also to global heart function (Jebran et al. 2024 [in press]). Detailed *post mortem* histopathological analyses confirmed cardiomyocyte retention and vascularization of ENHPM grafts, which was dependent on immune suppression with tacrolimus and methylprednisolone.

Based on these data and the average heart wall thickness of 6-10 mm in human (Kawel et al. 2012), administration of 5x EHM as starting dose (minimal effective dose - MED) for target heart wall augmentation was anticipated for clinical administration in patients with heart failure in BioVAT-HF. Data from 13 patients treated in Part A of BioVAT-HF confirmed that the administration of 5-20x EHM is safe, with first signs for dose-dependent efficacy according to the proposed mode of action resulting (1) target heart wall augmentation (thickening), (2) enhanced global heart function (increase in ejection fraction), and (3) improvement of symptoms assessed by patient reported outcome measures (KCCQ-23 Overall Summary Score) at the according to the study protocol maximal feasible dose of 20x EHM (refer to IB for details).

1.2 Overview of investigational medicinal product(s) (IMP(s))

The designated IMP is Engineered Human Myocardium (EHM) for applications as Biological Ventricular Assist Tissue (BioVAT). EHM are constructed from induced pluripotent stem cell (iPSC)-derived cardiomyocytes and stromal cells mixed at a defined ratio and suspended in a bovine collagen type I hydrogel. The iPSCs are derived from a master cell bank (MCB) created by Lonza (USA) on behalf of the *US National Institutes of Health* (NIH). The iPSCs were prepared via a plasmid-based reprogramming technique using CD34⁺ cells from cord blood of an anonymous male donor. iPSCs from the MCB were imported via Repairon GmbH according to the requirements of the German Medicinal Products Act ("Arzneimittelgesetz") and the competent authority (Paul-Ehrlich-Institut - PEI). On behalf of Repairon GmbH, BioNTech GmbH was contracted to produce a working cell bank (WCB). MCB and WCB were created according to GMP as starting material for EHM production. EHM are produced individually under GMP-conditions at the University Medical Center Göttingen (UMG) under the auspices of the Institute of Transfusion Medicine. Manufacturing authorization according to the German Medicinal Products Act was granted by the local competent authority (LCA; Gewerbeaufsichtsamt Braunschweig) in September 2020. The BioVAT-HF clinical trial (NCT04396899) was approved in December 2020 by the PEI and the competent ethics committee. The first patient was treated in March 2021. The dose finding part of the BioVAT-HF FIH study (Part A) was completed in December 2022 after 28 days of uneventful follow-up after treatment of 8 patients. 5 additional patients were treated since then in a Part A extension (total number of patients treated in Part A is 13 as of November 2024). No dose limiting toxicities were observed regardless of the tested dose level. Data from 13 patients treated in Part A of BioVAT-HF confirmed that the administration of 5-20x EHM is safe, with first signs for dose-dependent efficacy according to the proposed mode of action resulting in (1) target heart wall augmentation (thickening), (2) enhanced global heart function (increase in ejection fraction), and (3) improvement of symptoms assessed by patient reported outcome measures (KCCQ-23 Overall Summary Score) at the according to the study protocol maximal feasible dose of 20x EHM (refer to IB for details).

Five additional clinical trials, investigating the delivery of pluripotent stem cell-cardiac progenitors or cardiomyocytes to the heart of patients with heart failure are ongoing (registered at ClinicalTrials.gov):

(1) the ESCORT trial (NCT02057900) tested the delivery of 5-10 million embryonic stem cell (ESC)-derived cardiac progenitor cells (identified by the expression of the SSEA1 surface marker) immobilized in a fibrin patch to the heart (n=6 patients). The study is completed and the data have been published (Menasche et al., 2018);

(2) the HEAL-CHF trials (NCT03763136, NCT04982081, NCT05566600) are recruiting patients for intramyocardial injection of 100-400 million iPSC-derived cardiomyocytes in patients with heart failure since 2021 (total of 72 patients planned; clinical trial protocol published in Zhang et al. 2022).

(3) the *CellSheet* trial (NCT04696328) is recruiting patients for epicardial implantation of cell monolayers constructed from 100 million iPSC-derived cardiomyocytes in patients with heart failure since 2019 (total of 10 patients planned; data from 3 patients reported recently (Kawamura et al., 2023)).

(4) the HECTOR trial (NCT05068674) is recruiting patients for intramyocardial injection of 50-300 million embryonic stem cell-derived cardiomyocyte in patients with heart failure since 2022 (total of 18 patients planned; no data published).

(5) the LAPiS trial (NCT04945018) is recruiting patients for intramyocardial injection of spheres aggregated from induced pluripotent stem-derived cardiomyocyte in patients with heart failure (total of 10 patients planned; no data published).

For further details on the IMP please refer to section 9.

1.3 Trial purpose and rationale

To counter the irreversible and progressive loss of cardiomyocytes and effectively remuscularize the failing heart, cardiomyocyte therapeutics are being developed (refer to section 1.1. and 1.2). Human cardiomyocytes can be derived from embryonic (ESC) and induced pluripotent (iPSC) stem cells using scalable processes with reproducible quality (Chen et al., 2015). The suggested clinical route of administration is either by direct intramyocardial injection (Chong et al., 2014; Liu et al., 2018a; Romagnuolo et al., 2019; Shiba et al., 2016; Zhao et al., 2018) or by epicardial implantation of tissue engineered heart muscle (Didie et al., 2013; Riegler et al., 2015; Tiburcy et al., 2017; Weinberger et al., 2016; Zimmermann et al., 2006). A prerequisite for therapeutically effective remuscularization of the human heart is cardiomyocyte survival and sustainable retention after implantation. Long term retention of intramyocardially injected cells, including cardiomyocytes, is negligible (<1%; (Nguyen et al., 2016)). Substantially more cardiomyocytes are retained after delivery via an epicardial patch approach ((Riegler et al., 2015); refer also to own data from additional rat and NHP model data in IMPD). Approximately 0.8-1 billion cardiomyocytes (i.e., 25% of the left ventricle) are lost in patients with advanced stage heart failure (Gepstein, 2002). Replacing the lost cardiomyocytes and thereby restoring heart function is the primary goal of cardiomyocyte therapeutics.

For the epicardial delivery, we propose a tissue engineered product, namely EHM, for epicardial application as BioVAT. The EHM-IMP is composed of defined mixtures of iPSC-derived cardiomyocytes and stromal cells seeded in a collagen type I hydrogel, cast into custom-made molds to obtain a desired geometry, and conditioned by exposure to mechanical load to simulate the hemodynamic loading conditions during a natural contraction-relaxation cycle of the heart (Tiburcy et al., 2017) (refer to IMPD for details).

The proposed primary mode of action (MoA) is functional remuscularization of the targeted hypokinetic heart wall (Fujita and Zimmermann, 2017a), resulting in (1) enhanced target heart wall thickness and (2) enhanced target heart wall thickening fraction.

The primary target patient population comprises subjects with advanced heart failure with a reduced ejection fraction (HFrEF; $EF \leq 35\%$) despite optimal medical therapy (OMT) and segmental hypo- or dyskinesia of the left (LV) or right (RV) ventricular free wall or a dilated heart chamber. This patient population is according to guideline recommendations protected from sudden cardiac death by for example implanted cardioverter/defibrillator (ICD) devices. Patients may be listed for heart transplantation, but are unlikely to receive a heart transplant due to for example age (>65 of age), blood group, and co-morbidities or other exclusion criteria. By targeting the hypokinetic heart wall or dilated heart chamber with contractile EHM, we aim at a functional remuscularization, which will, if successful, improve local and global heart function and patient outcome (mortality and quality of life).

Preclinical allograft (Didie et al., 2013; Zimmermann et al., 2002; Zimmermann et al., 2006) and xenograft (Qin et al., 2016; Riegler et al., 2015; Tiburcy et al., 2017) studies in rodents as well as auto- and allograft studies in Rhesus macaque with and without heart failure (Jebran et al. 2024 [in press]) have demonstrated the feasibility of EHM implantation onto the beating heart via a left-

lateral mini-thoracotomy, using a similar route of administration as used for surgical Transapical Aortic Valve Implantation (TAVI), Minimally Invasive Direct Coronary Artery Bypass (MIDCAB) surgery, or surgical placement of epicardial pacemaker leads. This will expose the target patient population to a minimal surgical risk with no anticipated mortality (own experience and experience from others (Puglisi et al., 2004)). As of November 2024, 13 patients have been treated with no mortality related to the surgical procedure or the EHM-graft. 4 patients passed away due to (1) multiorgan failure, (2) COVID related cardiopulmonary exhaustion, (3) type-A dissection of the thoracic aorta, and (4) urothelial cancer. The conclusion from the completed dose finding study and ongoing study extension is that the administration of 5-20x EHM is safe, with first signs for dose-dependent efficacy according to the proposed mode of action resulting (1) target heart wall augmentation (thickening), (2) enhanced global heart function (increase in ejection fraction), and (3) improvement of symptoms assessed by patient reported outcome measures (KCCQ-23 Overall Summery Score) at the according to the study protocol maximal feasible dose of 20x EHM (refer to IB for details). Heart failure related kidney dysfunction has to be monitored carefully to counter worsening of kidney function under immune suppression with calcineurin inhibitors, such as Tacrolimus. Adaptation of the immune suppression protocol as common practice in heart transplantation in patients with worsening of kidney function is considered (Velleca et al. 2023). Immune suppression, preferably Tacrolimus and Methylprednisolone, is essential to ensure allograft retention (Jebran et al. 2024 [in press]).

Surveillance of graft retention is by echocardiography, cardiac CT and/or cardiac MRI as well as biomarkers, including allograft-derived cell-free circulating DNA, which we aim to establish as an “online monitor” for graft retention/rejection (Beck et al., 2015). Patients are on the BioVAT-HF study protocol for 12 months with frequent clinical investigations (biomarkers, MRI or CT/echocardiography, quality of life assessments; refer to Visit schedule above). After the 12-month study period, consenting patients enter into the VAT-registry until end-of-life. In line with our preclinical and first clinical data, we anticipate that remuscularization of the failing heart will be sustained and as such improve heart failure symptoms, quality of life, and ultimately mortality in patients with heart failure. The therapeutic effects of EHM implantation require EHM engraftment, self-organization and maturation of the cardiomyocyte grafts and are accordingly anticipated to set in 3-12 months after implantation. Continuous cardiomyocyte growth and maturation as well as related efficacy is anticipated beyond the 12 month study period.

Whereas dose finding has established the first in human evidence for a safe and efficacious administration on the left ventricular free wall BioVAT-HF Part A, extension of BioVAT-HF Part B will focus on providing additional data for left ventricular heart wall augmentation by 20x EHM allografts as well for the first time also right ventricular (RV) augmentation in patients with compromised right ventricular function, a condition for which a clear unmet need exists. 20x EHM were considered an appropriate dose also for RV augmentation by the dose determining committee (DDC) which was further endorsed by the DSMB.

1.4 Rational for choice of control interventions/comparators

This is a first-in-patient study to obtain safety and efficacy data from patients with advanced heart failure. The lowest EHM dose was chosen as a minimally effective dose (MED) based on the observed effect in the pivotal preclinical non-human primate study (Jebran et al. 2024 [in press]; refer also to IMPD). Data from the BioVAT-HF trial will be compared to the data from patients included in the VAT-registry that were not yet treated. Given the intention to treat design of the BioVAT-HF first-in-patient trial it is not acceptable to include a placebo control intervention.

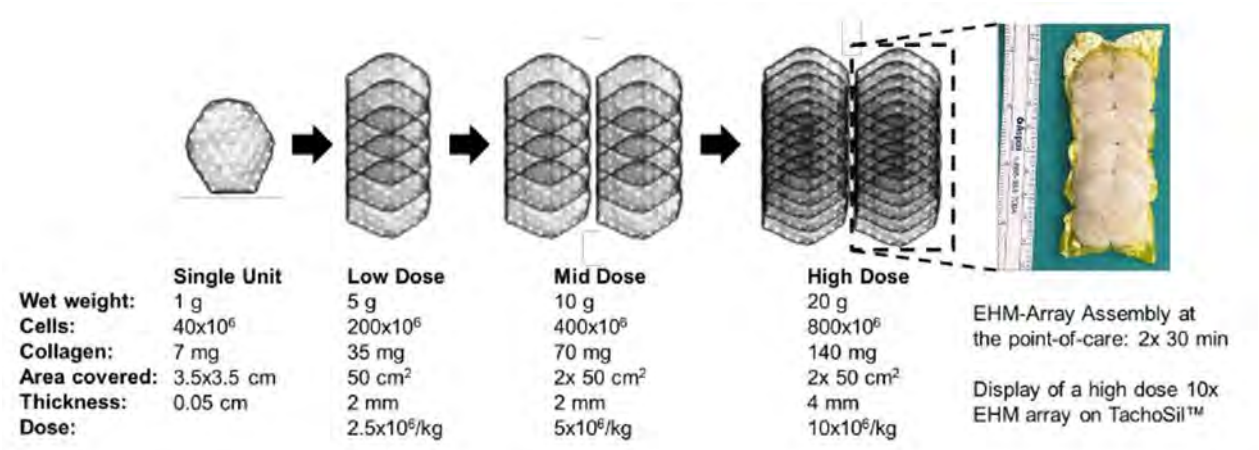
1.5 Rational for dose selection

We have completed a dose finding study (BioVAT-HF Part A 5x-20x EHM; refer to Figure 1 below) with the identification of 20x EHM as the maximal feasible dose (MFD). 10x EHM increased the heart wall thickness by 1-2 mm (Jebran et al. 2024 [in press]). The MFD (1) increased the target heart wall by approximately 4 mm, (2) enhanced global heart function (left ventricular ejection fraction by echocardiography) by approximately 10%, and (3) improved symptoms assessed by KCCQ-23 OSS by approximately 25 points 12 months after implantation. Based on the experience from surgical administration of EHM on the left ventricle in BioVAT-HF Part A, we anticipate that 20x EHM will also be suitable for the support of the right ventricle. The is proceeding has been suggested by the DDC and endorsed by the DSMB. The EHM dose will be recommended individually prior to implantation on the LV and RV and may be adjusted for example according to the patient body weight.

In the BioVAT-HF study, EHM patches are sutured to cover hypokinetic myocardium with suture points in the surrounding healthy myocardium, similarly as performed in previous studies (Didie et al., 2013; Riegler et al., 2015; Tiburcy et al., 2017; Zimmermann et al., 2002) and further refined in the completed Rhesus macaque study and the completed dose finding study of BioVAT-HF (Jebran et al. 2024 [in press]; refer to IMPD for a detailed description of the implantation procedure). To prevent potential epicardial bleeding events, EHM implants are stabilized with a 4.8x4.8 cm fibrinogen (5.5 mg cm²) and thrombin (2 I.U./cm²) coated equine collagen membrane (TachoSil®; BLA125351, EU/1/04/277/001-005; Takeda), which is fully resolved over time.

EHM-patch assemblies will be prepared by an overlapping sandwich format strategy. The Figure below depicts an individual EHM patch as well as schematic examples for 5x EHM (low dose), 10x EHM (mid dose) and 20x EHM (high dose) assemblies (Fig. 1):

Figure 1 EHM dosing



The BioVAT stacking strategy can be adapted according to individual clinical needs. Dose escalation in BioVAT-HF was according to recommendations by the dose determining committee (DDC). The 20x EHM dose for RV administration was suggested by the DDC and endorsed by the DSMB..

Patients are under optimized guideline-directed heart failure therapy. Rejection of EHM allografts is prevented by clinically established (in OHT) immune suppression (Velleca et al., 2023), including calcineurin inhibition (Tacrolimus: 5-10 ng/ml) with or without mTOR (Everolimus) or proliferation signal inhibition (Mycophenolat mofetil [MMF]) in case of calcineurin inhibitor induced

nephrotoxicity and corticosteroids (methylprednisolone: 0.15 mg/kg bodyweight * day) starting 7±3 days before EHM implantation. Adaptations of immune suppression may be acceptable according to clinical recommendations. Specific criteria are defined for stopping immunosuppression and thus initiating graft rejection, in case of immune suppression-related complications or lack of a defined treatment outcome. In the ESCORT trial (Menasche et al., 2018), with a weaning of immune suppression 1-2 months after cell implantation, and in our own studies in Rhesus macaque after withdrawal of immune suppression in a study subcohort (Jebran et al. 2024 [in press]; refer also to IMPD) no evidence for rejection related events were observed.

1.6 Risk-benefit assessment

Administration of immune suppression is started 7±3 days before EHM implantation. This allows for an individual dose adjustment of the calcineurin inhibitors to steady state levels at the day of EHM implantation (Visit 5) and the identification of potential side effects (e.g., liver damage [increase in ALT/AST], kidney damage [increase in creatinine]) prior to EHM implantation (Visit 5). Calcineurin inhibition will be continuously applied throughout the study and prescribed beyond the 12 month study period if clinical follow-up suggests efficacy of EHM implantation without safety concerns. Corticoids (preferably methylprednisolone) will be maintained at low dose to avoid Cushing symptoms and weaned after 3-6 months in line with the proceeding in heart transplant patients. The treating physicians together with the study coordinating team will review the individual patient data obtained during the study and make a recommendation to either continue prescription of the immune suppression or to stop immune suppression.

The surgical procedure related risk of EHM implantation via a minimal left lateral thoracotomy is best compared to the low risk associated with the surgical fixation of epicardial pacemaker leads (experience of the participating surgical teams and for example (Puglisi et al., 2004)). Typical surgical complications include bleeding, adhesions, and impaired wound healing.

The immune suppression protocol and surgical procedure were simulated in a pivotal preclinical Rhesus macaque allo- and autograft study (Jebran et al. 2024). First clinical experience and the continuous risk-benefit assessments in BioVAT-HF agree with the preclinical experience. The 20x EHM MFD in BioVAT-HF resembles a 2x EHM graft in macaques considering heart weight (10-16 g in Rhesus macaque [own data] vs 80-120 g in human [Yeon et al. 2014]) and 10x EHM graft in macaques considering target heart wall thickness (2-5 mm in Rhesus macaque [own data] vs 4-10 mm in human [Kawel et al. 2021]). No critical unwanted effects (no deaths, no tumor, no arrhythmia, no perturbation of heart function, no immune suppression related complications) were observed to be related to the tested doses. For details refer to IMPD.

Patients in BioVAT-HF Part A remained hospitalized for 2 weeks after EHM implantation to closely monitor potential side effects. Arrhythmia (ventricular tachycardia) represent the main complications in preclinical studies, testing iPSC-derived cardiomyocytes injections (Chong et al., 2014; Liu et al., 2018b; Romagnuolo et al., 2019; Shiba et al., 2016). Conversely, graft related arrhythmia were never observed in preclinical studies of tissue engineered heart repair and also not in to date 13 patients treated in BioVAT-HF. This is most likely explained by the fundamental difference of IMP preparation and administration (Jebran et al. 2024 [in press]).

Patients will remain under standard of care, which may include inotrope treatment, mechanical circulatory support, and OHT as clinically indicated.

A DSMB, comprised of external independent experts with expertise in cell-based heart repair studies is charged with the oversight of the clinical trial: Prof. P. Menasche. Paris (Cardiothoracic

Surgeon), Prof. S. Janssens, Leuven (Cardiologist), Prof. S. Zohar, Paris (Statistician). The DSMB has regularly reviewed the data from BioVAT-HF Part A and recommended continuation with BioVAT-HF Part B, including left and right ventricular administration of EHM. The DSMB requested to review the 28 day post first-in-patient RV implantation data. In addition, the compiled data after 5 RV administration of EHM will be presented to PEI for review.

The DSMB may recommend that the sponsor suspends enrolment, amends the study or discontinues the study at any time. Clinical investigators will report all events to the principle investigator (PI). PI will report all events to the sponsor and the DSMB. DSMB will review events and recommend continuation, modification, or discontinuation of the study. Discontinuation will be by stopping the immune suppression and thus initiating graft rejection. Discontinuation will be according to pre-specified stopping criteria such as (1) immune suppression related complications (e.g., sepsis, kidney failure, liver failure), (2) lack of EHM patch retention (e.g., no evidence for enhanced thickness of the target hypokinetic heart segment or thinned heart wall due to heart chamber dilation, (3) graft related perturbations of heart function (e.g., sustained arrhythmia, decrease of EF, end-organ failure due to low-output syndrome), or (4) exaggerated disease progression (e.g., increased frequency in recurrent hospitalizations due to worsening of heart failure, need for sustained inotrope support).

Preclinical studies have provided no palpable evidence for toxicity or tumorigenicity of EHM implantation (refer to IMPD). Potential risks associated with the nature of the IMP and companion treatment (immune suppression), include arrhythmia (as a result of faulty cardiomyocyte integration), tumor formation (as a result of pluripotent stem cell impurities), perturbation of heart function (as a result of mechanical compression or constriction of the heart), and immune suppression related side effects.

Participation in the BioVAT-HF study has no negative effect on the patient's place on the transplantation list or heart transplantation (Jebran et al. 2024 [in press] with data from a BioVAT-HF patients subjected to heart transplantation 3 months after 10x EHM implantation).

During the course of the study echocardiography, cardiac CT and cardiac MRI will be used to examine the heart's target wall thickness and thickening fraction (local heart wall function supported by EHM implant) as well as global heart function parameters, such as ejection fraction. Echocardiography will be performed at all study visits. Because cardiac MRI was not feasible in most patients of BioVAT-HF Part A, Cardiac CT will be performed instead at baseline (Visit 2) and follow-up visits 8 and 10. In patients without devices, cardiac MRI will be considered instead of cardiac CT at baseline and follow-up visits 8, 9, and 10. Cardiac CT assessments are considered clinically acceptable against the background of the patient's age and the underlying disease with a 5-year mortality of 50%. The benefit is of particular importance both for assessing the effect of the EHM patch and for the patient as confirmation of successful target heart wall strengthening. The advantage of the EHM patch treatment is that the investigators can show the patient the evidence of the strengthening of the heart wall directly on the cardiac MRI or CT image. This is on the one hand an important motivation for the patients in the BioVAT-HF study and on the other hand pivotal information for the treating physician as conformation of the mode of action of the EHM grafts.

For more information see also Appendix 19.4 "Overall risk and benefit assessment".

1.6.1 Information regarding COVID-19 pandemic

The decision as to inclusion in the BioVAT-HF study is made exclusively by the treating investigators at the participating centers on the basis of the assessment of a therapeutic benefit through implantation of the EHM and the procedure related risks also in light of the COVID-19 pandemic.

Patients with severe heart failure are identified via the heart failure outpatient units of the participating clinics. Patients will be included after informed consent and scheduled for a baseline investigation (Visit 2). Data obtained at the baseline (Visit 2) will be individually discussed in a dedicated case conference held before Visit 3 (start of immune suppression) together with the team of the participating cardiac surgeons, cardiologists, IMP manufacturer, radiologists, scientific coordinator, the LKP and the CI. In each case, the risks associated with the COVID-19 pandemic will be considered, before triggering the start of immunosuppression (Visit 3) and release of the IMP by the manufacturer (Institut of Transfusion Medicine) for clinical administration.

Study subjects of the BioVAT-HF first-in-patient trial are despite optimal medical therapy in a clinically symptomatic and in stage III or IV according to the New York Heart Association (NYHA) or in Stage D according to Classification of the American Heart Association. These patients are considered insufficiently managed despite optimal medical therapy. In the BioVAT-HF study, a new therapeutic approach aiming at the remuscularization of the failing heart is being tested, a therapeutic mode of action which is currently not achievable by any alternative therapeutic approach and which addresses directly the need for additional contractile elements (cardiomyocytes) in the terminally failing heart. The one-year-mortality in the target patient population is 20% and thus there is a clear unmet need for therapy irrespective of the COVID-19 pandemic.

According to the EMA / PEI guidelines for the implementation of FIP studies with ATMPs, a minimally effective dose (MED) has to be chosen as a starting dose. According to preclinical studies in Rhesus macaque, considerable thickening of the target heart wall (by +5 mm), substantial remuscularization (histological evidence), and an improvement of local (target heart wall) and global (LV ejection fraction) heart function is anticipated at the proposed MED, i.e., a 5x EHM assembly.

Due to the lack of alternatives for this group of patients and the anticipated low procedural risk, we see the need to start the BioVAT-HF study irrespective of the COVID-19 pandemic, but of course considering carefully the associated risks in each individual patient. Patients are informed individually about preventive measures to avoid COVID-19 infections. If a COVID-19 infection occurs in the course of the study, close clinical monitoring will be carried out and discontinuation of the immunosuppression accompanying the study will be considered.

In case of a lock down with restrictions as to operative care at the involved clinics, EHM implantations will be postponed as medically justified.

If an effective vaccine against COVID-19 becomes available, immunization will be offered to BioVAT-HF patients at any time point during the study.

2 Objectives and endpoints

Table 2 Objectives and related endpoints

	Objective	Endpoint
Safety (Part A + B)		
Part A of this study has been completed		
Primary	Identification of a safe maximal dose (SMD)	<ul style="list-style-type: none"> Adverse events related to the procedure, including in particular arrhythmic events and worsening of disease progression within 28 days (Part A) and the whole study duration (Part B)
Secondary	<p>Identification of a safe dose range.</p> <p>Identification of dose related toxicity/efficacy to inform an individual risk-benefit assessment.</p> <p>Gathering of evidence to support the application of a safe maximal dose (SMD) in RV applications of EHM.</p>	<ul style="list-style-type: none"> Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death) Frequency and severity of arrhythmic events Incidence of immune rejection (DSA, CK/CK-MB, hs-cTnT, circulating cell-free allograft DNA) Incidence of mechanical perturbation of ventricular function by EHM graft
Efficacy (Part B)		
Primary	Identification of the efficacious therapeutic utility of engineered human myocardium (EHM) in patients with heart failure with reduced ejection fraction ($EF \leq 35\%$).	<ul style="list-style-type: none"> Structural endpoint: Change of target heart wall thickness (TWTh in mm) Echo or cCT or cMRI Functional endpoint: Change of LV/RV-ejection fraction (LV/RV-EF in %) Echo or cCT or cMRI Patient reported outcome Change of KCCQ-23 OSS (Overall Summary Score)
Secondary	<p>To assess effects of EHM-grafts on disease-specific events and symptoms</p> <p>Identification of an optimally effective dose range (Part A)</p>	<p><u>Key secondary endpoint:</u> Frequency of recurrent hospitalizations for heart failure</p> <p><u>Further secondary endpoints:</u></p> <ul style="list-style-type: none"> Time to mechanical circulatory assist device implantation. Time to heart transplantation.

		<ul style="list-style-type: none"> • Functional status in patients as determined by cardiopulmonary stress testing (VO₂max), six-minute walk test (6MWT) - distance, and hand-grip strength measurements • Patient reported outcomes assessed by NYHA classification, quality of life score (EQ-5D-5L), and study adherence motivation (HADS, MoCA, medication adherence, B-IPQ, TEX-Q) • All-cause and cardiovascular mortality
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2.1 Evaluation of primary / secondary objectives and endpoints

Safety (Part A + B) and efficacy (Part B) of EHM implantation will be key outcome measures. These will inform the strategy to advance EHM-based heart repair from early clinical testing towards a pivotal clinical trial, application under hospital exemption §4b AMG, and routine clinical application. The dose finding cohort (Part A) aimed at defining the Safe Maximal Dose (SMD). In Part A of BioVAT-HF, safety was confirmed for the as per clinical trial protocol tested dose range from 5x to 20x EHM. 20x EHM was determined as safe maximal dose (SMD) for administration to support a target heart wall. Testing in Part A was strictly on the left ventricular (LV) heart wall guided by experience of the clinical teams at the primary study sites in Göttingen and Lübeck, reviewed by the Dose Determining Committee (DDC) and the Data Safety Monitoring Board (DSMB). The DSMB endorsed continuation as Part B with 20x EHM administration on the left or right ventricle. In light of the a positive risk-benefit assessment, a substantial amendment to approve continuation of BioVAT-HF Part B is submitted to PEI and the primary Ethics Committee.

Primary efficacy outcome measures will be determined primarily by echocardiography, supported by cardiac CT and cardiac MRI (heart wall thickness and left ventricular as well as assessment of of heart failure symptoms). Note that the adjustment of the primary efficacy endpoints is necessary because in BioVAT-HF Part A it became apparent that the target heart wall thickness and thickening fraction cannot be measured precisely by cardiac MRI, because of commonly observed device artifacts. As a consequence, we implemented cardiac CT imaging as an alternative to MRI, which can be applied to precisely measure heart wall thickness, but cannot be used similarly frequently as echocardiography. Accordingly, we will use echocardiography as the key measure at study visits 2 (Baseline), 4, 6, 7, 8, 9, 10 and cardiac CT as well as cardiac MRI (if possible as alternative to cardiac CT) as supportive measures at study visits 2 (Baseline), 8, 10 (cardiac CT which may be performed as PET/CT if possible) and 2 (Baseline), 8, 9, 10 (cardiac MRI). Longitudinal and multimodal assessments of cardiac function is important to gain precise information and confidence as to efficacy of EHM. In addition, KCCQ-23 OSS will be assessed for a precise documentation of patient symptoms. All measures have been applied in Part A and were found to be reliable in assessing structural, functional and symptomatic changes. Safety outcome measures (AE and SAE quality and quantity) is supported by ECG and if clinically indicated pulmonary artery pressure telemetry recordings (e.g., with a CardioMEMS device) and biomarkers (CK/CK-MB, hs-TnT2, NT-proBNP). As key secondary efficacy endpoint, recurrent heart failure

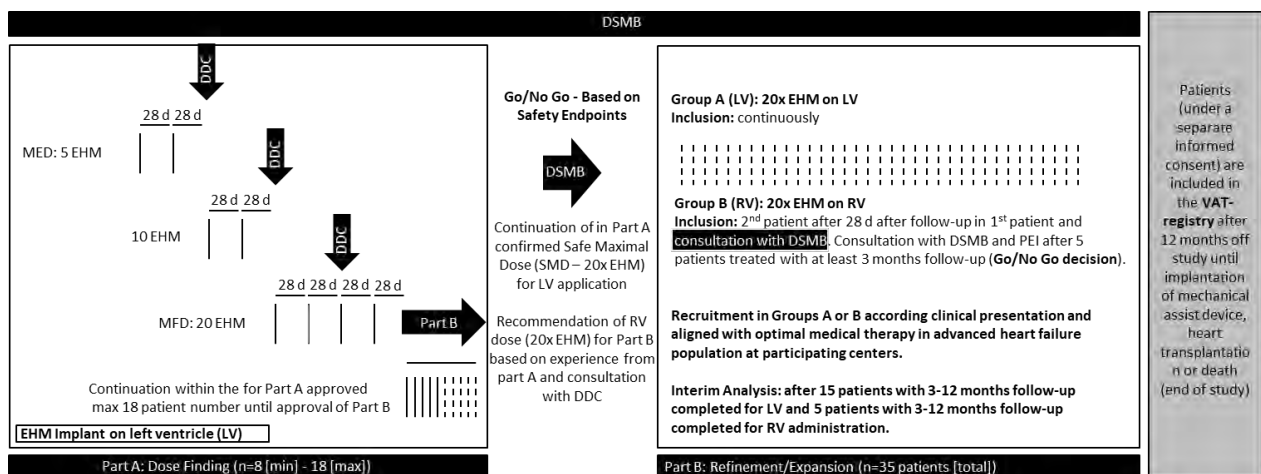
hospitalization was chosen because it may best represent the trajectory of disease progression and quality of life in the proposed patient population.

3 Clinical trial plan

3.1 Trial design

This is a combined, open-label, 2-stage, phase I/II safety and efficacy study investigating induced pluripotent stem cell-derived engineered human myocardium (EHM) as biological ventricular assist tissue (BioVAT) in patients with terminal heart failure. 53 patients will be recruited over 30 months. The study consists of two consecutive parts (Fig. 2). Dose Finding has been completed with the identification of the SMD (Part A); treatment is continued in Part A and shall be converted into Part B upon approval by PEI and the CEC.

Figure 2 Trial design



Abbreviations: MED: Minimally effective dose; MFD: Maximal feasible dose; DSMB: Data Safety Monitoring Board; DDC: Dose Determining Committee; EHM: Engineered human myocardium; LV: Left ventricle, RV: Right ventricle, TAPSE: Tricuspid Annular Plane Systolic Excursion used as diagnostic measure of RV function. Solid lines represent treated patients (n=13 as of November 2024); striped lines represent patients to be treated according to the clinical trial protocol.

Patient populations: LV EF≤35% and previous heart failure hospitalization
in case of RV administration in addition: TAPSE <16 mm (combined LV and RV failure)
All patients are on optimal guideline-directed therapy
LV-implantation as standalone procedure
RV-implantation on top of elective surgery on LV (e.g., VAD, CABG, valve repair/replacement)

3.2 Treatment parts

Patients in the BioVAT-HF trial receive induced pluripotent stem cell (iPSC)-derived EHM to support the failing heart by remuscularization.

Within **Part A** a dose finding regimen to determine the Optimally Effective Dose Range and if possible the Safe Maximal Dose (SMD) of EHM will be applied (Fig. 2). A minimum of 8 and a maximum of 18 patients will be included with data from the first 4 patients treated with the identified MFD reaching the 28-day endpoint reported to the DSMB for the assessment as SMD and continuation into Part B of the BioVAT-HF trial. Safety and efficacy data obtained in patients with

the SMD in Part A will be included into the interim analysis of Part B of the BioVAT-HF trial. The interim analysis will be performed 3 months after 15 patients treated with the SMD have reached 3 months follow-up. 5x EHM assemblies will be applied as starting dose. 5x EHM assemblies resulted in an augmentation of the target heart wall by ~5 mm in a pivotal macaque study and thus are considered a minimally effective dose (MED) in the BioVAT-HF study. Dose escalation may be recommended after review of the clinical data obtained during the first 4 weeks after treatment of the first two patients in each dosing cohort by the Dose Determining Committee (DDC). Multiples of 5x EHM assemblies, i.e. 5, 10, 15, 20x EHM assemblies are considered as dosing intervals (refer to IMPD and page 26 for details). A minimal number of 2 patients must be included in each dose escalation step. The earliest time point for inclusion of a second patient in each dose cohort is 4 weeks after treatment of the first patient in the respective dose cohort.

At latest after 28-day follow-up (Visit 7) of the 4th patient treated with the MFD or determination of the Safe Maximal Doses (SMD) study Part A is completed; interim results will be evaluated by the DSMB. The DSMB will make a recommendation regarding the continuation of the BioVAT-HF study with the SMD as outlined (Fig. 2) for Part B. Afterwards, the interim results of Part A and the recommendations of the DSMB will be submitted to the competent authority (Paul-Ehrlich-Institut; PEI) and the competent ethics committee (CEC) in order to receive an approval for a substantial amendment by the PEI and positive vote of the CEC for study Part B. Only after this approval study Part B will be initiated. For study Part B further patients (n = 35) will be included to collect proof-of-concept data as to efficacy of EHM mediated assistance/augmentation of the LV or RV (Fig. 2).

In **Part B** EHM treatment of the left ventricle (LV) will be extended with the SMD and compared to EHM treatment of the right ventricle (RV; Fig. 2). At least 5 patients will receive EHM-implantation on the LV or RV. RV treatment in part B addresses the unmet need for RV support in patients referred to a surgical intervention on the LV (i.e., CABG, LVAD, valve reconstruction) with concurrent RV failure. The 2nd patient in the RV study cohort will be included after 28 days follow-up in the 1st patient and endorsement by the DSMB after review of the safety data. After completion of 3 month follow-up in the 5th RV patient, the DSMB will review all associated AEs and recommend continuation, dose adaptation or stopping of RV treatment. Recruitment for LV EHM administration will be unaffected by the RV treatment consideration and continuous after endorsement and approval of Part B by the DSMB, PEI and CEC as outlined above.

3.3 Treatment duration

According to experience from BioVAT-HF Part A implantation of 20x EHM-patches takes approximately 120 minutes. Immunosuppressive treatment is started 7±3 days prior to the scheduled EHM implantation and continued for 12 months unless unwanted effects require withdrawal of immune suppression. Immune suppression will be continued off-study if patients present evidence for efficacy (i.e., enhanced heart wall thickness, improved ejection fraction, improved symptoms) without safety concerns. The decision to prescribe immune suppression off-study will be by the responsible clinical PI after individual data review and patient consent. Patients will be included in the VAT-registry off-study until death, heart transplantation or implantation of a mechanical assist device. Data from routine heart failure management will be recorded.

3.4 Number of patients

Up to 53 patients are planned to be enrolled in the trial (Part A: max. 18, Part B: 35).

3.5 Participating sites

The 9 participating sites (8 in Germany / 1 in the Netherlands) are highly specialized centers for heart failure therapy:

3.6 Recruitment rate

The participating centers run active heart failure programs, including specific programs for OHT and/or LVAD implantation. Other physicians may refer patients to the study via consultation with the primary study center in Göttingen. The participating centers have agreed to recruit 3-12 patients/year to the BioVAT-HF trial.

3.7 Trial timetable

Planned dates:

Enrolment of first patient, first patient in (FPI)	1st quarter 2021
Enrolment of last patient, last patient in (LPI)	4 th quarter 2026
End of trial for last patient, last patient last visit (LPLV)	4 th quarter 2027
Final statistical analysis	2 nd quarter 2028
Planned interim analysis	4 th quarter 2025
	After 28 days follow-up inclusion of the 4 th patient in part A (Safety) was completed.
	After 15 (LV) and 5 (LV) patients with the SMD have completed at least 3 months follow-up in Part B.

4 Trial population and selection criteria

4.1 Target population

Patients will only be allowed to enter the trial if they provide written informed consent to their participation (following full explanation of the trial) (see section 5.1).

4.1.1 Health condition studied

Patients with terminal heart failure will be enrolled into this trial; eligible patients must present with reduced left ventricular ejection fraction ($EF \leq 35\%$) and a history of hospitalization for heart failure

despite optimized guideline directed therapy. A hypo- or dyskinetic free heart wall or dilated heart chamber must be identified as target for EHM implantation.

4.1.2 Gender distribution

There will be no selection of patients according to gender. No evidence for differences in outcome are anticipated for male and female subjects. In agreement with other clinical trials in patients with endstage heart failure, we anticipate an overrepresentation of male subjects.

4.2 Inclusion criteria

Patients eligible for this trial must meet all of the following criteria:

1. Symptomatic heart failure (NYHA II-IV) with reduced ejection fraction (HFrEF with LVEF $\leq 35\%$) as assessed by echocardiography.
2. Patients on guideline-directed medical therapy
3. NT-proBNP >300 pg/mL for patients in sinus rhythm or >900 pg/mL if in atrial fibrillation
4. History of previous heart failure hospitalization in the past 12 months
5. At least one hypo- or dyskinetic segment or dilated heart chamber to demark the implant target area
6. **(A)** Stable disease condition allowing for an elective left-lateral mini-thoracotomy (for LV applications) **or (B)** open-chest surgery (for RV applications) for a clinically indicated intervention on the LV (e.g., coronary bypass surgery, valve repair, mechanical circulatory support device implantation) with concomitant RV dysfunction, diagnosed using the Tricuspid Annular Plane Systolic Excursion (TAPSE) index <16 mm (Rudski et al. 2010).
7. 18-80 years of age
8. Willingness and ability to give written informed consent
9. Female subjects of childbearing potential must agree to use acceptable method(s) of contraception for the full study duration.

4.3 Exclusion criteria

Patients eligible for this trial must not meet any of the following criteria:

1. Contraindication to immunosuppressive drugs (e.g. known history of unresolved cancer, hepatitis B/C, HIV, HTLV1)
2. Contraindication to TachoSil® (e.g. hypersensitivity to human fibrinogen, human thrombin, horse collagen, human albumin, Riboflavin, Natriumchloride, Natriumcitrate, L-Arginin-Hydrochloride)
3. Hypertrophic cardiomyopathy (HCM)
4. Terminal kidney failure (stage 4; GFR <30 ml/min) at the time of enrolment
5. Terminal liver failure (Child-Pugh stage C; score >10) at the time of enrolment
6. History of disabling stroke
7. Reduced life expectancy in the short term due to non-cardiac disease
8. Any condition that excludes adherence to study protocol (in particular lack of adherence to prescribed medication)
9. Simultaneous participation in another interventional trial
10. Pregnant or breastfeeding females
11. Known or suspected alcohol and/or drug abuse

5 Enrolment and patient registration

5.1 Patient eligibility

The investigator will inform the patient about the trial and ask the patient for his/her written consent prior to inclusion and scheduling of Visit 2. The patient has at least 24 h to familiarize her*himself with the patient information and provide his*her informed consent. The signed informed consent form can be returned to the investigator by mail, facsimile or email (the originally signed informed consent form can be returned to the investigator at a scheduled baseline Visit 2). Patients waiting for EHM implantation will be asked to enrol into the VAT-registry (under a separate informed consent) to obtain information as to disease progression.

It is imperative that written consent is obtained prior to any trial-specific procedures as part of Visit 2. After informed consent is provided, the investigator will record the details of the trial patients in the following trial-specific list:

Subject identification log: A confidential log of the names of all trial patients with the identification code. Sponsor representatives, clinical research associates (CRAs), auditors and representatives of competent authorities (CA) will be allowed to inspect the list on request.

Only if the patient has signed the informed consent and meets all of the inclusion criteria and none of the exclusion criteria at the baseline visit 2 the patient is eligible for the study.

Furthermore, the investigator will ask the patient to provide biological samples for the DZHK-Biobank project. A separate consent form will need to be signed for drawing biological samples for the DZHK-Biobank project. This consent process is independent from the BioVAT-HF study and patients can participate in the BioVAT-HF trial without consenting to the DZHK-Biobanking.

5.2 Patient registration

Patient registration will be done within the eCRF (secuTrial). After registration an individual Patient Identification number will be assigned to each patient

6 Treatment plan and procedure

6.1 Dosing regimen and IMP administration

Patients in the BioVAT-HF trial are under immune suppression (typically the calcineurin inhibitor Tacrolimus combined for 3-6 months with a corticoid such as methylprednisolone) for the whole duration of the trial to prevent EHM allograft rejection. The immune suppressants will be provided to the patients by the treating study center. The immune suppression protocol may be adapted to clinical recommendations. After a dedicated case conference held by the participating cardiac surgeons, cardiologists, IMP manufacturer, radiologists, scientific coordinator, and the clinical PI for a review of the baseline Visit 2 data, the patient is informed to start immune suppression 7±3 days (Visit 3) prior to the scheduled EHM implantation. The patient is asked to document the daily *per os* intake of immune suppression. Upon administration to the treating clinic (Visit 4) therapeutic drug monitoring is performed. The calcineurin-inhibitor dose will be adjusted prior EHM implantation to reach target blood concentrations. Dosing to reach target blood levels is according

to standard protocols in patients with heart transplantation, i.e., Tacrolimus (5-15 ng/ml) or Ciclosporine A (150-375 ng/ml) and Methylprednisolone (~0.15 mg/kg bodyweight * day; typically, 5-10 mg/day). Trough levels for the administered calcineurin inhibitor will be assessed every 2-3 days from the day of hospital administration until the release from the hospital after Visit 6 and at every Visit thereafter. Immune suppression dose may have to be adjusted individually during the course of the study.

Adaptations of immune suppression, for example administration of other calcineurin inhibitors or other immune suppressants in clinical use in organ transplantation (e.g., Everolimus, MMF), may be acceptable if clinically indicated and recommended by the treating physician.

EHM will be delivered individually from the manufacturing site (University Medical Center Göttingen) to the point-of-care and assembled at the point-of-care (refer to IMPD). EHM assemblies will be sutured to a TachoSil® membrane (refer to IMPD) and then sutured to the target heart wall via a left lateral thoracotomy.

Table 3 Dosing schedule

Study parts	ATMP form and route of administration	Dose	Regimen
Part A: Dose finding cohort	Implantation of EHM assemblies to the LV composed of increasing EHM layers	5x EHM 10x EHM 20x EHM Dosing intervals recommended by the DDC and endorsed by DSMB after safety data review	<u>8 patients:</u> Patient I: 5x EHM Patient II: 5x EHM Patient III: 10x EHM Patient IV: 10x EHM Patient V: 20x EHM Patient VI: 20x EHM Patient VII: 20x EHM Patient VIII: 20x EHM <u>Extension recommended by the DDC, endorsed by the DSMB and approved by the PEI and the CEC (to a maximum of 18 patients treated):</u> Patients IX-XVIII: 20x EHM Extension may be used to investigate EHM production protocol changes (requires a substantial amendment of the protocol)
Part B: Refinement /Expansion cohort	Implantation of EHM patches to LV and RV	Safe Maximal Dose (SMD) for LV as established in Part A	Patients will be included in LV and RV cohorts according to eligibility for LV or RV augmentation.

Study parts	ATMP form and route of administration	Dose	Regimen
		Dose for RV as suggested by DDC, endorsed by DSMB after interim data review and approved by PEI and CEC	

6.2 Dose modification and dose delay / or dose reduction

Calcineurin-inhibitor dose will be adapted according to therapeutic drug monitoring data to achieve the target blood levels according to the proceeding in orthotopic heart transplantation (ISHLT Guidelines; Velleca et al. 2023) with a preference for a combined use of Calcineurin inhibitors (CNI - Tacrolimus) and mTOR inhibitors (Everolimus) with Methylprednisolone:

Tacrolimus:

8-10 ng/ml at the time of implantation maintained for 3-6 month (Baran et al. 2011) and then (1) continued at a reduced dose to maintain trough levels of 5-8 ng/ml (in case of no drug related side effects), (2) reduced to 3-8 ng/ml in combination with a mTOR-inhibitor (in case of drug related side effects) or (3) discontinued (CNI-free protocol; in case of intolerable CNI-related side effects).

Everolimus:

mTOR-inhibitors, preferably Everolimus, in combination with CNI are adjusted to trough concentrations of 3-8 ng/mL. In case of CNI-free protocol Everolimus maintenance trough levels of 6-10 ng/ml are targeted. Prescription of mTOR-inhibitors only after completion of wound healing, i.e., typically 1 months after EHM implantation.

Mycophenolat mofetil

Proliferation signal inhibitors (PSIs), preferably mycophenolat mofetil (MMF), may be added according to ISHLT-guidelines (Velleca et al. 2023) and the reported proceeding in the TICTAC trial for 2 weeks post operatively (NCT00299221; Baran et al. 2011) at the discretion of the treating physician.

Methylprednisolone:

Methylprednisolone will be administered at a maintenance dose (0.15 mg/kg bodyweight * day, i.e., typically 5-10 mg/day) until weaning 3-6 months after EHM implantation.

Immune suppression may be further adapted according to clinical routine proceedings in case of evidence for immune suppression related toxicity (liver, kidney) or rejection.

Withdrawal of immune suppression may be recommended in case of intolerable unwanted effects.

Immune suppression dose reduction or withdrawal or change in prescription must be recorded on the appropriate eCRF page. Patients are requested to document their daily immune suppression intake and return unused immune suppressants to the study center at the UMG.

Immune suppression will be regularly prescribed off-study in case of a perceived benefit of EHM implantation and patient consent.

6.2.1 Concomitant treatment/medication

6.2.2 Permitted prior/concomitant treatment/medication

Treatment with transfusions (red blood cells and platelets) and supportive care are permitted after the initiation of study treatment.

The patient must notify the investigational site of any new medication he/she starts taking after the start of the trial medication. All medications (other than IMP) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts treatment with IMP must be listed in the eCRF.

6.2.3 Guidelines for rescue medications and/or non-drug therapies or supportive care

Patients should receive treatment/medication appropriate to their clinical condition in an emergency.

Withdrawal of immune suppression may be recommended by the investigator to initiate rejection of EHM implants in case of intolerable side effects (for details see section 6.2).

EHM implants may be surgically removed if required as an emergency measure.

6.2.4 Permitted concomitant therapy requiring caution and/or action

Calcineurin inhibitors (tacrolimus and cyclosporine) are substrates of CYP3A4. Pharmacokinetic interactions with other CYP3A4 substrates, inhibitors and activators (such as for example HMG-CoA reductase inhibitors or St. John's wort) must be considered according to standard clinical practice.

6.2.5 Prohibited concomitant therapy

CYP3A4 accelerating drugs (e.g., St. John's Wort) must not be administered. Patients must be informed about the potential need for dose modification if CYP3A4 inhibitors (e.g., macrolide antibiotics, HMG-CoA reductase inhibitors) are concurrently administered. Control of trough levels of calcineurin inhibitors must be performed according to routine practice and dosing adjusted accordingly.

6.3 Unblinding of treatment assignment

Not applicable, trial is unblinded.

6.4 Treatment and Follow-up after end of the trial

Upon completion of the BioVAT-HF trial with 12 months follow-up, study subjects will be included in the VAT-registry off-study until death, mechanic assist device implantation or heart transplantation. Immune suppressive therapy will be continued according to the ISHLT-guidelines

(adopted by the “Deutsche Gesellschaft für Thorax-, Herz- und Gefäßchirurgie” – DGTHG) in patients with heart transplantation to prevent rejection of EHM.

7 Visit schedule and assessments

7.1 Flow and visit schedule

A detailed Flowchart is provided in Table 1 Visit schedule and assessments – Flowchart

The schedule of assessment lists all of the assessments and indicates with an “x” when they have to be performed. All data obtained from these assessments must be available in the patient’s source documentation.

7.2 Visit and assessment windows

During the course of the trial, visits and test procedures should occur on schedule whenever possible; visits that occur within a certain timeframe from the scheduled date will not constitute a protocol deviation. These timeframes are defined in Table 1 and the following descriptions.

7.3 Baseline 1 (Visit 1, min. 24 h before Baseline 2)

The investigator is obliged to give the patient thorough information about the trial and the trial related assessments, and the patient should be given ample time to consider his or her participation. The investigator must not start any trial-specific procedure before Informed Consent Form (ICF) is signed and dated by both the patient (and impartial witness, if applicable) and the investigator. The investigator must keep the original signed ICF (a signed copy is given to the patient), (see section 15.3). This visit is performed at least 24 h before Baseline 2 (Visit 2).

7.4 Baseline 2 (Visit 2, Month -3 to week -2)

Patients considered eligible by the investigator will be included and registered to the trial (see section 5.2).

The data that will be collected at baseline include the following (please refer to section 7.9 for a precise definition of assessments):

- Inclusion / Exclusion criteria
- Psychosocial analysis to assess medication adherence
- Medical history
- DZHK basic data set
- Concomitant heart failure medications

Laboratory assessments:

CBC with differentials and platelet count

- CRP, Procalcitonine, IL6
- Liver panel
- Albumin
- Serum Creatinine, BUN, cystatin C

- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- hs-TnT, CK, CK-MB (at the discretion of the clinical PI also if CK is not elevated)
- NT-proBNP
- HBsAg, Anti-HCV, Anti-HIV Anti-HTLV-1
- Pregnancy Test (for females of childbearing potential)
- Blood draw for allograft DNA assessment
- HLA/KIR-Typing
- Donor Specific Antibodies

Further assessments / Scores:

- 12-lead ECG
- Echocardiography (preferably transthoracic echocardiography; in case of poor image quality with contrast agent)
- Cardiac-CT or cardiac-MRI (MRI instead of CT if high data quality can be anticipated)
- Cardiopulmonary exercise testing
- Hand grip strength
- 6 minute walking test
- EuroSCORE II
- INTERMACS

Questionnaires (patient reported outcome): quality of life

- KCCQ-12
- EQ-5D-5L

Questionnaires (patient reported outcome): study adherence motivation

- HADS
- MoCA
- Medication adherence
- IPQ-B
- TEX-Q

Treatment / therapy:

- ICD/CRTD-event recorder readout (if implanted)
- DZHK-Biobanking Data Set (not mandatory, only if separate Consent available)

Results will be reviewed during dedicated case conference and patient cleared for implantation before the start of immune suppression (Visit 3). The EHM Production Unit (Qualified Person) will be informed at latest 8 days before the scheduled implantation to initiate the release process.

7.5 Start of Immunosuppression (Visit 3, Day -10 to -4)

Immunosuppression (calcineurin inhibitor combined with a corticoid) will be started 7 ± 3 days prior to the scheduled EHM implantation. Dosing of the calcineurin inhibitor and corticoid will be daily *per os* according to standard protocols in patients with heart transplantation, i.e., Tacrolimus (5-15 ng/ml) or Ciclosporine A (150-375 ng/ml) and Methylprednisolone (0.15 mg/kg bodyweight * day, i.e., typically 5-10 mg/d).

Further data to be collected beginning from the first admission of immunosuppression:

- Adverse events
- TDM Calcineurin Inhibitors

7.6 Hospital administration (Visit 4; Day -6 to -1)

Patient will be hospitalized at the clinical PI's discretion at latest on the day before EHM implantation.

- Reduced DZHK basic data set according to chapter 7.9.3
- Concomitant heart failure medications
- Adverse events

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, Procalcitonine, IL6
- Liver panel
- Albumin
- Serum Creatinine, BUN, cystatin C
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- hs-TnT, CK, CK-MB (at the discretion of the clinical PI also if CK is not elevated)
- NT-proBNP

Further assessments / Scores:

- 12-lead ECG
- Echocardiography (preferably transthoracic echocardiography; in case of poor image quality with contrast agent)

Treatment / therapy:

- ICD/CRTD-event recorder readout
- TDM Calcineurin Inhibitors (see chapter 7.5) – level assessment and dose adjustment as needed

Anaesthesia protocol planning and preparation and, if necessary, chest x-rays (posterior-anterior and lateral projections) to exclude an acute, new-onset pneumonia. The standard mean radiation exposure for this X-ray examination is 0,1 mSv.

7.7 Implantation (Visit 5; Day 0)

On this day, EHM patch implantation will be performed and AEs will be documented.

7.8 Follow up

Following implantation of EHM as BioVAT (Visit 5), in-hospital monitoring will be according to SOP in heart transplant patients. The patient will be release from hospital at the discretion of the clinical PI. After release from the hospital, patients are scheduled for follow-up visits 1, 3, 6 and 12 months after BioVAT implantation. For details see sections below.

7.8.1 Assessments at Visit 6 (1-2 days prior to the release from hospital)

- Reduced DZHK basic data set according to chapter 7.9.3
- Concomitant heart failure medications
- Adverse events

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, Procalcitonine, IL6
- Liver panel
- Albumin
- Serum Creatinine, BUN, cystatin C
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- hs-TnT, CK, CK-MB (at the discretion of the clinical PI also if CK is not elevated)
- NT-proBNP
- Blood draw for allograft DNA assessment

Further assessments / Scores:

- 12-lead ECG
- Echocardiography (preferably transthoracic echocardiography; in case of poor image quality with contrast agent)

Treatment / therapy:

- ICD/CRTD-event recorder readout
- TDM Calcineurin Inhibitors (see chapter 7.5) - level assessment and dose adjustment as needed

7.8.2 Assessments at Visit 7 (Month 1 \pm 7 days), Visit 8 (Month 3 \pm 7 days), and Visit 9 (Month 6 \pm 7 days), and Visit 10 (Month 12 \pm 7 days)

- Reduced DZHK basic data set according to chapter 7.9.3
- Concomitant heart failure medications
- Adverse events

Laboratory assessments:

- CBC with differentials and platelet count
- CRP, Procalcitonine, IL6
- Liver panel
- Albumin
- Serum Creatinine, BUN, cystatin C
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- hs-TnT, CK, CK-MB (at the discretion of the clinical PI also if CK is not elevated)
- NT-proBNP
- Blood draw for allograft DNA assessment
- Donor Specific Antibodies

Further assessments / Scores:

- 12-lead ECG
- Echocardiography (preferably transthoracic echocardiography; in case of poor image quality with contrast agent)
- Cardiac-CT (Visits 8 and 10) or cardiac-MRI (Visits 8, 9 and 10; MRI instead of CT if high data quality can be anticipated)
- Cardiopulmonary exercise testing (visits 8,9,10)
- Hand grip strength (visits 8,9,10)
- 6-minute walk test (6MWT; visits 8,9,10)
- INTERMACS (at visit 10)

Questionnaires (patient reported outcome): quality of life at 3 and 12 months follow-up

- KCCQ-12 (visits 8 and 10)
- EQ-5D-5L (visits 8 and 10)

Questionnaires (patient reported outcome): study adherence motivation at 3 and 12 months follow-up

- HADS (visits 8 and 10)
- MoCA (visits 8 and 10)
- Medication adherence (visits 8 and 10)

Treatment / therapy:

- ICD/CRTD-event recorder readout
- TDM Calcineurin Inhibitors (see chapter 7.5) - level assessment and dose adjustment as needed

7.9 Assessments and specifications

7.9.1 Psychosocial analysis to assess medication adherence

Psychosocial analysis according to standard procedures in patients with heart transplantation or ventricular assist device implantation to assess medication adherence and support patient inclusion by excluding the risk of a lack of adherence to prescribed medication, in particular to the in the BioVAT-HF study prescribed immune suppression to prevent rejection of EHM implants.

7.9.2 Medical history

Terminal heart failure patients with a documented history of LVEF $\leq 35\%$ and a history of previous hospitalization for heart failure (HFH) despite optimized guideline-directed therapy are eligible to enrol in the BioVAT-HF trial. LVEF will be re-evaluated by echocardiography (ECHO; refer to description in 7.8.7) to confirm LVEF $\leq 35\%$ at the baseline visit (Visit 2). Cardiac computerised tomography (cCT; refer to description in 7.9.11) or as an alternative by cardiac magnetic resonance imaging (cMRI; refer to description in 7.9.9) will be performed to in detail investigate heart structure and function at the baseline visit (Visit 2). Medical history is gathered according to DZHK-SOPs:

DZHK-SOP-K-01 „Basisdatensatz Anamnese/Klinische Diagnosen/Körperliche Untersuchung“

DZHK-SOP-K-02 „Anamnese/Klinische Diagnosen“

Data will be reported according to the eCRF modules in DZHK-SOP-K-01 and DZHK-SOP-K-02.

7.9.3 DZHK basic data set

For the DZHK basic data set data collection is performed according to DZHK-SOP_K_01 “Basisdatensatz – Anamnese/Klinische Diagnosen/Körperliche Untersuchung”. For baseline visit all mentioned examinations have to be performed. For all other visits a reduced data set will be collected. Items not necessary are marked in the distributed DZHK-SOP_K_01 “Basisdatensatz – Anamnese/Klinische Diagnosen/Körperliche Untersuchung”.

7.9.4 Concomitant heart failure medications

Patients will be under optimal medical therapy, according to the most recent guidelines ESC guidelines (i.e., at present the 2023 updated ESC Guidelines).

7.9.5 Pregnancy Test (for females of childbearing potential)

Recommendation by the Clinical Trial facilitation Group as to contraception and pregnancy testing will be followed (www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf).

Women of childbearing potential will be subjected to urinary pregnancy testing at baseline (visit 2) to confirm eligibility in the trial (see Table 1). During the study period women of childbearing potential must adhere to appropriate contraceptive measures (i.e. low failure rate less than 1% per year; e.g. oral contraceptives, intra-uterine device [IUD] or transdermal contraceptive patch).

The trial treatment cannot be withdrawn after administration. The concomitant immune suppression can be terminated, which will result in IMP rejection.

In case of pregnancy, the pregnancy will be followed up by the sponsor to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence/absence of any birth defects, congenital abnormalities or maternal and newborn complications.

7.9.6 Laboratory assessments

Collection of blood and urine will be according to DZHK-SOP-B01 for obtaining:

- (A) a DZHK Basic Set
- (B) a DZHK Study Set

From the DZHK Study Set following blood tests and urine analysis will be performed:

Blood tests:

- CBC with differentials and platelet count
- CRP, Procalcitonine, IL6
- Liver panel

- Albumin
- Serum Creatinine, BUN, cystatin C
- PTT or PT/INR as applicable
- Plasma free haemoglobin; Iron
- hs-TnT, CK, CK-MB (at the discretion of the clinical PI also if CK is not elevated)
- NT-proBNP
- Blood draw for allograft DNA assessment
- HLA/KIR typing
- Donor specific antibodies (DSA)

Urine analysis:

Pregnancy test

Blood and urinalysis from the DZHK Study Set will be performed by the accredited central laboratories of the participating study sites.

Details on all laboratory procedures, collections, shipment of samples and reporting of results, alerting of extreme values and notable values to the principle investigator will be provided to investigators in the laboratory manual (see appendix 19.3). The central laboratories of the study sites will provide the sponsor with a copy of the laboratory certification and tabulation of reference ranges for the individually measured parameters.

Details on collection and handling of laboratory samples are provided in the laboratory manual/instruction.

7.9.7 ECG

A resting 12-lead surface electrocardiogram (ECG) will be performed at all visits according to DZHK-SOP-K-03. Each ECG tracing will be kept in the source documents at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K03.

7.9.8 Echocardiography

Transthoracic echocardiography will be performed according to DZHK-SOP-K-08. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K08.

7.9.9 Cardiac-CT

Cardiac-CT will be performed at the baseline (Visit 2) and follow-up visits (Visits 8 and 10) according to standard procedures to obtain data on systolic and diastolic heart wall and EHM implant thickness as well as chamber dimensions and thereby assess local as well as global heart contractility. The source data will be documented at the investigational site. Similar data as obtainable by MRI (DZHK-SOP-K06) will be reported in an accordingly modified eCRF module. The standard mean radiation exposure for each CT is 15 mSv. For all three CTs the exposure is 45 mSV.

Patients can still participate, although they selectively decline CT-examinations. In these cases the ejection fraction will be estimated by via echocardiography and cardiac MRI if possible. The primary endpoint (Identification of safe maximal dose) does not depend on heart imaging.

If manifest hyperthyroidism or an allergy to contrast media is found in the course of the study, these patients will be excluded from the CT examination, but will remain in the study.

7.9.10 Chest x-rays

Chest x-rays will be performed at the day of hospital administration (Visit 4) and within 2 h after EHM implantation (Visit 5) as well as 2 h after removal of the chest drainage according to standard procedures. The Visit 4 chest x-rays (posterior-anterior and lateral projections) will be performed optionally if requested by a study physician to exclude acute onset pneumonia. The chest x-rays (anterior-posterior or posterior-anterior projections) after EHM-implantation and chest drainage removal will be performed to detect potential bleeding or pneumothorax complications. The image data will be documented at the investigational site.

7.9.11 Cardiac-MRI

Cardiac MRI will be performed instead of MRI, if feasible and diagnostically valuable images can be anticipated, at the baseline (Visit 2) and follow-up visits (Visits 8,9,10) according to DZHK-SOP-K-06. Patients with devices may not be eligible to MRI investigations or cannot be diagnostically evaluated because of artifacts. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K06.

7.9.12 Cardiopulmonary exercise testing

Spiroergometry will be performed at the baseline (Visit 2) and follow-up visits (Visits 8-10) according to DZHK-SOP-K-07 if possible, i.e., if patients can be subjected to the spiroergometry. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K07. VO2 max will be reported as secondary endpoint.

7.9.13 EuroSCORE II

The surgical risk will be estimated at the baseline visit (Visit 2) using the EuroSCORE II risk calculator and/or logistic EuroScore I if implemented as a standard at the participating study sites (<http://euroscore.org/calc.html>).

7.9.14 INTERMACS

Patients will be stratified according to the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profiles (Alba et al. 2009; Schulze et al. 2022) at baseline (Visit 2) and at follow-up visit 10 (12-month endpoint of the study).

7.9.15 Hand grip strength

Hand grip strength is measured with a hand dynamometer. Maximum hand grip strength correlates well with total muscle mass. Loss in muscle mass or cachexia are associated with poor prognosis. Hand grip strength measurements will be done according to a SOP by the Nutritional Assessment Platform.

7.9.16 6 minute walk test (6MWT)

The 6MWT will be performed at the baseline (Visit 2) and follow-up visits (Visits 8,9,10) according to DZHK-SOP-K-04 if possible, i.e., if patients can be subjected to the 6MWT. The source data will be documented at the investigational site. Data will be reported according to the eCRF module in DZHK-SOP-K04.

7.9.17 Assessment of quality of life and study adherence motivation

The **Quality of life** of the patients will be evaluated using the following Quality of Life Questionnaire:

KCCQ-23 (Kansas City Cardiomyopathy Questionnaire)

The HF-specific KCCQ is a 23-item self-administered questionnaire that quantifies multiple domains such physical limitations, symptom frequency, QoL, and social limitations. These domains can be combined into a Clinical Summary (KCCQ-CS) score including the physical limitation and total symptom score to mirror the NYHA functional class from the patient's perspective, and into an overall summary (KCCQ-OS) score that combines the total symptom, physical and social limitation, and health-related QoL to provide an overall summary of the patients' health status. Scores for each domain range from 0-100 (0=the worst health status; 100=the best health status). The KCCQ-23 has been validated in stable and acute heart failure recovery patients (Spertus and Jones 2015) and full KCCQ has been validated in German heart failure patients (Faller et al. 2005). In the HF population, the KCCQ is independently associated with mortality, hospitalizations, and costs (Chan et al., 2009; Pokharel et al., 2017).

EQ-5D-5L

The EuroQoL five-dimensional (EQ-5D) questionnaire is an instrument for measuring quality of life and determining Quality Adjusted Life Years in health economic studies. It has been validated previously (Hurst et al. 1997) and also found valid in CAD patients (Ellis et al. 2005) and in German depressed patients (König et al. 2005). The EQ-5D-5L covers five dimensions mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, each rated on a 5-point scale and has been used as a valid instrument with acceptable sensitivity to change that can also be used to evaluate cost-effectiveness.

The **study adherence motivation** will be evaluated using the following questionnaires:

Medication adherence

Pharmacological treatment is one of the main interventions for improving outcomes in heart failure (Forsyth et al. 2015), but its effectiveness is limited by the patients' ability and willingness to take their medications regularly. Medication adherence is a result of multiple factors such as cognitive abilities, intentions, social circumstances, behaviors, or interactions with health care providers (WHO, 2003). The range of non-adherence to pharmacological therapy in heart failure was reported from 2% to 90% (Wu et al. 2008). This variation is caused

by differences in methodological approach, such as the definitions and measures of non-adherence, and patient selection criteria within each individual study. There is no 'gold standard' measure for medication adherence. The patient's adherence to medication - regarding the handling of all previously prescribed medicines – will be assessed using a three-item self-report scale. Patients are asked to check the frequency of their general past and present behaviors concerning medication intake described on a three-point Likert scale.

HADS (Hospital Anxiety and Depression Scale)

The HADS (Zigmond and Snaith, 1983) is well validated and has been used extensively to assess symptoms of depression and anxiety in patients with various medical conditions, including heart failure. It is used in BioVAT-HF to cover the two most common psychological symptom areas found in medical patients and affecting both, treatment adherence and quality of life. It comprises 14 items (7 items each for anxiety and depression), with a score ranging between 0 and 21 for the anxiety and depression subscales. Scores between 8 and 10 indicate a moderate presence of symptoms, whereas a score greater than 11 indicates a significant number of symptoms that likely correspond with a clinical diagnosis. In a review of 747 studies in which HADS was used for various purposes, a mean Cronbach alpha of 0.82 for the "anxiety" subscale and 0.83 for the "depression" subscale was described (Bjelland et al. 2002; Petermann 2011; Smith et al. 2007).

MoCA (Montreal Cognitive Assessment)

The MoCa is a validated cognitive instrument that consists of 30 questions and is suitable for detecting mild cognitive impairment and early stages of dementia (sensitivity: 90%; specificity: 87%) (Nasreddine et al., 2005). Cognitive abilities, such as short-term memory, visual-spatial skills, attention, concentration, working memory, language and orientation to place and time are examined. A maximum of 30 points can be achieved, with a total value of ≤ 26 points indicating cognitive impairment (Luis et al., 2009; Nasreddine et al., 2005; Smith et al., 2007).

B-IPQ (Brief Illness Perception Questionnaire)

The IPQ-B is a questionnaire with eight items that records the cognitive perception of illness, the emotional perception of illness and the understanding of illness (Broadbent et al., 2006). To score the B-IPQ questionnaire, each item is rated on a 0-10 scale, with higher scores indicating a more threatening perception of the illness. The total score is calculated by summing the scores of all eight items where items 3, 4, and 7 are inversed. The possible range of scores is 0-80. The B-IPQ is a reliable and valid tool for assessing cognitive and emotional representations of illness in various populations.

TEX-Q (Treatment Expectation Questionnaire)

The TEX-Q is a generic, multidimensional measure to assess patient expectations of medical and psychological treatments (Alberts et al., 2020; Shedden-Mora et al., 2023). The short version consists of 15 items and it aims to record patients' treatment expectations for upcoming heart surgery using a ten-point scale. The TEX-Q assesses expectations of treatment benefit, positive impact, adverse events, negative impact, process and behavioural control with a total of 15 items. Results for the subscales and the sum score indicated good internal consistency ($\alpha = 0.71-0.92$), moderate to high test-retest reliability ($r = 0.39-0.76$).

The KCCQ-23, EQ-5D-5L, HADS, MoCA, B-IPQ, TEX-Q and "Medication Adherence" questionnaires

will be performed during visit 2, 8 and 10.

7.9.18 ICD/CRTD-event recorder readout

ECG with potential arrhythmia events will be monitored by ICD or CRT-D devices with in-build event recorders. Data will be documented at visit 2, 4 and 6-10.

7.10 Additional biological specimen collection

7.10.1 Biological specimen collection for translational program

Biological samples will only be collected during the course of the trial if the patient consents to the DZHK-Biobanking project. Collection of biological samples is not mandatory for participation in the BioVAT-HF-study.

Detailed instructions on sample collection, processing, handling and shipment are provided in the DZHK-SOP-B-02 "Biomaterial Processing Basic Set". Following sample are included in this SOP:

- Serum
- EDTA-Plasma
- Citrate-Plasma
- Urine
- Buffy Coat

7.10.2 Blood samples collection for the assessment of circulating cell-free allograft DNA

In order to analyse if the established technique to detect circulating cell-free DNA will also detect circulating cell-free DNA released from EHM allografts as a sign of EHM rejection, blood samples will be collected during the course of the trial if the patient consents. For assessment of circulating cell-free EHM-allograft DNA, blood samples will be sent after pseudomysation to the Chronix Biomedical GmbH in Göttingen (www.chronixbiomedical.de). At Chronix Biomedical GmbH the detection of unique DNA sequences of the EHM allograft will be attempted and the data returned to the Sponsor for further analysis. No analysis of patient DNA will be performed. The obtained results will not be used for further diagnostics and treatment of the patient. Detailed instructions on the sample collection, processing, handling and shipment are provided in Appendix 19.3.

Collection of these blood samples is not mandatory for participation in the BioVAT-HF-study.

8 Discontinuation criteria

8.1 Premature termination of one of the treatment arms or the entire trial

The sponsor/coordinating investigator is under obligation to monitor the progress of the clinical trial with regard to safety-relevant developments and, if necessary, initiate the premature

termination of a treatment arm or the entire clinical trial. The sponsor/coordinating investigator will be supported in this responsibility by the DSMB, if necessary.

A treatment arm or the entire clinical trial must be terminated prematurely if:

- the benefit-to-risk ratio for the patients changes markedly,
- the sponsor/coordinating investigator OR the DSMB considers that the termination of the trial is necessary,
- indications arise that the trial patients' safety is no longer guaranteed,
- the question(s) addressed in the trial can be clearly answered on the basis of an interim analysis,
- the questions(s) addressed in the trial can be clearly answered on the basis of results of another trial on the same subjects,
- an insufficient recruitment rate makes a successful conclusion of the clinical trial unrealisable/no longer feasible.

If the clinical trial is prematurely terminated or suspended for any reason, the investigator should promptly inform the trial patients and ensure appropriate therapy and follow-up for the patients. Where required by the applicable regulatory requirements, the CA(s) and the IEC(s) will also be informed (this is usually done by the sponsor).

8.2 Premature termination of the trial at one of the trial sites

Both the investigator and the sponsor have the right to terminate the trial at one of the sites.

The clinical trial can be terminated prematurely at his site by the investigator if, for instance unforeseeable circumstances have arisen at the trial site which preclude the continuation of the clinical trial, the investigator considers that the resources for continuation are no longer available, the investigator considers that the continuation of the trial is no longer ethically or medically justifiable.

The sponsor/coordinating investigator can initiate the exclusion of a site from further participation if, for instance, patient recruitment is inadequate, serious problems arise with regard to the quality of the collected data which cannot be resolved.

Premature termination at one of the trial sites does not automatically mean a termination of the trial for already enrolled trial patients. A separate decision on further treatment must be made for each patient, depending on the overall situation. Adequate further treatment and follow-up of already enrolled trial patients must be ensured. The documentation of already enrolled trial patients will be reviewed for completeness and plausibility. Queries may be raised for further clarification before the site is closed. These queries must be answered properly by the site. The CA(s) and IEC(s) must be duly notified of the site's closure, including reasons, within the specified period. The trial site concerned will be closed in stages by the CRA when a decision has been made on the further treatment of the patients concerned.

8.3 Discontinuation of trial treatment or trial participation for individual patients

It has to be distinguished if trial treatment of a patient has been stopped prematurely (by withdrawal of immune suppression or surgical removal of the EHM implant) or if the trial participation of a patient was stopped prematurely.

In the case trial treatment of a patient has been stopped prematurely, further follow-up visits and the assessment of the trial endpoints are essential to enable an analysis of the full analysis set according to the intention-to-treat principle. Further visits, follow-up and documentation should always be striven for/ensured in this case. This includes the follow-up of AEs, the time of termination, the results available at that time and, if known, the documentation of the termination of treatment on the eCRF and in the medical record, giving reasons, a final examination and documentation according to the protocol (if possible).

In the case trial participation of a patient was stopped prematurely, the conduct of further follow-up visits is no longer possible. The documentation should be completed as far as possible under these circumstances, e.g. a final examination and documentation according to the protocol (if possible), a documentation of the premature trial termination on the CRF and in the medical record, giving reasons, appropriate further treatment and follow-up outside the trial should be ensured; inform general practitioner of the termination, if necessary (provided that the patient agrees). In studies that assess the survival status, an attempt should at least be made to assess the patient's survival status by telephone follow-up (unless informed consent for documentation has been withdrawn).

8.3.1 Premature discontinuation of trial treatment

After implantation the EHM patch can be removed by a discontinuation of immunosuppressive treatment or by surgical excision in case of unwanted effects.

8.3.2 Premature termination of trial participation

The trial patient can withdraw his/her consent at any time, without having to give reasons, and have his/her entire trial participation terminated prematurely. However, the prerequisite for this is that the patient actively terminates trial participation by withdrawing his/her consent for the follow-up and documentation.

The responsible investigator may only withdraw a patient from participation in the trial for the following reasons:

Extreme circumstances arise which make any trial-relevant follow-up impossible (see section 6.2)

9 Investigational medicinal product (IMP)

9.1 Engineered Human Myocardium (EHM) background information

Engineered Human Myocardium (EHM) in a clinically translatable format was introduced in 2017 (Tiburcy et al., 2017). The production protocol was optimized for GMP production and application as BioVAT. The EHM protocol originates from ~30 years of preclinical development (Didie et al., 2013; Fujita and Zimmermann, 2017a, b, 2018; Riegler et al., 2015; Tiburcy et al., 2017; Zimmermann et al., 2006). Jebran et al. 2024 (in press)

9.1.1 Preclinical data

Feasibility, safety, and efficacy of tissue engineered heart repair was thoroughly investigated in 3 preclinical animal species (rat, mouse, and Rhesus macaque):

- 1) Rat model with uncompromised heart function (Zimmermann et al., 2002)
- 2) Rat model of chronic (severe) heart failure after permanent LAD occlusion (Zimmermann et al., 2006)
- 3) Mouse model of acute myocardial infarction by permanent LAD occlusion (Didie et al., 2013)
- 4) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Riegler et al., 2015)
- 5) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Qin et al., 2016)
- 6) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (Tiburcy et al., 2017)
- 7) Rat model of chronic (mild) heart failure after ischemia/reperfusion injury (extension of Tiburcy et al., 2017 with focus on EHM patch retention; data presented in IMPD)
- 8) Rhesus macaque model with uncompromised heart function (Jebran et al. 2024 [in press]; data presented in IMPD)
- 9) Rhesus macaque model with post-myocardial infarction chronic heart failure (Jebran et al. 2024 [in press]; data presented in IMPD)

Additional pilot studies were performed in pig models, but found to be of no predictive value because of limited xenograft retention, despite administration of comprehensive immune suppression regimens.

These studies collectively provide the rationale for the BioVAT-HF trial. Study 1 demonstrated feasibility and safety of Engineered Rat Myocardium (ERM) implantation under immune suppression in a healthy rat model. Study 2 demonstrated electromechanical integration, safety and efficacy of ERM allografts in a rat model of chronic heart failure. Safety and efficacy was further confirmed in a mouse model of subacute myocardial infarction and the application pluripotent stem cell-derived Engineered Mouse Myocardium (EMM) allografts (Study 3). Studies 2 and 3 employed MRI and echocardiography to document efficacy of ERM and EMM allograft-based heart repair by detection of an enhancement of thickness and contractility of the target heart wall, in line with the proposed mode of action. Studies 4-7 established and validated a human GMP-compatible EHM as well as retention upon implantation. In a pivotal Rhesus macaque allograft study (study 8), a Engineered Non-Human Primate Myocardium (ENHPM) graft dose dependent increase in target heart wall thickness by approximately 1 and 5 mm was observed as a result of the implantation of 1x and 5x ENHPM assemblies, respectively, in line with the observations from allograft studies 2 and 3. The augmentation of the target heart wall was sustained for the whole study duration (investigated for up to 6 months in the 5x ENHPM group) with no evidence for unwanted effects (no arrhythmia, no tumor, no perturbation of heart performance, no immune suppression related side effects). In an extension of study 8, ENHPM (2X ENHPM and 5x ENHPM) were safely implanted into Rhesus macaque with chronic heart failure. Similar as in study 8 (ENHPM in healthy Rhesus macaques), a sustained augmentation of the target heart wall was observed for the whole study duration (6 months) with no evidence for arrhythmia, tumor formation, perturbation of heart performance, and immune suppression related side effects. Whereas ENHPM target heart wall contractility was unaffected in control animals enhanced target heart wall thickening fraction and of left ventricular ejection fraction was observed

suggesting a contribution of the ENHPM patch not only to local, but also to global heart function (Jebran et al. 2024 [in press]).

The preclinical data is in agreement with the data obtained from 13 patients in Part A of BioVAT-HF.

9.1.2 Pharmacokinetics

Not applicable.

9.1.3 Pharmacodynamics

We considered the 5x EHM assembly as minimal effective dose (MED) for the BioVAT-HF trial and anticipated a similar thickening of the target heart wall as observed in the Rhesus macaque study, improved target heart wall thickening fraction, and improved left ventricular ejection fraction as observed in the extension of the pivotal Rhesus macaque trial. The dose finding study confirmed that 20 x EHM assemblies thicken the human heart similarly as 5x ENHPM assemblies in the Rhesus macaque.

Considering the differences in heart and body size in Rhesus macaque and human (10-fold for heart weight and 2-fold for heart wall thickness) and applying allometric scaling a 5x EHM assembly in Rhesus macaque resembles a 50x or 10x EHM assembly in human. At a 20x EHM dose no graft related safety concerns and signs for efficacy were observed in patients with advanced heart failure.

9.1.4 Adverse reactions

Arrhythmia (in case of irregular electromechanical integration), tumor formation (in case of the contamination with pluripotent stem cells), perturbation of heart performance (in case of compression or stiffening of the target heart wall), and immune suppression related side effects may be anticipated. Preclinical studies did not provide evidence for clinically relevant adverse reactions (hyperglycaemia was observed as a common side effect of tacrolimus without a need for antidiabetic treatment). BioVAT-HF Part A data did not find EHM-related adverse reaction. Immune suppression related kidney damage, a common and anticipated adverse reaction to calcineurin inhibitors was observed in some patients. Reduction in Tacrolimus levels (Part A 5-15 ng/ml to Part B 3-10 ng/ml trough levels) and a replacement of Tacrolimus with the mTOR inhibitor Everolimus in case of evidence for kidney damage is introduced as mitigation strategy in accordance with the most recent guidelines of the ISHLT for immune suppression in patients with heart transplantation (Velleca et al., 2023).

9.2 IMP pharmaceutical characteristics

The IMP used in this trial is characterised as follows, according to the applicable IB:

Proprietary name:	Engineered Human Myocardium (EHM)
Name of substance:	Human heart muscle comprised of iPSC-derived cardiomyocytes and stromal cells supported to self-organize into heart muscle with

	structural, functional, and molecular properties of juvenile myocardium by a bovine collagen type I hydrogel environment
Manufacturer:	University Medical Center Göttingen
Approved indications:	IMP not yet approved
Dosage form:	Refer to Figure 1
Strength:	Not applicable
Total daily dose:	Not applicable

For further characteristics, see current version of the corresponding IB.

9.3 Packaging and labelling

EHM is packaged as single patch mounted on a holder in a screw container filled with 50 ml transport medium. EHM will be delivered directly to the point-of-care at the required quantity.

Medication labels will be in German and comply with GMP Annex 13 and legal requirements in Germany.

9.4 Supply and ordering

EHM will be ordered from the University Medical Center Göttingen. EHM will be ordered 1 - 4 months prior to implantation by E-mail to the following address:

BioVAT-HF.Bestellung@med.uni-goettingen.de

9.5 Receipt and storage

EHMs will be delivered as individual patches in a sterile screw-cap container to the point-of-care. After receipt, EHM will be left in the unopened screw-cap containers at room temperature until implantation on the day of delivery.

Delivery of the EHM patch to the point-of-care is further described in a separate protocol.

9.6 Preparation of EHM patch

EHMs will be delivered as individual patches in sterile screw-cap containers to the point-of-care. Upon opening the screw-cap container at the point-of-care under aseptic conditions, EHM mounted on stretch devices with transport medium will be transferred into a container (e.g., a kidney dish). EHM will then be released from the stretch devices using a custom-made fork and transferred into a second container (e.g., a kidney dish) filled with Ringer Acetate solution (200 ml) for a first washing step using a custom-made shovel. After 5 min EHM will be transferred into a third container (e.g., a kidney dish) filled with Ringer Acetate solution (200 ml) for a second washing step (5 min) using a custom-made shovel and subsequent assembly into the desired EHM doses in custom-made assembly devices. Assembled EHM will be sutured onto a TachoSil™ membrane and subsequently transferred onto the heart. EHM should be implanted within 30-60 min after opening of the screw container.

Preparation of EHM patch is further described in a separate protocol.

9.7 EHM Implantation

EHM assemblies will be sutured to the target left ventricular heart wall via a left lateral thoracotomy. The surgical access route and fixation procedure is similar to the proceeding in epicardial pacemaker lead implantations. After the thoracotomy the pericardium is opened horizontally anterior to the phrenic nerve allowing a full access to the anterior and lateral wall of the left ventricle. The EHM patch is then fixed to the target left ventricular wall using 5/0 prolene sutures. Hereafter, the pericardium is closed with interrupted sutures and a chest tube is inserted into the left thoracic cavity to evacuate blood, air or pleural effusions, which may occur in the first postoperative days. To detect these adverse events a thoracic X-ray (anterior-posterior projection) will be performed within 2 h after the operation. The chest tube is usually removed within 3 days after surgery. Removal of the chest tube can cause bleeding or pneumothorax. Therefore, a thoracic X-ray will be performed ~2 h after removal of the chest tube (anterior-posterior or posterior-anterior projection). The standard mean radiation exposure for each X-ray is 0,05 mSv.

The implantation on the right ventricle takes place during a planned surgical procedure with a medial thoracotomy.

The implantation of a heart patch does not require the support of a heart-lung machine. Accordingly, the left ventricular application to the beating heart is carried out without the use of a heart-lung machine. In case of an implantation onto the right ventricle, the use of a heart-lung machine may be obligatory because of the primary elective intervention on the left ventricle. In the case of right ventricular implantation as an add on to an elective left ventricular procedure, patients will firstly be asked for informed consent as to the elective surgical intervention and secondly asked for informed consent as to the concomitant EHM implantation to support the right ventricle.

The study-related operating time from induction of anaesthesia to weaning from anaesthesia is ~120 minutes for left ventricular surgery and an anticipated ~30 minutes of additional operating time for right ventricular surgery. No patch implantation associated risk is assumed.

EHM implantation is further described in a specific protocol depicting the surgical procedures.

9.8 Return and destruction

Unused IMP can be destroyed at the study site.

9.9 Drug compliance and accountability

The investigator or designee must maintain records of the delivery of the IMP, the use in individual trial patients, and the disposal of unused IMP(s). The investigator must ensure that the IMP is only used according to this protocol.

The investigator bears the responsibility for the proper storage in an appropriate place to which unauthorised persons have no access.

The investigator may only use the IMP for implantation in patients who have been enrolled in the study. Usage of the IMP for implantation in patients outside of this clinical trial is not permitted.

The investigator or designee should explain the IMP implantation to each trial patient and check at regular intervals whether the IMP has been rejected or not.

The investigator should take notice of the IMP Handling Manual, if applicable.

9.10 Treatment adherence

Not applicable as EHM patch is surgically implanted and cannot be removed by the patient.

10 Safety monitoring and reporting

10.1 Adverse Events (AEs)

10.1.1 Definition of AEs

An adverse event (AE) is any untoward medical occurrence in a patient administered any dose of a pharmaceutical product and which does not necessarily have to have a causal relationship with the use of the product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not related to the product.

- In order to monitor the conditions of the patients from the time the patients receive the first dose of immunosuppressive medication the investigator is requested to report any untoward clinical event on the AE-page of the eCRF. Any untoward medical occurrence, which occurs after the period of patient follow-up defined in the protocol, is not considered an AE.
- Irrespective of any causal relationship, all AEs spontaneously reported by the patient or observed by the investigator will be continuously documented in the medical record and on the designated case report form (AE eCRF page).
- All AEs must be described by diagnosis or, if an underlying diagnosis is not known, by symptoms or medically significant laboratory or instrumental abnormalities. The AEs will be documented as shown in section 10.1.2. Please note that medical or surgical procedures (e.g., tooth extraction, transfusion, surgery) performed are not AEs *per se*; the medical condition that leads to the procedure is an AE.
- Symptoms, medically significant laboratory, or instrumental (e.g. electrocardiographic) abnormalities of a pre-existing disease are not to be considered an AE. Occurrences of new symptoms or laboratory or instrumental abnormalities, as well as worsening of pre-existing ones, are considered AEs.
- All AEs, no matter how intense, are to be followed up by the investigator in accordance with ICH-GCP until resolved or judged no longer clinically relevant, or in case of a chronic condition, until it is fully characterised.
- Overdose without clinical sequelae is not to be considered an AE. For the purposes of this study, an overdose is defined as a single dose of IMP that exceeds the prescribed dose for each age range.

10.1.2 Documentation of AEs

Adverse events have to be documented in the eCRF starting from first intake of immunosuppressive medication (Visit 3) until the last study visit (Visit 10).

The following data need to be documented:

- Characterization of the event (diagnosis; if not available, symptoms)
- Onset date / date of resolution
- Severity of event ("mild, moderate, severe, life-threatening or fatal")
- Relationship to the IMP(s) (related/not related), The expression "related" means, that there is evidence or argument to suggest a reasonable causal relationship between the event and the administration of the study drug, e.g. close temporal connection, exclusion of other causes. The assessment "not related" is appropriate, if the AE is clearly or most likely explained by other causes even if a potential relationship between study drug and the AE cannot be completely excluded.
- Serious / non-serious
- Action taken with regard to IMP (continued/stopped/interrupted)
- Outcome of AE

10.2 Serious Adverse Events (SAEs)

10.2.1 Definition of SAEs

A Serious Adverse Event (SAE) is any AE that at any dose

- results in death,
- is life-threatening,
- requires hospitalization or prolongation of existing hospitalization (excluding those for study therapy and elective or pre-planned treatment/surgery)
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect,
- other medically important event: events that may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered as 'serious' in accordance with the definition and have to be reported as SAE.
- In addition, cases of misuse, abuse, medication error, overdose of study medication, pregnancy and product deficiencies should also be reported like SAEs (please refer to section 10.2.3.4, 10.2.3.5, and 10.2.3.6).

Please note:

- term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

10.2.2 Documentation of SAEs

All SAEs (with the exception of the special situation described in section 10.3.1.2) starting from first administration of immunosuppressive concomitant medication until the patient's last study visit (patient's individual study end date) will be documented in the eCRF and on the provided SAE reporting form.

The SAE reporting form will be processed as described in the section below.

10.3 Investigator reporting requirements

10.3.1.1 Reporting policy

The following events must be reported by investigator to the sponsor:

- SAEs
- Pregnancies (10.2.3.4)
- Special situations (10.2.3.5)
- Product deficiency / complaint concerning study medication (10.2.3.6)

The events above must be reported by fax to the following address within 24 hours after knowledge by the investigator:

Pharmacovigilance
Clinical Trials Unit
Medical Center - University of Freiburg
Elsaesser Str. 2, 79110 Freiburg
SAE Fax No.
+49 761 270-74 390

If only limited data are initially available, a follow-up report is required. If new information including outcome becomes available or e.g. relationship to IMP is reconsidered, a SAE follow-up report should be sent within 24 hours using the same procedure as for transmitting the initial SAE report (details will be provided in SAE reporting manual).

10.3.1.2 Specific protocol exceptions to expedited SAE reporting

As this trial involves patients suffering from severe heart failure (NYHA Class III or IV) associated with significant mortality/morbidity, and as the frequency of recurrent hospitalizations for worsening of heart failure is one of the secondary endpoints (i.e. anticipated clinical outcomes) thoroughly collected on the specific eCRF pages and taking into consideration recommendations of the CIOMS working group VI concerning management of safety information from clinical trials, the following events have not to be notified to the sponsor as SAEs:

“worsening of heart failure”, except if the investigator evaluates worsening of heart failure as being related to the IMP, this case has to be reported to the Sponsor as a SAE;

Hospitalization due to study therapy and/or assessments, placement of an indwelling catheter, social/convenience admissions, respite care, elective or pre-planned treatment/surgery

10.3.1.3 Reporting of patient death

According to 10.2.1 "Definition of SAEs", "Death" is always considered an SAE and needs to be reported to the sponsor. Please note that "death" is usually an SAE outcome, only in cases where the clinical circumstances before the death are unknown (i.e. patient died without a determinable cause of death), then the diagnosis "death" itself should be reported as an SAE. In case of fatal outcome of an already-registered SAE, a follow-up notification must be done.

In case of patient's death the investigator must submit on demand all information to the competent IEC, the other IEC(s) involved, the CA and the sponsor, that is required for the fulfilment of their duties (note that personal data must be transmitted using the trial-specific patient identification number, i.e. in pseudonymised form).

10.3.1.4 Reporting of Pregnancies

Any pregnancy (female trial participant or female partner of male trial participant) that occurs during trial participation must be reported. To ensure patient safety each pregnancy must be reported to Pharmacovigilance CTU on the pregnancy reporting form within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence/absence of any birth defects, congenital abnormalities or maternal and new-born complications.

10.3.1.5 Reporting of Special situations

In addition to serious adverse events, the following special situations which affect the safety of study participants have to be reported to the sponsor without undue delay. For reporting, please refer to EMA Guideline on Good Pharmacovigilance Practices, Annex I, defines these special situations as follows:

Medication error

An unintended failure in the drug treatment process that leads to, or has the potential to lead to, harm to the patient.

Misuse of a medicinal product

Situations where a medicinal product is intentionally and inappropriately used not in accordance with the terms of the marketing authorisation.

Abuse of a medicinal product

Persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects [DIR 2001/83/EC Art 1(16)].

Overdose

Administration of a quantity of a medicinal product given per administration or cumulatively which is above the maximum recommended dose according to the authorised product information. When applying this definition, clinical judgement should always be applied.

10.3.1.6 Reporting of product deficiencies / complaints concerning study medication

Product deficiencies or complaints about study medication, i.e. IMP quality or labelling, are reported in the same way as SAEs, pregnancies or special situations.

10.4 SUSARs

The sponsor's reporting requirements are divided into expedited reporting and reporting that must be performed on request or annually.

10.4.1.1 Definition of SUSARs / Reference safety information

The sponsor's expedited reporting requirements are particularly relevant to suspected unexpected serious adverse reactions (SUSARs). SUSARs are all SAEs that are considered as both possibly related to study medication by investigator or sponsor and which are unexpected based on the information provided by the reference safety information (RSI).

As this is the first in man clinical study, all SAEs judged by the investigator to be related to the IMP will be classified as a SUSAR.

10.4.1.2 Reporting of SUSARs

The sponsor has to report SUSARs without delay electronically to EudraVigilance according to instructions given in EU Individual Case Safety Report (ICSR) Implementation Guide in an expedited fashion to EMA and investigators.

The sponsor's expedited reporting requirements comprise the following:

All SUSARs that are life-threatening or result in death must be reported within 7 days after sponsor knowledge, all other SUSARs must be reported within 15 days after sponsor knowledge.

The information contained in SUSAR reporting to EudraVigilance will obey CTR (EU) 536/2014 Annex III 2.3. The details on the implementation of these requirements are described in a study specific SAE-manual.

10.5 Reporting of Serious breaches and urgent safety measures

According to Art. 52 of the Regulation (EU) No 536/2014, a serious breach is defined as any breach of the Regulation EU No 536/2014 and/or the version of the clinical trial protocol applicable at the time of the breach which is likely to affect to a significant degree the safety and the rights of a subject and/or the reliability and robustness of the data generated in the clinical trial.

According to Art. 54, urgent safety measures are taken by the sponsor when an unexpected event is likely to seriously affect the benefit-risk balance of the study.

When the trial site identifies a critical non-conformity which might be or might result in a serious breach or urgent safety measure, the trial site is responsible to inform the sponsor of the clinical trial immediately at:

E-mail: sz-umg.sponsor-qm@med.uni-goettingen.de

Phone: + 49 (0) 551 39 60812

The sponsor is responsible to perform a risk assessment and to notify the competent authorities of the Member States concerned via CTIS without undue delay but not later than seven days after becoming aware of that breach in case the reported critical non-conformity is considered a serious breach or an urgent safety measure.

According to Art. 53, unexpected events which affect the benefit-risk balance of the clinical trial, but are not SUSARS, have to be reported within 15 days by the sponsor.

10.6 Development Safety Update Report (DSUR)

In addition to the expedited reporting, the sponsor shall submit through CTIS an annual report once a year or on request throughout the clinical trial period, according to ICH guideline E2F. The aim of the DSUR is to concisely describe all new safety information relevant for one or several clinical trial(s), to assess the safety conditions of subjects included in the concerned trial(s) and to evaluate whether the benefit / risk ratio is still favourable.

11 Data handling and data management

11.1 Data confidentiality

Information about trial patients will be kept confidential and managed under the applicable laws and regulations. Those regulations require a signed patient authorization informing the patient of the following:

- what protected health information (PHI) will be collected from patients in this trial;
- who will have access to that information and why;
- who will use or disclose that information;
- the rights of a research patient to revoke their authorization for use of their PHI.

In the event that a patient revokes authorisation to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For patients that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the patient is alive) at the end of their scheduled trial part.

The data collection system for this trial uses built-in security features to encrypt all data for transmission in both directions, preventing unauthorised access to confidential participant information. Access to the system will be controlled by individually assigned user identification codes and passwords, made available only to authorised personnel who have completed prerequisite training.

11.2 Documentation of trial data

11.2.1 Documentation in medical records

The investigator will record the participation in the trial, the frequency of the trial visits, the relevant medical data, the concomitant treatment and the occurrence of adverse events in the medical record of each trial patient.

11.2.2 Documentation in eCRF

An electronic data capture (EDC) system will be used in this trial (called e-CRF). All data collected during the trial will be entered on the trial-specific e-forms by the responsible investigator, or designated person, as timely as possible. Data entry and data corrections on e-forms are automatically tracked in the audit trail created by the EDC system.

Data corrections in the e-CRF due to queries are performed by the responsible investigator, or designated person, as timely as possible.

11.3 Data management

Data handling

The Department of Medical Informatics at the University Medical Centre Göttingen manages the medical data for the DZHK (Data Handling). For this purpose, the secuTrial® system is being provided in which electronic case report forms (eCRF) are being modeled. The DZHK aims to standardise data collect on amongst its clinical studies in order to enable secondary use of the data across studies. Therefore, Standard Operating Procedures (SOPs) as well as data capture modules for common cardiological assessment procedures are available. The DZHK item catalogue comprises all currently standardised data capture modules.

For future expansion of the BioVAT-HF study as a multinational-regional clinical trial according to ICH E17 it might be necessary to transfer data to third party countries (e.g., USA).

secuTrial

Data capture using secuTrial software is achieved by entering the items queried during collection using the eCRFs created and provided by the Data Handling (DH) unit. With secuTrial it is possible to conduct multicentre clinical studies and post-marketing studies. secuTrial allows direct, decentralised electronic capture of study data (remote data entry) in a central database. Operation of secuTrial is completely browser-based, which means it is not necessary to install software for either management or data capture. Authorised users can define the studio setup, manage participants, export data and enter patient data from any internet-enabled PC. Within the application there is both a separate test area (setup) and a productive area. This means that prior to the start of the actual study or in the case of changes implemented after the go-live, any function can be tested before it is unlocked for users. The tested study setup can go live after it has successfully completed testing. The changes are subject to constant version control. secuTrial complies with all regulatory standards (CRF, GCP) and is certified for all FDA-compliant functions, such as audit trail, a roles and rights concept and electronic signature.

Data Collection

Although the data collection process runs parallel to the data capture process in most cases, this is not absolutely necessary from a technological point of view. The collected data can initially be stored intermediately on printed questionnaires. The collected data is captured in the eCRFs provided and is usually captured by a study nurse or study doctor. The medical data (MDAT) and identifiable information (IDAT) are separated before data collection. This is achieved by incorporating an IDAT input mask provided by the Independent Trusted Third Party (TTP) before

the MDAT is actually entered. The IDAT is entered directly on the TTP servers. This tunnelling ensures that the IDAT is at no time known to the secuTrial system.

secuTrial also implements query management within studies, registers and cohorts. Queries are designed to help monitors and other authorized users to investigate unclear entries. Study doctors and study nurses can read, review, answer (if necessary) and close queries. This process can also only be accessed by users who are set up in the system.

Data Management

In addition to providing suitable capture tools, another core task of the Central Data Management unit is the long-term storage and management of the collected data. A non-bypassable audit trail is created for the corresponding dataset as early as the data capture stage. This audit trail saves which person (login information in secuTrial) with which role (derived from the login information) conducted which operation at which time, on which date and on which dataset. This allows consistent version control and traceability for all amendments. The aforementioned query management is also recorded in its entirety in the audit trail. The audit trail offers an overview of all changes made to the data and saved in the up-to-date form. It can be accessed after the form has been saved for the first time. Entering and saving comments, conducting and answering queries, conducting (Source Data Verification) SDV, reviews and form-locking actions, and ending data capture are all storage processes for the respective form. For this reason, all these actions are illustrated in the storage history in the upper part of the audit trail. Every storage operation documents the current project version so that changes to the project setup can be traced here as well. It also displays whether an e-signature was used for saving and if it is still valid.

Details on data management (e.g., software, procedures, responsibilities) will be described in a data management plan prior to the trial. During the trial, the performance of data management and any deviations from the data management plan will be documented in a data management report. Technical specifications of the trial data base and all data checks will be documented in a data validation plan.

The trial data base has been fully validated before any data entry will be performed. Data entry personnel will not be given access to the trial data base until they have been trained. An audit trail provide a data history which data were entered, changed or deleted, by whom and when.

11.4 Data coding

Concomitant treatments or procedures entered into the database will be coded using the WHO Drug Reference List.

Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

12 Quality assurance

The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted, data are generated, documented, and reported in compliance with the protocol, ICH-GCP, and the applicable regulatory requirement(s).

12.1 Monitoring procedure

Monitoring is performed by the CRAs of the CTU, University Medical Center Göttingen. Risk-based monitoring will be done according to ICH-GCP E6 and standard operating procedures (SOP) to verify that patients' rights and wellbeing are protected, reported trial data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with the currently approved protocol/amendment, with ICH-GCP and with the applicable regulatory requirements to ensure safety and integrity of clinical trial data.

The investigator will accept monitoring visits before, during and after the clinical trial. Prior to the trial, a site initiation visit at each site is conducted in order to train and introduce the investigators and their staff to the trial protocol, essential documents, handling of IMP and related trial specific procedures, ICH-GCP and national/local regulatory requirements.

During the trial, the CRA will visit the site regularly to monitor recruitment rate and quality of data. During these on-site visits, the CRA verifies that the trial is conducted according to the trial protocol, trial specific procedures, ICH-GCP and national/local regulatory requirements. The presence of signed informed consents, eligibility of patients, primary endpoint, handling of IMP and documentation/reporting of safety data (e.g., AE/SAE) will be verified by the CRA. The CRA performs also source data verification and drug accountability to ensure that the clinical trial data which are recorded in the source data and CRFs are complete and accurate. Extent of source data verification and monitor visit frequency will be adapted for individual sites in case of lack of data quality or a high number of protocol violations. All trial specific monitoring procedures, monitoring visit frequency and extent of SDV will be predefined in a trial specific monitoring manual. The investigator must maintain source documents for each patient in the trial, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments (see section 7). All information recorded on CRFs must be traceable to source documents in the patient's file as defined in section 11.2. The investigator must also keep the original signed informed consent form (a signed copy is given to the patient).

The investigator must give the CRA access to all relevant source documents to confirm their consistency with the CRF entries.

12.2 Source data verification (SDV)

Source data as defined by ICH-GCP include original documents, data, and records such as hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial.

12.3 Auditing procedures and inspections

According to the ICH-GCP guidelines, audits may be performed as a quality measure. Audits may be conducted by the sponsor or an independent external party, inspections by CA(s).

The investigator needs to inform the CTU immediately of an inspection requested by a regulatory authority. The investigator is responsible for providing / giving access to source data/documents to auditors/inspectors.

13 Biostatistical planning and analysis

Before the final analysis (see section 13.6) a detailed statistical analysis plan (SAP) will be prepared. This will be completed before data base lock, at the latest. If the SAP contains any changes to the analyses outlined in the trial protocol, they will be marked as such, and reasons for amendments will be given.

All statistical programming for analysis will be performed with the Statistical Analysis System (SAS) or the statistical software package R.

13.1 Trial design

For details on trial design see section 3.1 of the protocol.

13.2 Objectives and endpoints

For details on endpoints see section 2 of the protocol.

13.3 Sample size calculation

The required number of patients to be enrolled results as a total of the numbers needed for the three parts of the trial. A maximum of n=18 patients in dose finding cohorts of n=2-4 patients is planned for the first part, the Dose Finding stage. This number of patients is not uncommon for phase I dose-escalation studies and can be justified through simulation studies. In the second part, the Refinement/Expansion stage, n=35 patients in two cohorts (min. 5 per LV and RV cohort) are needed and will focus on the most feasible, safe, and effective approach. In the Refinement/Expansion stage, sample size planning is based on the primary endpoint heart wall thickness and systolic thickening fraction. In a pre-post comparison of means a sample size of 30 patients yields a power of 80% (90%) to detect a standardized mean difference (Cohen's d) of 0.47 (0.55) at a two-sided significance level of 10% given α . As this is an early study to evaluate trends the slightly larger than usual significance level is justified (Kianifard and Islam, 2011). A number of n=35 patients are needed for the second part. Summing up a total n=53 patients have to be enrolled in the entire study. Calculations were done using the statistical software nQuery (version 9.2.1.0).

13.4 Definition of populations included in the analyses

Primary analysis will be performed on all patients with complete observations at baseline and complete or imputed observation at month 12. This will be referred to as the intention-to-treat population (ITT). If not mentioned otherwise, all analyses are based on the ITT population. Imputation will be performed using predictive mean matching.

Sensitivity analysis will be performed on all patients with complete observations at baseline and month 12. This will be referred to as the per-protocol (PP) population.

A CONSORT (consolidation standards of reporting trials) flow chart will be provided to report disposition of patients. Safety of patients and interim analyses will be supervised by the DSMB.

13.5 Methods of analysis

13.5.1 Patient demographics/other baseline characteristics

Demographic and other baseline data (including disease characteristics) will be summarised descriptively using the FAS.

Continuous data will be summarised by arithmetic mean, standard deviation, minimum, 25% quantile, median, 75% quantile, maximum, and the number of complete and missing observations. If appropriate, continuous variables will also be presented in categories.

Categorical data will be summarised by the total number of patients in each category and the number of missing values. Relative frequencies are displayed as valid % (number of patients divided by the number of patients with non-missing values).

13.5.2 Trial medication

Numbers of patients in the dose cohorts of the Dose Finding stage of the trial with corresponding dose levels will be reported.

13.5.3 Concomitant medication

The concomitant medications will be summarised by ATC level 1/3/5. In each table, patients will be counted once, if they took at least one medication from the respective ATC level. The number of patients and the percentage of the total number of patients in the respective population will be given.

13.5.4 Primary endpoint

Part A investigated safety at dose levels of 5x (2 ± 0.1 million cells/kg body weight; n=2), 10x (4 ± 1.6 million cells/kg body weight; n=2) and 20x (10 ± 2 million cells/kg body weight; n=9) EHM as primary endpoint. Efficacy data was obtained for information. Part B design is informed by the Part A experience, which resulted in efficacy endpoint adjustment. Accordingly, we will obtain structural, functional and symptomatic using in the patient population of BioVAT-HF robustly applicable measures. This is:

- (1) measurement of target heart wall thickness (structural endpoint) by echocardiography supported by cCT and cMRI to confirm EHM graft retention and target heart wall augmentation if possible
- (2) measurement of left and right ventricular ejection fraction (functional endpoint), depending on the EHM implantation site, by echocardiography supported by cCT and cMRI to confirm EHM graft contribution of heart function

(3) measurement of patient reported outcome (symptomatic endpoint) by KCCQ-23

Primary efficacy analyses are based on the changes between baseline and 3 months, 6 months and 12 months after implantation. To test for a time-effect a linear mixed model will be employed for each of the three primary endpoints. Due to the explorative character of the efficacy analysis testing will be performed at a 10% two-sided significance level. Mean differences will be reported along with 90% confidence intervals.

13.5.5 Secondary endpoints for efficacy

The key secondary endpoint (**frequency of recurrent heart failure hospitalizations**) will be compared to data collected from patients in the VAT-registry waiting for EHM implantation and tested at a two-sided 10% significance level as well due to the explorative character of the trial.

Functional status

The functional status is measured by three endpoints

- Six-minute walk test (6MWT)
- Hand-grip strength
- Cardiopulmonary stress testing

Changes over time will each be analysed with a generalized linear mixed model comparing measurements at baseline and 2 weeks, 1 month, 3 months, 6 months and 12 months after implantation. Subsequent Dunnett-type pairwise comparisons to baseline may be performed in case of significant ANOVA results. Effects will be reported with 90% confidence intervals.

Patient reported outcomes

Change in patient reported outcomes is assessed by

- Quality of life score (KCCQ-23, EQ-5D-5L)
- Compliance determining score (HADS, MoCA, B-IPQ, TEX-Q, Medication adherence)
- NYHA classification

KCCQ-23 (23 items), EQ-5D-5L (5 items), HADS (14 items), MoCA (30 items), and medication adherence changes over time will each be analysed with a generalized linear mixed model comparing measurements at baseline (visit 2) and 6 months (visit 8) and 12 months (visit 10) after implantation. Subsequent Dunnett-type pairwise comparisons to baseline may be performed in case of significant ANOVA results. Mean differences will be reported along with 90% confidence intervals. B-IPQ (8 items) and TEX-Q (15 items) information will be obtained at the baseline visit to characterize the BioVAT-HF patient population.

NYHA classification change over time will be analysed giving proportions of patients with NYHA Class I/II at time points baseline and 1 month, 3 months, 6 months and 12 months after implantation. Proportions are accompanied by 90% confidence intervals.

Time to Mechanical Assist Device implantation

Time to mechanical assist device implantation will be displayed showing Kaplan-Meier curves with either pointwise 90% confidence intervals or 90% confidence bands and, if a sufficient number of events occurred, analysed using a Cox proportional hazards model. Data will be compared the data from patients included in the VAT-registry that were no yet treated.

Time to heart transplantation

Time to heart transplantation will be displayed showing Kaplan-Meier curves with either pointwise 90% confidence intervals or 90% confidence bands and, if a sufficient number of events occurred, analysed using a Cox proportional hazards model. Data will be compared the data from patients included in the VAT-registry that were no yet treated.

Mortality

Both all-cause mortality and cardiovascular mortality will be displayed showing Kaplan-Meier curves with either pointwise 90% confidence intervals or 90% confidence bands and analysed using a Cox proportional hazards model exploring the prognostic quality of the biomarkers assessed at baseline. The baseline mortality in the target patient population is 20-50% within 12 months (Dunlay et al., 2021; Hsich et al., 2016).

13.5.6 Safety parameters

Safety is assessed by frequencies of adverse events including:

- major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke and cardiovascular death – further defined by a clinical endpoint adjudication committee - EAC)
- Frequency and severity of arrhythmic events
- Incidence of immune rejection (allograft DNA, CK/CK-MB, hs-cTnT)
- Incidence of mechanical perturbation of ventricular function by EHM graft

All safety parameters will be listed and displayed in summary tables. Adverse events (AEs) are displayed in summary tables as follows:

The total number of AEs, the minimum, maximum and mean number of AEs per patient, the total number of follow-up days (number of days in the observation period), the number of AEs per FU-day (total number of AEs divided by the total by the number of follow-up days), and the number of patients who had at least one AE.

The incidence of AEs defined by preferred term (PT) according to MedDRA will be calculated as the number of patients who experienced at least one AE with the respective PT in percentage of the total number of patients in the safety population. In the incidence tables the PTs will be grouped by system organ class (SOC) according to MedDRA. Additionally, the incidence of AEs defined by SOC will be calculated as the number of patients who experienced at least one AE in the respective SOC as percentage of the total number of patients in the safety population.

Incidences of AEs will be calculated with 95%-confidence intervals.

Laboratory data will be presented in the measured units (or in SI units, being converted from the original units, if necessary). Values outside the investigator's reference range will be flagged as

above or below the reference range in the listings. Shift tables for all parameters will also be generated.

13.6 Interim analyses

A interim analysis of part Part B data is planned for the time point when 15 patients have been implanted on the LV or 5 patients have been implanted on the RV with at least 3 months follow-up completed. The interim analysis and the interim report will describe patient recruitment, treatment compliance as well as safety and efficacy for the patients in this period. An adjustment of the type I error will be performed using a type I error-spending function.

The results of the interim analysis will be reported only to the independent data safety monitoring board (DSMB), see section 14. The DSMB will give advice to the Coordinating Investigator concerning further conduct of the trial.

Naturally, each transfer from one study part to another is accompanied by an interim evaluation.

14 Scientific steering and data monitoring committees

All applicable DZHK-SOPs will be used in the proposed study. Data management and biosampling will be according to DZHK rules. Data will be monitored by Dose Determining (DDC) and Data Safety Monitoring Board (DSMB) comprised of members with appropriate scientific and medical expertise to monitor the study. The DDC and DSMB will be charged with ensuring the safety of the subjects. The DDC is charged primarily with determining the appropriate dose escalation steps according to hands-on experience gained within the study. The DSMB is composed of independent experts and charged with the whole study oversight. The DSMB may recommend that the Sponsor suspends enrolment, amends the study, or discontinues the study at any time.

14.1 Dose Determining Committee (DDC)

A trial related dose determining committee (DDC) will be appointed by the sponsor prior to the start of the trial comprising of 3-5 investigators participating in the trial and sponsor representatives from the clinical trial team and representatives from the CTU including the responsible biostatistician.

The DDC will be involved in the development of the protocol and will ensure transparent management of the trial according to the protocol through recommending and approving modifications as circumstances require. The DDC will review protocol amendments as appropriate. The DDC members will also constitute the clinical endpoint adjudication committee – EAC,

14.2 Data Safety Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) has been established. The members of the DSMB are given in section “Responsibilities”. The function of the DSMB is to monitor the course of the trial and if necessary to give a recommendation to the sponsor, coordinating investigator and the DDC for discontinuation, modification or continuation of the trial. The underlying principles for the DSMB are ethical and safety aspects for the patients. It is the task of

the DSMB to examine, whether the conduct of the trial is still ethically justifiable, whether security of the patients is ensured, and whether the process of the trial is acceptable. For this the DSMB has to be informed about the adherence to the protocol, the patient recruitment, and the observed adverse events. The DSMB will receive the corresponding reports twice a year and at the time of the planned interim analyses. The composition and responsibilities of the DSMB, the structure and procedures of its meetings, and its relationship to other key trial team members (DDC), are laid down in a DSMB charter.

15 Ethical and legal principles

15.1 Regulatory and ethical compliance

This clinical trial was designed, shall be implemented and reported in accordance with the ICH-GCP, with applicable local regulations (including European Directive 2001/20/EC and Regulation 536/2014/EC), and with the ethical principles laid down in the Declaration of Helsinki.

Before initiating the clinical trial, the sponsor/coordinating investigator should submit the CTP and any required application(s) to the appropriate competent authority for review, acceptance, and/or permission, as required by the applicable regulatory requirements.

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Independent Ethics Committee (IEC) before trial start. A signed and dated statement that the protocol and informed consent have been approved by the IEC must be available prior to initiation of the trial.

15.2 Responsibilities of the investigator

Before the start of the trial, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the trial in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to sponsor CRAs, auditors, sponsor Clinical Quality Assurance representatives, designated agents of sponsor, IECs and CA(s) as required.

15.3 Informed consent procedures

Before enrolment in the clinical trial, the patient will be informed that participation in the clinical trial is voluntary and that he/she may withdraw from the clinical trial at any time without having to give reasons and without penalty or loss of benefits to which the patient is otherwise entitled.

The treating physician will provide the patient with information about the treatment method and the possible risks involved. At the same time, the nature, significance, implications, expected benefits and potential risks of the clinical trial and alternative treatment will be explained to the patient. During the informed consent discussion, the patient, if applicable, will also be informed about the insurance cover that exists and the insured's obligations. The patient will be given ample time and opportunity to obtain answers to any open questions. All questions relating to the clinical trial should be answered to the satisfaction of the patient and/or his/her legal representative. In addition, the patient will be given a patient information sheet which contains all the important information in writing.

The patient's written consent must be obtained before any trial-specific tests/treatments.

For this purpose, the written consent form will be personally dated and signed by the trial patient and the investigator conducting the informed consent discussion.

Two consent forms are signed in original by study patient and investigator. By signing the consent form, the patient agrees to voluntarily participate in the clinical trial and declares his/her intention to comply with the requirements of the clinical trial and the investigator's instructions during the clinical trial. By signing the form, the patient also declares that he/she agrees to the recording of personal data, particularly medical data, for the trial, to their storage and codified ("pseudonymised") transmission to the sponsor, CA(s), and further agrees that authorised representatives of the sponsor, who are bound to confidentiality, national or foreign CA(s) may inspect his/her personal data, particularly medical data, which are held by the investigator.

After signing, the patient will be given one of the two originally signed and dated written consent forms and any other written information to be provided to the patients. The other originally signed and dated consent form is filed in the Investigator Site File.

In the case of substantial amendments, the patient must be informed with an appropriate revised patient information/consent form. Changed trial procedures can only be carried out if they have been approved by the CA and the leading IEC, and if the patient has been appropriately informed and has given his/her written consent.

Fertile men and women of child bearing potential should be informed that taking the IMP may involve unknown risks to the foetus if pregnancy were to occur during the trial and agree that in order to participate in the trial they must adhere to the contraception requirement for the duration of the trial. The patients have to agree to data collection related to pregnancy and its outcome. If there is any question that the patient will not reliably comply, they should not be entered in the trial.

Separately, the patients will be informed about the DZHK project regarding Data & Biospecimen collection. Consent to DZHK Biospecimen collection is not mandatory for participation in the BioVAT-HF-study.

The patient informed consent will be adjusted, if the risk-benefit-ratio changes during dose escalation in study part A, as well as after the interim analysis at the end of study part A. Updated informed consent forms need to be evaluated and voted positively by the competent ethic commission before any other patient is included in the BioVAT-HF study.

15.4 Patient insurance

Subject insurance (minimum: € 500,000 per subject) according to applicable law has been taken out with

Newline Europe Versicherung AG

Schanzenstraße 28a

51063 Köln

for all subjects participating in the clinical trial.

The investigator, or an individual who is designated by the investigator, will inform the subject of the existence of the insurance, including the obligations arising from it. The trial subjects must be

afforded access to insurance documents and provided with a copy of the general conditions of insurance on request.

15.5 Confidentiality of trial documents and patient records

The investigator must ensure anonymity of the patients; patients must not be identified by names in any documents submitted to sponsor. Signed informed consent forms and patient enrolment log must be kept strictly confidential to enable patient identification at the site.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded identification number (see section 5.1) only to maintain participant confidentiality.

15.6 Financial disclosure

Financial disclosures should be provided by trial personnel who is directly involved in the treatment or evaluation of patients at the site - prior to trial start.

16 Trial documents and archiving

16.1 Trial documents/investigator site file

The investigator will be given an investigator site file containing all the necessary essential trial documents for the initiation of the trial at his/her site. The essential documents include a list on which the investigator will enter all appropriately qualified persons to whom he/she has delegated important trial-related tasks.

The investigator, or an individual who is designated by the investigator, will be responsible for the maintenance and completeness of the trial documents during the clinical trial. At the request of the CRA, auditor, IEC or CA(s), the investigator shall make available all the requested trial-related records for direct access. Essential documents must not be removed permanently.

16.2 Archiving

After completion of the clinical trial, the essential trial documents - as defined by ICH-GCP E6 section 8 - will be retained at the trial site for a sufficient period so that they will be available for audits and inspections by the CA(s).

The investigator will be responsible for the storage. The following retention periods will apply after the completion/termination of the clinical trial:

- The above-mentioned essential documents must be retained for at least 30 years (§15 Transplantationsgesetz).
- The medical records and other source documents must be retained for the longest possible period allowed by the hospital, the institution or the private practice.

The investigator/the institution should take measures to prevent accidental or premature destruction of these documents. The sponsor will notify the investigator in writing when the trial-related essential documents are no longer required.

16.3 Access to trial data

The trial investigators and all authors of the main publications of the trial result have access to the full trial dataset in order to ensure that the validity of the results can be verified.

17 Protocol adherence and amendments

17.1 Protocol adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact sponsor or its agents, if any, monitoring the trial to request approval of a protocol deviation, as no authorised deviations are permitted. If the investigator feels a protocol deviation would improve the conduct of the trial this must be considered a protocol amendment, and unless such an amendment is agreed upon by sponsor and approved by the IEC it cannot be implemented.

17.2 Amendments to the protocol

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by sponsor, CA where required, and the IEC.

Only changes of the protocol that are required for patient safety may be implemented prior to IEC approval.

Regardless of the need for approval of formal protocol amendments, the investigator is expected to take immediate action required for the safety of any patient included in this trial, even if this action represents a deviation from the protocol. In such cases, the sponsor has to be notified as soon as possible of this action; the IEC should be informed correspondingly.

Information regarding important protocol modifications will be provided in due time to further relevant parties (e.g. investigators, trial participants, trial registries, journals).

17.3 Protocol deviations

Details will be described in the Monitoring Manual.

18 Administrative Agreements

18.1 Financing of the trial and role of funders

The clinical trial will be financed/financially supported by Deutsches Zentrum für Herz-Kreislauf-Forschung e.V (DZHK). Costs for the IMP production will be covered by Repairon GmbH.

18.2 Trial agreement- investigator compensation

According to ICH-GCP 4.9.6, a trial agreement on the conduct of the clinical trial and the compensation for conducting the trial will be signed between the sponsor of the clinical trial and the investigators including their heads of administration.

18.3 Reimbursement of trial patients

There is no payment planned for patients.

18.4 Trial reports

After completion of the analysis by the responsible biostatistician, the coordinating investigator will prepare and sign the final integrated medical and statistical report, a synopsis of the results and publication(s) containing the results of the study jointly with the biostatistician.

Except when required by law, no one will disclose a result of the clinical trial to third parties unless all parties involved have first agreed on the results of the analysis and their interpretation.

The final trial report will be written and signed in co-operation between the coordinating investigator, the coordinating scientist and the CTU of the University Medical Center Göttingen.

18.5 Clinical trials registry

The sponsor ensures that the key design elements of this protocol will be posted in publicly accessible clinical trials registries: clinicaltrials.gov.

18.6 Publication of trial protocol and results

The sponsor assures that the key design elements of this protocol will be posted in a publicly accessible clinical trials registry (see section 18.5). In addition, upon trial completion the results of this trial will be submitted for publication and posted in a publicly accessible database of clinical trial results irrespective of the results of the trial.

Reporting guidelines will be taken into account (see www.equator-network.org), the CONSORT statement will be adhered to in the preparation of papers on the results of randomised studies.

Each publication of trial results will be in mutual agreement between the principal investigator and the other investigators involved. All data collected in connection with the clinical trial will be treated in confidence by the coordinating investigator and all others involved in the trial, until publication. Interim data and final results may only be published (orally or in writing) with the agreement of the coordinating investigator and the coordinating scientist.

18.7 Authorship in publications of trial protocol and results

Authorship criteria defined by the International Committee of Medical Journal Editors will be adhered to. Investigators with a substantive contributions to the design, conduct, interpretation, and reporting of a clinical trial are recognized through the granting of authorship on the trial report. Individual contributions will be acknowledged in publications according to the publisher's

regulations. The coordinating investigator and the coordinating scientist will be responsible to mediate in case of disputes as to authorship.

19 Appendices

Appendix 1 New York Heart Association “Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels”

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

19.1 Relevant Guidelines and Laws

[List and reference to websites; delete not applicable links]

Declaration of Helsinki	http://www.wma.net/en/30publications/10policies/b3/
ICH E6 - GCP Guideline	http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/good-clinical-practice.html
ICH E8 – General considerations for clinical trials	http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/general-considerations-for-clinical-trials.html
ICH E2F - DSUR	http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2F/Step4/E2F_Step_4.pdf
EMA Guidelines	http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/landing/human_medicines_regulatory.jsp&mid=WC0b01ac058001ff89
CTR (EU) 536/2014	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0536&from=DE
Eudravigilance	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0536&from=DE
Recommendations related to contraception and pregnancy testing in clinical trials- Heads of Medicines Agencies (HMA)	http://www.hma.eu/fileadmin/dateien/Human_Medicines/01_-_About_HMA/Working_Groups/CTFG/2014_09_HMA_CTF_G_Contraception.pdf
2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure	https://leitlinien.dgk.org/2016/2016-esc-guidelines-for-the-diagnosis-and-treatment-of-acute-and-chronic-heart-failure/

19.2 Summary of translational project


The combined Phase I/II (open label, non-randomized) BioVAT-HF Early Clinical Study is designed to obtain first clinical data on feasibility, safety and efficacy of epicardial EHM implantation in patients with end-stage heart failure and reduced ejection fraction (HFrEF; EF $\leq 35\%$). It will comprise of two parts: (A) a dose finding cohort (n=8-18 patients) starting with the anticipated minimally effective dose (i.e., EHM-assemblies constructed from 200 million induced pluripotent stem cell-derived cardiomyocytes and stroma cells [iPSC-CM/StC]) up to an anticipated maximal feasible dose (MFD; i.e., EHM-assemblies constructed from 800 million iPSC-CM/StC); (B) a refinement/expansion cohort (n=35) to test for optimal applications on the left (min. 5) and right (min. 5) ventricles and to obtain clinical proof-of-concept for the most suitable indication and dosing according to the data obtained in A. Route of administration will be via minimal left-lateral thoracotomy as a standalone intervention in LV applications and concomitant to an open-chest LV intervention in RV applications.

19.3 Blood draw for allograft DNA assessment (Liquid biopsy)

Liquid Biopsy:

Blutentnahme

Blutentnahme – Beschriftung – Versand




Die Abnahme der Blutproben erfolgt in speziellen Abnahmeröhrchen (CELL-FREE DNA BCT®) der Firma Streck. Die abgenommene Blutprobe bleibt in diesen Röhrchen bis zu 14 Tage bei Temperaturen von 6-30°C stabil.

Pro Studienteilnehmer/in sollen zwei Röhrchen mit jeweils ca. 10 mL Vollblut befüllt werden (insgesamt 20 mL pro Abnahme).

Die Blutproben sollten möglichst per Venenpunktion entnommen werden. Eine Abnahme über einen zentralvenösen Zugang (Port) ist ebenfalls möglich, in diesem Fall muss aber zwingend darauf geachtet werden, dass die Blutprobe nicht mit Heparin aus Spüllösungen kontaminiert werden kann. Wenn eine Heparin-Kontamination nicht sicher ausgeschlossen werden kann, ist die Entnahme per Venenpunktion vorzuziehen.

Bei den o.g. Röhrchen handelt es sich um sogenannte Vacutainer Abnahmesysteme. Die Abnahme sollte mit G21 oder G22 Nadeln erfolgen. Die Gummikappe der Röhrchen muss nach folgendem Schema durchstoßen werden:



Nach dem Durchstechen der Gummikappe warten bis der Blutfluss automatisch durch das nachlassende Vakuum stoppt.

Die Abnahme muss direkt in das Röhrchen erfolgen, die Gummikappe darf nicht entfernt werden.

Die Röhrchen sollten zu ca. 2/3 befüllt sein.

Sollte ein Röhrchen unterfüllt sein, ist die Abnahme zu wiederholen.

Unmittelbar nach der Abnahme muss das Röhrchen zehnmal über Kopf geschwenkt werden.



Liquid Biopsy:

Blutentnahme – Beschriftung – Versand

Dokumentation und Beschriftung

Alle klinischen Informationen und personenbezogenen Daten verbleiben bei den Studienleitern der klinischen Studienzentren.

Die Proben werden pseudonymisiert verschickt.

Hierzu werden Etiketten mit vorgedruckten Strichcodes verwendet. Um Probenverwechslungen zusätzlich auszuschließen werden die Etiketten außerdem vom Entnehmer handschriftlich mit einem Probenkürzel und dem jeweiligen Abnahmedatum beschriftet.

Die zu verwendenden Probenkürzel setzen sich wie folgt zusammen:

KürzelStudie_KürzelKlinik_LaufendeNummerPatient_LaufendeAbnahmenummerProPatient

Wobei gilt:

KürzelStudie: HP20

KürzelKlinik: G = UM Göttingen; O = Klinik Bad Oeynhausen; L = Universitätsklinikum Schleswig-Holstein – Campus Lübeck

Laufende Nummer Patient/in: Pro Studienteilnehmer soll eine eindeutige Nummer vergeben werden, die sich im Laufe der Studie (für Folgeabnahmen) nicht ändern darf. (Schema: 001, 002, 003, etc)

LaufendeAbnahmenummerProPatient: Zur Unterscheidung serieller Abnahmen eines/r Patienten/in (z.B. prä-OP und post-OP) erhält jede Abnahme eine laufende Nummer nach folgendem Schema _001, _002 etc.

Beispiel Probenbeschriftung (bitte die Unterstriche als Trenner beachten):

HP20_G_001_001

Beide Röhrchen einer Abnahme können mit dem gleichen Probenkürzel versehen werden.



Probenversand

Blutproben verschiedener Studienteilnehmer/innen können über den Zeitraum einer Woche:

z.B. von Dienstag bis Dienstag

zunächst im jeweiligen klinischen Studienzentrum gesammelt werden.

Der Versand aller Proben einer Woche kann dann gesammelt z.B. an jedem Mittwoch erfolgen.

Die Proben sollten bis zum Versand liegend bei Raumtemperatur (ca. 20-22°C) gelagert werden.

Die Proben bitte nicht länger lagern!

Der Versand erfolgt in entsprechender biologischer Sicherheitsverpackung mit einem entsprechenden Kurierdienst.

Der Versand der Proben erfolgt an die Adresse:

Chronix Biomedical GmbH

Burckhardtweg 2

37077 Göttingen

19.4 Overall risk and benefit assessment

iPSC-derived EHM	Benefit-risk assessment
Benefit-risk assessment	
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Table 1: Risk management and mitigation approach 13

List of abbreviations

ATMP	Advanced therapy medicinal product
CM	Cardiomyocyte
CRT-D	Cardiac resynchronization therapy-defibrillator
DDC	Dose determining committee
DSMB	Data safety monitoring board
ECG	Echocardiogram
EF	Ejection fraction
EHM	Engineered human myocardium
FIH	First-in-human
GLP	Good laboratory practice
ICD	Implantable cardioverter defibrillator
IMP	Investigational medicinal product
iPSC	Induced pluripotent stem cell
LVAD	Left ventricular assist device
MACE	Major adverse cardiovascular events
MFD	Maximal feasible dose
MI	Myocardial infarction
MRI	Magnetic resonance imaging
NHP	Nonhuman primate
OHT	Orthotopic heart transplantation
OMT	Optimal medical therapy
StC	Stromal cell
TEP	Tissue engineered product

1 Potential benefits of product

1.1 Unmet medical need for treatment of disease

Approximately 2 million patients in Germany, 6.5 million patients in Europe, and 23 million patients globally are diagnosed with heart failure. The incidence of heart failure is reported to be ~300,000 new cases/year in Germany. Mortality is 20% within 1 and 50% within 5 years upon the initial diagnosis “heart failure”, independent of disease severity and despite optimal medical therapy (OMT) ([Lopez-Sendón & Montoro, 2015](#)). The numbers of patients living with heart failure are increasing as a result of, e.g., (1) better acute therapy of myocardial infarction (MI survivors), (2) increases in cardiovascular morbidities (e.g., diabetes, obesity, hypertension), (3) demographic changes due to an aging population, (4) improved survival in pediatric patients with cardiac malformations, and (5) survival under cardiotoxic treatments (e.g., in patients living with cancer). Inotrope dependent patients listed for heart transplantation have an average life expectancy of 1.1 years if eligible and 9.4 months if ineligible for orthotopic heart transplantation (OHT) ([Long et al., 2014](#)). Less than 300 patients per year receive an OHT with an estimated need for OHT in ~8,000 patients per year in Germany. Left ventricular assist devices (LVADs) are used in these patients as bridge-to-transplantation, rarely as bridge-to-recovery, and increasingly also as destination therapy. LVAD recipients demonstrate 1-, 2-, and 3-year survival rates of 75%, 63%, and 54%, but are despite technical improvements subject to commonly occurring serious side effects, including stroke, gastrointestinal bleeding, driveline infections, and device failure ([Atluri et al., 2013](#)). Moreover, LVAD candidates present commonly with right heart failure, which is associated with poor outcome and may even preclude the option of LVAD implantation ([Lampert & Teuteberg, 2015](#); [Turner, 2018](#)). Therefore, new treatment options apart from heart transplantation and LVAD are desperately needed.

In addition to greatly reduced life expectancy, quality of life in patients living with heart failure is greatly and similarly reduced as in patients with stroke ([Nieminen et al., 2015](#)). Ischemic heart disease, the main cause of heart failure in the target patient population of the BioVAT-HF trial, is the leading contributor to disability-adjusted life years ([Murray et al., 2012](#)). The socioeconomic burden of heart failure is anticipated to increase markedly; e.g., direct and indirect cost associate with heart failure were \$28 billion in the United States in 2010 with an anticipated increase to \$78 billion by 2030 ([Lopez-Sendón & Montoro, 2015](#)) – a similar burden applies to Europe. Taken together, heart failure is already today the most common cause of death and will continue to be a further growing health and socioeconomic burden.

Engineered human myocardium (EHM) is an advanced therapy medicinal product, in particular a tissue engineered product (ATMP-TEP). It is being developed as regenerative therapeutic to repair myocardial damage in patients with heart failure with a reduced ejection fraction (EF, $\leq 35\%$). EHM is intended to provide a novel, cell based treatment utilizing human cardiomyocytes (CMs) and stromal cells (StC), both derived from a cell bank of human induced pluripotent stem cells (iPSC).

Delivery and reintroduction of CMs by EHM implantation is designed to compensate for the loss in heart muscle structure and function, leading to enhanced myocardial performance via sustained remuscularization, which is not possible by optimal medical therapy. EHM patches

have a size of approximately 3.5 x 3.5 x 0.05 cm and are constructed from 4×10^7 cells. Multiple patch assemblies created by stacking of individual EHM patches (up to 20) allow for dose adaptation.

1.2 Preclinical pharmacology results

The rationale for use of EHM is based on several preclinical studies performed by the Sponsor with the clinical EHM product and surrogate products manufactured with mouse, rat and nonhuman primate iPSC-derived cells. Preclinical studies in rodent and NHP models have established evidence for functional myocardial remuscularization by EHM allografts and demonstrated the:

- Feasibility and safety of an allograft EHM surrogate (ERM) implantation under immune suppression in a healthy rat model ([Zimmermann et al., 2002](#)).
- Efficacy of EHM surrogate allografts (ERM and EMM) in acute and chronic heart failure models demonstrated by MRI- and echocardiographically-detected enhancement of heart wall thickness and contractility (thickening fraction) ([Didie et al., 2013](#); [Zimmermann et al., 2006](#)).
- Establishment and validation of a human GMP-compatible EHM format in vitro and in vivo ([Qin et al., 2016](#); [Riegler et al., 2015](#); [Tiburecy et al., 2017](#)).
- Long-term engraftment of CMs if administered as EHM and at least 5-fold higher retention of CM compared to intramyocardially injected CMs ([Riegler et al., 2015](#)).
- Feasibility of surgical application of human heart sized EHM patches onto the beating heart in Rhesus macaque models with evidence for an EHM dose dependent augmentation of the targeted heart wall in line with the proposed mode of action.
- Dose dependent increases in (1) target wall thickness, (2) target heart wall systolic thickening fraction as a sign for locally enhance contractility, and (3) left ventricular ejection fraction (LV EF) as a sign for globally enhanced heart function upon surrogate EHM implantation in Rhesus macaque models with chronic heart failure after induced MI. In contrast, continuous deterioration in LV ejection fraction in control animals.

These results collectively provide the scientific rationale for the BioVAT-HF trial.

2 Potential risks

2.1 Potential risks of product

For the risk identification and the design of the risk mitigation approach the Applicant has considered the anticipated mode of action, the results of preclinical studies, the product development rationale as well as required treatment related procedures such as surgery and immunosuppression. Furthermore, the EU guideline on strategies to identify and mitigate risks for first-in-human (FIH) and early clinical trials with investigational medicinal products (EMA/CHMP/SWP/28367/07 Rev. 1, 2017) was taken into consideration during the development of the synopsis for the proposed clinical study.

2.1.1 Possible adverse events associated with the immune suppression regimen

Patients will receive immune suppression, using a similar protocol as found effective in the NHP study (Feasibility and safety study of ENHPM patch allo- and autografting in NHPs with 6 months follow-up), confirmed in an extension of the NHP study and clinically established in OHT (according to ISHLT guidelines; Costanzo et al. (2010)), including calcineurin inhibition (Tacrolimus: 5-15 ng/ml or Cyclosporine A: 150-325 ng/ml) and corticosteroids (5-10 mg/day) starting 7 ± 3 days before EHM implantation. Calcineurin inhibition (e.g., Tacrolimus, Cyclosporine) will be continuously applied throughout the study and beyond the 12 month study period if clinical follow-up suggests efficacy of EHM implantation without safety concerns. Corticoids (preferably methylprednisolone) will be maintained at low dose to avoid Cushing symptoms and weaned after 3 - 6 months in line with the proceeding in heart transplant patients.

Potential side effects of the immune suppression regimen include infections, liver toxicity (increase in alanine transaminase/aspartate aminotransferase – also referred to as transaminitis), nephrotoxicity (increase in creatinine), hyperglycaemia, hyperlipidaemia, hypertension, malignancy (in particular skin cancer after long-term exposure), bone marrow suppression and drug interactions (especially if co-medication is metabolized via Cyp3A4). All side effects are well known from clinical practice with patients under immune suppression (e.g., patients with organ transplants or autoimmune disease) and can be monitored using standard clinical assessments. Hyperglycaemia, hyperlipidaemia and transaminitis were observed in a subset of animals in the NHP study extension (2, 2 and 3 of 8 animals, respectively), receiving 18 ± 1 ng/mL Tacrolimus on average over 168 days (Mean \pm SEM from n=8 and 87 time points). At clinical target concentrations of 5 - 15 ng/mL (as tested in the original NHP study with no such observed adverse events) low rate of Tacrolimus related adverse events is anticipated and will be handled according to clinical standard of care, which may include complete withdrawal of immune suppression if adverse immune suppression related effects are found to be clinically not tolerable by the study subjects. Hyperglycaemia is a commonly observed side effect of Tacrolimus intake (≥ 1 of 10 patients according to the SmPC), dose related, and reversible upon lowering of Tacrolimus doses to target maintenance trough levels of 5 - 10 ng/ml (Cho et al., 2003). Tacrolimus inhibits insulin transcription and secretion from pancreatic beta-cells and thereby triggers an enhanced gluconeogenesis with associated weight loss.

An observation of lymphocyte proliferation in lymph nodes (Lnn. mediastinales craniales and Lnn. tracheobronchiales), spleen and tonsils in 1 NHP (#2907) receiving 16 ± 1 ng/mL Tacrolimus (Mean \pm SEM; from 11 time points in #2907) on average over 168 days was investigated by independent pathologists at the German Primate Center (Dr. med. vet. Eva Gruber-Dujardin) and the University Medical Center Göttingen (PD Dr. med. Felix Bremmer und Prof. Dr. med. Philip Ströbel). A graft or immune suppression related malignancy was excluded. The observed finding is best described as reactive lymphocytosis, which is commonly observed in macaques under immune suppression (refer to pathology expert statement as Annex to NHP Study Extension Report).

Importantly, none of the immune suppression related observations led to clinically symptomatic side effects and all observations were well within expectations and experience with the clinical use of Tacrolimus. In clinical practice, a dose reduction or an exchange of Tacrolimus for Cyclosporine (incidence of hyperglycaemia is reported as $\geq 1/100$ and $< 1/10$ according to the SmPC), or the use of alternative immune suppressants such as MMF (Mycophenolate mofetil) can be considered. In some patients, anti-hyperglycaemic medication, including insulin supplementation may have to be considered. The management of posttransplant diabetes mellitus (PTDM) in allograft organ recipients is well established.

Administration of immune suppression will start 7 ± 3 days before EHM implantation. This allows for an individual dose adjustment of the calcineurin inhibitors to steady state levels and the identification of acute unwanted effects before EHM implantation. The study will be conducted at centers experienced in organ transplantation and concomitant immune suppressive therapies.

Patients will be closely monitored by routine post heart transplant therapeutic drug monitoring as well as monitoring for ventricular hypertrophy, kidney dysfunction, liver function, metabolites, red and white blood cells, and blood pressure at each study visit (before EHM implantation as well as 2 weeks, 1 month, 3 months, 6 months, and 12 months after EHM implantation). Monitoring will also include screening for skin malignancies.

In case of infection, broad-spectrum antibiotics will be applied as needed. Reduction of immune suppression dose according to general clinical standards for the treatment of patients with immune suppression may be applied. Defined immunosuppression stopping criteria, which would also result in graft rejection, are in place. Preclinical data suggest no compromise in heart function upon controlled rejection (NHP study data). In addition, withdrawal of immune suppression has been performed per protocol in clinical trials with PSC-derived cardiac progenitors (NCT02057900), in cell sheets comprised of iPSC-derived cardiomyocytes (UMIN000032989), and iPSC-derived cardiomyocytes (NCT03763136) with apparently no palpable side effects.

Co-medication will be critically assessed as to drug-drug interaction potential and dosing adjusted accordingly.

2.1.2 Possible adverse events associated with the surgical procedure

The surgical procedure related risk of EHM implantation via a minimal invasive left lateral thoracotomy is best compared to the low risk associated with the surgical fixation of epicardial pacemaker leads (experience of the participating surgical teams and others, for example [Puglisi](#)

et al. (2004)). Potential general surgical complications include bleeding, adhesions, and impaired wound healing.

Surgeries will be performed at highly specialized centers and surgical teams experienced with minimal invasive left lateral thoracotomy. The surgeons will be the same as in the preclinical NHP study and will receive, together with additionally involved cardiothoracic surgeons, receive additional training in EHM implantation at the primary study center (University Medical Center Göttingen) as found necessary. Patients will be selected for negligible risk for procedural complications. Patients will stay hospitalized for 2 weeks after EHM-implantation to ensure close monitoring of potential unwanted effects and enable rapid mitigation procedures if needed (e.g., in case of the occurrence of arrhythmia). In addition, the first two patients in each dose cohort will be enrolled sequentially, i.e., 4 weeks apart, to extend the monitoring time and ensure EHM administration with solid evidence for no acute or subacute procedure related unwanted effects.

Both the surgical procedure and immune suppression protocol were simulated in a pivotal preclinical NHP study at dose levels equal to the starting dose for the BioVAT-HF trial (5x EHM assemblies). No-complications were observed in preclinical studies in a healthy nonhuman primate (NHP) model (implanted with 1x [n=7] and 5x [n=7] EHM) and a NHP model with chronic heart failure (in an extension of the study), which closely resembles the clinical scenario (implanted with 2x [n=3] and 5x [n=1] EHM). In Rhesus macaques, this dose represents a 50x EHM assembly dose in human according to allometric scaling (body and heart weight in Rhesus macaque is approximately 1/10 of the body and heart weights in human subjects). No unwanted effects (no surgical procedure related unwanted effects, no deaths, no tumor, no arrhythmia, no perturbation of heart function, no clinically concerning immune suppression related complications) were observed at the tested doses, establishing a safety margin of 2.5 compared to the maximal feasible dose (MFD; 20x EHM). A continuation of NHP implant studies in parallel to the clinical trial, such as already started with the NHP study extension, is planned to continuously collect additional safety data and further refine the treatment algorithm.

2.1.3 Possible adverse events associated with EHM implantation

Potential risks associated with the nature of the investigational medicinal product (IMP) are:

- Non-engraftment/rejection
- Fibrotic tissue degradation/formation
- Arrhythmia (as a result of faulty cardiomyocyte integration)
- Major adverse cardiovascular events (MACE, i.e. non-fatal MI, non-fatal stroke, cardiovascular death)
- Perturbation of heart function (as a result of mechanical compression or constriction of the heart)
- Teratoma formation or unwanted, e.g. osteochondral, cell differentiation (as a result of pluripotent stem cell impurities).

General monitoring

To guarantee patient safety, all identified risks are reflected in the study protocol and the following measures are in place:

Patients will remain hospitalized for 2 weeks after EHM implantation and will be closely monitored for detrimental effects on heart function or abnormal growth after EHM implantation. Patients will be monitored before EHM implantation as well as 2 weeks, 1 month, 3 months, 6 months, and 12 months after EHM implantations by 17 segment high-resolution echocardiography (ECG) and cardiac magnetic resonance imaging (MRI) analyses (MRI if possible, i.e., if not compromised by device implants or other contraindications such as claustrophobia). Patients will be under constant telemetric monitoring via ideally MRI-compliant implantable cardioverter defibrillator (ICD)- or cardiac resynchronization therapy-defibrillator (CRT-D)-devices with event recorders for the whole duration of the study. Patients will remain under standard of care, which may include inotrope treatment, mechanical circulatory support, and OHT as clinically indicated.

A data safety monitoring board (DSMB), comprised of external independent experts with expertise in cell based heart repair studies will be charged with the oversight of the clinical trial: Prof. P. Menasche, Paris (Cardiothoracic Surgeon), Prof. S. Janssens, Leuven (Cardiologist), Prof. S. Zohar, Paris (Statistician) have agreed to be on the DSMB. The DSMB may recommend that the Sponsor suspends enrolment, amends the study, or discontinues the study at any time. Investigators will report all serious adverse events (SAE) to the Sponsor. Sponsor will report all SAEs to the Coordinating investigator (CI) and the DSMB. DSMB will review events and recommend continuation, modification, or discontinuation of the study. Discontinuation will be by stopping the immune suppression and thus initiating graft rejection. Discontinuation will be according to pre-specified stopping criteria, such as immune suppression related complications (e.g., sepsis, kidney failure, liver failure), lack of EHM patch retention (e.g., no evidence for enhanced thickness of the target hypokinetic heart segment), graft related perturbations of heart function (e.g., sustained arrhythmia, end-organ failure due to low-output syndrome), or exaggerated disease progression (e.g., increased frequency in recurrent hospitalizations due to worsening of heart failure, accelerated decrease of EF, need for sustained inotrope support). Special considerations apply in light of the COVID-19 pandemic, which may, in case of a COVID-19 infection, require transient or permanent withdrawal of immune suppression.

Non-engraftment/rejection

Graft rejection has been observed in a rat allograft (synergic graft) model without immune suppression. Graft retention has been documented under tacrolimus and cyclosporine in combination with methylprednisolone and azathioprine in rat and under methylprednisolone only on MHC-matched mouse models. In a pivotal NHP study, allograft rejection has been observed in case of insufficient immunosuppression (Tacrolimus only), controlled withdrawal of immune suppression (i.e., after 3 months of combined tacrolimus and methylprednisolone treatment), and under co-administration of apparently insufficiently (for the NHP model) dosed cyclosporine in combination with methylprednisolone.

Patients will undergo pre-implant (Visit 2) and follow-up (1, 3, 6, and 12 months after EHM implantation) testing for donor specific antibodies (DSA) in serum from patients against CMs

and StCs from the iPSC line used for EHM production. Results will be subject to the first scheduled interim analysis 4 weeks after inclusion of the last patient in Part A of the BioVAT-HF early clinical trial. The PI, sponsor representatives, and DSMB will further evaluate whether or not to adjust the inclusion criteria for Part B of the BioVAT-HF trial by considering preformed DSA as an additional exclusion criterion.

Rejection of EHM allografts will be prevented by clinically established (in OHT) immune suppression (according to ISHLT guidelines; [Costanzo et al. \(2010\)](#)), including calcineurin inhibition (Tacrolimus: 5 - 15 ng/ml or Ciclosporine A: 150 - 325 ng/ml) and corticosteroids (5 - 10 mg/day) starting 7 ± 3 days before EHM implantation. Adaptations of immune suppression may be acceptable according to clinical recommendations and results from the DSA analyses. The first two patients in the respective dose escalation cohorts will be enrolled sequentially (4 weeks apart).

Fibrotic tissue formation/degradation

In the context of regulatory agency interactions, concerns were raised on the potential fibrotic degradation of EHM patches or on the formation of fibrotic tissue in the patches in areas outside of the host-graft interface.

Importantly, neither degradation nor excessive fibrotic tissue formation were observed during nonclinical studies with NHPs receiving iPSC-derived surrogate ENHPM products. Extracellular matrix (ECM) is a key component (produced by its essential stromal cell component) of the IMP, comprising 70-80% of its volume at the time of implantation. The ECM component is key for the IMP tissue integrity. Despite this large ECM component, EHM exhibit similar viscoelastic properties as reported for the healthy heart (Young's modulus of ~10 kPa). Accordingly, implantation of EHM does not result in stiffening or diastolic dysfunction of the heart (assessed for example in [Riegler et al. \(2015\)](#) and with no evidence for diastolic dysfunction in the NHP studies).

Even in case of complete cardiomyocyte rejection, which was assessed in 4 NHPs during nonclinical studies, no evidence suggesting that the pliant ENHPM ECM remnants would contribute to myocardial dysfunction could be obtained. Conversely, nonclinical studies have repeatedly shown that even avital, fibrose-tissue-rich EHM grafts ([Didie et al., 2013](#); [Riegler et al., 2015](#); [Zimmermann et al., 2006](#)) would counter the natural course of disease progression. Thus, even in the worst case of complete cell loss and instead EHM graft ECM retention, a positive rather than negative outcome would be expected. This effect is however anticipated to be inferior to the proposed mode of action, namely remuscularization, for the IMP in the proposed BioVAT-HF study.

In summary, there is no evidence (not in healthy nor in NHPs with chronic heart failure or any previous preclinical model) that a compromising fibrosis would occur by EHM or EHM surrogate implantation. In fact, the obtained preclinical data indicates that even in case of a rejection, supportive ECM would be retained epicardially. Thus, if anything the benefit/risk ratio in this regard is considered further improved by obtained data from the NHP extension study which investigated the implantation of ENHPM onto the intrinsically chronically scarred heart after myocardial infarction.

Arrhythmia

Arrhythmia (in particular ventricular tachycardia) represent the main complications in preclinical studies, testing iPSC-derived cardiomyocytes injections (Chong et al., 2014; Liu et al., 2018; Romagnuolo et al., 2019; Shiba et al., 2016). Arrhythmia were not reported in clinical trials, testing low cardiomyocyte doses (100 million; UMIN000032989, NCT03763136). In preclinical studies of EHM based heart repair, arrhythmia were not observed, which is most likely explained by the implantation of a preformed functional syncytium with high electrophysiological stability, rather than individual mostly spontaneous active unstructured/unconnected injected CMs, each being because of intrinsic pacemaker activity a potential source for ectopy. The observation of ectopy rather than re-entry in NHP models with intramyocardial CM injection is in agreement with this assumption (Liu et al., 2018).

Study centers and investigators in the BioVAT-HF study are experienced in heart failure clinical trials. Patients will be under OMT, which includes ICD/CRT-D for protection from sudden cardiac death. Patients will further be under constant telemetric monitoring via ICD- or CRT-D-devices with event recorders for the whole duration of the study.

If arrhythmia occur, management should be initiated according to the clinical judgement of the treating investigator. Defined immunosuppression stopping criteria are in place to enable controlled rejection of EHM grafts.

MACE

The occurrence of MACEs (i.e. non-fatal MI, non-fatal stroke, cardiovascular death) is a risk inherently linked with the patient population (patients with heart failure with a reduced EF; $\leq 35\%$). MACE definition can be further adapted according to advice of the DSMB.

As discussed for arrhythmia, study centers and investigators in the BioVAT-HF study are experienced in heart failure clinical trials. Patients will be under constant telemetric monitoring via devices with event recorders and receive OMT, including ICD/CRT-D for protection from sudden cardiac death (according to guidelines in patients with HF and EF $< 35\%$) for the whole duration of the study.

Perturbation of heart function

Perturbation of heart function as a result of mechanical compression or constriction of the heart is a theoretical risk linked to the nature of the IMP (heart patches implanted onto the heart). Preclinical studies have not shown any perturbations of heart function.

Dose levels in the preclinical NHP allo- and autograft study represented the starting dose for the BioVAT-HF trial (5x EHM assemblies). In Rhesus macaques, this dose represents a 50x EHM assembly dose in human according to allometric scaling (body and heart weight in Rhesus macaque is approximately 1/10 of the body and heart weights in human subjects). No unwanted effects (no surgical procedure related unwanted effects, no deaths, no tumor, no arrhythmia, no perturbation of heart function, no clinically symptomatic immune suppression related complications) were observed at the tested doses, establishing a safety margin of 2.5 compared to the MFD (20x EHM).

The same monitoring approaches as for arrhythmia and MACEs apply. Furthermore, EHM grafts are clearly visible in by echocardiography and MRI assessments. Echocardiography will be performed 2 weeks as well as 1, 3, 6, 12 months after EHM implantation (refer to clinical follow-up plan), and cardiac MRI 2 weeks as well as 1, 3, 6, 12 months after implantation (refer to clinical follow-up plan). Results will be discussed by clinical investigators, advice will be thought from DSMB in case of perturbations and measures taken accordingly: (1) no change in protocol, (2) controlled withdrawal of immune suppression to induce rejection of allograft, or (3) surgical revision with removal of EHM implant followed by pathological analysis.

The first two patients in the respective dose escalation cohorts will be enrolled sequentially (4 weeks apart).

Teratoma formation or unwanted cell growth

Potential teratoma formation or unwanted cell differentiation resulting in ectopic cell growth are risks resulting from the iPSC-derived nature of the IMP, as pluripotent stem cell or multipotent and unipotent progenitor cell impurities may lead to the formation of teratoma or unwanted ectopic growth. No preclinical studies with human PSC-derived EHM products have shown evidence of any abnormal growth. Furthermore, teratoma formation or unwanted ectopic growth have so far not been reported in any of the registered clinical trials employing human PSC products (reporting period 2010-2019 ([17. Tätigkeitsbericht ZES, 2019](#))).

Osteochondral differentiations were observed in 7 of 18 NHPs receiving iPSC-derived surrogate ENHPM products. In an extensive follow-up and risk assessment supported by single cell nucleus RNA sequencing of both Rhesus and human iPSC-derived CM and StC populations, these occurrences were linked to a not NHP-optimized iPSC-CM differentiation protocol, resulting not only in a lower cardiomyocyte purity (~80% in Rhesus iPSC-derived CM vs. >90% defined as release criterion in human iPSC-derived CM populations), but also high transcriptional heterogeneity with evidence for the presence of osteochondral (progenitor) cells based on a newly developed osteochondral single cell transcriptome signature. Optimizations of the NHP iPSC-CM derivation protocol according to the IMP protocol resulted in higher CM purity (~90%) and consequently a 50-fold lower osteochondral load in a NHP study with ENHPM allograft implantation in Rhesus macaques with chronic heart failure after myocardial infarction. An even higher purity and markedly lower transcriptional heterogeneity of human iPSC-derived CMs was also confirmed by single cell nucleus RNA sequencing and is in agreement with the absence of osteochondral differentiations or ectopic cell growth, including teratoma growth, in all preclinical studies testing EHM. Analogous analysis of NHP and human iPSC-derived StCs confirmed a high purity with no evidence for osteochondral cells/progenitors originating from the iPSC-StC populations. Based on these comprehensive *in vitro* and *in vivo* observations, the relevance of the finding of osteochondral differentiations in the completed NHP-study for the proposed clinical trial was assessed to be low.

Irrespective of the assessment of a low risk, patients will be closely monitored for unwanted growth by echocardiography and cardiac MRI (2 weeks as well as 1, 3, 6, and 12 months after implantation; refer to clinical follow-up plan) to be in the position to mitigate associated risks if they should occur as early as possible. Results and risk mitigation strategies will be discussed amongst the clinical investigators; advice will be thought from the DSMB in case of signs for unwanted growth and measures taken accordingly: (1) no change in protocol, (2) controlled

withdrawal of immune suppression to induce rejection of allograft, or (3) surgical revision with removal of EHM implant followed by pathological analysis.

2.2 Summary of proposed risk management and mitigation approach

Table 1: Risk management and mitigation approach

Risk/Uncertainty	Assessment	Proposed Measure / Mitigation Approach
Immunosuppression	<p>Potential risks related to immunosuppressive regimen:</p> <ul style="list-style-type: none"> • Infection • Nephrotoxicity • Liver toxicity • Hyperglycaemia • Hypertension • Malignancy • Bone marrow suppression • Unfavorable drug-drug interactions (especially if co-medication is metabolized via Cyp3A4) 	<ul style="list-style-type: none"> • Immune suppression as clinically established (in OHT) (according to ISHLT guidelines; Costanzo et al. (2010)), including calcineurin inhibition (Tacrolimus: 5-15 ng/ml or Ciclosporine A: 150-325 ng/ml) and corticosteroids (5-10 mg/day) starting 7±3 days before EHM implantation. • The study will be conducted at centers experienced in organ transplantation and concomitant immune suppression therapy • Application of broad-spectrum antibiotics as needed • Close monitoring of patients by routine post heart transplant therapeutic drug monitoring • Monitoring for ventricular hypertrophy, kidney function, liver function, metabolites, red and white blood cells, and blood pressure at each study visit • Monitoring for skin malignancies • Reduction of immune suppression dose if clinically indicated according to general clinical standards for the treatment of patients with immune suppression. Potential exchange of Tacrolimus for Cyclosporine (incidence of hyperglycaemia is reported as $\geq 1/100$ and $< 1/10$ according to the SmPC), or the use of alternative immune suppressants such as MMF • Defined immunosuppression stopping criteria, which would result in graft rejection, are in place. Preclinical data suggest no compromise in heart function upon controlled rejection (NHP study data). In addition, withdrawal of immune suppression has been performed per protocol in clinical trials with PSC-derived cardiac progenitors (NCT02057900), in cell sheets comprised of iPSC-derived cardiomyocytes (UMIN000032989), and iPSC-derived cardiomyocytes (NCT03763136) with apparently no palpable side effects • Critical assessment of co-medication and dosing according to therapeutic drug monitoring
Surgical procedure	<p>Potential risks associated with surgical procedure:</p> <ul style="list-style-type: none"> • No-complications in healthy NHP model (implanted with 1x [n=7] and 5x [n=7] EHM in completed Feasibility and Safety Study). • No-complications in NHP model with chronic heart failure, which closely resembles the clinical scenario (implanted with 2x [n=3] 	<ul style="list-style-type: none"> • Limited number of highly specialized centers and surgical teams • Study centers experienced with minimal invasive left lateral thoracotomy (surgical TAVI route) • Selection of patients with negligible risk for procedural complications • Surgeons received training in EHM implantation procedure (same surgeons as for NHP study)

iPSC-derived EHM

Benefit-risk assessment

Risk/Uncertainty	Assessment	Proposed Measure / Mitigation Approach
	<ul style="list-style-type: none"> and 5x [n=1] EHM in extension of Feasibility and Safety Study). Low risk profile according to experience with procedurally similar surgical placement of epicardial pacemaker leads in a similar patient cohort 	<ul style="list-style-type: none"> 2 weeks post EHM implantation in-hospital monitoring of patients Sequential enrolment for first two patients in the respective dose escalation cohorts (4 weeks apart) Surgical procedure according to SOP
Non-engraftment / rejection	<p>Based on preclinical EHM surrogate allograft studies:</p> <ul style="list-style-type: none"> Graft rejection has been observed in a not immune suppressed rat allograft (synergic graft) model Graft retention has been documented under tacrolimus and cyclosporine in combination with methylprednisolone and azathioprine in rat and methylprednisolone only under MHC-matching in mouse models Graft rejection has been observed in NHP due to insufficient immunosuppression regimen (Tacrolimus only), controlled withdrawal of immune suppression (i.e., after 3 months of combined tacrolimus and methylprednisolone treatment), and under co-administration of apparently insufficiently (for the macaque model) dosed cyclosporine in combination with methylprednisolone 	<ul style="list-style-type: none"> Patients will undergo pre-implant (Visit 2) and follow-up (1, 3, 6, and 12 months after EHM implantation) testing for donor specific antibodies (DSA) in serum from patients against CMs and StCs from the iPSC line used for EHM production. Results from DSA assay will be subject to the first scheduled interim analysis 4 weeks after inclusion of the last patient in Part A of the BioVAT-HF early clinical trial. The PI, sponsor representatives, and DSMB will further evaluate whether or not to adjust the inclusion criteria for Part B of the BioVAT-HF trial by considering preformed DSA as an additional exclusion criterion. Immunosuppression regimen as in heart transplantation, but limited to calcineurin inhibitor and methylprednisolone; immune suppression may be adapted according to clinical indications and results from the DSA tests. 2 weeks post EHM implantation in-hospital monitoring of patients with intense monitoring of calcineurin inhibitor trough level and if needed dose adjustment. Sequential enrolment for first two patients in the respective dose escalation cohorts (4 weeks apart).
Arrhythmia	<ul style="list-style-type: none"> Commonly observed during the first 4 weeks after treatment in preclinical studies testing delivery of cardiomyocytes by intramyocardial injection of high cardiomyocyte doses (800-1.000 million) Not reported in clinical trials, testing lower cardiomyocyte doses (100 million; UMIN000032989, NCT03763136) Animals in preclinical studies using EHM and EHM surrogates showed no arrhythmias Arrhythmia appear to be dependent on the dose and route of administration EHM implant design principle with electrically synchronized cardiomyocyte syncytia/networks, in contrast to unstructured/unconnected cellular grafts in direct CM injection studies, with high electrophysiological stability 	<ul style="list-style-type: none"> Study centers experienced in heart failure management 2 weeks post EHM implantation in-hospital monitoring of heart rate and rhythm Sequential enrolment Continuous telemetric monitoring via ICD- and CRT-D event recorders for whole study duration Patients protected from sudden cardiac death by ICD or CRT-D (ICD or CRT-D implantation indicated according to guidelines in patients with HF and EF ≤35%) Defined immunosuppression stopping criteria in place to enable controlled rejection.
MACE (i.e. non-fatal myocardial infarction, non-fatal stroke, cardiovascular death)	Potential risk associated with patient population and procedure	<ul style="list-style-type: none"> MACE definition can be further adapted according to advice of DSMB Study centers experienced in heart failure management

iPSC-derived EHM

Benefit-risk assessment

Risk/Uncertainty	Assessment	Proposed Measure / Mitigation Approach
		<ul style="list-style-type: none"> Study centers and investigators experienced in heart failure clinical trials 2 weeks post EHM implantation in-hospital observation period Sequential enrolment Continuous telemetric monitoring via ICD- and CRT-D event recorders for whole study duration Patients protected from sudden cardiac death by ICD or CRT-D (ICD or CRT-D implantation indicated according to guidelines in patients with HF and reduced EF ($\leq 35\%$)).
Perturbation of heart function	<p>Potential risk associated with the nature of the product:</p> <ul style="list-style-type: none"> Preclinical experiments do not show any perturbations of heart function. MFD was defined as 20 patches based on preclinical experience in NHP, allometric scaling considerations and assumptions regarding pericardial volume (20 patches are equivalent to a volume of 20 mL) Cell dose is equivalent to typical tissue damage found in human heart after a hemodynamically relevant myocardial infarction EHM grafts are clearly visible in echocardiography/MRI follow-up 	<ul style="list-style-type: none"> Patients will be closely monitored for perturbations of heart function by ECG-monitoring (continuously via ICD or CRT-D device with event recorder), echocardiography and cardiac MRI (2 weeks as well as 1, 3, 6, and 12 months after implantation; refer to clinical follow-up plan). Results will be discussed amongst clinical investigators, advice will be thought from DSMB in case of perturbations and measures taken accordingly: (1) no change in protocol, (2) controlled withdrawal of immune suppression to induce rejection of allograft, or (3) surgical revision with removal of EHM implant followed by pathological analysis 2 weeks post EHM implantation in-hospital observation period Sequential enrolment
Teratoma growth	<p>Potential risk for pluripotent stem cell based therapeutic approaches depending on the presence of undifferentiated, proliferating pluripotent stem cells:</p> <ul style="list-style-type: none"> iPSC-derivatives are developed following stringent quality control measures, which include assays for the presence of pluripotent stem cells. Long maturation protocol (70 plus days) reduces the risk to maintain a pluripotent cells contamination. Ectopic growth, including teratoma formation, has not been reported in registered clinical trials (reporting period 2010-2019 (17. Tätigkeitsbericht ZES, 2019)). Teratoma formation has not been observed in preclinical studies with clinical and clinical-like EHM, including a completed GLP tumorigenicity study. 	<ul style="list-style-type: none"> Patients will be closely monitored for unwanted growth by echocardiography and cardiac MRI (2 weeks as well as 1, 3, 6, and 12 months after implantation; refer to clinical follow-up plan). Results will be discussed by clinical investigators, advice will be thought from DSMB in case of hints for unwanted growth and measures taken accordingly: (1) no change in protocol, (2) controlled withdrawal of immune suppression to induce rejection of allograft, or (3) surgical revision with removal of EHM implant followed by pathological analysis. Sequential enrolment

DSA: Donor specific antibodies; ECG: Echocardiogram; EF: Ejection fraction; EHM: Engineered human myocardium; MRI: Magnetic resonance imaging; NHP: Nonhuman primate; iPSC: Induced pluripotent stem cell; MI: Myocardial infarction; MFD: Maximal feasible dose; OHT: Orthotopic heart transplantation; DSMB: Data safety monitoring board; ICD: Implantable cardioverter defibrillator; CRT-D: Cardiac resynchronization therapy device; MACE: Major adverse cardiac events

3 Conclusion

Considering the high unmet medical need in patients with heart failure, despite the available standard of care treatment options, an acceptable balance of benefit and risk is underlined by favorable preclinical data balanced against the risk associated with the immunosuppressive regiment, the surgical procedure, and the nature of the IMP (i.e., non-engraftment/rejection, arrhythmia, perturbation of heart function, MACE, teratoma formation or unwanted growth). The risks are thoroughly addressed by an appropriate risk management, monitoring, and mitigation approach. The study will be performed at highly specialized centers by physicians experienced in heart failure clinical trials to minimize potential safety risks in the treated patients. Furthermore, comprehensive preclinical testing and a rigorously analyzed GMP EHM production process ensure safety of the target patient population and delivery of cardiomyocytes in a preformed and structurally as well as functionally well-defined heart muscle format to enable clinically relevant and sustainable remuscularization.

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19.5 Handout for emergency physicians and treating physicians (to be adapted by study site)

BioVAT-HF	GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN STIFTUNG ÖFFENTLICHEN RECHTS UNIVERSITÄTSMEDIZIN GÖTTINGEN Ressort Forschung und Lehre – Studienzentrum UMG Von-Bar-Str. 2/4, 37075 Göttingen Tel.: 0551-39171347	 Page 1/17
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Handreichung
für Notarzt und weiterbehandelnde Klinikärzte

BioVAT-HF
Engineered Human Myocardium (EHM)
bei Patienten mit terminaler Herzmuskelschwäche


Therapeutic area	Terminale Herzinsuffizienz
Development Phase	Phase I/II
Sponsor	Universitätsmedizin Göttingen vertreten durch die Leitung des Studienzentrums UMG Von-Bar-Str. 2/4, 37075 Göttingen
Coordinating Investigator "Leiter der Klinischen Prüfung/LKP" (nach deutschem Arzneimittelgesetz)	Prof. Dr. Tim Seidler Universitätsmedizin Göttingen Robert-Koch-Str. 40 37075 Göttingen

BioVAT-HF	GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN STIFTUNG ÖFFENTLICHEN RECHTS UNI- VERSITÄTSMEDIZIN GÖTTINGEN Ressort Forschung und Lehre – Studienzentrum UMG Von-Bar-Str. 2/4, 37075 Göttingen Tel.: 0551-39171347	 Page 2/17
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STUDIENTITEL	Sicherheit und Wirksamkeit von aus induzierten, pluripotenten Stammzellen hergestelltem menschlichem Herzmuskelgewebe (Engineered Human Myocardium) als biologisches ventrikuläres Unterstützungsgewebe bei terminaler Herzmuskelschwäche	
KURZTITEL	BioVAT-HF	
INDIKATION	Terminale Herzinsuffizienz	
PHASE	Phase I/II	
STUDIENZIELE	<p>Primäres Ziel:</p> <ul style="list-style-type: none">Bewertung der Sicherheit und Wirksamkeit von Engineered Human Myocardium (EHM) bei Patienten mit terminaler Herzinsuffizienz (HFrEF EF ≤ 35%) mit oder ohne RV-Dysfunktion (TAPSE <16 mm) <p>Sekundäres Ziel:</p> <ul style="list-style-type: none">Bewertung der Auswirkungen von EHM-Transplantaten auf krankheitsspezifische Ereignisse und Symptome	
CHARAKTERISTIKA DER PRÜFSUBSTANZ	Name	Engineered Human Myocardium (EHM)
	Name der Substanz	Menschliches Herzmuskelgewebe hergestellt aus einem Gemisch aus Kardiomyozyten und Stromazellen aus induzierten pluripotenten Stammzellen (iPSC) in einem Rinderkollagen Typ I-Hydrogel
	Hersteller	University Medical Center Göttingen
	Zugelassene Indikation	Noch nicht zugelassen
WIRKMECHANISMUS	Im Rahmen umfangreicher vorklinischer Prüfungen wurde eine strukturelle und funktionelle Stärkung des Zielmyokards beobachtet.	
BEHANDLUNG	<p><u>Experimentelle Intervention / Indextest:</u> Implantation von EHM auf dysfunktionales links- oder rechtsventrikuläres Myokard bei Patienten mit HFrEF (EF ≤ 35%).</p> <p>Die epikardiale Implantation erfolgt bei LV-Applikation über eine minimalinvasive linksseitige Thorakotomie als eigenständiges Verfahren. Die RV-Applikation erfolgt im Rahmen einer geplanten Operation am LV am offenen Brustkorb (z.B. im Rahmen einer Bypass Operation, der Implantation eines LV-Herzunterstützungssystems oder einer Herzklappenoperation).</p> <p>Die Behandlung erfolgt unter Einnahme folgender Immunsuppressiva:</p> <ul style="list-style-type: none"><u>Calcineurin-Inhibitor:</u> Tacrolimus: 0,05-0,1 mg/kg/Tag p.o.<u>Ciclosporin:</u> 4-8 mg/kg/Tag p.o.<u>Corticosteroid:</u> Methylprednisolon 5-10 mg/Tag p.o. <p><u>Hinweis:</u> Die Methylprednisolon Dosierung (5-10 mg/Tag p.o.) erfolgt unter der individuellen Cushing-Schwelle. Die Verabreichung von Methylprednisolon wird nach 3-6 Monaten gemäß Richtlinien für die Immunsuppression bei Herztransplantation beendet.</p>	

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	<p>Calcineurin-Inhibitor Äquivalenzdosis bei p.o. und i.v. Verabreichung: Tacrolimus: 0,05-0,1 mg/kg/Tag p.o. = 0,01-0,02 mg/kg/Tag i.v. Ciclosporin: 4-8 mg/kg/Tag p.o. = 1-2 mg/kg/Tag i.v.</p> <p>Die Calcineurin-Inhibitor Dosierung wird gemäß gewünschtem Talspiegel angepasst (Vorgehen gemäß ISHLT Leitlinien bei orthotoper Herztransplantation):</p> <p>Tacrolimus: 10-15 ng/ml zum Zeitpunkt der Implantation für 2 Monate 8-12 ng/ml bis Monat 6 nach Herzpflaster Implantation 5-10 ng/ml bei stabilen Patienten 6 Monate nach Implantation</p> <p>oder</p> <p>Ciclosporin A: 275-375 ng/ml zum Zeitpunkt der Implantation für 6 Wochen 200-350 ng/ml bis Woche 12 nach Herzpflaster Implantation 150-300 ng/ml bis Monat 6 nach Herzpflaster Implantation 150-250 ng/ml bei stabilen Patienten 6 Monate nach Implantation</p>
MÖGLICHE UNERWÜNSCHTE EREIGNISSE UND RISIKEN	<p>Im Rahmen dieser Studie erfolgt weltweit erstmalig die Testung von aus Stammzellen hergestelltem Herzmuskelgewebe zur Behandlung der Herzmuskelschwäche. Im Rahmen umfangreicher Prüfungen vor der Erstanwendung im Menschen sind keine klinisch relevanten unerwünschten Wirkungen aufgetreten.</p> <p>Aufgrund theoretischer Überlegungen sind folgende unerwünschte Wirkungen bei der Anwendung von aus pluripotenten Stammzellen hergestellten Herzmuskelpräparaten möglich:</p> <ol style="list-style-type: none">1) Herzrhythmusstörungen (auch potentiell lebensbedrohliche),2) Verschlechterung der Herzfunktion durch mechanische Fehlbelastung der behandelten Herzwand (z.B. bei unerwünschtem Zellwachstum oder Bindegewebebildung),3) Tumorwachstum (sogenannte Teratome oder Teratocarcinome). <p>Das Auftreten dieser Nebenwirkungen kann Dosis-abhängig sein. In präklinischen Anwendungen sind diese Nebenwirkungen auch bei erheblichen höheren Dosierungen der Herzpflasterpräparate, als im Rahmen der BioVAT-HF Studie zu testen ist, nicht aufgetreten.</p> <p>Darüber hinaus sind vor allem Nebenwirkungen der immunsuppressiven Begleitbehandlung zu berücksichtigen. Im Einzelnen sind dies Funktionsstörungen der Niere und der Leber, gesteigerte Infektanfälligkeit, Blutdruckanstieg,</p>

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	<p>Erhöhung der Blutzucker- und Fettwerte, Zu- und Abnahme des Körpergewichts bei Einnahme von Tacrolimus oder Ciclosporin und Methylprednisolon. Eine detaillierte Übersicht der Nebenwirkungen und Wechselwirkungen von Tacrolimus oder Ciclosporin und Methylprednisolon finden Sie im Anhang I.</p> <p>Aufgrund der Anwendung eines Allografts (Herzpflaster mit TachoSil® Versiegelungsmatrix) kann es zu einer Immunisierung mit Antikörperbildung und Abstoßung kommen. Eine detaillierte Übersicht der Nebenwirkungen und Wechselwirkungen von TachoSil® finden Sie im Anhang II.</p> <p>Bei einer Abstoßung des Herzpflasters geht der aktive Effekt (Kontraktilität) durch Remuskularisierung verloren. Eine passive Stärkung (Stabilisierung) der Herzwand bleibt auch bei Abstoßung der Herzmuskelzellen im Sinne von zusätzlichem epikardialen Bindegewebe erhalten. Ein Abbau des allogenen Restgewebes ist über die Zeit (Monate) zu erwarten. Eine akute Dekompensation ist bei Abstoßung nicht zu erwarten.</p> <p>Bei klinisch nicht akzeptablen Nebenwirkungen des Herzpflasters oder der Immunsuppression wird die Immunsuppression reduziert oder je nach klinischer Notwendigkeit abgesetzt; dies führt zu einer „kontrollierten“ Abstoßung der mit dem Herzpflaster implantierten Zellen.</p> <p>Besteht weiterhin der Bedarf einer Entfernung des nicht abgestoßenen Restgewebes wird eine chirurgische Entfernung bei individueller Abwägung des Risiko-Nutzenverhältnisses erwogen.</p>
MAßNAHME IM NOTFALL	<p>Notfallbehandlungen erfolgen grundsätzlich gemäß gängigen Leitlinien.</p> <p>Es ist zu erwarten, dass sich Patienten mit Herzmuskelschwäche mit Dekompensation der Herzmuskelschwäche oder mit Herzrhythmusstörungen präsentieren. Darüber hinaus besteht die Möglichkeit, dass diese Symptome durch die Verabreichung der notwendigen Immunsuppression verstärkt werden; Infektionserkrankungen können unter Immunsuppression vermehrt und mit einem lebensbedrohlichen Verlauf (Sepsis) auftreten:</p> <p>Bei Herzinsuffizienz-bedingter Dekompensation:</p> <ol style="list-style-type: none"> 1) Kontaktaufnahme mit Prüfer oder Vertreter 2) Aufnahme auf die Überwachungsstation 3) Behandlung der Herzmuskelschwäche bei Dekompensation gemäß Leitlinien 4) Fallkonferenz (Kardiologie - Herzchirurgie) 5) Entscheidung: <ol style="list-style-type: none"> a) Absetzen der Immunsuppression b) Fortsetzung der Immunsuppression 6) Entlassung aus stationärer Behandlung nach Rekompensation. <p>Bei Herzrhythmusstörungen:</p> <ol style="list-style-type: none"> 1) Kontaktaufnahme mit Prüfer oder Vertreter

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	<ul style="list-style-type: none">2) Aufnahme auf die Überwachungsstation3) Behandlung von Rhythmusstörungen gemäß Leitlinien4) Fallkonferenz (Kardiologie - Herzchirurgie)5) Entscheidung:<ul style="list-style-type: none">a) Absetzen der Immunsuppressionb) Fortsetzung der Immunsuppression6) Entlassung aus stationärer Behandlung nach Rhythmisierung <p>Bei Verdacht auf Sepsis:</p> <ul style="list-style-type: none">1) Kontaktaufnahme mit Prüfer oder Vertreter2) Aufnahme auf die Überwachungsstation/Intensivstation3) Absetzen der Immunsuppression4) Behandlung gemäß SOP bei Sepsis5) Entlassung aus stationärer Behandlung nach Behandlung
KONTAKTDATEN	<p>Zuständiger Prüfer: [Name] [Kontaktdaten]</p> <p>Stellv. Prüfer: [Name] [Kontaktdaten]</p> <p>Zuständige Study Nurse: [Name] [Kontaktdaten]</p> <p>24/7 Notfallnummer: [Name] [Kontaktdaten (z.B. Stationsnummer)]</p> <p>Leiter der klinischen Prüfung: Prof. Dr. T. Seidler Universitätsmedizin Göttingen Innere Medizin / Klinik für Kardiologie und Pneumologie Tel.: 0551- 39 62266 E-Mail: tim.seidler@med.uni-goettingen.de</p>

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Anhang I

Nebenwirkungen und Wechselwirkungen mit gängigen Herzmedikamenten der immunsuppressiven Medikation (Tacrolimus, Ciclosporin, Methylprednisolon)

Entnommen aus den Fachinformationen für 1) Tacrolimus Heumann 0,5 mg/-1 mg/-5 mg Hartkapseln, 2) Sandimmun® 50 mg/ml (Ciclosporin) und 3) Urbason® 8 mg Tabletten (Methylprednisolon)

1) Tacrolimus Heumann 0,5 mg/-1mg/-5mg Hartkapseln

Das Nebenwirkungsprofil von Immunsuppressiva lässt sich oft wegen der Grunderkrankungen des Patienten und der Behandlung mit einer Vielzahl anderer Medikamente nicht genau feststellen. Viele der nachstehend angeführten Nebenwirkungen sind reversibel und/oder sprechen auf eine Herabsetzung der Dosis an. Bei einer oralen Behandlung dürfte die Häufigkeit von Nebenwirkungen geringer sein als bei intravenöser Gabe. Nachfolgend werden die Nebenwirkungen von Tacrolimus nach ihrer Häufigkeit in absteigender Reihenfolge angeführt:

- sehr häufig (≥1/10)
- häufig (≥1/100 bis <1/10)
- gelegentlich (≥1/1.000 bis <1/100)
- selten (≥1/10.000 bis <1/1.000)
- sehr selten (<1/10.000)
- nicht bekannt (Häufigkeit auf Grundlage der verfügbaren Daten nicht abschätzbar)

Infektionen und parasitäre Erkrankungen

Wie bekanntermaßen bei anderen hochwirksamen Immunsuppressiva ist bei Patienten, die mit Tacrolimus behandelt werden, die Anfälligkeit für Infektionen (virale, bakterielle, mykotische und protozoale) häufig erhöht. Bereits bestehende Infektionen können sich verschlechtern. Infektionen können sich lokal oder systemisch manifestieren. Fälle von BK-Virus-assoziiierter Nephropathie und JC-Virus-assoziiierter progressiver multifokaler Leukoenzephalopathie (PML) wurden bei Patienten unter Immunsuppressionstherapie, einschließlich Therapie mit Tacrolimus, berichtet.

Gutartige, bösartige und unspezifische Neubildungen (einschl. Zysten und Polypen)

Bei Patienten, welche mit Immunsuppressiva behandelt werden, erhöht sich das Risiko einer Tumorentwicklung. Es wurde über gutartige oder bösartige Neoplasmen einschließlich EBV-assoziierte lymphoproliferative Erkrankungen und Hauttumoren unter Behandlung mit Tacrolimus berichtet.

Erkrankungen des Blutes und des Lymphsystems:

- Häufig: Anämie, Leukozytopenie, Thrombozytopenie, Leukozytose, abnorme Erythrozytenwerte
- Gelegentlich: Blutgerinnungsstörungen, abnorme Gerinnungs- und Blutungswerte, Panzytopenie, Neutropenie
- Selten: Thrombotische thrombozytopenische Purpura, Hypoprothrombinämie, thrombotische Mikroangiopathie

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Nicht bekannt: PureRed Cell Aplasia (Erythroblastopenie), Agranulozytose, hämolytische Anämie

Erkrankungen des Immunsystems:

Unter der Anwendung von Tacrolimus wurden allergische und anaphylaktoide Reaktionen beobachtet.

Endokrine Erkrankungen:

Selten: Hirsutismus

Stoffwechsel- und Ernährungsstörungen:

Sehr häufig: Hyperglykämische Zustände, Diabetes mellitus, Hyperkaliämie

Häufig: Hypomagnesiämie, Hypophosphatämie, Hypokaliämie, Hypocalcämie, Hyponatriämie, Flüssigkeitsüberbelastung, Hyperurikämie, verminderter Appetit, metabolische Azidose, Hyperlipidämie, Hypercholesterinämie, Hypertriglyceridämie, andere Elektrolytstörungen

Gelegentlich: Dehydratation, Hypoproteinämie, Hyperphosphatämie, Hypoglykämie

Psychiatrische Erkrankungen:

Sehr häufig: Schlaflosigkeit

Häufig: Angsterscheinungen, Verwirrtheit und Desorientiertheit, Depression, depressive Verstimmung, affektive Störungen und Störungen des Gemütszustandes, Albträume, Halluzinationen, Geisteskrankheiten

Gelegentlich: Psychotische Störung

Erkrankungen des Nervensystems:

Sehr häufig: Tremor, Kopfschmerzen

Häufig: Krampfanfälle, Bewusstseinsstörungen, Parästhesien und Dysästhesien, periphere Neuropathien, Schwindelgefühl, Schreibstörung, Störungen des Nervensystems

Gelegentlich: Koma, Blutungen im Zentralnervensystem und Apoplexie, Paralyse und Parese, Enzephalopathie, Sprech- und Sprachstörungen, Amnesie

Selten: erhöhter Tonus

Sehr selten: Myasthenie

Augenerkrankungen:

Häufig: Verschwommenes Sehen, Photophobie, Augenerkrankungen

Gelegentlich: Katarakt

Selten: Blindheit

Nicht bekannt: Neuropathie des Nervus opticus

Erkrankungen des Ohrs und des Labyrinths:

Häufig: Tinnitus

Gelegentlich: Hörschwäche

Selten: Neurosensorische Taubheit

Sehr selten: Eingeschränktes Hörvermögen

Herzerkrankungen:

Häufig: Ischämische Störungen der Herzkranzgefäße, Tachykardie

Gelegentlich: Kammerarrhythmien und Herzstillstand, Herzversagen, Kardiomyopathie, Kammerhypertrophie, supraventrikuläre Arrhythmien, Palpitationen

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Selten: Perikarderguss
Sehr selten: *Torsades de Pointes*

Gefäßerkrankungen:

Sehr häufig: Hypertonie
Häufig: Hämorrhagie, thromboembolische und ischämische Störungen, periphere Gefäßerkrankungen, hypotensive Gefäßerkrankungen
Gelegentlich: Infarkt, tiefe Venenthrombose, Schock

Erkrankungen der Atemwege, des Brustraums und Mediastinums:

Häufig: Dyspnoe, Erkrankung des Lungenparenchyms, Pleuraerguss, Pharyngitis, Husten, Anschwellung und Entzündung der Nasenschleimhaut
Gelegentlich: Atemversagen, Erkrankung der Atemwege, Asthma
Selten: Akutes Atemnotsyndrom

Erkrankungen des Gastrointestinaltrakts:

Sehr häufig: Durchfall, Übelkeit
Häufig: gastrointestinaler Entzündungszustand, Magen-Darm-Geschwür und -Perforation, Blutungen aus dem Magen-Darm-Trakt, Stomatitis und Ulzeration, Aszites, Erbrechen, Schmerzen im Magen-Darm-Bereich und Abdomen, dyspeptische Zeichen und Symptome, Obstipation, Flatulenz, Blähungen und Aufgebläetheit, lockerer Stuhl, Zeichen und Symptome im Magen-Darm-Bereich
Gelegentlich: Ileus paralyticus, akute und chronische Pankreatitis, gastroösophagealer Reflux, beeinträchtigte Magenentleerung
Selten: Subileus, Pankreaspseudozyste

Leber- und Gallenerkrankungen:

Häufig: Cholestase und Ikterus, Leberzellschaden und Hepatitis, Cholangitis
Selten: Thrombose der Leberarterie, mit Venenverschluss einhergehende Lebererkrankung
Sehr selten: Leberversagen, Gallengangstenose

Erkrankungen der Haut und des Unterhautzellgewebes:

Häufig: Pruritus, Exanthem, Alopezie, Akne, verstärktes Schwitzen
Gelegentlich: Dermatitis, Photosensibilität
Selten: Epidermolysis acuta toxica (Lyell-Syndrom)
Sehr selten: Stevens-Johnson-Syndrom

Skelettmuskulatur-, Bindegewebs- und Knochenerkrankungen:

Häufig: Gelenkschmerzen, Muskelkrämpfe, Schmerz in den Extremitäten, Rückenschmerzen
Gelegentlich: Gelenkerkrankungen
Selten: beeinträchtigte Beweglichkeit

Erkrankungen der Nieren und Harnwege:

Sehr häufig: Nierenfunktionsstörung
Häufig: Nierenversagen, akutes Nierenversagen, Oligurie, Tubulusnekrose, toxische Nephropathie, Veränderungen des Harns, Symptome von Harnblase und Harnröhre
Gelegentlich: Anurie, hämolytisch-urämisches Syndrom

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Sehr selten: Nephropathie, hämorrhagische Blasenentzündung

Erkrankungen der Geschlechtsorgane und der Brustdrüse:

Gelegentlich: Dysmenorrhö und Uterusblutung

Allgemeine Erkrankungen und Beschwerden am Verabreichungsort:

Häufig: asthenische Zustände, fieberhafte Erkrankungen, Ödem, Schmerzen und Beschwerden, gestörtes Empfinden der Körpertemperatur

Gelegentlich: multiples Organversagen, grippeartige Erkrankung, Temperaturunverträglichkeit, Druckgefühl in der Brust, Zitterigkeit, Krankheitsgefühl

Selten: Durst, Sturz, Beklemmung in der Brust, Ulkus

Sehr selten: Zunahme des Fettgewebes

Untersuchungen

Häufig: Veränderungen der Leberenzymwerte und Leberfunktion, erhöhte Blutspiegel der alkalischen Phosphatase, Gewichtszunahme

Gelegentlich: erhöhte Amylasewerte, anormales EKG, anormale Herz- und Pulsfrequenz, Gewichtsverlust, erhöhte Laktatdehydrogenase-Konzentration im Blut

Sehr selten: anormales Echokardiogramm, QT-Verlängerung im Elektrokardiogramm

Verletzung, Vergiftung und durch Eingriffe bedingte Komplikationen:

Häufig: Funktionsstörung des Transplantats

Anwendungsfehler, einschließlich unachtsamer, unbeabsichtigter oder unbeaufsichtigter Umstellung zwischen Tacrolimus-Formulierungen mit unmittelbarer oder retardierter Freisetzung sind beobachtet worden. Es ist von einer Reihe von damit zusammenhängenden Transplantatabstoßungen berichtet worden (Häufigkeit auf Grundlage der verfügbaren Daten nicht abschätzbar).

Wechselwirkung von Tacrolimus mit gängigen Herzmedikamenten:

Tacrolimus ist als CYP3A4-Hemmer bekannt; daher kann die gleichzeitige Anwendung von Tacrolimus mit Arzneimitteln, die durch CYP3A4 metabolisiert werden (z.B. HMG-CoA-Reduktase Hemmer), deren Metabolismus beeinträchtigen. Besondere Vorsicht ist bei gleichzeitiger Einnahme von CYP3A4 induzierenden Substanzen wie zum Beispiel Johanniskraut geboten; hier kann es zu einem Abfall der Tacrolimus Spiegel mit Abstoßung der Herzpflaster kommen.

Da es unter Tacrolimus zu einer Hyperkaliämie oder zur Verstärkung einer bereits bestehenden Hyperkaliämie kommen kann, ist eine hohe Kaliumzufuhr oder die Verwendung kaliumsparender Diuretika (z. B. Amilorid, Triamteren oder Spironolacton) zu vermeiden.

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2) Sandimmun® 50mg/ml (Ciclosporin)

Zusammenfassung des Sicherheitsprofils

Die primären Nebenwirkungen, die in klinischen Studien beobachtet wurden und mit der Anwendung von Ciclosporin in Zusammenhang stehen, umfassen Nierenfunktionsstörung, Tremor, Hirsutismus, Hypertonie, Diarrhoe, Anorexie, Übelkeit und Erbrechen. Viele Nebenwirkungen einer Therapie mit Ciclosporin sind dosisabhängig und sprechen auf eine Dosisreduktion an. Das Gesamtspektrum der Nebenwirkungen ist bei den verschiedenen Indikationen im Wesentlichen das gleiche; es gibt allerdings Unterschiede in der Häufigkeit und im Schweregrad. Aufgrund der nach einer Transplantation erforderlichen höheren Initialdosen und der längeren Erhaltungstherapie sind die Nebenwirkungen bei Transplantationspatienten häufiger und normalerweise auch stärker ausgeprägt als bei Patienten, die für andere Indikationen behandelt werden. Anaphylaktoide Reaktionen wurden nach intravenöser Verabreichung beobachtet

Infektionen und parasitäre Erkrankungen

Bei Patienten mit einer immunsuppressiven Therapie, einschließlich Ciclosporin und Ciclosporin-haltiger Therapien, besteht ein erhöhtes Risiko für Infektionen (virale, bakterielle, parasitäre oder Pilzinfektionen). Es können sowohl generalisierte als auch lokale Infektionen auftreten. Ebenso können sich bestehende Infektionen verstärken und die Reaktivierung einer Polyomavirus Infektion kann zu einer Polyomavirus-assoziierten Nephropathie (PVAN) oder einer JC-Virus-assoziierten progressiven multifokalen Leukoenzephalopathie (PML) führen. Es wurden Fälle mit schwerwiegendem und/oder tödlichem Ausgang berichtet.

Gutartige, bösartige und unspezifische Neubildungen (einschließlich Zysten und Polypen)

Bei Patienten mit einer immunsuppressiven Therapie, einschließlich Ciclosporin und Ciclosporin-haltiger Therapien, besteht ein erhöhtes Risiko für die Entwicklung von Lymphomen oder lymphoproliferativen Erkrankungen und anderer Malignome, insbesondere solcher der Haut. Die Häufigkeit solcher Malignome erhöht sich mit der Intensität und der Dauer der Therapie. Einige Malignome können tödlich verlaufen.

Tabellarische Zusammenfassung der Nebenwirkungen aus klinischen Studien


Nebenwirkungen aus den klinischen Studien (Tabelle 1) werden nach MedDRA-Systemorganklassen angeführt. Innerhalb jeder Systemorganklasse werden die Nebenwirkungen nach Häufigkeit gereiht, wobei die häufigsten Nebenwirkungen zuerst angeführt werden. Innerhalb jeder Häufigkeitskategorie werden die Nebenwirkungen nach abnehmendem Schweregrad gereiht. Zusätzlich beruht die entsprechende Häufigkeitskategorie für jede Nebenwirkung auf den folgenden Definitionen (CIOMS II):

- sehr häufig (≥ 1/10);
- häufig (≥ 1/100, < 1/10);
- gelegentlich (≥ 1/1.000, < 1/100);
- selten (≥ 1/10.000, < 1/1.000);
- sehr selten (< 1/10.000),
- nicht bekannt (Häufigkeit auf Grundlage der verfügbaren Daten nicht abschätzbar).

Tabelle 1: Nebenwirkungen aus klinischen Studien

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Erkrankungen des Blutes und des Lymphsystems	
Häufig	Leukopenie
Gelegentlich	Thrombozytopenie, Anämie
Selten	Hämolytisch-urämisches Syndrom, mikroangiopathische hämolytische Anämie
Nicht bekannt*	Thrombotische Mikroangiopathie, thrombotische thrombozytopenische Purpura
Stoffwechsel- und Ernährungsstörungen	
Sehr häufig	Hyperlipidämie
Häufig	Hyperglykämie, Anorexie, Hyperurikämie, Hyperkaliämie, Hypomagnesiämie
Erkrankungen des Nervensystems	
Sehr häufig	Tremor, Kopfschmerzen
Häufig	Konvulsionen, Parästhesie
Gelegentlich	Enzephalopathie einschließlich posteriores reversibles Enzephalopathiesyndrom (PRES), Zeichen und Symptome wie Konvulsionen, Verwirrtheit, Desorientiertheit, verminderte Reaktivität, Agitiertheit, Schlaflosigkeit, Sehstörungen, kortikale Blindheit, Koma, Parese und zerebelläre Ataxie
Selten	Motorische Polyneuropathie
Sehr selten	Ödem der Sehnervpapille einschließlich Papillenödem, mit möglicher Sehstörung in der Folge einer benignen intrakraniellen Hypertonie
Nicht bekannt*	Migräne
Gefäßerkrankungen	
Sehr häufig	Hypertonie
Häufig	Flush
Erkrankungen des Gastrointestinaltrakts	
Häufig	Übelkeit, Erbrechen, Bauchbeschwerden/Bauchschmerzen, Diarrhoe, Gingivahyperplasie, Magengeschwüre
Selten	Pankreatitis
Leber- und Gallenerkrankungen	
Häufig	Anormale Leberfunktion
Nicht bekannt*	Hepatotoxizität und Leberschäden einschließlich Cholestase, Gelbsucht, Hepatitis und Leberversagen, in einigen Fällen mit tödlichem Ausgang
Erkrankungen der Haut und des Unterhautzellgewebes	
Sehr häufig	Hirsutismus
Häufig	Akne, Hypertrichose
Gelegentlich	Allergischer Ausschlag
Skelettmuskulatur-, Bindegewebs- und Knochenerkrankungen	
Häufig	Myalgie, Muskelkrämpfe
Selten	Muskelschwäche, Myopathie
Nicht bekannt*	Schmerzen der unteren Extremitäten
Erkrankungen der Nieren und Harnwege	
Sehr häufig	Nierenfunktionsstörung
Erkrankungen der Geschlechtsorgane und der Brustdrüse	
Selten	Menstruationsstörungen, Gynäkomastie
Allgemeine Erkrankungen und Beschwerden am Verabreichungsort	
Häufig	Fieber, Müdigkeit
Gelegentlich	Ödeme, Gewichtszunahme

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* Nebenwirkungen aus der Erfahrung nach Markteinführung, für die die Häufigkeit mangels eines realen Bezugswerts nicht bestimmt werden kann.

Andere Nebenwirkungen aus der Erfahrung nach Markteinführung

Es liegen auch Berichte sowie Spontanmeldungen über Hepatotoxizität und Leberschäden, einschließlich Cholestase, Gelbsucht, Hepatitis und Leberversagen, bei mit Ciclosporin behandelten Patienten vor. Die meisten Meldungen betrafen Patienten mit signifikanten Begleitkrankheiten, Grundkrankheiten und anderen Begleitfaktoren wie etwa infektiösen Komplikationen und Begleitmedikationen mit hepatotoxischem Potenzial. In einigen Fällen, vor allem bei Transplantatpatienten, wurde ein tödlicher Ausgang beschrieben.


Akute und chronische Nephrotoxizität

Bei Patienten mit einer Therapie mit einem Calcineurin-Inhibitor (CNI), einschließlich Ciclosporin und Ciclosporin-haltiger Therapien, besteht ein erhöhtes Risiko für akute oder chronische Nephrotoxizität. Es gibt Berichte aus klinischen Studien und aus der Erfahrung nach Markteinführung in Verbindung mit der Anwendung von Sandimmun. In Fällen von akuter Nephrotoxizität wurden Störungen der Homöostase, wie Hyperkaliämie, Hypomagnesiämie und Hyperurikämie, berichtet. Fälle, die chronische morphologische Veränderungen beschrieben, umfassten Arteriolenhyalinose, tubuläre Atrophie und interstitielle Fibrose

Wechselwirkung von Ciclosporin mit gängigen Herzmedikamenten:

Für verschiedene Wirkstoffe ist bekannt, dass sie die Konzentration von Ciclosporin im Plasma oder im Vollblut erhöhen oder vermindern, üblicherweise durch Hemmung oder Induktion von Enzymen, die an der Metabolisierung von Ciclosporin beteiligt sind, insbesondere CYP3A4 (z.B. HMG-Co-Reduktase Hemmer und Johanniskraut).

Ciclosporin ist ebenfalls ein Inhibitor von CYP3A4, des Multidrug-Efflux-Transporter-P-Glycoproteins und der Organo-Anion-Transporter-Proteine (OATP) und kann die Plasmaspiegel von Begleitmedikationen erhöhen, die Substrate dieses Enzyms und/ oder der Transporter sind.

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3) Urbason® 4 mg/8 mg/16 mg/40 mg Tabletten (Methylprednisolon)

Bei den Häufigkeitsangaben zu Nebenwirkungen werden folgende Kategorien zugrunde gelegt:

Sehr häufig ($\geq 1/10$)
Häufig ($\geq 1/100$ bis $< 1/10$)
Gelegentlich ($\geq 1/1.000$ bis $< 1/100$)
Selten ($\geq 1/10.000$ bis $< 1/1.000$)
Sehr selten ($< 1/10.000$)
Nicht bekannt (Häufigkeit auf Grundlage der verfügbaren Daten nicht abschätzbar).

Die im Folgenden genannten Nebenwirkungen sind ohne Häufigkeitsangaben aufgeführt, das heißt, die Häufigkeit ist nicht bekannt (Häufigkeit auf Grundlage der verfügbaren Daten nicht abschätzbar).

Hormonersatzbehandlung

Geringes Nebenwirkungsrisiko bei Beachtung der empfohlenen Dosierungen.

Pharmakotherapie

In Abhängigkeit von Therapiedauer und Dosis können folgende Nebenwirkungen auftreten:

Erkrankungen des Blutes und des Lymphsystems

Leukozytose, Lymphopenie, Eosinopenie, Polyglobulie, Thrombozytopenie, Thrombozytoseeigung.

Erkrankungen des Immunsystems

Schwächung der Immunabwehr mit Erhöhung des Infektionsrisikos (bestimmte virusbedingte Erkrankungen, z. B. Varizellen, Herpes simplex oder – während der virämischen Phase – Herpes zoster, können einen schweren, manchmal auch lebensbedrohlichen Verlauf nehmen), Maskierung von Infektionen, Exazerbation latenter Infektionen, allergische Reaktionen.

Endokrine Erkrankungen


Phäochromozytom-Krise, adrenale Suppression oder Atrophie und Induktion eines Cushing-Syndroms (typische Symptome Vollmondgesicht, Stammfettsucht und Plethora), Wachstumshemmung bei Kindern, Störungen der Sexualhormonsekretion (Amenorrhö, Hirsutismus, Impotenz).

Stoffwechsel- und Ernährungsstörungen

Reversible epikardiale oder mediastinale Lipomatosen, epidurale Lipomatose. Natriumretention mit Ödembildung, vermehrte Kaliumausscheidung mit möglicher Hypokaliämie (cave: Rhythmusstörungen!), verminderte Glucosetoleranz, Diabetes mellitus, Hypercholesterinämie und Hypertriglyceridämie, verstärkter Eiweißabbau.

Psychiatrische Erkrankungen

Schwere Depressionen, Gereiztheit, Persönlichkeitsänderungen, Stimmungsschwankungen, Euphorie, Antriebs- und Appetitsteigerung, Psychosen, Schlafstörungen.

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Erkrankungen des Nervensystems

Pseudotumor cerebri (insbesondere bei Kindern), Manifestation einer latenten Epilepsie und Erhöhung der Anfallsbereitschaft bei manifester Epilepsie, Schwindel, Kopfschmerzen.

Augenerkrankungen

Katarakt, insbesondere mit hinterer subkapsulärer Trübung, Glaukom, Chorioretinopathie, Verschlechterung der Symptome bei Hornhautulkus, Begünstigung viraler, fungaler und bakterieller Entzündungen am Auge, verschwommenes Sehen.

Herzerkrankungen

Progression der Stauungslunge bei Linksherzinsuffizienz, hypertrophische Kardiomyopathie bei Frühgeborenen.

Gefäßerkrankungen

Hypertonie, thrombotische Ereignisse, Erhöhung des Arteriosklerose- und Thromboserisikos, Vaskulitis (auch als Entzugssyndrom nach Langzeittherapie).

Erkrankungen des Gastrointestinaltrakts

Magen-Darm-Ulzera mit der Gefahr einer Perforation (mit z. B. Peritonitis), gastrointestinale Blutungen, Pankreatitis, Oberbauchbeschwerden.

Leber- und Gallenerkrankungen

Erhöhung von Leberenzymen.

Erkrankungen der Haut und des Unterhautzellgewebes

Striae rubrae, Atrophie, Teleangiektasien, erhöhte Kapillarfragilität, Petechien, flächige Hautblutungen, Ekchymosen, Hypertrichose, Steroidakne, verzögerte Wundheilung, Rosazea-artige (periorale) Dermatitis, Änderungen der Hautpigmentierung, Überempfindlichkeitsreaktionen, z. B. Arzneimittellexanthem.

Skelettmuskulatur-, Bindegewebs- und Knochenkrankungen

Muskelatrophie und -schwäche, bei Myasthenia gravis reversible Zunahme der Muskelschwäche bis hin zur myasthenischen Krise, Auslösung einer akuten Myopathie bei zusätzlicher Anwendung von nicht depolarisierenden Muskelrelaxanzien, Osteoporose (dosisabhängig, auch bei nur kurzzeitiger Anwendung möglich), in schweren Fällen mit der Gefahr von Knochenbrüchen, aseptische Knochennekrosen (Kopf des Oberarm- und Oberschenkelknochens), Sehnenruptur.

Hinweis

Bei zu rascher Dosisreduktion nach lang dauernder Behandlung kann es zu Beschwerden wie Muskel- und Gelenkschmerzen kommen.

Erkrankungen der Nieren und Harnwege

Sklerodermiebedingte renale Krise.

Hinweis

Das Auftreten Sklerodermie bedingter renaler Krisen variiert in den verschiedenen Subpopulationen. Das höchste Risiko wurde bei Patienten mit diffuser systemischer Sklerose berichtet. Das niedrigste Risiko wurde bei Patienten mit begrenzter systemischer Sklerose (2 %) und juveniler systemischer Sklerose (1 %) berichtet.

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Untersuchungen
Gewichtszunahme.

Wechselwirkung von Methylprednisolon mit gängigen Herzmedikamenten:

Herzglykoside
Die Glykosidwirkung kann durch Kaliummangel verstärkt werden.

Saluretika/Laxanzien
Die Kaliumausscheidung kann erhöht werden.

ACE-Hemmstoffe
Es besteht ein erhöhtes Risiko des Auftretens von Blutbildveränderungen.

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Anhang II

Nebenwirkungen von TachoSil®

Entnommen aus der Fachinformation für TachoSil® Versiegelungsmatrix

Bei Patienten, die mit Fibrinkleber/Hämostatika behandelt wurden, kann es in seltenen Fällen zu Hypersensitivität oder allergischen Reaktionen kommen (inklusive Angioödem, Brennen und Stechen an der Applikationsstelle, Bronchospasmus, Schüttelfrost, Flush, generalisierte Urtikaria, Kopfschmerz, Nesselausschlag, Hypotonie, Lethargie, Übelkeit, Ruhelosigkeit, Tachykardie, Engegefühl in der Brust, Kribbeln, Erbrechen, keuchende Atmung). In Einzelfällen können diese Reaktionen bis zur schweren Anaphylaxie führen. Derartige Reaktionen können insbesondere bei wiederholter Anwendung oder bei Patienten mit bekannter Überempfindlichkeit gegenüber einem der Bestandteile des Präparates auftreten.

Immunogenität:
Antikörper gegen Komponenten von Fibrinkleberprodukten/Hämostatika können in seltenen Fällen auftreten. Allerdings konnten in einer klinischen Studie mit TachoSil® bei Leberoperationen, in denen die Patienten hinsichtlich der Bildung von Antikörpern untersucht wurden, bei 26 % der 96 mit TachoSil® behandelten und getesteten Patienten, eine Bildung von Antikörpern gegen Pferdekollagen nachgewiesen werden. Die Pferdekollagen-Antikörper, die sich in einigen Patienten bildeten, nachdem sie mit TachoSil behandelt wurden, reagierten nicht mit humanem Kollagen. Ein Patient entwickelte Antikörper gegen humanes Fibrinogen. Es gab keine unerwünschten Ereignisse, die auf die Bildung von Antikörpern gegen humanes Fibrinogen oder Pferdekollagen zurückzuführen waren. Es liegen nur sehr begrenzte klinische Daten zu einer erneuten Exposition mit TachoSil® vor. Zwei Patienten wurden in einer klinischen Studie mit TachoSil® reexponiert und zeigten keine immunbezogenen unerwünschten Ereignisse. Allerdings ist ihr Antikörper-Status bezüglich Kollagen oder Fibrinogen nicht bekannt. Bei intravaskulärer Anwendung kann es zu thromboembolischen Komplikationen kommen

Zusammenfassung des Sicherheitsprofils:
Das Sicherheitsprofil von TachoSil® spiegelt die mit den im Rahmen der klinischen Studien durchgeführten chirurgischen Eingriffen allgemein einhergehenden postoperativen Komplikationen sowie die Grunderkrankung der Patienten wider. Die Daten aus acht kontrollierten klinischen Studien, die der Zulassungsinhaber durchgeführt hat, wurden in einem Datenpaket gepoolt. In die Analyse wurden 997 mit TachoSil® behandelte Patienten und 984 Patienten mit einer vergleichenden Behandlung einbezogen. Aus praktischen Gründen (Vergleich zur chirurgischen Standardversorgung und Standardblutstillung) war eine Verblindung in den TachoSil®-Studien nicht möglich, folglich wurden diese in einem offenen Design durchgeführt.

Tabellarische Zusammenfassung der Nebenwirkungen:
Folgende Nebenwirkungen wurden nach der Zulassung berichtet. Die Häufigkeit der unten aufgelisteten Nebenwirkungen wurde als „Nicht bekannt (Häufigkeit auf Grundlage der verfügbaren Daten nicht abschätzbar)“ kategorisiert.

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Systemorganklasse	Häufigkeit nicht bekannt
Erkrankungen des Immunsystems	Anaphylaktischer Schock, Überempfindlichkeit
Gefäßerkrankungen	Thrombose
Erkrankungen des Gastrointestinaltrakts	Darmverschluss (in der Bauchchirurgie)
Allgemeine Erkrankungen und Beschwerden am Verabreichungsort	Adhäsionen

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Summary of Changes to the Protocol

The previous version of this protocol (Version 6, 13 August 2024) was amended to create the current version (Version 7, 20 November 2024). The protocol history is summarized below:

Protocol History	
Version and Date of Protocol	Comments
Version 1, 28 May 2020	Original Version
Version 2, 28 September 2020	<ul style="list-style-type: none">• Change of Coordinating Investigator• Addition of Coordinating Scientist• Description of treatment groups was amended from “Phase I (Dose Finding Cohort), II (Refinement Cohort), and III (Expansion Cohort)” to “Part A (Dose Finding Cohort) and Part B (Refinement/Expansion Cohort)”• Clarifications as to the duration of the intervention• Clarification as to follow-up measures:<ul style="list-style-type: none">○ Deletion of FDG-PET at 12 months if clinically indicated○ Monitoring of pulmonary artery pressure with CardioMEMs HF Device if previously implanted• Clarification as to cell-free allograft DNA analysis for the monitoring of rejection to be conducted as substudy• The following inclusion criteria was deleted:<ul style="list-style-type: none">○ No realistic chance or not eligible for heart transplantation• The following exclusion criteria were added:<ul style="list-style-type: none">○ Contraindication to TachoSil® (e.g. hypersensitivity to human fibrinogen, human thrombin, horse collagen, human albumin, Riboflavin, Natriumchloride, Natriumcitrate, L-Arginin-Hydrochloride)○ Any condition that excludes adherence to study protocol (in particular lack of adherence to prescribed medication)• The following exclusion criterion was deleted:<ul style="list-style-type: none">○ Alloimmunisation against EHM implant cells• The following inclusion criteria were clarified:<ul style="list-style-type: none">○ Terminal kidney failure (stage 4; GFR <30 ml/min) at the time of enrolment○ Terminal liver failure (Child-Pugh stage C; score >10) at the time of enrolment• Clarification and amendments of the study endpoints in Part A and Part B in the “Study Synopsis” as well as “Objectives and endpoints section” (Table 2), “Clinical trial plan” (Figure 2), “Treatment plan and procedure” (Table 3), and “Trial design - Sample size Calculation”:<ul style="list-style-type: none">○ Primary Safety Endpoints

	<ol style="list-style-type: none"> 1) Part A (Dose Escalation steps): Adverse events related to the procedure, including in particular arrhythmic events, and worsening of disease progression within 28 days (based on a comparison of data obtained during visit 2 and visit 7) 2) Part B: Adverse events related to the procedure, including in particular arrhythmic events and worsening of disease progression within the whole study duration <ul style="list-style-type: none"> ○ Secondary Safety Endpoints <ol style="list-style-type: none"> 1) Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke and cardiovascular death) 2) Frequency and severity of arrhythmic events 3) Incidence of immune rejection (allograft DNA, CK/CK-MB, cTnT, DSA) 4) Incidence of mechanical perturbation of ventricular function by EHM graft ○ Primary Safety Endpoints <ol style="list-style-type: none"> 1) Evidence for structural and functional muscular augmentation of target myocardium determined as enhanced target heart wall thickness (HWT) and thickening fraction (HWTF) ○ Key secondary endpoint: <ol style="list-style-type: none"> 1) Recurrent HF hospitalizations ○ Further secondary endpoints <ol style="list-style-type: none"> 1) Left ventricular ejection fraction (EF) 2) Change in heart failure medication 3) Functional status in patients as determined by cardiopulmonary stress testing (VO2max), six-minute walk test (6MWT), and hand-grip strength measurements 4) Patient reported outcomes assessed by NYHA classification, quality of life score (KCCQ, EQ-5D, QoL-VAD), and study adherence motivation (PHQ-9, HAF-17, ESSI, LOT-R, ULS-8, medication adherence, Trust/Mistrust in medical staff) 5) All-cause and cardiovascular mortality <ul style="list-style-type: none"> • Clarification of the Sample Size in Part B: <ul style="list-style-type: none"> ○ Part B: n=35 (min. 5 with LV and min 5 with RV EHM placement; max. 30 per LV or RV indication) • Dates for planned enrolment adjusted. • Clarification of the time point for interim analysis: <ul style="list-style-type: none"> ○ Interim analysis will be performed after end of study part A and after 15 of the patients in Part B with either LV or RV
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	<p>administration of EHM have completed the 12 month follow-up.</p> <ul style="list-style-type: none"> • Addition of Repairon GmbH as funder • Adjustment of the “Visit schedule and assessments–Flowchart” (Table 1) • Additions to: <ul style="list-style-type: none"> ○ Background and rationale (1) specification of heart failure prevalence and (2) target patient population ○ Scientific Background: (1) data from a Rhesus macaque study (heart failure model) ○ Overview of IMP: (1) information on induced pluripotent stem cell source material ○ Rationale for dose selection: (1) reference to data from a Rhesus macaque study (heart failure model), (2) examples of EHM dose levels displayed in Figure 1 ○ Risk-benefit assessment: (1) additional information as to the rationale for pre-implantation initiation of immune suppression, (2) clarification that the “Participation in the BioVAT-HF study has no negative effect on the patient's place on the transplantation list”, (3) addition of Appendix 19.4 with detailed “Benefit-Risk-Assessment” • Addition of “Information regarding COVID-19 pandemic” • Clarification as to informed consent procedure: <ul style="list-style-type: none"> ○ Patient will be given at least 24 h to provide informed consent before a scheduled baseline visit (Visit 2 according to study protocol) for baseline investigations and to determine patient eligibility. • Clarification as to the start, dosing (guideline-directed), monitoring (therapeutic drug monitoring), and patient documentation of immune suppression • Addition information as to rescue procedures: <ul style="list-style-type: none"> ○ EHM implants may be surgically removed if required as an emergency measure • Quality of life questionnaires amended: <ul style="list-style-type: none"> ○ Deleted: MLHFQ and SF-36 ○ Added: QoL-VAD, PHQ-9, HAF-17, ESSI, LOT-R, ULS-8, Medication adherence, Trist/Mistrust in medical staff • Amendments (underlined) of the “Visit Schedule and assessment”: <ul style="list-style-type: none"> ○ <u>Screening Visit</u> / Study registration (Visit 1, Month <u>-6</u> to week <u>-2</u>) ○ Baseline (Visit 2, Month <u>-1</u> to week <u>-2</u>) ○ <u>Hospital administration</u> (Visit 4; Day <u>-1</u>) ○ Implantation (Visit <u>5</u>; Day 0)
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	<ul style="list-style-type: none"> ○ Assessments at Visit <u>6</u> (Week 2 ± 2 days) ○ Assessments at Visit <u>7</u> (Month 1 ± 7 days), Visit <u>8</u> (Month 3 ± 7 days), and Visit <u>9</u> (Month 6 ± 7 days), and Visit <u>10</u> (Month 12 ± 7 days) • Addition of recommendation to review baseline investigation data at latest 8 days prior to scheduled implantation (day 0) to inform EHM Production Unit (Qualified Person) to initiate the EHM (IMP) release process • Additional information to specify “appropriate contraceptive measures” • Clarification that EuroSCORE II is to be documented at baseline (Visit 2) • Description as to the Quality of Life and Study Adherence Motivation studies added or amended • Information as to pharmacodynamics amended • Information as to adverse reactions amended • Information as to volume of EHM transport medium amended • More details as to EHM implantation procedure provided • Wording corrected (underlined): <ul style="list-style-type: none"> ○ The investigator or designee <u>must</u> maintain records of the delivery of the IMP, the use in individual trial patients, and the disposal of unused IMP(s). The investigator <u>must</u> ensure that the IMP is only used according to this protocol. • Clarification of adverse event documentation: <ul style="list-style-type: none"> ○ starting from the first intake of immunosuppressive medication (Visit 3) ○ Definition of adverse events documentation amended • Clarification that “Death” is always considered a serious adverse event • Clarification that in case of an expansion to a multinational-regional clinical trial according to ICH E17 data transfer to third party countries may be necessary • Amendment as to Methods of analysis of primary and secondary (patient-reported outcomes) endpoints • Clarification that the patient informed consent document will be updated if changes in the benefit-risk-assessment become apparent
Version 3, 22 December 2020	<ul style="list-style-type: none"> • Dates for planned enrolment adjusted • Amendment of the timing for the Screening Visit (Baseline 1 – Visit 1) to any time, but no later than 24 h before the Baseline 2 (Visit 2), i.e., the minimal time allowed for informed consent decision by a prospective BioVAT-HF patient • Clarification that patient registration will be after informed consent at Baseline 2

	<ul style="list-style-type: none"> • Psychosocial analysis to assess medication adherence added to Baseline 2 investigation; description for psychosocial anamnesis added under “Assessments and specifications” • Addition of HBsAg, Anti-HCV, Anti-HIV, and Anti-HTLV-1 testing to Baseline 2 (Visit 2) • Clarification as to blood sample collection for the assessment of circulating cell-free allograft DNA added • Deletion of handling descriptions at point-of-care deleted, because available to investigators in a separate protocol (Assembly log) • Amendment of Data Archiving information according to §15 Transplantationsgesetz (German Transplant Act)
Version 4.2, 3 March 2021	<ul style="list-style-type: none"> • Addition of pre-surgery chest x-ray to Visit 4 (Hospital Administration) to exclude acute, new-onset pneumonia if clinically suspected • Addition of post-surgery chest x-ray investigations ~2 h after EHM implantation and ~2 h after chest drainage removal • Extension of the description of the EHM Implantation procedure
Version 5.1, 3 November 2021	<ul style="list-style-type: none"> • Replacement of cardiac MRI by cardiac CT in patients with MRI artefacts due to metal implants or other ineligibilities (e.g., claustrophobia); description added under “Assessments and Specifications”
Version 5.2, 10 November 2021	<ul style="list-style-type: none"> • Change of sponsor representative • Clarification that a minimum of 2-4 patients must be included in each dose step of Part A (Dose Escalation) • Clarification that: <ul style="list-style-type: none"> ○ data from Part A (Dose Escalation) after treatment of 4 patients with the as per protocol maximal feasible dose (MFD) will be reported to the DSMB for the assessment as safe maximal dose (SMD) and continuation into Part B ○ safety and efficacy data obtained in patients with the SMD in Part A will be included into the interim analysis of Part B. The interim analysis will be performed after 15 patients treated with the SMD have reached 3 months follow-up (FU 3).
Version 6, 13 August 2024	<ul style="list-style-type: none"> • Mandatory transition from EudraCT (2019-000885-39) to CTIS (EMA Clinical Trials Information System) with assignment of EU CT No: 2024-515708-38-01 requiring the following changes to the protocol: <ul style="list-style-type: none"> ○ Deletion of Coordinating Investigator role ○ Clarification of Adverse Event (AE) documentation, Serious Adverse Event (SAE) Definition (including Suspected

	<p>Unexpected Serious Adverse Reactions [SUSARs]), and Reporting policies</p> <ul style="list-style-type: none"> ○ Amendment of sections on “Reporting of Pregnancies”, “Reporting of Special Situations”, “Reporting of product deficiencies / complaints concerning study medication”, “Reporting of Serious breaches and urgent safety measures”, and “Development Safety Update Reports (DSUR)” according to EMA guidelines. • Dates for planned enrolment adjusted • Change of the for the IMP manufacturing responsible and qualified person • Clarification that two informed consent forms must be signed in original by the study patient and the investigator; one duly signed informed consent form will be kept by the patient and one duly signed informed consent form will be added to the Investigator Site File.
Version 7, 20 November 2024	<ul style="list-style-type: none"> • Clarification as to the use of guideline-directed immune suppression including combinations of Calcineurin-inhibitors and mTOR-inhibitors as well as Calcineurin-inhibitor free protocols in patients with cardiorenal syndrome • Clarification as to the use of guideline-directed use of antibiotics • Clarification as to the utility of cardiac MRI (limited in the target patient population) and cardiac CT • Clarification as to the assessment of CK-MB only if CK is elevated • Clarification as to the assessment of hs-cTnT • Addition of cystatin C for the assessment of renal function • The following inclusion criteria were modified (underlined) for the Part B protocol according to the clinical trial investigators’ recommendations to better allocate the target patient population with advanced heart failure in agreement with current guidelines: <ul style="list-style-type: none"> ○ <u>Symptomatic</u> heart failure (NYHA <u>II-IV</u>) with reduced ejection fraction (HFrEF with LV-EF $\leq 35\%$) as assessed by echocardiography ○ <u>Patients under guideline-directed medical therapy</u> ○ <u>NT-proBNP >300 pg/mL for patients in sinus rhythm or >900 pg/mL if in atrial fibrillation</u> ○ <u>History of previous heart failure hospitalization in the past 12 months</u> ○ At least one hypo- or dyskinetic segment <u>or dilated heart chamber</u> to demark the implant target area

	<ul style="list-style-type: none"> ○ <u>The requirement for previous implantation of an ICD or CRT-D with event recorder was deleted</u> • The following inclusion criterion was deleted as per clinical study physician request: <ul style="list-style-type: none"> ○ Autoimmune disease – study physicians will consider autoimmune diseases on a case-by-case basis as condition that may exclude adherence to study protocol (exclusion criterion #8) • Adjustment of primary endpoints in consideration of (1) in clinical practice reliably measurable parameters and (2) measures appropriate for a subsequent pivotal trial: <ul style="list-style-type: none"> ○ <u>Structural endpoint</u>: Change from baseline in EHM target heart wall thickness (HWT) measured by echocardiography or cardiac CT or cardiac MRI over 12 months ○ <u>Functional endpoint</u>: Change from baseline in left/right ventricular ejection fraction (LV-EF/RV-EF) measured by echocardiography or cardiac CT or cardiac MRI in the LV/RV cohorts over 12 months ○ <u>Patient reported outcome/quality of life</u>: Change from baseline in Kansas City Cardiomyopathy Questionnaire-23 Overall Summary Score (KCCQ-23 OSS) over 12 months • Adjustments (underlined) of secondary endpoints in consideration (1) in clinical practice reliably measurable parameters and (2) measures appropriate for a subsequent pivotal trial: <ul style="list-style-type: none"> ○ <u>Time to mechanical circulatory assist device implantation</u> ○ <u>Time to heart transplantation</u> ○ Functional status in patients as determined by cardiopulmonary stress testing (VO2max), six-minute walk test (6MWT) - distance, and hand-grip strength measurements ○ Patient reported outcomes assessed by NYHA classification, quality of life score (EQ-5D-5L), and study adherence motivation (<u>HADS, MoCA, IPQ-8, TEX-Q</u>, medication adherence) ○ All-cause and cardiovascular mortality • The following primary and secondary endpoints were deleted: <ul style="list-style-type: none"> ○ Target heart wall thickening fraction – questionable whether reliably measurable in clinical routine practice (will be assessed as an exploratory endpoint) ○ Left Ventricular-Ejection Fraction – adjusted to co-primary endpoint
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	<ul style="list-style-type: none"> ○ Change in heart failure medication – recommended heart failure medication similar in NYHA I-IV (will be continued as an exploratory endpoint) ○ Patient reported outcome measures – QoL-VAD, PHQ8, HAF 17, Trust/Mistrust in medical staff – deleted to reduce the to workload on patients and clinical investigators • Clarification of statistical analysis: <ul style="list-style-type: none"> ○ Baseline 2 (Visit 2) as reference time point ○ independent testing of the hypothesis of a positive trend over 12 months for the three primary endpoints with type-1 error control using the Hochberg procedure ○ assessment of time to event outcomes such as the time to mechanical assist device implantation or the time to heart transplantation will be displayed using Kaplan-Meier curves. If sufficient number of events occurred, Cox regression analyses will be applied • Adjustment of the estimated duration of the surgical intervention per patient in Part B according to experience gained in Part A • Indication of a scheduled increase in participating sites in and outside Germany • Adjustment of the “Visit schedule and assessments – Flowchart” (Table 1) • Update and clarifications of: <ul style="list-style-type: none"> ○ Background and rationale (1) specification of heart failure prevalence and (2) target patient population (advanced heart failure) ○ Scientific Background: (1) data from a Rhesus macaque study (heart failure model) and (2) experience from 13 patients treated in Part A of BioVAT-HF ○ Overview of IMP: (1) experience from 13 patients treated in BioVAT-HF Part A and (2) registered clinical trials testing pluripotent stem cell implantation in heart failure ○ Trial purpose and rationale: (1) targeting of LV and RV (in Part B) with hypo- or dyskinesia or a dilated heart chamber, and (2) endorsement of continuation into Part B by the DDC and DSMB with LV and RV treatment. ○ Choice of control interventions/comparators: outcome in BioVAT treated patients will be compared to outcome in patients that were recruited (in the VAT-registry) and/or found eligible (completed Baseline 2), but were not treated ○ Dose selection: rationale for SMD selection for Part B LV and RV administration
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	<ul style="list-style-type: none"> ○ Risk-benefit assessment: experience from preclinical and clinical investigations added ○ Evaluation of primary / secondary objectives and endpoints: experience from 13 patients treated in BioVAT-HF Part A ○ Trial design: extension of Part A protocol until treatment of in total 18 patients (14 treated with the SMD) before transition into Part B protocol. ○ Preclinical data: additional Rhesus macaque data included ○ Pharmacodynamics: additional experience from preclinical and BioVAT-HF Part A included ○ Adverse reactions: experience from BioVAT-HF Part A included • Clarification as to the proceeding with RV treatment in Part B of BioVAT-HF • Clarification as to the decision to continue immune suppression (under regular prescription) off-study by the responsible clinical investigator after individual data review and patient consent as well as inclusion in VAT-registry (under a separate informed consent) for continuous monitoring until end-of-life, heart transplantation or implantation of a mechanical assist device • Clarification that patients waiting for EHM implantation will be asked to enrol into the VAT-registry (under a separate informed consent) to obtain information as to disease progression • Amendments (underlined) of the “Visit Schedule and assessment”: <ul style="list-style-type: none"> ○ Baseline (Visit 2, <u>Month -3</u> to week -2) ○ Hospital administration (Visit 4; Day <u>-6</u> <u>[or at the clinical investigator’s discretion]</u> to day -1) ○ Assessments at Visit 6 (<u>1-2 days before release from the hospital at the clinical investigator’s discretion</u>) • Clarification as to laboratory assessments: (1) additional assessment of procalcitonine as a measure of inflammation, (2) additional assessment of BUN and cystatin C to assess kidney function, (3) assessment of CK-MB at the discretion of the clinical investigator if CK is not elevated • Clarification as to the preferred mode of echocardiography (transthoracic) and in case of poor image quality addition of contrast agent • Clarification as to the preference for MRI only if high quality data can be anticipated
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	<ul style="list-style-type: none"> • Additional documentation of INTERMACS at baseline (Visit 2) and follow-up 5 (Visit 10); description of the proceeding included in protocol • Clarification that Baseline (Visit 2) data will be reviewed (case conference) before the start of immune suppression; deletion of the previous recommendation to hold the case conference at latest 8 days prior to implantation • Omission of (1) echocardiography, (2) cardiac MRI, (3) 6-minute walk test, (4) cardiopulmonary exercise testing, (5) hand-grip strength assessment, (6) quality of life and patient reported outcome questionnaires at Visit 6, i.e., prior to release from hospital to reduce the burden on patients and investigators • Adjustment of follow-up to reduce burden and workload on patients and clinical investigators: <ul style="list-style-type: none"> ○ Cardiac CT at visits 8 and 10, i.e., 3 and 10 months post implant ○ Cardiac MRI at visits 8, 9, and 10, i.e., 3, 6, and 10 months post implantation at the clinical investigator's discretion instead of cardiac CT if found to provide reliable information (limited in patients with devices, metal implants) ○ 6-minute walk test at visits 8, 9, and 10 i.e., 3, 6, and 10 months post implantation ○ Cardiopulmonary exercise testing at visits 8, 9, and 10 i.e., 3, 6, and 10 months post implantation ○ Hand grip strength assessment at visits 8, 9, and 10 i.e., 3, 6, and 10 months post implantation ○ KCCQ-23 at visits 8 and 10 i.e., 3 and 10 months post implantation ○ EQ-5D-5L at visits 8 and 10 i.e., 3 and 10 months post implantation ○ HADS at visits 8 and 10 i.e., 3 and 10 months post implantation ○ MOCA at visits 8 and 10 i.e., 3 and 10 months post implantation ○ Medication adherence at visits 8 and 10 i.e., 3 and 10 months post implantation • Clarification that KCCQ-23 and not KCCQ-12 is assessed • Clarification that the DDC member constitute the clinical endpoint adjudication committee (EAC)
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Statistical Analysis Plan – Interim Analysis for Part A + B

Version: 1.0 Date: 17.09.2025

Safety and Efficacy of Induced Pluripotent Stem Cell-derived Engineered Human
Myocardium as Biological Ventricular Assist Tissue in Terminal Heart Failure

BioVAT-HF

Study Protocol	Version 7.0 / 20.11.2024
EudraCT No.	2024-515708-38-01
Clinicaltrials.gov-No.	NCT04396899
FOMA-ID	02289
Therapeutic area	Terminal heart failure
SAP Version	1.0/17.09.2025
Sponsor	University Medical Center Göttingen Robert-Koch-Straße 40 37075 Göttingen, Germany
Coordinating Scientist	Prof. Dr. Wolfram-Hubertus Zimmermann University Medical Center Göttingen Department of Pharmacology and Toxicology Robert-Koch-Straße 40 37075 Göttingen, Germany

Approval of the Statistical Analysis Plan

BioVAT-HF

Clinicaltrials.gov-No.: 2024-515708-38-01

SAP Version No: 1.0/17.09.2025

Prof. Dr. Wolfram-Hubertus
Zimmermann

17. Sept. 2025

Coordinating Scientist

Date

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17 SEP 2025

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List of Abbreviations

AAD	Antiarrhythmic drug
ADE	Adverse device effect
AE	Adverse event
AF	Atrial fibrillation
BioVAT	Biological Ventricular Assist Tissue
CPET	Cardiopulmonary exercise testing
CT	Computed tomography
CWTh	Contralateral (untreated) heart wall thickness
EHM	Engineered Human Myocardium
LV-EF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
RV-EF	Right ventricular ejection fraction
KCCQ-23	Kansas City Cardiomyopathy Questionnaire – 23
OSS	Overall Summary Score
pVO ₂	peak oxygen consumption
SMD	Safe maximal dose
TAPSE	Tricuspid annular plane systolic excursion
TWThd	(EHM) Target heart wall thickness in diastole
FU	Follow Up
MMRM	Mixed Model Repeated Measures
IA	Interim Analysis

1 Introduction

1.1 Background and Rationale

[see study protocol, V7.0, dated 20.11.2024]

This SAP specifies the statistical analyses for the interim analysis including patients of Parts A and B of the study. The interim analysis is triggered when 15 patients (including patients from Part A) have been implanted on the LV with the SMD or 5 patients have been implanted on the RV with at least 3 months follow-up completed after the 15th patient with the SMD is treated.

All available data on safety and efficacy will be presented, while hypothesis testing will focus on data up to 3 months follow-up as this will already be available for all patients treated with SMD at the timepoint of the IA.

1.2 Objectives and Endpoints

1.3 Primary objective and endpoint

1.3.1 Safety

The primary safety objective of BioVAT-HF is the identification of a safe maximal dose (SMD). The **primary safety endpoint** is defined as **any adverse event related to the EHM implant**, including in particular arrhythmic events and worsening of disease progression within 28 days (Part A) and the whole study duration (Part B).

1.3.2 Efficacy

The primary efficacy objective is the identification of the efficacious therapeutic utility of engineered human myocardium (EHM) in patients with advanced heart failure (HFrEF EF $\leq 35\%$) despite optimal guideline-directed therapy without (in case of left ventricular [LV] administration) or with (in case of left ventricular [RV] administration) RV dysfunction (TAPSE < 16 mm). The **primary efficacy endpoint** is defined as evidence for structural and functional augmentation of target heart wall by remuscularization as well as patient reported outcome (symptoms) as described by three individually measured primary endpoints:

- **Structural endpoint:**
Change from baseline in EHM target heart wall thickness in diastole (TWThd) measured by echocardiography or cardiac CT or cardiac MRI at 3, 6, and 12 months FU
- **Functional endpoint:**
Change from baseline in left ventricular ejection fraction (LV-EF) measured by echocardiography or cardiac CT or cardiac MRI in the LV/RV cohorts at 3, 6, and 12 months FU
- **Patient reported outcome/quality of life:**

Change from baseline in Kansas City Cardiomyopathy Questionnaire-23 Overall Summary Score (KCCQ-23 OSS) at 3 and 12 months FU

1.4 Secondary objectives and endpoints

1.4.1 Safety

The **secondary safety endpoints** are defined as

- Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death)
- Frequency and severity of arrhythmic events
- Frequency of mechanical perturbation of ventricular function by EHM graft
- Frequency of immune rejection (DSA, CK/CK-MB, cTnT, circulating cell-free allograft DNA [exploratory substudy])

1.4.2 Efficacy

The secondary efficacy objectives of BioVAT-HF are

- To assess effects of EHM-grafts on disease-specific events and symptoms
- Identification of an optimally effective dose range (Part A)

The **key secondary efficacy endpoint** is

- Frequency of recurrent hospitalizations for worsening of heart failure

Further **secondary efficacy endpoints** are

- Change in functional status in patients as determined by six-minute walk test (6MWT; key endpoint: six-minute walk distance), cardiopulmonary exercise testing (CPET; key endpoint pVO₂), and hand-grip strength
- Change in patient reported outcomes assessed by NYHA classification, quality of life score (KCCQ-23, EQ-5D-5L, QoL-VAD) and study adherence motivation (PHQ-9, HAF-17, ESSI, LOT-R, ULS-8, Medication adherence, Trust/Mistrust in medical staff)
- Time to mechanical circulatory assist device implantation.
- Time to heart transplantation
- All-cause and cardiovascular mortality

2 Study methods

2.1 Trial design

This is a combined, open-label, 2-stage, phase I/II safety and efficacy study investigating induced pluripotent stem cell-derived engineered human myocardium (EHM) as biological ventricular assist tissue (BioVAT) in patients with advanced heart failure. A maximum of 53 patients will be recruited over 60 months. The study consists of two consecutive parts (see study protocol, V7.0, dated 20.11.2024, Fig. 2).

Within Part A, a dose finding regimen to determine the Optimally Effective Dose Range and if possible the Safe Maximal Dose (SMD) of EHM will be applied (Fig. 2). A minimum of 8 and a maximum of 18 patients will be included with a minimum of 4 patients treated with the identified MFD.

The dose finding part of the BioVAT-HF FIH study (Part A) has been completed in December 2022 after 28 days of uneventful follow-up after treatment of 4 patients with the MFD.

DDC, DSMB, CEC, PEI approved extension of Part A to a maximum of 18 patients.

DSMB, PEI and CEC endorsed transition into Part B in Q1/2025. They endorsed treatment of the RV with the same dose determined as SMD for the LV.

First patient treated under Part B protocol (CTP V7) was in June 2025. 2 Patients have been treated under the Part B protocol (V7). All others under CTP V6.

As of September 2025 there has been no treatment of RV so far.

2.2 Sample Size

18 patients were included in Part A with 14 patients treated with the identified MFD and with 12 patients with at least 28 days of follow-up. Up to 53 patients are planned to be treated in the trial.

The required number of patients to be enrolled results as a total of the numbers needed for the two parts of the trial. A maximum of $n=18$ patients in dose finding cohorts of $n=2-4$ patients is planned for the first part (Part A). This number of patients is not uncommon for phase I dose-escalation studies and can be justified through simulation studies. In the second part (Part B), $n=35$ patients in two cohorts (min. 5 per LV and RV cohort) will be treated with a focus on the most feasible, safe, and effective approach. In Part B, sample size planning is based on the three individually measured co-primary endpoints: (1) change of target heart wall thickness in diastole, (2) change of left (in case of LV treatment) or right (in case of RV treatment) ventricular ejection fraction, and (3) patient reported outcome assessed by KCCQ-23 OSS. In a pre-post comparison of means a sample size of 30 patients yields a power of 80% (90%) to detect a standardized mean difference (Cohen's d) of 0.47 (0.55) at a two-sided significance level of 10%. As this is an early study to evaluate trends the slightly larger than usual significance level is justified (Kianifard and Islam, 2011). A total number of up to $n=35$ patients will be recruited in Part B. Collectively, a total of $n=53$ patients may be treated in the entire study. Calculations were done using the statistical software nQuery (version 8.3).

2.3 Timing of the Interim Analysis

The interim analysis will be carried out when 15 patients (including patients from Part A) have been implanted on the LV with the SMD or 5 patients have been implanted on the RV with at least 3 months follow-up completed after the 15th (LV implantation) or 5th (RV implantation) patient is treated with the in Part A determined SMD.

2.4 Timing of Outcome Assessments

There are 9 planned trial visits (V2-V10 after the screening visit [V1]) at which outcomes are assessed (timepoint [time slot]):

- V1: Informed consent (at least 24 h before V2)
- V2: Baseline (day -84 to day -14)
- V3: Start of Immunosuppression (day -10 to day -4)
- V4: Hospital Admission (day -6 to day -1)
- V5: Implantation (day 0)
- V6: Follow Up 1 (before release from the hospital)
- V7: Follow Up 2 (day 28±7 – 1 month visit)
- V8: Follow Up 3 (day 84±7 – 3 months visit)
- V9: Follow Up 4 (day 168±7 – 6 months visit)
- V10: Follow Up 5 (day 336±7 – 12 months visit)

Note: 28 days are considered one month.

3 Statistical Principles

3.1 Confidence intervals and p-values

Testing is performed to a two-sided significance level of 10% and a one-sided significance level of 5%, respectively.

P-values ≥ 0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as “<0.001”. The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures, and will be accompanied by 90%-confidence intervals whenever possible.

3.2 Analysis population for the interim analysis

The interim analysis will present data of all patients that received an EHM implant (20). The 4 low and middose patients will only be included in the descriptive statistics and safety analyses, while 16 patients treated with the SMD up to the timepoint of this interim analysis will also be part of the efficacy analyses. One of the 16 patients was implanted with 19xEHM instead of 20xEHM and will be analysed among those “treated with SMD” as the intention was to treat this patient with the highest dose.

4 Trial population

4.1 Screening data

[see study protocol, V7.0, dated 20.11.2024, for inclusion and exclusion criteria]

4.2 Eligibility

[see study protocol, V7.0, dated 20.11.2024, for inclusion and exclusion criteria]

4.3 Withdrawal/follow-up

[see study protocol, V7.0, dated 20.11.2024, for inclusion and exclusion criteria]

4.4 Baseline patient characteristics

All continuous variables will be summarised using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all data will be listed, sorted by site, treatment and subject, and when appropriate by visit number within subject. All summary tables will be structured with a column for each treatment and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

Summary statistics will be provided for variables summarizing subject disposition, protocol deviations, demographic characteristics, baseline characteristics, concurrent illnesses and medical conditions, prior and concurrent medications.

5 Analysis

5.1 eCRF Forms

The interim analysis presents all data points captured in the eCRF (secuTrial) as listed in the following forms (captured at)

- Inclusion and Exclusion Criteria (Baseline)
- Anamnesis and Clinical Diagnosis (Baseline)
- Physical examination (Hospital Admission, FU1, FU2, FU3, FU4, FU5)
- Laboratory diagnostics (Baseline, Hospital Admission, FU1, FU2, FU3, FU4, FU5)
- Blood draw for allograft DNA assessment (Baseline, FU1, FU2, FU3, FU4, FU5)
- Electrocardiogram (Baseline, Hospital Admission, FU1, FU2, FU3, FU4, FU5)
- Echocardiography* (Baseline, Hospital Admission, FU1, FU2, FU3, FU4, FU5)

- Magnetic resonance imaging and Computed Tomography** (Baseline, FU1, FU3, FU4, FU5)
- 6MWT (Baseline, FU1, FU2, FU3, FU4, FU5)
- Spiroergometry (Baseline, FU1, FU2, FU3, FU4, FU5)
- Hand grip strength (Baseline, FU1, FU2, FU3, FU4, FU5)
- EuroScore II (Baseline)
- Quality of life questionnaires (Baseline, FU1, FU3, FU5)
- Surgical Procedure (Implantation)
- ICD/CRTD-event recorder readout (Baseline, Hospital Admission, FU1, FU2, FU3, FU4, FU5)
- TDM Calcineurin Inhibitors (Immunosuppression, Hospital Admission, FU1, FU2, FU3, FU4, FU5)
- Hospitalization for heart failure decompensation (any time)
- Concomitant medications (any time)
- Concomitant therapy (any time)
- Protocol deviation (any time)
- End of study (permanent withdrawal of immune suppression, death, heart transplantation) (any time)
- Adverse Event (any time)
- Serious Adverse Event (any time)

*Echocardiography data will be evaluated at the study centers (LV-EF) and by a Core-Lab (Leuven).

**Magnetic resonance imaging and Computed Tomography will be analysed by a Core-Lab (Göttingen).

5.2 Analysis methods

5.2.1 Descriptive statistics

Outcomes in the above mentioned eCRF forms are either captured only at one visit, e.g. the Baseline visit or at several visits (Baseline, Hospital Admission, FU1, FU2,..., FU5).

In case of single measurements at only one visit, data will be summarized in a table containing each data point for each patient and each item of the corresponding eCRF form as well as summary statistics (e.g. mean, standard deviation for continuous variables/items, frequencies and percentages for categorical variables). For example, the Anamnesis Form is only captured at baseline. Date of examination is a nominal variable, therefore for each patient the examination dates will be shown, but now summary statistic calculated. Sex is a categorical variable. Each input (male, female) will be presented as well as frequencies (percentages) summarizing all

patients. Age is a continuous variable and will be summarized using the mean and standard deviation. Table 1 gives an example.

Table 1. Example for summarizing baseline data providing data on individual participants and summary statistics (5 patients)

Item	Pat1	Pat2	Pat3	Pat4	Pat5
1. Date of examination	2021-02-04	2021-05-07	2021-06-11	2021-10-31	2022-03-20
2. Sex	female	male	male	female	male
- female: 2 (40%)					
- male: 3 (60%)					
3. Age [years]	49	61	63	66	52
- Mean (SD): 58.20 (7.33)					
4.					

eCRF Forms that capture longitudinal measurements, e.g. laboratory measurements at Baseline, FU1, FU2,...,FU5, will be presented showing each patients progress as well as the number of documented observations at each visit and a suitable summary statistic. Continuous variables will be visualized by line plots showing each patients progress over time along with the number of observations and the mean progress accompanied by pointwise 95% confidence intervals. For example Haemoglobin is a laboratory item captured at Baseline, Hospital Admission, and each of the five Follow Up visits. It is a continuous variable. Table 2 shows the raw data presentation while Figure 1 gives a visualization.

Table 2. Example for summarizing longitudinal data (5 patients)

Item	Summary	Baseline	Hosp Adm	FU 1	FU2	FU3	FU4	FU5
1. Haemoglobin [g/dl]	- Mean (SD)	43.10 (3.36)	45.08 (4.76)	42.62 (5.76)	44.64 (2.37)	39.56 (5.26)	39.88 (5.08)	41.43 (0.21)
	- N	5	5	5	5	5	4	3
	Pat_01	45.7	48.4	36.3	45.4	43.7	41.6	#NV
	Pat_02	40.1	43	46.8	46.8	44.6	42.7	41.5
	Pat_03	40.4	37.9	45.4	44.4	36	#NV	#NV
	Pat_04	47.6	46.3	36.5	45.9	32.3	32.3	41.6
	Pat_05	41.7	49.8	48.1	40.7	41.2	42.9	#NV

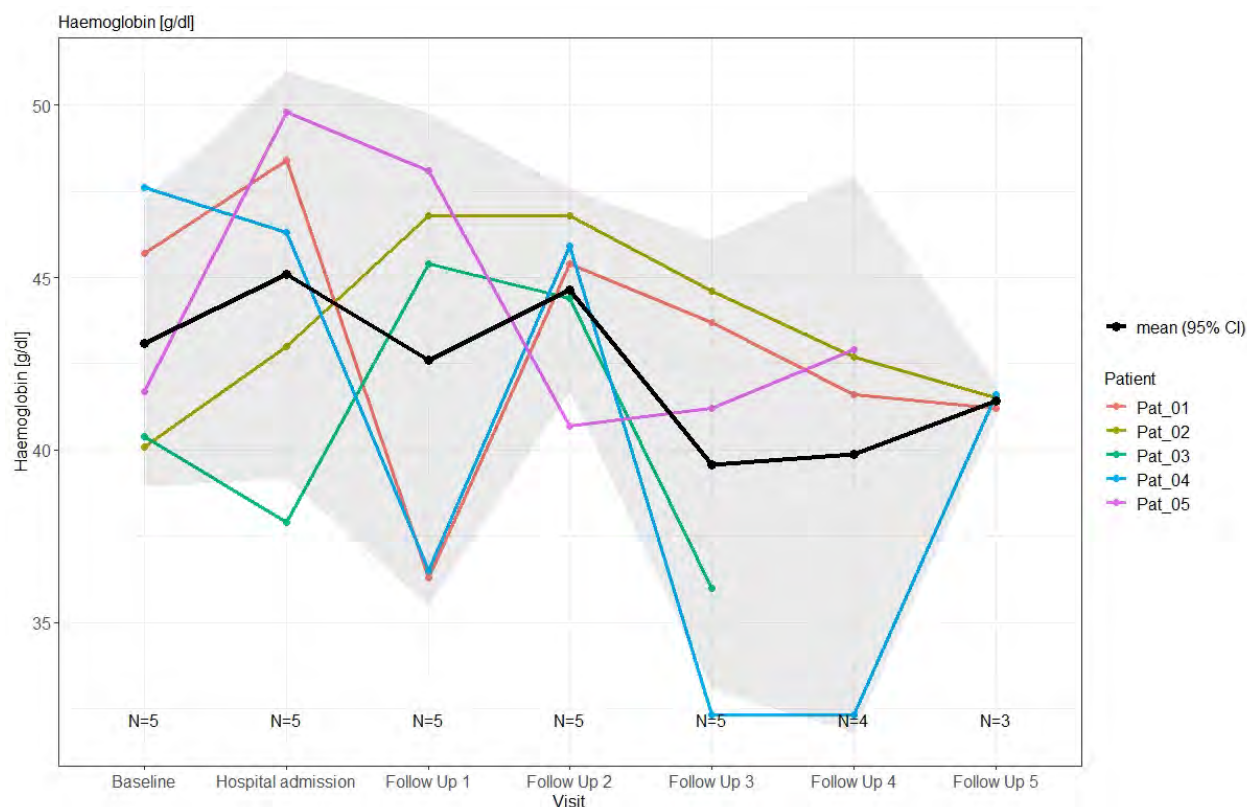


Figure 1. Example for visual presentation of longitudinal data (5 patients)

Forms without visit affiliation, e.g. Adverse Events, Serious Adverse Events, are presented as line listings of all documented cases. Items that can be summarized will be shown in an accompanying table giving frequencies and percentages.

5.2.2 Adjustments for Multiple Testing

Error control across multiple looks

Since hypothesis testing in this interim analysis focusses on all available data up until the three months follow up while the final analysis is focussed on outcomes at 12 months follow up, we will not employ type I error spending functions to ensure type I error rate control.

Error control across multiple endpoints

There are three primary endpoints that will be assessed independently.

The three **primary endpoints** will be tested two-sided at a significance level of 10%. Type I error rate control across those three tests will be ensured by using the **Hochberg procedure** (Hochberg, 1988). The primary outcomes are:

- Change from baseline in EHM target heart wall thickness in diastole (TWThd) measured by echocardiography or cardiac CT or cardiac MRI at 3 months FU

- Change from baseline in left (in case of LV EHM implantation) or right (in case of RV EHM implantation) ventricular ejection fraction (LV-EF/RV-EF) measured by echocardiography or cardiac CT or cardiac MRI in the LV/RV cohorts at 3 months FU
- Change from baseline in Kansas City Cardiomyopathy Questionnaire-23 Overall Summary Score (KCCQ-23 OSS) at 3 months FU

Secondary endpoints will only be tested if all three primary hypotheses were rejected.

The **secondary endpoints** are:

- Recurrent hospitalizations for worsening of heart failure (complete FU)
- Change in 6-minute walk distance from baseline at 3 months FU
- Change in VO2max from baseline at 3 months FU
- Change in hand-grip strength from baseline at 3 months FU
- NYHA functional class over 3 months FU
- Change in EQ-5D-5L VAS from baseline at 3 months FU
- Other Patient-reported outcomes (HADS, KCCQ subscales, MoCA, medication adherence, B-IPQ, TEX-Q) at 3 months FU
- Time to mechanical circulatory assist device implantation
- Time to heart transplantation

All secondary endpoints will be tested at a nominal significance level of 10% two-sided.

5.2.1 Safety endpoints

Frequencies and proportions of patients with one of the following safety endpoints will be reported with Clopper-Pearson exact confidence intervals.

- All-cause mortality
- Cardiovascular mortality
- Any adverse event related to the EHM implant
- Any adverse event related to the surgical procedure
- Any adverse event related to the immune suppression protocol
- Frequency of major adverse cardiac events (MACE; non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death)
- Frequency and severity of arrhythmic events
- Frequency of mechanical perturbation of ventricular function by EHM graft
- Frequency of immune rejection (DSA, CK/CK-MB, cTnT, circulating cell-free allograft DNA)

```
bt <- binom.test(x, n, conf.level = 1 - alpha) #x=No_success, n=sample size
bt$conf.int    # exact Clopper-Pearson interval
bt$estimate    # sample proportion
```

5.2.2 Primary efficacy endpoints

Change from baseline in EHM target heart wall thickness in diastole (TWThd), change from baseline in left (in case of LV EHM implantation) or right (in case of RV EHM implantation) ventricular ejection fraction (LV-EF or RV-EF) as well as change from baseline in KCCQ-23 OSS at 3 months FU will be analyzed using Gaussian linear models for repeated measures (so-called MMRM). These models use all available data (even beyond 3 months, but analyses will focus on 3 months FU). Assessments of changes are available at the following visits

- EHM target heart wall thickness in diastole (TWThd) (Baseline [alternatively Hospital admission], FU2, FU3, FU4, FU5)
- Left or right ventricular ejection fraction (LV-EF or RV-EF; Baseline [alternatively Hospital admission], FU2, FU3, FU4, FU5)
- KCCQ-23 OSS (Baseline, FU3, FU5)

and will be fitted with time (FU visit) as factor, and baseline values as covariate. The error terms are assumed to follow a multivariate normal distribution with unstructured covariance.

The null and alternative hypotheses are stated as (as an example for KCCQ-23 changes from baseline):

$H_0: \mu_{KCCQ,t} = 0$ vs.

$H_1: \mu_{KCCQ,t} \neq 0,$

where $\mu_{KCCQ,t}$ represent the mean change from baseline in KCCQ-23 at time points $t=FU3, FU5$.

Least squares means will be reported with 90% confidence intervals (CI) and p-value for testing the hypothesis at FU3

```
library(mmr)
## MMRM for differences
mod.change<-mmrm(KCCQ_change ~ VISIT + KCCQ_BL + us(VISIT|SUBJID),
                  primend.data, method="Kenward-Roger")

# LS-means of change at FU3 and FU5
emm <- emmeans(mod.change, ~ VISIT)
emm

# Pairwise contrast
contrast(emm, method = "pairwise")
```

5.2.3 Secondary endpoints

Recurrent hospitalizations for worsening of heart failure

The number of **recurrent hospitalizations for worsening of heart failure** will be analysed using a negative binomial regression model accounting for between-patient heterogeneity (Rogers et al, 2014). The model accounts for varying follow-up times. The estimated event rate will be given per person-year with 90% confidence intervals.

```

library(MASS)

# Negative binomial regression
fit_nb <- glm.nb(
  hosp ~ 1 + offset(log(fup_time)),
  data = df
)

# Estimated event rate (per person-year)
lambda_hat <- exp(coef(fit_nb)[1])
lambda_hat

# 95% CI for event rate
confint(fit_nb)
exp(confint(fit_nb)) # back-transformed from log-rate

```

Change in 6-minute walk distance from baseline at 3, 6, and 12 months FU

Will be analysed along the same lines as the primary endpoints (MMRM), see above.

Available timepoints: Baseline, FU2, FU3, FU4, FU5. P-value for testing the hypothesis at FU3 will be given.

Change in VO2max from baseline at 3, 6, and 12 months FU

Will be analysed along the same lines as the primary endpoints (MMRM), see above.

Available timepoints: Baseline, FU2, FU3, FU4, FU5. P-value for testing the hypothesis at FU3 will be given.

Change in hand-grip strength from baseline at 3, 6, and 12 months FU

Will be analysed along the same lines as the primary endpoints (MMRM), see above.

Available timepoints: Baseline, FU2, FU3, FU4, FU5. P-value for testing the hypothesis at FU3 will be given.

NYHA functional class at 3, 6, and 12 months

Longitudinal (repeated) measures of the NYHA class will be analysed using a mixed-effects proportional odds model with visit and baseline NYHA class as factors. The correlation in longitudinal assessments within the same patient will be accounted for by a random patient effect.

The null and alternative hypotheses are stated as:

H_0 : $OR_{NYHA, t} \leq 1$ vs.

H_1 : $OR_{NYHA, t} > 1$,

where $OR_{NYHA, t}$ denotes the odds ratio (vs baseline) at time points $t = \text{FU2, FU3, FU4, FU5}$, respectively.

The intervention effect will be reported as odds ratios (OR) with 90% confidence intervals and a p-value for testing the hypothesis at FU3.

```
library(ordinal)

# Fit mixed-effects proportional odds model
fit <- clmm(
  nyha ~ visit + nyha_BL + (1 | id),
  data = df,
  link = "logit"    # proportional odds
)

summary(fit)

# Estimated odds ratios for visits (vs reference visit)
exp(coef(fit))

# Likelihood ratio test for visit effect
anova(fit)
```

Change in EQ-5D-5L from baseline at 3 months FU

Will be analysed along the same lines as the primary endpoints (MMRM), see above.

Available timepoints: Baseline, FU3, FU5. P-value for testing the hypothesis at FU3 will be given.

5.2.1 Safety endpoints

All-cause mortality (if reasonable number of events)

A Kaplan-Meier curve will be shown along with survival probability estimates (Kaplan-Meier estimates) at 3 months, 6 months and 12 months with 95% confidence intervals. Here the `bpcp` package in R implements several methods for exact or nearly exact confidence intervals for Kaplan-Meier survival probabilities at fixed time points. It implements a linear rank-based (LR) method described by Fay & Shaw (2010). This approach provides more exact coverage than the standard Greenwood/log-log CIs that come from `survfit()`. It inverts exact binomial-type tests (like Clopper-Pearson does for proportions), adapted for censored survival data.

```

library(survival)
library(survminer)
library(bpcp)

fit <- survfit(Surv(time, status) ~ 1, data = df)

# Plot KM curve with 95% CI
ggsurvplot(
  fit,
  data = df,
  conf.int = TRUE,
  xlab = "Time (days)",
  ylab = "Survival probability"
)

# Extract exact confidence intervals at timepoints of interest
km_ci <- tidykmciLR(
  fit,
  time = c(90, 365), # time points of interest
  conf.level = 0.95
)

km_ci

```

Cardiovascular mortality (if reasonable number of events)

Here cumulative incidence function curves will be shown reporting the cumulative incidence of CV death, with non-CV death as the competing event.

```

library(cmprsk)

# CIF analysis: CV death (1) with non-CV death (2) competing
cif_fit <- cuminc(
  ftime = df$time,
  fstatus= df$status,
  cencode= 0
)

# Show cumulative incidence estimates
cif_fit

# Plot CIF for CV death and non-CV death
plot(cif_fit, xlab = "Days", ylab = "Cumulative incidence")

```

Time to mechanical circulatory assist device implantation (if reasonable number of events)

Same as CV mortality (here all-cause death as competing).

Time to heart transplantation (if reasonable number of events)

Same as CV mortality (here all-cause death as competing).

5.3 Statistical software

All analyses will be performed using R, Version 4.3.1 or higher, a language and environment for statistical computing [1], or SAS, Version 9.4 or higher.

5.4 References

1. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
2. Hochberg YA (1988). Sharper Bonferroni procedure for multiple significance testing. *Biometrika* 1988; 75: 800–802.
3. Rogers JK et al. (2014). Analysing recurrent hospitalizations in heart failure: a review of statistical methodology, with application to CHARM-Preserved. *Eur. J. Heart Fail.* 16, 33–40 (2014).
4. Clopper, C. J., & Pearson, E. S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika*, 26(4), 404–413.
5. Fay, M. P., & Shaw, P. A. (2010). Exact and asymptotic weighted logrank tests for interval censored data: the interval R package. *Journal of Statistical Software*, 36(2), 1–34.