Estimating versus **M**easuring **P**lasm**a** Volume and **K**idney Func**t**ion in Acute Decompensated **C**ongestive **H**eart **F**ailure

EMPAKT-CHF

Statistical Analysis Plan

Version 1.0 (final) of 3rd February 2020

Investigational medicinal product:	Visible fluorescent injectate (VFI) (FD004)
Comparator:	Not applicable
Indication:	Hospitalized Patients with acute decompensated congestive heart failure
Sponsor:	Charité – Universitätsmedizin Berlin
Financial support:	
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Protocol Version:	5.0 from 11th February 2019

Version history

Version	Changes
0.1	Initial draft
0.2	Inclusion of formulas for plasma volume and blood volume, Details for regression models Addition of baseline parameters
0.3	Clarification of analyses and time points to be used
1.0	Inclusion of Cystatin C formulas for estimation of GFR

Approved by

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Principal Investigator

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Abbreviations

Abbreviation	Term
AE	Adverse Event
AKI	Acute Kidney Injury
CHF	Congestive Heart Failure
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
eGFR	estimated Glomerular Filtration Rate
ePV	estimated Plasma Volume
eTBV	estimated Total Blood Volume
FAS	Full Analysis Set
GFR	Glomerular Filtration Rate
mGFR	measured Glomerular Filtration Rate
mPV	measured Plasma Volume
mTBV	measured total blood volume
NYHA	New York Heart Association
PPP	Per protocol population
PV	Plasma Volume
PVS	Plasma Volume Status
TBV	total blood volume
TBVS	Total Blood Volume Status
VFI	Visible fluorescent injectate
WOCBP	Women of child-bearing potential

1 Background

1.1 Trial objective

This is an investigator-initiated, one-armed phase II clinical trial designed to primarily evaluate the safety and functionality of the FAST PV and measured Glomerular Filtration Rate (mGFR) Technology in Congestive Heart Failure (CHF) patients.

- Safety will be assessed by determination of the absolute and relative frequencies of AEs and SAEs related to Visible fluorescent injectate (VFI).
- Function of the FAST PV measurement will be assessed by determining the plasma stability of the FD003 high molecular weight marker over the 15, 30, and 60 minute blood draws and applying the following criteria:
  - The FAST PV measurement is considered as stable, if the mean plasma concentration of FD003 at 30 minutes is not more than 10% lower than the mean plasma concentration at 15 minutes AND if the mean plasma concentration at 60 minutes is not more than 10% lower than the mean plasma concentration at 30 minutes
  - We will determine the percentage of patients which show a decline in the plasma concentration of FD003 of more than 10% from 15min to 30min and separately from 30 min to 60min. This percentage should be ideally close to 0.

Secondary objectives are the following:

- To evaluate how estimated plasma volume (ePV) and estimated total blood volume (eTBV) assessments on days 1 and 3 (by established measures as Nadler's formula (eTBV), Kaplan-Hakim formula (ePV), Strauss formula (ePV Change)) predict measured PV (mPV; as assessed by FAST methodology) and measured TBV (mTBV; calculated from mPV and measured hematocrit) at these time points.
- To evaluate how a clinical evaluation (including a reasonable subset of the variables age, gender, BMI, hematocrit, edema grade, presence of pulmonary rales, presence of jugular venous congestion, arterial blood pressure, New York Heart Association (NYHA) stage, respiratory rate) on days 1 and 3 predicts mPV and mTBV at these time points.
- To evaluate whether estimated Glomerular Filtration Rate (eGFR) (calculation by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, sMDRD, Cystatin C formula, and Creatinine-Cystatin C formula) on days 1 and 3 provides an accurate estimate of measured GFR (mGFR; assessed by FAST methodology) in heart failure patients undergoing active fluid management.
- To evaluate whether patients with a low mGFR/eGFR ratio on days 1 and 3 are at higher risk of developing acute kidney injury (AKI) within the following 48-72 hours.
- To evaluate whether ranges of mPV (low mPV, normal mPV, high mPV) and/or mTBV (low mTBV, normal mTBV, high mTBV) on days 1 and 3 are associated with subjectively reported symptoms (PGA, dyspnea, dizziness, nausea) determined using patient symptom assessment sheets within the following 24-48 hours.

- To evaluate whether patients with low mPV or low mTBV on day 1 or on day 3 are at risk of developing low-output complications within the next 24-48 hours (subjectively reported dizziness/nausea, PGA, hypotension, need for iv-fluid therapy).
- To evaluate whether patients with a high mPV or a high mTBV on day 1 and/or day 3 are at risk of being refractory to diuretic therapy (e. g. require dosage increases of furosemide/torasemide, require ultrafiltration/RRT within the next 24-48 hours, fail to improve subjectively in PGA and dyspnea scales).
- To evaluate whether the time course of ePV and eTBV from day 1 to day 3 adequately reflect the time course of mPV and mTBV at these time points.
- To evaluate whether the change of mPV and mTBV from day 1 to day 3 is predictive of length of stay, dialysis/ultrafiltration requirement, or rehospitalization within 30 days, or mortality.
- To evaluate whether the time course of mGFR from day 1 to day 3 correlates temporally with or predicts the time course of eGFR within the next 24-48 hours.
- To evaluate (by an adjudication committee) whether clinical decision making would have been affected by adding FAST GFR and PV measurements to clinical routine including the following questions:
    - Would FAST GFR and PV measurements have changed clinical management overall?
    - Would the FAST GFR and PV measurement on day 1 have led to applying a different diuretics dosage between day 1-3?
    - Would the FAST GFR and PV measurement on day 3 have led to applying a different diuretics dosage between day 3-5?
    - Would the FAST GFR and PV measurement on day 1 have led to choosing dialysis/ultrafiltration instead of diuretics escalation between day 1-3?
    - Would the FAST GFR and PV measurement on day 1 have led to choosing diuretics escalation instead of dialysis/ultrafiltration between day 1-3?
    - Would the FAST GFR and PV measurement on day 3 have led to choosing dialysis/ultrafiltration instead of diuretics escalation between day 3-5?
    - Would the FAST GFR and PV measurement on day 3 have led to choosing diuretics escalation instead of dialysis/ultrafiltration between day 3-5?

1.2 Trial design

This is an investigator-initiated, human clinical trial designed to evaluate the safety and functionality of the FAST PV and mGFR Technology in CHF patients.
Patients enrolled in the study will be administered VFI (Day 1) and with a second dose occurring 48h (+/- 5h) after the initial dose (Day 3).
After consenting to be enrolled in the study, patients meeting the enrollment criteria will receive a single dose of VFI. Blood draws (approximately 2 mL) will be collected pre-dose and at

15 (± 5), 30 (± 5), 60 (+30), and 180 (+120) minutes post-dose. The physician will remain present at the bedside of the patient for 10 minutes after administering the FAST VFI. Thereafter, the patient will be monitored on an ongoing basis, with clinical staff assessing the patient's condition along with the 15, 30, and 60 minutes blood draws specified in the protocol.

Patients will be treated according to standard of care throughout the time of their hospitalization. A second dose of VFI will be administered 48h (+/- 5h) after the initial dosing. Blood draws (approximately 2 mL) will be collected pre-dose and at 15 (± 5), 30 (± 5), 60 (+30), and 180 (+120) minutes post-dose.

Prior to administering the first and second dose of VFI, the physician will be asked to complete a very brief survey to provide a qualitative assessment of the patient's perceived volume status and renal function prior to initiating the FAST PV and mGFR measurements.

Laboratory values and assessments (see Table 1) will be captured on each day that the VFI is administered and these data will be entered into the case report form. Any AEs determined by the investigator will be captured. Patients will also receive a follow up phone call 7 days (± 2 days) after the first dose of VFI was administered, and a second follow up call 30 days after the first dose of VFI was administered as an end-of-study follow up call. Any AEs determined by the investigator will be captured during the follow up calls and will be followed to resolution. Additionally, in-person follow-up visits may be scheduled as needed.

Table 1: Schedule of Study Events/Examinations

Study Timepoint	Data collected from chart prior to enrolling	Patient Enrolled and Dosed	Data collected between 1st and 2nd Dose	Patient 2nd VFI Dose	Data Collected after 2nd Dose	Data Collected Before Discharge	Follow up in person or by phone call	Follow up phone call/ Follow up Visit for Pregnancy Test if WOCBP
Visit	**Pre-Dose**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 10**	**Day 30**
Informed Consent	X							
Inclusion/ Exclusion Criteria Assessment	X							
Structural Heart Disease Criteria Assessment		X						
Demographics		X						
Comorbidities		X						
Full medication list on enrollment into the study and during follow-up		X	X	X	X	X		
Patient Symptom Assessment Survey		X	X	X	X	X		
Clinical Presentation/ Exam		X	X	X	X	X		
Treating Physician Survey		X		X				
Study-Related Laboratory Measurements		X	X	X	X	X		
Routine Clinical Parameters	X*	X	X	X	X	X		
Clinical Data	X*	X	X	X	X	X		
Adverse events		X	X	X	X	X	X	X
Blood specimen for marker analysis [a]		X		X				
Discharge from study								X
Adjudication Committee Evaluation								X
WOCBP Pregnancy test	X			X				X

[a] Blood Specimens for the determination of the FAST PV and mGFR will be taken pre-dose and at 15 (±5), 30 (±5), 60 (+ 30) and 180 (+ 120) minutes post dose on Day 1 and Day 3. Note that the 30 minute blood draw must be at least 10 minutes after the 15 minute blood draw, and the 180 minute blood draw must be at least 100 minutes after the 60 minute blood draw.

*Will be collected retrospectively after study inclusion

Treatment

The visible fluorescent injectate (VFI) agent is comprised of a mixture of two different molecular weight carboxymethyl dextran molecules (5 kD and 150 kD) with different fluorescent dye molecules attached. The 5 kD carboxymethyl dextran, (FD001), is labeled with 5-aminofluorescein and the 150 kD carboxymethyl dextran, (FD003), is labeled with 2-sulfohexamine rhodamine. These fluorescent labels are covalently attached to the dextran through the carboxymethyl moiety. Please see the chemical structures for FD001 and FD003, which are shown in Figure 1 and Figure 2 , respectively.

The high molecular weight labeled carboxymethyl dextran is not rapidly cleared from the vasculature and is not rapidly cleared by passive filtration in the kidneys; therefore, its concentration in the blood stream after injection provides a direct measurement of the total blood volume. The low molecular weight labeled carboxymethyl dextran is also not patient to rapid metabolism but is freely filtered by the kidneys.

The VFI is administered intravenously through a bolus injection. The anticipated single dose for a 70 kg human will consist of 35 mg of FD001 and 12 mg of FD003 diluted at 15.67 mg/mL mg/mL for a total injection of 3 mL of VFI.

There is no placebo/reference medication for this study as this is a one-armed study

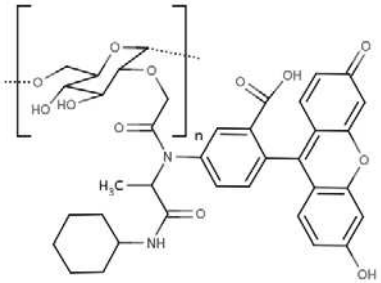

Figure 1: Structure of FD001 (5 kD carboxymethyl dextran with covalently bound 5 aminofluorescein)

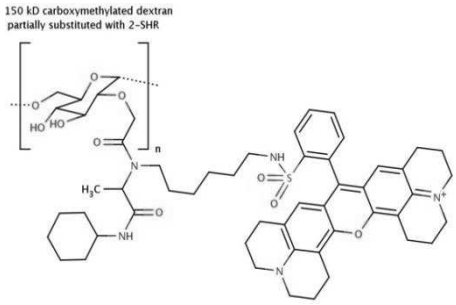

Figure 2: Structure of FD003 (150 kD carboxymethyl dextran with covalently bound 2 sulfohexamine rhodamine)

Randomization

Not applicable since this is a one-armed study.

Blinding and Unblinding

Data from the FAST PV and mGFR Technology will not be evaluable by the treating physician,

but will be made available to an adjudication committee for consideration after patient enrollment has concluded or at predetermined intervals during the course of the study.

Sample size

The primary objective is to assess the safety and function of the FAST PV and mGFR Technology in hospitalized patients with heart failure. This is a phase II trial. It is planned to enroll 50 eligible patients. No formal hypothesis test is performed to assess the primary objective. This is justified as follows:

1. Both function and safety cannot be uniquely assessed by one specific endpoint, but there exist several relevant endpoints and endpoint-related criteria which must be investigated in order to assess the global function and safety.
2. For reasons of feasibility, the studies sample size is limited to 50 patients which is an adequate number in the context of a phase II trial. However, this limited number does not allow to address a complex multiple testing problem with adequate power.

Therefore, all analyses are purely descriptive.

Inclusion criteria

- Written informed consent indicating that they understand the purpose of and procedures required for the study and are willing to participate in the study prior to any study related measures present
    - Hospitalized patient with acute decompensated heart failure, diagnosed on the basis of the presence of at least one symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography) of heart failure.
    - Subject: ≥ 18. Male with female partners of childbearing potential gave agreement to practice abstinence or use condoms from enrollment through 90 days after administration of the last dose of study drug present.
- A female subject is eligible to enter the study if she is:
    - Not pregnant or nursing
    - Of non-childbearing potential (i.e., post-menopausal defined as having been amenorrheic for at least 1 year prior to screening, or has had bilateral tubal ligation at least 6 months prior to administration of study drug or bilateral oophorectomy or complete hysterectomy)
    - If of childbearing potential, must have a negative urine or serum pregnancy test prior to drug administration and be using a highly effective means of contraception during study participation and until 1 month after the last dose of study drug. Highly effective contraception methods include:
        - Total abstinence (when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods

of contraception
- Combination of any two of the following (i+ii or i+iii, or ii+iii): (i) use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate < 1%), for example hormone vaginal ring or transdermal hormone contraception.
  (ii) placement of an intrauterine device (IUD) or intrauterine system (IUS)
  (iii) barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps)
- In case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking study treatment

- Partner of a male patient: Agreement to use a medically acceptable method of contraception (a barrier method, intrauterine device, or hormonal contraception) from enrollment through 90 days after administration of the last dose of study drug present.
- Male patient agreed to not donate sperm from enrollment through 90 days after administration of the last dose of study drug present
- Patient is able to communicate effectively with the study personnel.
- Patient is informed of the nature and risks of the study and give written informed consent prior to enrollment.

Exclusion criteria

- New or ongoing myocardial infarction or instable angina present at the time of planned study inclusion.
- Patient shows evidence of severe infection other than pneumonia, or active internal bleeding (characterized by recent decrease of blood hemoglobin concentration by more than 2 g/dl).
- Patient experiences new onset atrial fibrillation.
- Patient has an elective surgery planned during the 30 days they are enrolled in the study.
- Patient has a psychiatric disease or a history of illicit drug use that would prohibit them from complying with study requirements
- Prior exposure to VFI present.
- History of any clinically significant allergic or negative reactions, side effects, or anaphylaxis to fluorescent dyes, or dextran molecules present.
- Patient requires intravenous vasodilators or inotropic agents (other than digoxin or digitoxin) for heart failure.
- Patient undergoes chronic dialysis (for example peritoneal or hemodialysis) treatments.
- Patient is in cardiogenic shock or on vasopressors.
- Hypotension as defined by blood pressure < 90 systolic and/or < 50 mm Hg diastolic exists.
- Patients suffering from significant non-cardiac diseases of other organ systems (e. g. Malignancies, significant neurological diseases).
- Patient does not have a working telephone.

- Patient is a pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin laboratory test.
- Lack of willingness to storage and disclosure of pseudonymous disease data in the context of the clinical trial present.
- Subject has a participation in another interventional clinical trial during this study or within 30 days (or longer) before entry into this trial (as a minimum; 5 x elimination half-life / terminal elimination of an investigational medicinal product).
- Subject is legally detained in an official institution.
- Subject is dependent on the sponsor, the investigator or the trial sites.

2 Analysis sets

2.1 Definitions

The full analysis set (FAS) comprises all subjects who received at least one dose of VFI. The FAS will be based on the intention-to-treat principle.
The per-protocol population (PPP) will include all subjects in the FAS without major protocol violations. Prior to the analysis, the protocol violations will be evaluated and for each patient a decision regarding the allocation to the analysis populations will be made during the data review meeting (see section 6).
There will be no separate safety analysis set since there are only two dosing visits within two days.

2.2 Application

All analyses including safety evaluations will be done in the FAS. For the efficacy endpoints the analysis will also be done in the PPP as a sensitivity analysis.

3 Trial centres

50 patients with acute decompensated heart failure were enrolled at the Nephrology and Cardiology departments of Charité - Universitätsmedizin Berlin as well as at the Kerckhoff-Klinik Bad Nauheim.

4 Analysis variables

4.1 Demography and baseline characteristics

- Age at inclusion (years)
- Gender
- Ethnicity
- Height (cm)
- Weight (kg)

- BMI (kg/m^2)
- Paroxysmal nocturnal dyspnea during previous 3 days
- Night cough
- Cardiomegaly (clinical or on chest X-ray or echocardiography)
- S3 gallop
- Hepatojugular reflux
- Hepatomegaly
- Pleural effusion
- Last stable body weight before admission ("best body weight")(kg)
- NYHA-Stage
- Systolic Blood Pressure
- Diastolic Blood Pressure
- Mean Arterial Pressure
- SpO2
- Heart rate
- Respiratory rate
- Comorbidities (Chronic Kidney Disease stage, previous myocardial infarction, hypertension, preexisting coronary heart disease, preexisting congestive heart failure, previous hospitalization for acute decompensated heart failure, Diabetes mellitus, Cerebral vascular disease, Peripheral arterial occlusive disease, Other comorbidities)
- Medication on admission (ACE inhibitor, AT1 receptor blocker, AT1/Neprilysininhibitor, Loop diuretic, Furosemide p.o. including dosage (mg) per 24h prior to dosing, Torasemide p.o. including dosage (mg) per 24h prior to dosing, Thiazide diuretic, Aldosterone antagonist, beta-Blocker, Nitrates, Digoxin/Digitoxin)
- Structural Heart disease Criteria (Last documented left ventricular ejection fraction (%), evidence of left ventricular hypertrophy, evidence of left atrial enlargement, evidence of diastolic dysfunction, other cause of heart failure)
- Baseline Serum creatinine and Baseline eGFR
- eGFR Day1
- Serum creatinine Day 1
- NT-proBNP
- Hematocrit
- Serum Sodium from routine lab

4.2 Primary variables

Global aim is to assess the safety and function of the FAST PV and mGFR Technology in hospitalized patients with heart failure.

- Absolute and relative frequencies of AEs and SAEs related to VFI.
- Plasma stability of the FD003 high molecular weight marker over the 15, 30, and 60 minute blood draws at day 1 and 3

4.3 Secondary variables

4.3.1 Efficacy

- Estimated PV (by Kaplan-Hakim's formula, see chapter 7.2) and measured PV (assessed by FAST methodology) on days 1 and 3
- Estimated TBV (by Nadler's formula, see chapter 7.2) and measured TBV (mTBV: calculated from mPV and measured hematocrit, see chapter 7.2) on days 1 and 3
- Estimated GFR (CKD-EPI formula, sMDRD, Cystatin C formula, and Creatinine-Cystatin C formula, see chapter 7.2) and measured GFR (assessed by FAST methodology) on days 1 and 3 in heart failure patients undergoing active fluid management
- Development of AKI within 72 hours after day 1 and 48 hours after day 3 respectively
- Current level and level within last 24 hours of well-being, dyspnea, dizziness, nausea reported on day 1 and day 3
- Subjectively reported symptoms (PGA, dyspnea, dizziness, nausea) determined using patient symptom assessment sheets within the following 24 and 48 hours on day 1 and following 24 and 48 hours on day 3
- Low-output complications within the next 24 and 48 hours (e. g. subjectively reported dizziness/nausea, PGA, hypotension (Systolic <100, diastolic <60mmHg), need for iv-fluid therapy) at day 1 and within the next 24 and 48 hours at day 3
- Risk of being refractory to diuretic therapy (e. g. require dosage increases of furosemide/torasemide, require ultrafiltration/RRT within the next 24-48 hours, fail to improve subjectively in PGA and dyspnea scales) within 24 and 48 hours on day 1 and day 3

4.3.2 Safety/Tolerability

- Length of stay, dialysis/ultrafiltration requirement, or rehospitalization within 30 days, or mortality
- Laboratory values (Serum creatinine (mg/dl), Hematocrit (%), creatine phosphokinase (CPK) (U/l), NT-proBNP (ng/l), Alanine aminotransferase (ALT) (U/l), Aspartate aminotransferase (AST) (U/l), Alkaline phosphatase (AP) (U/l), bilirubin, total and direct (mg/dl), INR (dimensionless)) collected on day 1, 3, 5 (except for serum creatinine which is collected on all days and hematocrit which is only collected on day 1)
- Occurrence of AEs and SAEs
- Routine clinical parameters (Serum creatinine on admission (mg/dl), Baseline serum creatinine (nadir from inspection of previous, lab values or by primary care physician) (mg/dl), Serum creatinine (mg/dl), Serum sodium (mmol/l), Serum potassium (mmol/l), eGFR (CKD-EPI) (ml/min/1,73 m2), Hemoglobin (g/dl), Serum-Albumin (g/dl), Urine output (0:00 - 24:00 on the indicated day) (ml/24 h) (if, incomplete, extrapolate the available time period to 24 hours), Chest X-ray: evidence of pulmonary congestion, Chest X-ray: evidence of pleural effusion, Chest X-ray: evidence of cardiomegaly, Alanine aminotransferase (ALT) (U/l), Aspartate aminotransferase (AST) (U/l), Alkaline phosphatase (AP) (U/l), Bilirubin, total and direct (mg/dl))

5 Handling of missing values and outliers

5.1 Missing values

For the safety assessment of the AEs and SAEs, completeness of the data (severity, relationship, and seriousness) is required. The rate of missing values for the concentrations of FD003 is expected to be low. A decision on how to handle missing value for these values will be made during the data review (see section 6). Serum creatinine values missing at day 1 or 3 will be replaced by the respective values of day 0 and day 4 or the routine measurements at these days, if the aforementioned values are not available. For secondary parameters, missing values will not be replaced.

5.2 Outliers

No specific criteria for outliers will be applied. Rather, relevant parameters (Serum creatinine, Hematocrit, weight, NT-Pro-BNP, eGFR, and all measurements done by FAST) will be inspected by tables and figures (including histograms, kernel density plots, normal Q-Q-plots, boxplots) during the data review (see section 6). Further, for each patient the time series of weight, serum creatinine, and hematocrit will be displayed graphically to identify outliers or data errors.

6 Data review meeting

After the data base lock (i.e. after all queries are solved within the data management process) and before the analysis, a data review meeting will be held in order to assign patients to the analysis populations, to determine the amount of missing values for the concentrations of FD003, and to identify any remaining data errors and decide on their handling. The results of this meeting and possible changes or additions to the analysis including reasons will be documented in a data review report. Prior to the data review a set of tables, listings and figures will be defined to be used.

7 Statistical analyses / methods

7.1 General remarks

The primary objective is to assess the safety and function of the FAST PV and mGFR Technology in hospitalized patients with heart failure. This is a phase II trial. No formal hypothesis test is performed to assess the primary objective. This is justified as follows: 1. Both function and safety cannot be uniquely assessed by one specific endpoint, but there exist several relevant endpoints and endpoint-related criteria which must be investigated in order to assess the global function and safety. 2. For reasons of feasibility, the studies sample size is limited to 50 patients which is an adequate number in the context of a phase II trial. However, this limited number does not allow to address a complex multiple testing problem with adequate power.

Therefore, all analyses including p values are purely descriptive. The aim is to generate new hypotheses to be tested in subsequent phase III trials.
For continuous variables descriptive statistics include mean, standard deviation, median, inter quartile range and range. For categorical variables descriptive statistics include absolute and relative frequencies.
Due to the low number of patients in one of the centers, no adjustment for center will be done in the analyses.

7.2 Derived Parameters

The following formulas will be used for determination of estimated and measured TBV, ideal TBV, estimated and measured total blood volume status (eTBVS and mTBVS), ePV, estimated PV change, ideal PV, estimated and measured plasma volume status (ePVS and mPVS), and eGFR on day 1 and day 3:

Nadler formula for eTBV (Sharma & Sharma, 2020):

- Men: eTBV [L] = (0.3669 × Height [m]^3) + (0.03219 × Weight [kg]) + 0.6041
- Women: eTBV [L] = (0.3561 × Height [m]^3) + (0.03308 × Weight [kg]) + 0.1833

mTBV will be calculated as follows:

- mTBV [mL] = mPV [mL] / (1 – hematocrit [%]/ 100)

Lemmens-Bernstein-Brodsky formula for ideal TBV (iTBV) (Lemmens, Bernstein, & Brodsky, 2006):

- iTBV [mL/kg] = 70/[sqrt(body mass index/22)] – the lowest body weight during the days 1 to 5 will be used for calculation of BMI
- iTBV [mL] = iTBV [ml/kg] × weight [kg] – the lowest body weight during the days 1 to 5 will be used for calculation

Estimated and measured total blood volume status:

- eTBVS [%] = Percentage change from iTBV: (eTBV - iTBV)/iTBV
- mTBVS [%] = Percentage change from iTBV: (mTBV - iTBV)/iTBV

Kaplan-Hakim Formula for ePV (Ling et al., 2015):

- Men: ePV [mL] = (1 – hematocrit [%]) × [1530 + (41 x weight [kg])]
- Women: ePV [mL] = (1 – hematocrit [%]) × [864 + (47.9 x weight [kg])]

Strauss formula for plasma volume change (Strauss, Davis, Rosenbaum, & Rossmeisl, 1951):

- PV_2/PV_1 × 100 [%] = hemoglobin_1 [g/dL]/hemoglobin _2 [g/dL] × (1 - hematocrit_2 [%])/(1 - hematocrit 1 [%]) × 100

Plasma volume change will also be calculated via the ratios of ePV by the Kaplan-Hakim-Formula

Harrison formula for ideal PV (iPV) (from Ling 2015):

- Men: iPV [mL] = 39 [mL/kg] × weight [kg] – the lowest body weight during the days 1 to 5 will be used for calculation
- Women: iPV [mL] = 40 [mL/kg] × weight [kg] – the lowest body weight during the days 1 to 5 will be used for calculation

Estimated and measured plasma volume status (ePVS and mPVS):

- ePVS [%] = Percentage change from iPV: (ePV - iPV)/iPV
- mPVS [%] = Percentage change from iPV: (mPV - iPV)/iPV

The eGFR will be calculated using CKD-EPI formula (Levey et al., 2009):

- eGFR [mL/min/1.73 m²] = 141 x min(serum creatinine [mg/dL]/κ, 1)$^{\alpha}$ x
  max(serum creatinine [mg/dL]/κ, 1)$^{-1.209}$ x 0.993$^{\text{age [years]}}$ x
  1.018 [if female] x 1.159 [if Black]
- κ = 0.7 (females) or 0.9 (males)
- α = -0.329 (females) or -0.411 (males)

The eGFR will also be calculated by the CKD-EPI cystatin C formula (Inker et al., 2012):

- eGFR [mL/min/1.73 m²] = 133 x min(cystatin C [mg/L]/0.8, 1)$^{-0.499}$ x
  max (cystatin C [mg/L]/0.8, 1)$^{-1.328}$ x 0.996$^{\text{Age [years]}}$ x
  0.932 [if female]

The eGFR will additionally be calculated by the CKD-EPI creatinine-cystatin C formula (Inker et al., 2012):

- eGFR [mL/min/1.73 m²] = 135 × min(serum creatinine [mg/dL]/κ, 1)$^{\alpha}$ ×
  max(serum creatinine [mg/dL]/κ, 1)$^{-0.601}$ ×
  min(cystatin C [mg/L]/0.8, 1)$^{-0.375}$ ×
  max(cystatin C [mg/L]/0.8, 1)$^{-0.711}$ × 0.995$^{\text{Age [years]}}$ ×
  0.969 [if female] × 1.08 [if black]
- κ = 0.7 (females) or 0.9 (males)
- α = -0.248 (females) or -0.207 (males)

The eGRF will further be calculated by sMDRD (Levey et al., 2007):

- eGFR [mL/min/1.73 m²] = 175 × (serum creatinine [mg/dL])$^{-1.154}$ × (age [years])$^{-0.203}$ ×
  0.742 [if female] × 1.212 [if black]

The following dose equivalents for Furosemid i.v. will be used:

- 20 mg Furosemid i.v. = 40 mg Furosemid p.o.
- 20 mg Furosemid i.v. = 20 mg Torasemid p.o.

7.3 Demography and baseline characteristics

Demographic and baseline characteristics will be displayed descriptively.

7.4 Prior or concomitant medication and diseases

Prior or concomitant medication and diseases will be listed individually by patient and shown descriptively.

7.5 Exposition to treatment/Compliance

The frequency of patients having the FAST PV measurement done on day 1 and day 3 will be shown descriptively. Reasons for missing second doses will be listed.

7.6 Primary analysis

Safety will be assessed by determination of the absolute and relative frequencies of AEs and SAEs related to VFI. Related AEs and SAEs include all events that are sure, probable and possible related.

Function of the FAST PV measurement will be assessed by determining the plasma stability of the FD003 high molecular weight marker over the 15, 30, and 60 minute blood draws at day 1 and 3 and applying the following criteria:

- The FAST PV measurement is considered as stable, if the mean plasma concentration of FD003 at 30 minutes is not more than 10% lower than the mean plasma concentration at 15 minutes AND if the mean plasma concentration at 60 minutes is not more than 10% lower than the mean plasma concentration at 30 minutes. The frequency of patients with stable FD003 plasma concentration will be analyzed descriptively for day 1 and day 3.
- The absolute and relative frequency of patients which show a decline in the plasma concentration of FD003 of more than 10% from 15 minutes to 30 minutes and separately from 30 minutes to 60 minutes will be determined for day 1 and day 3.

Further, the change (absolute and relative) in plasma concentration of FD003 from 15 to 30 minutes, and from 30 to 60 minutes will be displayed descriptively for day 1 and day 3 separately.

7.7 Secondary analyses

7.7.1 Efficacy

To evaluate how estimated PV (ePV) and estimated total blood volume (eTBV) assessments on days 1 and 3 (by Nadler's formula, Kaplan-Hakim formula) predict measured PV (mPV; as assessed by FAST methodology) and measured TBV (mTBV; calculated from mPV and measured hematocrit) at these time points, linear regression models with the measured values at a given day as dependent variable and the estimated version as independent variable will be generated. The models will be adjusted for BMI, weight, and height separately.

Further, Bland-Altman plots will be generated comparing the estimated and measured parameters separately for day 1 and day 3. The defined limits of agreement are ±500ml for TBV and ±300ml and for PV. The amount of values within 15% and within 30% of the measured values will also be determined. In addition, the diagnostic test accuracy including 95% confidence interval as proposed by Obuchowksi (Obuchowski, 2005) will be calculated. The interpretation for a continuous reference standard is as follows: Of two randomly chosen patients, the diagnostic accuracy is the probability that the patient with the higher reference standard result has a higher diagnostic test result than the patient with the lower reference standard test result. In the case of the study, the measured PV and TBV will act as reference standard while the estimated versions serve the diagnostic test.

To evaluate how a clinical evaluation on days 1 and 3 predicts mPV and mTBV at these time points, linear regression models will be used.
Depending on the amount of available information and distribution, the following parameters will be considered for the analysis in the order of appearance: hematocrit (only PV), mean arterial blood pressure, BMI (only PV), weight, height (BMI, weight, height will be entered in 3 separate models), age, gender, NYHA stage, respiratory rate, edema grade, presence of pulmonary rales, jugular venous congestion. At least 10 observations will be needed per parameter that need to be estimated. Bland-Altman plots, frequencies within 15% and 30%, and diagnostic accuracy will be evaluated as stated before.

To evaluate whether eGFR calculation by the CKD-EPI formula and sMDRD formula on days 1 and 3 provides an accurate estimate of measured GFR (mGFR; assessed by FAST methodology) in heart failure patients undergoing active fluid management, linear regression models with the measured value at a given day as dependent variable and the estimated version as independent variable will be generated. The models will be adjusted for mPV and mPVS. Further, Bland-Altman plots will be generated comparing the estimated and measured parameters separately for day 1 and day 3. The defined limits of agreements are ± 6ml/min. Diagnostic accuracy including frequencies of values between 15% and 30% of the measured value will be determined as mentioned before.

To evaluate whether patients with a low mGFR/eGFR ratio on days 1 and 3 are at higher risk of developing AKI within the following 72 (day 1) and 48 (day 3) hours, logistic regression models with the AKI status as dependent variable and mGFR/eGFR ratio as independent variables will be generated. The models will be adjusted for mPVS, mTBVS. Three different versions of the mGFR/eGRF ratio will be evaluated: ratio < 1; ratio < first quartile; ratio as continuous parameter.
To evaluate whether ranges of mPVS (low mPVS (< -10%), normal mPVS (-10 to +10%), high mPVS (> +10%)) and/or mTBVS (low mTBVS (<-10%), normal mTBVS (-10 to +10%), high mTBVS (>+10%)) on days 1 and 3 are associated with subjectively reported symptoms (current PGA > 15, current dyspnea > 15, current dizziness > 15, current nausea > 15) determined using patient symptom assessment sheets within the following 24-48 hours, a logistic regression

model with occurrence of symptoms (PGA, dyspnea, dizziness, nausea) as dependent variable and the categorized measured value (mPVS, mTBVS) as independent variables will be generated. The models will be adjusted for mean arterial pressure.

To evaluate whether patients with low mPVS (< -10%) or low mTBVS (< -10%) on day 1 or on day 3 are at risk of developing low-output complications within the next 24-48 hours (subjectively reported dizziness > 15, nausea > 15, PGA < 25, hypotension (Systolic <100, diastolic <60 mmHg), need for iv-fluid therapy), logistic regression models using mPVS and mTBVS as independent variables will be used.

To evaluate whether patients with a high mPVS (> +10%) or a high mTBVS (> +10%) on day 1 and/or day 3 are at risk of being refractory to diuretic therapy (defined as any dosage increases of furosemide i.v. dose equivalent, adding thiazide after day 1, require ultrafiltration/RRT within the next 24-48 hours, fail to improve subjectively in PGA and dyspnea scales, fail of mean weight loss during the study period Day 1-Da y5 of at least 0,5 kg/d) logistic regression models using mPV and mTBV as independent variables will be used.

To evaluate whether the changes of ePV (using relative and absolute change as well as the Strauss' formula) and eTBV (using relative and absolute change) from day 1 to day 3 adequately reflects the changes of mPV and mTBV Bland-Altman plots will be generated. These plots will be analysed exploratory, since no appropriate values for limits of agreements are available. The amount of values within 10% and within 30% of the measured values will also be determined. In addition, the diagnostic test accuracy including 95% confidence interval as proposed by Obuchowksi will be calculated.

To evaluate whether the change of mPV and mTBV and changes of mPVS and mTBVS from day 1 to day 3 as well as the mPVS and mBVS on Day 1 and Day 3 is predictive of the remaining length of stay at the given day (i.e. remaining length of stay starting with day 1 or with day 3), dialysis/ultrafiltration requirement, or rehospitalization within 30 days, or mortality, linear (remaining length of stay), logistic (dialysis/ultrafiltration, rehospitalization within 30 days) or Cox-regression models (mortality) will be utilized using changes of mPV and mTBV from day 1 to day 3 (first model), changes of mPVS and mTBVS from day 1 to day 3 (second model), and mPVS and mTBVS at day 1 (third model) and day 3 (fourth model) as independent variables.

To evaluate whether the percentage changes of mGFR from day 1 to day 3 (<0 vs. ≥0; continuous) predicts the percentage changes of eGFR from day 2 to day 4 (CKD-EPI formula only; <0 vs. ≥0; continuous), a logistic as well as a linear regression model will be used.
The same will be done to investigate the changes in eGFR from day 1 to day 3 (all formulas) as the dependent variable. Further, exploratory Bland-Altman plots without predefined limits of agreement will be created.

To evaluate whether patients with a high PVS or TBVS on Day 1 (>+10%) and a decreasing PVS

or TBVS between Day 1 and 3 had significantly different percentage change of mGFR between Day 1 and Day 3 compared to patients with a high PVS or TBVS and an increasing PVS between Day 1 and 3, t-Tests or Mann-Whitney-U-tests will be used depending on the distribution of the values.

To evaluate whether Patients with a low PVS or TBVS on Day 1 (<-10%) and an increasing PVS or TBVS between Day 1 and 3 had significantly different percentage change of mGFR between Day 1 and Day 3 compared to patients with a low PVS or BCS and a decreasing PVS between Day 1 and 3, t-Tests or Mann-Whitney-U-tests will be used depending on the distribution of the values.

To evaluate whether patients with an improving (i.e. increasing) mGFR from day 1 to day 3 differ significantly from patients with a worsening (i.e. decreasing) mGFR regarding baseline characteristics (see chapter 4.1), mPVS, mTBVS, ePVS, eTBVS, and remaining length of stay appropriate tests depending on the distribution will be used (t-Test or Mann-Whitney-U-test for continuous variables and chi-square tests or Fisher-Boschloo-tests for categorical variables).

To evaluate whether disagreement between the physicians response to the question what they thought the intravascular volume of the patient was doing (increasing, decreasing, no change, treating physicians assessment Day 3) and the direction of measured change in TBVS is associated with a longer remaining length of stay, a t-test or Mann-Whitney-U-test depending on the distribution will be used. A measured change in TBVS of more than 5% between Day 1 and 3 was determined as a significant change.

To evaluate (by an adjudication committee) whether clinical decision making would have been affected by adding FAST GFR and PV measurements to clinical routine, the following questions were to be answered:

- How would you alter the intensity of Diuretic Therapy given on Day 1 and Day 3?
- How would you alter the intensity of RAASI Therapy given Day 1 and Day 3?
  How would you alter the intensity of Beta Blocker Therapy given Day 1 and Day 3?
- How would you alter the intensity of other GDMT given Day 1 and Day 3?

To evaluate this endpoint an adjudication committee will be convened to analyse the outputs of the study after its completion. The goal of the adjudication committee is to the best of their ability describe any changes is treatment they would have made to each patient had the mPV and mGFR metrics been available during the episode of care. No clinical decisions will be made based on the results generated by the FAST PV and mGFR Technology as results will not be made available to the treating physicians and will only be released to the adjudication committee after the last patient has been discharged.
The scale to define the intensity of changes reaches from 1 to 5 meaning from much less intense to much more intense (3 is no change).

Absolute and relative frequencies of the changes to the questions specified above will be calculated for the whole assessment and separately for Day 1 and Day 3.

7.7.2 Safety/Tolerability

Adverse events

AEs and SAEs will be regarded for safety analysis if they are treatment emergent, i.e. if they were not present prior to the first application of VFI or in case they were present and are worsening after the first application of VFI.

All AEs, whether or not they are considered to be related to treatment are reported.

They are listed by subject providing the following information:

- Age, sex, weight, BMI
- The adverse event (reported term by investigator, MedDRA preferred term, System Organ Class)
- Date of onset
- Duration of the adverse event
- Severity
- Seriousness (serious/non-serious)
- Action taken
- Outcome
- Causality assessment

The adverse events are displayed in summary tables as follows:

- A table of the preferred terms of all AEs will be presented including the number and percentage of subjects in whom at least one event with the respective preferred term (PT) occurred. The PTs will be grouped by MedDRA System Organ Class (SOC) and sorted by frequency.
- Additionally, the number and percentage of subjects in whom at least one event in the respective SOC occurred will be given.
- The tables above will be additionally divided into the defined severity categories grouped by relatedness of the event to VFI.

Every table will be produced for the following AE sets:

- All AEs
- AEs for which a relationship to formula intake was considered to be related by the investigator

Such AEs which were documented as SAEs will be reported in the same way as the AEs.

Every table will be produced for the following SAE sets:

- All SAEs
- SAEs for which a relationship to VFI cannot be ruled out
- All SAEs with outcome death

Additionally, all deaths during the study and deaths that resulted from a process that began during the study are listed by subject.

Laboratory parameters

Laboratory measurements that were study related will be analysed descriptively at each visit.

7.8 Planned subgroup analyses

No subgroup analyses are planned.

8 Changes from the protocol

In general, no variable selection will be done in the regression models due to limited sample size. Rather clinical expertise regarding the most important explanatory variables will be used to determine a fixed, hierarchical set of possible covariates.

The mPVS and mTBVS are used to determine patients with low, normal or high PV and TBV and thus, the status will be defined based on established estimation formulas for ideal PV and TBV incorporating weight and/or height.

The analysis of the time course of estimated and measured PV and TBV via t-tests was replaced by Bland-Altman-analyses since these analysis better reflects the underlying question of clinically equivalent measurements. Further, the curve characteristics (e.g. times when maximal and minimal volumes are reached and the areas under the curve) based on only few time points were deemed to be of minor meaningfulness.

Haemoglobin for estimating plasma volume changes by Strauss formula and cystatin C values to estimate Glomerular Filtration Rate were also recorded during the study and will also be utilized for the analysis in the respective estimation formulas.

9 Software

The software R (version 3.5.0 or higher) will be used for analysis.

10 References

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