EMPAKT-CHF

Estimating versus **M**easuring **P**lasma Volume and **K**idney Function in Acute Decompensated **C**ongestive **H**eart **F**ailure

EudraCT-Number: 2018-002638-18

Protocol

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Sponsor of the clinical study
Charité – Universitätsmedizin Berlin

Representative of the Sponsor and Principal Investigator

[redacted]

The following persons accept the content of this protocol and confirm to conduct this study in compliance with Good Clinical Practice and applicable regulatory requirements.

Representative of the Sponsor	[redacted]
Principal Investigator	

- ***Confidential*** -

The information contained in this protocol has to be kept strictly confidential. Therefore, the protocol is only provided to Investigators in confidence for review, to study staff, Independent Ethics Committee/Institutional Review Board, regulatory authorities and CROs (or KKS) and for obtaining written informed consent from patients.

STATISTICIAN SIGNATURE PAGE

Statistician: Address:	[redacted]
Tel: Email:	[redacted]

I, the undersigned, have reviewed this Protocol, and I will conduct my role in the clinical study as described and will adhere to GCP/ICH and all the ethical and regulatory considerations stated.

Signature: [redacted]

[redacted]

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Abbreviations

AE	Adverse Event
AKI	acute kidney injury
AUC	area under the concentration curve
CHF	Congestive Heart Failure
CKD	chronic kidney disease
CPK	creatine phosphokinase
CRF	Case Report Form
DAC	data adjudication committee
eGFR	estimated Glomerular Filtration Rate
ePV	estimated plasma volume
eTBV	estimated total blood volume
EOS	end of study
GFR	Glomerular Filtration Rate
hcg	human chorionic gonadotropin
IB	Investigators Broschure
ICU	Intensiv Care Unit

IMP	investigational medicinal product
ITT	Intent to treat Population
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
SAE	Serious Adverse Event
TBV	total blood volume
TEAE	treatment-emergent adverse event
VFI	Visible fluorescent injectate (FD004)
WOCBP	Women of child-bearing potential

1 Synopsis

Study title	**E**stimating versus **M**easuring **P**lasm**a** Volume and **K**idney Funct**i**on in Acute Decompensated **C**ongestive **H**eart **F**ailure (EMPaKt-CHF)
Type of project	This is an investigator-initiated, one-armed phase II clinical trial using an injectable fluorescent tracer to assay and evaluate measured Plasma Volume (mPV) and measured Glomerular Filtration Rate (mGFR) in Hospitalized Patients with acute decompensated congestive heart failure (CHF).
Sponsor	Charité Universitätsmedizin Berlin Charitéplatz 1, 10117 Berlin, Germany
Representative of the Sponsor and Principal Investigator **Deputies of the Principal Investigator** **Co-investigators**	[redacted]

Study Physician / member of the trial team **Trial Statistician**	
Hypotheses	It is hypothesized that utilizing the FAST PV and mGFR Technology™ is safe and functional in CHF patients. It is also hypothesized that quantitative measurements of intravascular plasma volume status and GFR will differ from standard clinical assessments and estimating equations, and that clinical decision making would have been improved in a subpopulation of patients had the quantitative measurements delivered by the FAST PV and mGFR Technology™ been available to the physician during the course of care.
Rationale	Previous clinical studies have shown the FAST technology to be safe and functional in patients with chronic kidney disease (CKD) and in healthy subjects. The technology has not been studied in CHF patients. In previous clinical studies, the technology has been demonstrated to accurately measure PV and mGFR in patients with CKD and healthy patients when compared to other clinical techniques including, estimated glomerular filtration rate (eGFR) calculations, Iohexol clearance and Nadler's formula. Additionally, previous studies have shown that the concentration of the PV marker remains relatively constant during the first two hours after injection, given that this high-molecular weight marker is predicted to exclusively occupy the intravascular compartment and exhibit a long half-life. In this study, the safety and functionality of the FAST Technology will be evaluated in CHF patients. The non-clinical and clinical history of the product provides confidence that the

	technology will be well tolerated and function correctly in the CHF population. The blood samples are timed to maximize the information gathered during the study. In addition to measurements of PV and GFR, FAST will determine the technology's functionality by comparing the concentration of the PV marker at the 15, 30 and 60 minute time points. It is not believed that the plasma volume will change significantly within that time period and thus the concentration of the PV marker should remain relatively constant.
Study medication (IMP) / Therapeutic strategy	Visible fluorescent injectate (VFI) (FD004), Powder for Solution for Injection, 47 mg. VFI is diluted with Water for Injection and a total of 3 mL of VFI is administered by bolus injection. There is 47 mg of VFI administered in this 3 mL dose (35 mg of FD001 and 12 mg of FD003). VFI is dosed on Day 1 and Day 3 approximately 48 hours apart.
Study design	This is an investigator-initiated, one-armed phase II clinical study designed to evaluate the safety and function of the FAST PV and mGFR Technology in patients with CHF. Data from the FAST PV and mGFR Technology will not be evaluable by the treating physician, but will be made available to the study investigators and to an adjudication committee. Patients enrolled in the study will be administered the Day 1 Dose of VFI after enrollment and the Day 3 dose approximately 48 hours after the initial dose. After consenting to be enrolled in the study, patients meeting the enrollment criteria will receive a single dose of VFI(Day 1). Blood draws (3 mLs) will be collected pre-dose and at 15 (±5 minutes), 30 (±5 minutes), 60 (+30 minutes), and 180 (+120 minutes) minutes post-dose which will be subsequently used to determine the patient's blood volume and mGFR using the FAST PV and mGFR Technology. Patients will be treated according to standard of care throughout the time of their hospitalization. A second dose of VFI will be administered 48 (±5 hours) hours after the initial dosing (Day 3). Again, blood draws (3 mLs) will be collected at 15 (±5 minutes), 30 (±5 minutes), 60 (+30 minutes), and 180 (+120 minutes) minutes post-dose In addition, study-related blood samples will be obtained on days 1, 2, 3, 4, 5 to measure serum creatinine (days 1-5), hematocrit (days 1, 3), and N-terminal pro-brain natriuretic peptide (NT-pro-BNP), Creatine phosphokinase (CPK), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) (U/l), Alkaline phosphatase (AP), Bilirubin total and direct, International Normalized Ratio (INR) (days 1,3,5), and

	will be stored for future testing of additional parameters in the research fields of acute decompensated heart failure and acute kidney injury. Furthermore, the available laboratory values from routine clinical practice (see *Table 1*) will be entered in the Case Report Form (eCRF). On days 1-5, the patient will be asked to complete a Patient Symptom Assessment Sheet (see Annex D). On days 1 and 3, the treating physician will be asked to complete a very brief survey (Annex E) to provide a qualitative assessment of the patient's perceived intravascular volume status and renal function. The study physician will conduct a non-invasive clinical assessment of the patient (including e. g. body weight, height, edema grade, blood pressure, heart rate, respiratory rate, jugular venous distension assessment, oxygen saturation) on days 1-5. In addition, the study physician will record in the eCRF detailed information regarding diuretics dosage, i. v. fluid application, and requirement of renal replacement therapy as ordered by the clinicians in the hospital. 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 eCRF. Any AEs determined by the investigator to be related or possibly related to the VFI will be captured. Patients will also receive a follow up phone call 7 days (± 2 days) after the second dose of VFI was administered, and a second follow up call 30 days after the second dose of VFI was administered. This second phone call will serve as the end-of-study (EOS) follow-up call. Any AEs determined by the investigator to be related or possibly related to the VFI will be captured during the follow up calls and will be followed to resolution. Additionally, in-person follow up visits may be scheduled, as determined by the investigator. During day 1-5, during follow-up phone calls or study visits and through information accessed within the hospital computer system or obtained from treating physicians or relatives, the following study outcomes will be recorded: mortality, length of hospital stay, readmission for heart failure, episodes of acute kidney injury, requirement of fluid resuscitation, episodes of symptomatic hypotension, successful reduction of body weight. Upon conclusion of the study, the recorded clinical data will be provided to an independent data adjudication committee (DAC) in a pseudonymized fashion. The DAC will be asked to

	review the available data at the time of the first dosing and the measured GFR and PV data for the first dosing day will be provided. The DAC will then be asked to fill out a survey asking whether the mGFR and PV data would have influenced their treatment decision, and if so, indicate what they would have done differently. Following the evaluation of mGFR and PV data at the time of the initial VFI dose, the mGFR and PV data determined from the second VFI dosing point will be provided to the DAC. The DAC will then be asked to fill out a separate survey asking how the actual measured PV and GFR differ from the clinical assessments, whether the mGFR and PV data would influence their treatment decision, and if so, indicate what they would have done differently. After all assessments have been completed, the DAC will review the results of their analyses and compile a summary report on the secondary endpoints as well as their assessment of the overall impact of the PV and mGFR on clinical decision making.
Total number of patients	We plan to enrol 50 eligible patients. Due to the explorative nature of this phase II study a formal sample size calculation is not possible. However, the sample size of 50 patients is sufficient to guarantee reasonable accuracy with respect to the main objectives of the trial as specified below.
Study population	Male and Female patients aged ≥18 admitted to the hospital ward with a diagnosis of congestive heart failure.
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 studyprior 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 years of age. • 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. • 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 • 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 (WOCBP), must have a negative urine or serum pregnancy test within 24h 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 • 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 $\leq$ 90 systolic and/or $\leq$ 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 (hCG) 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 IMP). • Subject is legally detained in an official institution. • Subject is dependent on the sponsor, the investigator or the trial sites.

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 [a]		X						
Demographics [b]		X						
Comorbidities [c]		X						
Full medication list on enrollment into the study and during follow-up [d]		X	X	X	X	X		
Patient Symptom Assessment Survey [e]		X	X	X	X	X		
Clinical Presentation/ Exam [f]		X	X	X	X	X		
Treating Physician Survey [g]		X		X				
Study-Related Laboratory Measurements [h]		X	X	X	X	X		
Routine Clinical Parameters [i]	X*	X	X	X	X	X		
Clinical Data [j]	X*	X	X	X	X	X		
Adverse events		X	X	X	X	X	X	X
Blood specimen for marker analysis [k]		X		X				
Discharge from study								X
Adjudication Committee Evaluation[l]								X
WOCBP Pregnancy test [m]	X			X				X

[a] See Table 2 for Structural Heart Disease Criteria

[b] See Table 3 for specific demographic parameters

[c] See Table 4 for specific instructions on collecting comorbidities

[d] See Table 5 for specific instructions of collecting medications

[e] See Annex D for the Patients Symptom Assessment Survey

[f] See Table 6 for Clinical presentation/exam parameters

[g] See Annex E for the Treating Physician Survey

[h] See Table 7 for Study Related Laboratory Measurements

[i] See Table 8 for Routine Clinical Parameters

[j] See Table 9 for Clinical Data collected

[k] 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. 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.

[l] See Table 10 for the Adjudication Committee Evaluation Questions; Adjudication Committee meets after all collected data available.

[m] See Table 11 for Details of Pregnancy test

*will be collected retrospectively after study inclusion

Study objectives / endpoints	Primary endpoint: Global aim is to assess the safety and function of the FAST PV and mGFR Technology in hospitalized patients with heart failure. • Safety will be assessed by determination of the absolute and relative frequencies of AEs and SAEs related to 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 Endpoints: • To evaluate how estimated PV (ePV) and estimated total blood volume (TBV) assessments on days 1 and 3 (by established measures such as Nadler's formula or Metropolitan Life Tables) 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, NYHA stage, respiratory rate) on days 1 and 3 predicts mPV and mTBV at these time points. • To evaluate whether eGFR calculation by the CKD-EPI formula on days 1 and 3 provides an accurate estimate of

	measured GFR (mGFR; by 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 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 (cutoffs potentially adjusted for some of the following variables age, gender, body height, body weight, optimal body weight (dry weight), hematocrit) on day 1 or on day 3 are at risk of developing low-output complications within the next 24-48 hours (e. g. subjectively reported dizziness/nausea, PGA, hypotension, need for iv-fluid therapy). • To evaluate whether patients with a high mPV or a high mTBV (cutoffs potentially adjusted for some of the following variables age, gender, body height, body weight, optimal body weight (dry weight), hematocrit) 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 estimated TBV (eTBV) from day 1 to day 3 adequately reflects 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

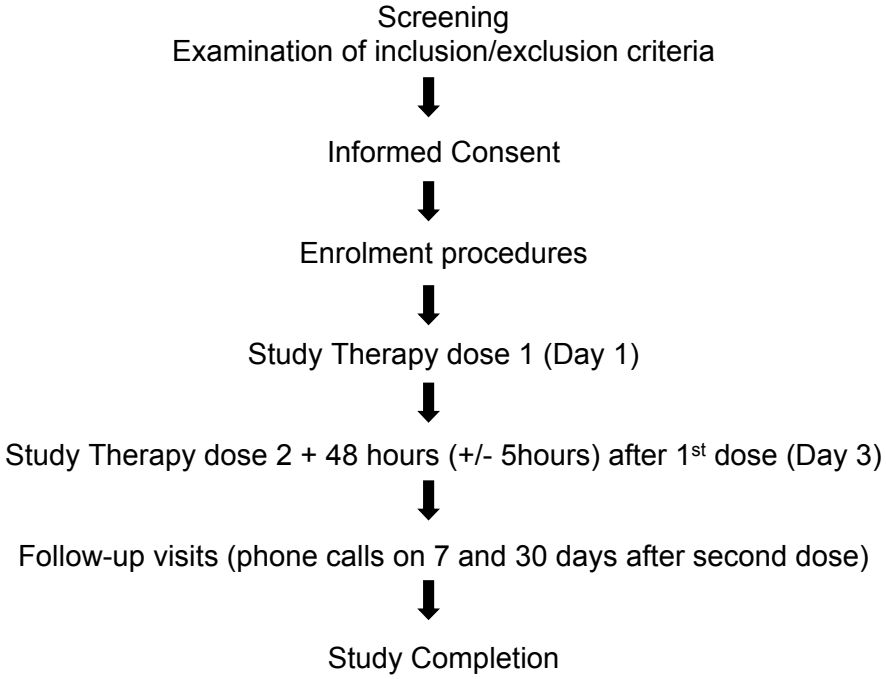
	routine including the following questions: o Would FAST GFR and PV measurements have changed clinical management overall? o Would the FAST GFR and PV measurement on day 1 have led to applying a different diuretics dosage between day 1-3? o Would the FAST GFR and PV measurement on day 3 have led to applying a different diuretics dosage between day 3-5? o Would the FAST GFR and PV measurement on day 1 have led to choosing dialysis/ultrafiltration instead of diuretics escalation between day 1-3? o Would the FAST GFR and PV measurement on day 1 have led to choosing diuretics escalation instead of dialysis/ultrafiltration between day 1-3? o Would the FAST GFR and PV measurement on day 3 have led to choosing dialysis/ultrafiltration instead of diuretics escalation between day 3-5? o Would the FAST GFR and PV measurement on day 3 have led to choosing diuretics escalation instead of dialysis/ultrafiltration between day 3-5?
Safety	Adverse events (AEs) will be collected and evaluated as they occur throughout the study.
Discontinuation criteria	**Individual Patient Discontinuation Criteria:** A patient is free to withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment according to standard clinical practice. The investigator may remove a patient if, in the investigator's judgment, continued participation would pose unacceptable risk to the patient or to the integrity of the study data. All procedures for early termination must be completed. Reasons for removal or withdrawal may include: • Admission to the ICU prior to either the first or second dose • The patient develops a stated exclusion criterion during the course of study • If the patient experiences an acute anaphylactoid reaction during the course of administering the FAST VFI, administration will stop immediately • Evidence of an anaphylactoid reaction (dyspnea, urticaria, flush, bronchospasm, or severe systemic hypotension) • Administrative decision by the investigator or sponsor • Significant protocol deviation • Patient noncompliance • Safety concern by the investigator or sponsor • Lost to follow-up • Pregnancy

	Patients who are withdrawn for reasons other than safety issues may be replaced at the discretion of the sponsor and investigator. In the event of a patient's withdrawal, the investigator makes every effort to complete the EOS assessments. All withdrawn patients with ongoing clinically significant clinical or laboratory findings will be followed until the finding is resolved or medically stable; reasonable attempts will be made to follow-up with patients. **Study Discontinuation Criteria:** • Unjustifiable risk and toxicity in risk-benefit analysis (decision taken by principal Investigator) • New scientific evidence provided during the study that could affect the patient's safety (benefit-risk analysis no longer positive) • Decision of the Sponsor or the Competent Authority that the study should be discontinued.
Statistical analysis	Descriptive statistics will be performed for safety assessments and for relationship between the FAST GFR and other measurements of renal function as well as between the FAST PV and other assessments of plasma volume.
Pharmacological-toxicological evaluation	Pharmacokinetics of the FAST VFI will not be evaluated as a part of this study, but these parameters have been investigated in previous clinical and nonclinical studies.
Risk-benefit analysis	<u>Risks of treatment methods:</u> Usual risks of participating in clinical trials, which are related to possible allergic reactions and blood drawing via venipuncture. <u>Risks (IMP) (according to the reference safety information)</u> Possible allergic reactions and risks associated with dosing and blood drawing via venipuncture. <u>Action steps for risks minimisation:</u> The FAST Technology has been previously used in three clinical studies. 87 patients have been dosed with the FAST VFI. There have been no reported SAEs and few minor AEs across all studies. Existing allergen risks in patients will be included as part of informed consent and staff will be well trained prior to the study initiation. <u>Remaining risk:</u> Possible allergic reactions and risks associated with dosing and blood drawing via venipuncture.

	Benefit: A rapid and accurate measurement of plasma volume (PV) and glomerular filtration rate (GFR) is important in chronic heart failure (CHF), acute kidney injury (AKI) and chronic kidney disease (CKD) for assessment of impairment, diagnosis, and prompt treatment. This study primarily serves the purpose of obtaining information about the tolerability and safety of the FAST PV and mGFR Technology in CHF patients. There are no perceived direct benefits to patients participating in this trial. However, thorough medical check-ups may be seen as an advantage. The safety of the patients will be observed during all study phases. Medical progress is based on research which ultimately must rest in part on experimentation involving humans. Eligible patients may consider participation in this clinical trial because they want to contribute to the advancement of medical knowledge. Still, considerations related to the well-being of the individual patients enrolled into this clinical study must take precedence over the interests of science and society. Based on available information and the design of the study, the sponsor and the investigator consider the trial to be ethically acceptable. The duration of hospitalization and the medical surveillance are considered adequate to ensure safety of the patients.
Contraindications:	The FAST VFI (FD004) should not be administered to patients with known hypersensitivity to dextran, 5 aminofluorescein dye, or 2-sulfohexamine rhodamine dye.
Procedures in case of emergency:	The FAST VFI (FD004) was well tolerated in the nonclinical studies conducted to date. Anaphylactic responses to dextrans, while rare, must be considered as a possibility for the high molecular weight dextrans being evaluated by FAST. As such, all testing facilities should have immediately available procedures to administer treatment for an anaphylactic response should it occur.
Treatment of overdose:	The FAST VFI is contained in single-dose vials, making significant overdose unlikely. Though the vials are overfilled with 4mL/62.6 mg in order to ensure that a full 3mL/47mg dose can be drawn, previous studies have evaluated the safety of the VFI using a 3mL/150mg dose, over two times the amount of material contained in the single-dose vials for this study. There is no known antidote for overdose of VFI. In the event of a suspected overdose, the patient should be monitored appropriately and should receive supportive therapy, as necessary.

Timetable	**Date of study / recruitment start (first patient first visit (FPFV):** January 2019 **End of recruitment:** Anticipated to be July 2019 **End of trial (last patient last visit, LPLV): Anticipated to be** August 2019 **Trial duration (for a patient):** 33 Days **Follow up:** Patients will receive a follow-up phone call on Day 7 and Day 30 after the second VFI dose
Funding	The study will be supported by a research grant from FAST BioMedical.
Key words	Congestive Heart Failure, Acute Kidney Injury, Plasma Volume, Clinical Managment, Glomerular Filtration Rate (GFR), Plasma Volume (PV), FAST VFI, FAST PV and mGFR Technology,

2 Flow-Chart



3 Introduction

3.1 Introduction and background

A rapid and accurate measurement of plasma volume (PV) and glomerular filtration rate (GFR) is important in acute kidney injury (AKI) and chronic kidney disease (CKD) for assessment of impairment, diagnosis, and prompt treatment.

Reductions in PV and/or GFR secondary to kidney injury, either acute or chronic, are accompanied by increases in blood urea nitrogen (BUN) and serum creatinine (S_{cr}) levels. Currently, either S_{cr}, or an equation based on S_{cr}, is used to determine a patient's estimated GFR (eGFR). Unfortunately, these 2 approaches are not reliable over the full range of GFR, and neither can be used for early detection or functional assessment in patients with a rapidly changing GFR, such as patients with AKI.

Recent data indicate that even very small changes in S_{cr}, which were previously thought to be clinically insignificant, are now known to predict an increased mortality rate. A technique to measure PV and GFR rapidly would be valuable to detect the loss of kidney function that leads to these acute changes in S_{cr} so that treatment can begin as soon as possible.

Glomerular filtration rate is the most clinically relevant metric for understanding renal function, as it is the rate by which the kidney is able to filter waste products in the bloodstream. Accurate measurement of GFR in acutely ill patients could also facilitate accurate dosing of drugs to maximize therapeutic efficacy while minimizing toxicity. Therefore, technical advances in this field are of major clinical importance, especially in high-risk patients where intense surveillance is necessary.

3.1.1 Investigational Device Background

The FAST PV and mGFR Technology™ is a direct measurement of PV and GFR that relies on reading the concentration of fluorescent markers attached to different size dextran molecules introduced into the bloodstream. The test is intended as an adjunct to current methods utilized to assess kidney function.

The FAST PV and mGFR Technology includes intravenously (IV) administered fluorescent markers, timed blood draws, a validated fluorometric assay, and a computed algorithm to integrate the results and calculate the GFR.

The FAST PV and mGFR Technology will aid in identifying and determining the extent of renal dysfunction, therefore promoting early treatment, including dialysis initiation, as well as enrollment and stratification for clinical studies. It could also be used to determine the effect of a clinical maneuver on GFR such as volume resuscitation.

In addition to measuring the GFR, the FAST PV and mGFR technology is capable of measuring the intervascular plasma volume of a patient. With a single dose, the PV can be monitored for change or therapeutic affects for up to 8 hours. It will rapidly determine intravascular volume allowing early pharmacologic and or IV volume expanding treatment, as well as enrollment and stratification for clinical studies, and prognostic information. Measurement of PV will allow for accurate therapeutic use of volume expansion or vasopressors in the ICU, especially in patients with endothelial injury as and increased vascular permeability as in sepsis.

3.1.2 Investigational Product Background

The IV administered visible fluorescent injectate (VFI)™ agent is comprised of a mixture of 2 different molecular weight carboxymethyl dextran molecules (5 kD and 150 kD) with different fluorescent dye molecules covalently 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. When combined in a 3:1 (mass) ratio of small to large molecules, these dextrans make up the VFI, termed FD004.

The high molecular weight labeled carboxymethyl dextran, FD003, 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 PV. The low molecular weight labeled carboxymethyl dextran, FD001, is also not patient to rapid metabolism but is freely filtered by the kidneys. The decline in the concentration of FD001 combined with the PV measured by FD003 provides a rapid and accurate assessment of GFR.

The VFI is administered IV through a bolus injection.

While the VFI is a substantially modified dextran-based compound, other dextran products have, on rare occasions, been associated with mild to severe, acute anaphylactic reactions including death. As a precaution, patients will be closely monitored for signs of allergy and anaphylaxis, and an emergency resuscitation kit

and team will be available within the clinical research unit (CRU) throughout the treatment period.

Fluorescent dyes such as fluorescein, which is also used in the VFI, are known to cause transient ocular light sensitivity, although this effect was not reported at any dose studied in any of the previous human clinical studies. Should this effect be observed, patients will be provided with disposable protective sunglasses to wear until the effect is reversed.

3.1.3 Summary of Findings to Date

3.1.3.1 Nonclinical Studies

Proof-of-concept nonclinical pharmacology studies have been conducted in dogs to confirm the assessment of GFR using the FAST mGFR Test™ (Wang 2011). The results of these studies provide evidence that the method, in combination with the VFI, provides an accurate GFR.

The liquid VFI agent (FD004) was evaluated in a full range of nonclinical studies, including pharmacology, safety pharmacology, pharmacokinetic (PK), single-dose toxicity, genotoxicity, and immunotoxicity in male and female rats and dogs at concentrations from 16 (FD003) to 26 (FD001) fold greater than the planned maximum human dose based upon observed and predicted area under the concentration-time curve (AUC) values. For more information please refer to the current Investigator's Brochure.

Observed effects of the VFI (FD001 and FD003) in pivotal single dose animal studies at higher doses (ranging from approximately 62 to approximately 100 times the planned human clinical dose of 47 mg [29 mg/m^2]) included emesis in dogs, discolored urine, and a species specific effect (rats not dogs) on tissue macrophages of the lymph nodes and spleen resulting in cell death, which did not reverse during the recovery period. The clinical relevance of the macrophage finding in rats is unknown.

Though the VFI has shown no pharmacological activity at the human dose in receptor screening, a cytochrome P450 (CYP) inhibition study has been performed to assess potential for interaction with concomitant medications. The ability of FD001 and FD003 to inhibit CYP isoforms, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5, was assessed to identify the potential of drug-drug interactions. FD001 and FD003 were each preincubated for 0 or 30 minutes at 7 concentrations (0.1, 0.3, 1, 3, 10, 30, and 100 µM) with human liver microsomes in the presence of probe substrates phenacetin, efavirenz, amodiaquine, diclofenac, mephenytoin, dextromethorphan, midazolam, and testosterone to determine if there was a direct effect on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5, respectively. Additionally, FD001 and FD003 were each incubated in the presence of dihydronicotinamide-adenine dinucleotide phosphate (NADPH) for up to 30 minutes at 37°C to assess the time-dependent inhibitory effects on CYP enzymes.

Results from the CYP inhibition studies suggest that:

- FD001 does not inhibit any CYP enzyme examined (ie, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 [using 2 different substrates midazolam and testosterone]).

- FD003 is a direct inhibitor of CYP2B6, CYP2C8, CYP2C19, and CYP3A4/5 (midazolam).

- The R1 values associated with the direct inhibition of CYP2B6, CYP2C8, CYP2C19, and CYP3A4/5 (midazolam) by FD003 are all less than 1.1, signifying little likelihood that FD003 may cause clinically relevant drug interactions due to direct inhibition in vivo.

- FD003 did not directly inhibit CYP1A2, CYP2C9, CYP2D6, or CYP3A4/5 (as measured by testosterone 6β-hydroxylation; testosterone).

- FD003 was a time-dependent inhibitor of CYP3A4/5 (as measured by testosterone 6β-hydroxylation) since the IC_{50} value shifted from > 100 µM with no preincubation to 49 µM after FD003 was preincubated with NADPH-fortified human liver microsomes for 30 minutes. However, this time-dependent inhibition was only apparent in the presence of concentrations of FD003 greater than 10 µM, or approximately 1500 times the total bound and unbound plasma maximum observed concentration (C_{max}) of 0.9 µg/mL (0.0066 µM).

- FD003 did not cause time-dependent inhibition of any other CYP enzyme examined (ie, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 [as measured by midazolam 1´-hydroxylation]).

3.1.3.2 First-in-Human Study

A FIH study, representing the first human use of the VFI, was concluded in 2012. Data from the study indicated that the FAST PV and mGFR Technology is safe and well tolerated. In this study, 32 patients were enrolled with 8 patients (6 VFI + 2 placebo) assigned to 1 of 4 dose groups. The VFI agent consisted of a mixture of 2 different molecular weight carboxymethyl dextran molecules (5 kD [FD001] and 150 kD [FD003]) with different fluorescent dye molecules attached. Four increasing doses were administered through a bolus injection: Group 1, 5 mg/0.1 mL VFI; Group 2, 15 mg/0.3 mL VFI; Group 3, 75 mg/1.5 mL VFI; Group 4, 150 mg/3 mL VFI.

All 32 patients completed the study as planned and were included in the safety analysis. Overall 9 patients (28.1%) experienced 15 treatment-emergent adverse events (TEAEs) which were considered by the investigator to be reasonably related to VFI administration or study procedures. Most related TEAEs were observed after administration of 15 mg VFI (9 TEAEs reported by 3 patients). No related TEAEs were observed after the highest dose. Only 2 patients (both 15 mg VFI) reported 6 TEAEs (pruritus and gastrointestinal disorders) which were considered related to VFI administration. All other related TEAEs were judged to be related to the study procedures (mainly injection site pain and hematoma at the blood sampling site). All TEAEs were of mild to moderate intensity. There were no serious adverse events

(SAEs); no patient discontinued the study due to adverse events (AEs). Safety laboratory parameters, vital signs, and electrocardiogram (ECG) parameters showed no clinically relevant time- or treatment-related effects or differences between dose groups.

All patients who received VFI were included in the PK analysis. Geometric mean AUC and C_{max} of FD001 and FD003 increased dose-proportionally. FD001 had a median time at which the maximum concentration was observed (t_{max}) of 0.25 hours for all doses; median t_{max} of FD003 was about 0.5 hours for the 2 lower doses and 2 hours for the 2 higher doses. FD001 was eliminated with a short elimination half-life ($t_{½}$) of about 1.5 to 4 hours; mean $t_{½}$ for FD003 was considerably longer ranging from 47 to 111 hours. Detailed PK data is available in the Investigator's Brochure for this study.

3.1.3.3 Phase 2a Study at University of Alabama at Birmingham

For the Phase 2a study completed in 2015 at the University of Alabama at Birmingham, 5 cohorts with a total of 33 patients were studied. The primary endpoint of the study was to establish safety of the IV administered VFI. This end point was achieved, and even with increasing severity of CKD, with its associated comorbidities, no SAEs were reported during the study. A relatively small number of AEs were reported as potentially related to the VFI. The FAST BioMedical measured GFR (mGFR) was evaluated by performing an Iohexol study along with standard estimates of GFR using creatinine and cystatin C values and both Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. The breakdown of patients and GFR ranges in each cohort is listed in the table below.

Phase II Patients by Cohort

Cohort	Type of Patient	eGFR
1	Normal	≥60 mL/min
2	CKD	30-59 mL/min
3	CKD	15-29 mL/min
4	AKI	AKI
5	Normal	≥60 mL/min

Following the enrollment of 1 hospitalized patient in Cohort 4 (AKI) the decision to stop enrolling patients into this cohort and stop the study was made. At that point, 33 of the originally planned 38 patients had been enrolled into the study. The decision for stopping enrollment was related to the anticipated time to enroll patients into the AKI cohort because in the opinion of the Principal Investigator and FAST management the enrollment criteria, developed to ensure the safety and appropriate evaluation of the CKD population, were too restrictive to enable enrollment of the originally planned AKI patients. Secondary rationale for stopping enrollment was the expiry of the clinical VFI manufactured in June of 2011, and the inability to extend the label dating due to purity issues with the reference materials used to calibrate the stability assay.

Subsequently, upon review of the medical history of the single patient enrolled in Cohort 4, it was determined that while this patient had a declining kidney function and was being treated clinically as an AKI patient, the patient did not meet either the Acute Kidney Injury Network stage "2" or Rifle (risk [class R], injury [class I] and

failure [class F] + 2 outcome classes [loss and end-stage kidney disease]) stage “I” enrollment criteria. This was noted as a protocol deviation.

A secondary endpoint of the study was to compare the kidney function quantified by the FAST VFI technique to that determined by existing measurements including Iohexol clearance (Cohorts 1, 2, 3, and 5) and serum biomarkers including cystatin C and creatinine for eGFR (calculated by the MDRD and CKD-EPI equations). Iohexol was omitted from Cohort 4, per protocol, because methodological differences between the 2-marker, 2-hour FAST BioMedical technology and the single-marker 6-hour Iohexol test would not provide comparable results in patients with a dynamically changing GFR, such as can be expected in AKI.

Both Iohexol and the FAST mGFR technologies utilized timed blood draws and subsequent concentrations to calculate a predicted total AUC. The dose of marker given was divided by this AUC to calculate the mGFR, with scaling based on body surface area. The FAST mGFR utilized 3 blood draws taken at early time points out to 2 hours. A fourth virtual point was calculated based on the PV at T0 (the starting concentration in the plasma just after the dose is given or time 0). The Iohexol GFR 6 test utilized a blank, plus a minimum of 6 additional blood draws taken over 6 hours to determine the AUC. Iohexol, having a much smaller molecular size, distributes differently within the extracellular space, so some difference in mGFR between the 2 markers was anticipated. FAST BioMedical expected to measure mGFR values slightly lower than Iohexol in normal patients, as most single marker systems like Iohexol have difficulty resolving the early decay phase when the clearance is rapid. It was expected that the FAST mGFR measurements would be within 10% in the lower GFR groups. Prior studies conducted by FAST BioMedical using both the VFI and Iohexol in anesthetized dogs, have shown there were differences between the 2 methods. Analysis of the single marker decay rate showed that it normally resolved to a falsely high volume of distribution making the resulting mGFR biased higher than the FAST 2 marker system, which quantifies the initial volume of marker distribution. This small error in AUC was only significant in mGFRs above 80 mL/min.

In addition to studying a range of GFRs using different measurement techniques, FAST BioMedical intended to evaluate FAST’s measured PV method against a standard clinical estimate of PV, specifically Nadler’s Formula (Nadler et al 1962).

Plasma Volume (males) = $0.3669 \times (\text{Height [m]})^3 + 0.03219 \times (\text{Body Weight [kg]}) + 0.6041$
Plasma Volume (females) = $0.3561 \times (\text{Height [m]})^3 + 0.03308 \times (\text{Body Weight [kg]}) + 0.1833$

The difference between a measured and estimated PV are between plus 20% to minus 30% (Feldschuh and Enson, 1977) using radioiodinated human serum albumin as the measured plasma marker. Since Nadler’s work was based primarily on measurements in healthy patients, it was expected there may be a greater range seen with the CKD population in this study.

Pharmacokinetic data analysis was performed on Cohorts 2, 3 and 5. The results indicated that the VFI performed as expected over the course of the study and corresponded well to the measured renal function and PV of the patients.

Following administration of 75 mg VFI by IV bolus injection to adults with preserved kidney function and impaired kidney function it was concluded that the mean plasma concentrations of FD001 exhibited little variability within study groups for samples collected from 0.25 to 24 hours. Plasma FD001 concentrations generally reached C_{max} following the end of the infusion and decreased in a multi-exponential, first-order elimination manner with early-phase distribution apparently complete at 8 to 12 hours and measureable concentrations observed in all patients at 24 hours. Median plasma FD001 t_{max} values were 0.25 hours, the time of the first post administration sample collection in all study groups. Mean plasma FD001 $t_{½}$ estimates were 5.64, 9.48, and 18.3 hours for Groups 5, 2, and 3, respectively, increasing with decreasing renal function. Mean FD001 CL decreased with decreasing renal function. No significant differences between groups were observed in FD001 C_{max} ($p = 0.6751$), t_{max} ($p = 0.7418$), V_{ss} ($p = 0.8425$), or C_{max}/Dose ($p = 0.4781$); and anticipated differences were observed in FD001 AUC_{last} ($p < 0.0001$), $t_{½,z}$ ($p = 0.0015$), CL ($p < 0.0001$), and AUC_{inf}/Dose ($p < 0.0001$).

Following administration of 75 mg VFI by IV bolus injection to adults with preserved kidney function and impaired kidney function, mean plasma FD003 concentrations exhibited little variability within study groups for samples collected from 0.25 to 504 hours. Plasma FD003 concentrations reached maximum concentrations within 1 hour in all study groups and decreased in a mono-exponential, first-order elimination manner with measureable concentrations observed in all patients at 504 hours. The observed half-life of FD003 ranged from 90 hours for healthy patients to 125 hours for patients with severely impaired kidney function (eGFR ≥ 15 and < 30 mL/min/1.73 m2) and were generally similar irrespective of renal function. Mean FD003 volume estimates indicated that FD003 appeared to occupy a volume space that corresponds with PV. Mean FD003 CL did not change with decreasing renal function. No significant differences were observed in FD003 C_{max} ($p = 0.0743$), t_{max} ($p = 0.0600$), AUC_{last} ($p = 0.8469$), AUC_{inf} ($p = 0.8684$), $t_{½,z}$ ($p = 0.0508$), V_z ($p = 0.2644$), V_{ss} ($p = 0.3305$), CL ($p = 0.5048$), C_{max}/Dose ($p = 0.1652$), or AUC_{inf}/Dose ($p = 0.5048$). A delay in the dispersion of the FD003 molecule initially after dosing resulting in a later than anticipated C_{max}. Complete PK data for the Phase 2a study is located in the Investigator's Brochure from this study.

3.1.3.4 Phase 2b Study at University of Alabama at Birmingham and ICON

The Phase 2b study was a prospective, open-label study designed to evaluate the safety, tolerability, PK, and pharmacodynamics (PD) of FAST PV and mGFR Technology in healthy patients and patients with varying degrees of renal impairment. A total of 32 patients were planned to be enrolled in 4 cohorts of up to 8 patients/patients each. Healthy patients in Cohorts 1 and 2 had an eGFR ≥ 60 mL/min/1.73 m^2; patients with impaired renal function in Cohort 3 had an eGFR ≥ 30 and < 60 mL/min/1.73 m^2; and patients with impaired renal function in Cohort 4 had an eGFR ≥ 15 and < 30 mL/min/1.73 m^2. Patients in Cohort 1 received a single dose of VFI followed 130 minutes later by a 350 mL infusion of 5% albumin in normal saline over 30 minutes on day 1. Patients in Cohort 2 received a dose of VFI followed 160 minutes later by a single dose of iohexol on day 1 and a second dose of VFI 24 hours following the initial dose of VFI. Patients in Cohorts 3 and 4 received a single dose of VFI followed 160 minutes later by a single dose of iohexol on day 1.

The pharmacokinetics of FD001 and FD003 showed that the clearance of FD001 was proportional to kidney function with urine clearances of 59.955 mL/min, 25.919 mL/min and 12.635 mL/min for cohorts 2, 3, and 4 respectively, when using 0 to 2 hour data. Following the end of the bolus injection, the plasma concentration of the FD001 decreased following a multi-exponent and first order elimination curve. Average measured GFR for cohorts 2, 3 and 4 were 87 mL/min, 48 mL/min, and 27 mL/min respectively.

The FD003 volume estimates indicated that FD003 appeared to occupy a volume of space equal to the plasma volume (Figure 4). FD003 had determined elimination half-lives of 95.3, 99.1, and 106 hours for Cohorts 2, 3, and 4, respectively. These rates were similar across the renal functions studied which indicates the marker's non-renal clearance is preserved in kidney failure patients.

Cohort 1 used FD003 to monitor a change in volume induced by the infusion of 350 ml of 5% albumin solution given over 30 minutes. At 5 minutes post completion of the infusion, the average plasma volume increase was 324 ml with an SD of 49mL, showing excellent accuracy for following a change in plasma volume. By 30 minutes post infusion, the average volume increase from baseline was reduced to 224ml showing that FD003 was being retained within the vasculature even though the 5% Albumin solution was slowly distributing into the interstitial space. This cohort was not used for PK due to the complication of accounting for the additional volume infused.

The FAST VFI was generally safe and well tolerated in healthy patients in Cohorts 1 and 2 and in patients with impaired renal function in Cohorts 3 and 4. No severe or serious TEAEs, TEAEs leading to study drug discontinuation, or deaths were reported during the study. Overall, the majority of TEAEs reported were mild in severity. Three patients in Cohort 4 had a TEAE of blood creatine phosphokinase increased related to clinically significant abnormal creatine kinase levels. All 3 TEAEs of blood creatine phosphokinase increased were mild in severity and considered by the investigator to be possibly related to study drug. There were no other clinically significant abnormal clinical laboratory values. There were no clinically significant abnormal ECG or vital signs measurements, nor were there clinically meaningful trends identified in observed values or changes from baseline. There were no clinically significant abnormal physical examination findings.

3.2 Rationale

3.2.1 Rationale for Dose Selection

Doses of VFI for this study were selected based on results of the Phase 1 FIH study in healthy patients and in the Phase 2a and 2b studies in patients with normal and compromised renal function, as well as from data obtained in nonclinical studies (additional information is provided in the Investigator's Brochure).

Data from the FIH study evaluating the safety of the FAST PV and mGFR Technology in healthy adult patients indicated the study dose of 75 mg/1.5 mL VFI was appropriate for the Phase 2a study. Data from the Phase 2a study and improvements to the FAST PV and mGFR Technology have indicated that, in the Phase 2b study, a single administration of VFI 47 mg/3 mL would be well tolerated and easily measured in all patients. Additionally, for Cohort 2, received 2 doses of VFI, each dose being 47 mg/and was well tolerated. As the FD001 marker is cleared

and the doses are separated by 24 hours, the maximum level of FD001 in the patients after the second dose did not exceed the dose of 52.5 mg of FD001 given in Phase 2a. Also, as the amount of FD003 had been reduced in the new VFI formulation, a second dose of VFI resulted in plasma exposure values close to the exposure previously observed in patients administered the high doses of FD003. In the Phase 2a clinical study a dose of 22.5 mg of FD003 was given, in the Phase 2b the maximum amount of FD003 following repeat dosing was 24 mg. This repeat-dose strategy was agreed to by the United States Food and Drug Administration in written correspondence dated February 24, 2016. The predose sample taken on Day 2 for Cohort 2 was analyzed and provided the residual amount of both FD001 and FD003. The FD003 residuals were then subtracted as was a normal predose sample from the values of the post-second-administration samples to allow for a clean calculation of PV. FAST has derived the mathematical equations for GFR to appropriately compensate for any residual FD001; however, the normal patients did not have a residual level of FD001 patients on Day 2 that would have affected the GFR calculation. Repeat doses of VFI are likely to be undertaken clinically as a method of evaluating treatments. These repeat measurements would not be done rapidly as a treatment required time to be effective. FAST chose 24 hours to separate VFI doses to mimic likely clinical situations. As discussed in section 3.1.3.4 the repeat administrations were generally safe and well tolerated by the 8 patients.

3.2.2 Rationale for Study Design

This is an investigator-initiated, one-armed phase II clinical trial designed to evaluate the safety and functionality of the FAST PV and mGFR Technology in CHF patients.

Previous clinical studies have shown the FAST technology to be safe and functional in patients with chronic kidney disease (CKD) and in healthy subjects. The technology has not been studied in CHF patients. In previous clinical studies, the technology has been demonstrated to accurately measure PV and mGFR in patients with CKD and healthy patients when compared to other clinical techniques including, eGFR calculations, Iohexol clearance and Nadler's formula. Additionally, previous studies have shown that the concentration of the PV marker remains relatively constant during the first two hours after injection, given that this high-molecular weight marker is predicted to exclusively occupy the intravascular compartment and exhibit a long half-life.
In this study, the safety and functionality of the FAST Technology will be evaluated in CHF patients. The non-clinical and clinical history of the product provides confidence that the technology will be well tolerated and function correctly in the CHF population.

The blood samples are timed to maximize the information gathered during the study. In addition to measurements of PV and GFR, FAST will determine the technology's functionality by comparing the concentration of the PV marker at the 15, 30 and 60 minute time points. It is not believed that the plasma volume will change significantly within that time period and thus the concentration of the PV marker should remain relatively constant.

4 Study objectives

4.1 Primary endpoints

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

- Safety will be assessed by determination of the absolute and relative frequencies of AEs and SAEs related to 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.

4.2 Secondary endpoints

- To evaluate how estimated PV (ePV) and estimated total blood volume (TBV) assessments on days 1 and 3 (by established measures such as Nadler's formula or Metropolitan Life Tables) 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, NYHA stage, respiratory rate) on days 1 and 3 predicts mPV and mTBV at these time points.

- To evaluate whether eGFR calculation by the CKD-EPI formula on days 1 and 3 provides an accurate estimate of measured GFR (mGFR; by 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 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 (cutoffs potentially adjusted for some of the following variables age, gender, body height, body weight, optimal body weight (dry weight), hematocrit) on day 1 or on day 3 are at risk of developing low-output complications within the next 24-48 hours (e. g. subjectively reported dizziness/nausea, PGA, hypotension, need for iv-fluid therapy).

- To evaluate whether patients with a high mPV or a high mTBV (cutoffs potentially adjusted for some of the following variables age, gender, body height, body weight, optimal body weight (dry weight), hematocrit) 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 estimated TBV (eTBV) from day 1 to day 3 adequately reflects 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?

4.3 Time point (for the evaluation of secondary endpoints)

- Secondary endpoints will be evaluated at the time points specified above.

4.4 Study designs

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. *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.*

Patients enrolled in the study will be administered VFI (Day 1) and with a second dose occurring 24 to 48 hours 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 mLs) will be collected pre-dose and at 15 (± 5), 30 (± 5), 60 (+30), and 180 (+120) minutes post-dose. 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.
As described in section 6.1.1.2 of this protocol, 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 no sooner than 43 hours and no later than 53 hours after the initial dosing. Blood draws (approximately 2 mLs) will be collected pre-dose and at 15 (± 5), 30 (± 5), 60 (+30), and 180 (+120) minutes post-dose. 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. The available laboratory values (see *Table 1*) will be entered in the Dosing Day Case Report Form (CRF), along with any copies of physician's notes and orders entered that day. 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 CRF. 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 (EOS) 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 [a]		X						
Demographics [b]		X						
Comorbidities [c]		X						
Full medication list on enrollment into the study and during follow-up [d]		X	X	X	X	X		
Patient Symptom Assessment Survey [e]		X	X	X	X	X		
Clinical Presentation/ Exam [f]		X	X	X	X	X		
Treating Physician Survey [g]		X		X				
Study-Related Laboratory Measurements [h]		X	X	X	X	X		
Routine Clinical Parameters [i]	X*	X	X	X	X	X		
Clinical Data [j]	X*	X	X	X	X	X		
Adverse events		X	X	X	X	X	X	X
Blood specimen for marker analysis [k]		X		X				
Discharge from study								X
Adjudication Committee Evaluation[l]								X
WOCBP Pregnancy	X			X				X

test [m]								

[a] See Table 2 for Structural Heart Disease Criteria

[b] See Table 3 for specific demographic parameters

[c] See Table 4 for specific instructions on collecting comorbidities

[d] See Table 5 for specific instructions of collecting medications

[e] See Annex D for the Patients Symptom Assessment Survey

[f] See Table 6 for Clinical presentation/exam parameters

[g] See Annex E for the Treating Physician Survey

[h] See Table 7 for Study Related Laboratory Measurements

[i] See Table 8 for Routine Clinical Parameters

[j] See Table 9 for Clinical Data collected

[k] 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.

[l] See Table 10 for the Adjudication Committee Evaluation Questions; Adjudication Committee meets after all collected data available.

[m] See Table 11 for Details of Pregnancy test

*will be collected retrospectively after study inclusion

Table 2: 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

Table 3: Demographics
ID
Date of admission
Date of inclusion (Day 1)
Date of birth
Age at inclusion (years)
Gender
Ethnicity

Table 4: Comorbidities
Free text documentation of all comorbidities
In addition, the following comorbidities are recorded systematically (by "yes"or "no" answers):
CKD 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

Table 5: Medication on admission
Free text documentation of all existing medication (1) upon admission, 3) upon inclusion into the study (day 1) and (3) during days 2-5, including name, dosage and timing of intake
In addition, the following medications are recorded systematically (by “yes”or “no” answers):
ACE inhibitor
AT1 receptor blocker
AT1/Neprilysininhibitor
Loop diuretic
Furosemide p.o. dosage (mg) per 24h prior to dosing (according to medication plan)
Torasemide p.o. dosage (mg) per 24h prior to dosing (according to medication plan)
Thiazide diuretic
Aldosterone antagonist
beta-Blocker
Nitrates
Digoxin/Digitoxin

Table 6: Clinical presentation/Exam Parameters
*Height (cm)
Weight (kg)
*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 (0-4)
Ankle/lower extremity edema (grade 0-4; according to Guelph General Hospital Congestive Heart Failure Pathway)
Pulmonary rales
Systolic blood pressure (mmHg) (morning, supine)
Diastolic blood pressure (mmHg) (morning, supine)
**Systolic blood pressure (mmHg) (morning, 30 sec after standing up)
**Diastolic blood pressure (mmHg) (morning, 30 sec after standing up)
Heart rate (beats per minute)(morning, supine)
Heart rate (beats per minute)(morning, 30 sec after standing up)
SpO2 (%) (morning)
Jugular venous distension (>4 cm above sternal angle with 45° upper body elevation) (morning)
Respiratory rate (breaths per minute) (morning)
O2 supply requirement (in L/min) (morning)

*Only assessed Day 1

*Only assessed Day 1 and Day 3

Table 7: Study-related laboratory measurements
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)
Storage of EDTA Plasma, Heparin-Plasma, Serum, whole blood, centrifuged urine (10 aliquots a 200 µl each) at ≤ -75°C

*Only Collected Day 1 and Day 3; For use in calculating an ePV from Nadler's formula

** Only Collected Day 1, 3 and 5

Table 8: 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)
INR (dimensionless)

*Only on Day 1

Table 9: Clinical data
Total 24h dose of i. v. furosemide (from 06:00am same day to 06:00 am next day) (mg)
Total 24h dose of i. v. torasemide (from 06:00am same day to 06:00 am next day) (mg)
Total 24h dose of p. o. furosemide (from 06:00am same day to 06:00 am next day) (mg)
Total 24h dose of p. o. torasemide (from 06:00am same day to 06:00 am next day) (mg)
Application of any thiazide diuretic?

Dialysis/ultrafiltration performed? (from 06:00am to 06:00 am next day)
iv. fluids (NaCl, balanced electrolytes) (from 06:00am to 06:00 am next day) (L/24 h)
Is the total dosage of diuretics (administered from 06:00 am same day to 06:00 am next day) increased compared to previous 24 h dosage? (e. g. increasing loop diuretics or adding thiazide diuretics or aldosterone antagonists)?
Is the total dosage of diuretics (administered from 06:00 am same day to 06:00 am next day) decreased compared to previous 24 h dosage? (e. g. decreasing loop diuretics or removing thiazide diuretics or aldosterone antagonists)?

Table 10: Adjudication Committee Evaluation
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 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?

*Responses will be collected as a Yes/No, with an additional free text section available to record details to substantiate the response

Table 11: Pregnancy Tests in WOCBP
Medically monitored pregnancy tests with a minimum sensitivity 25mU/ml will be assessed within 24 hours prior first study drug administration, on Day 3 and 30 in WOCBP

4.5 Timetable

- Recruitment and Enrolment to run (expected): January 2019-July 2019
- FPFV: After consent
- LPLV: 30 days following LPFD (Last patient First dose)

5 Study population

50 patients with acute decompensated heart failure will be enrolled at the nephrology and cardiology departments of Charité - Universitätsmedizin Berlin. There will be no specific gender-fractions as no gender specific differences concerning efficacy and safety of the investigational product are expected.

5.1 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 studyprior 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.

5.2 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 $\leq$ 90 systolic and/or $\leq$ 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 (hCG) 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 IMP).
- Subject is legally detained in an official institution.
- Subject is dependent on the sponsor, the investigator or the trial sites.

6 Plan for medical treatment

6.1 Description of study medication / investigational medicinal product

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 Figures 1 and 2 below, 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.

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)

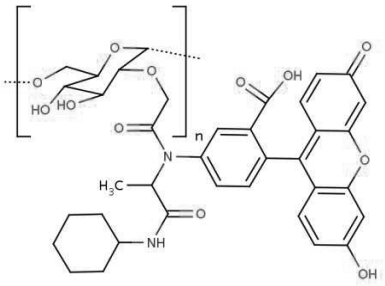


Figure 1 - FD001

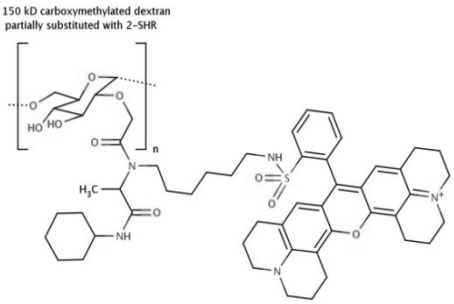


Figure 2 - FD003

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.

6.1.1 Adverse drug reactions and restrictions, contra-indications, drug interactions

6.1.1.1 Contraindications

The FAST VFI (FD004) should not be administered to patients with known hypersensitivity to dextran, 5-aminofluorescein dye, or 2-sulfohexamine rhodamine dye.

6.1.1.2 Precautions (overview, dextran safety, anaphylactoid reactions, patient monitoring)

The FAST VFI (FD004) was well tolerated in the nonclinical studies conducted to date. Anaphylactic responses to dextrans, while rare, must be considered as a possibility for the high molecular weight dextrans being evaluated by FAST. As such, all testing facilities should have immediately available procedures to administer treatment for an anaphylactic response should it occur.

Overview of The FAST BioMedical Visible Fluorescence Injectate (VFI)

The FAST BioMedical VFI (or FD004) contains 2 different molecular weight carboxymethyl dextran markers with 2 different fluorescent dye molecules attached. The small molecular weight marker, FD001 (approximately 5 kD), is conjugated with 5-aminofluorescein and the large molecular weight marker, FD003 (approximately 150 kD) is conjugated with 2-sulfohexamine rhodamine. These fluorescent labels are covalently attached to the dextran through the carboxymethyl moiety. Unlike clinical dextrans which are dosed in grams, FAST BioMedical has worked to minimize the dose of VFI. Since the 2013 Phase I study in Germany, the VFI dose has been reduced by almost 70%, moving from the initial maximum clinical dose of 150 mg to the current clinical dose of 47mg (35 mg of FD001 and 12 mg of FD003).

The larger marker, FD003, was designed not to be rapidly cleared from the vasculature and is also not rapidly cleared by passive filtration in the kidneys. Therefore, its concentration in the bloodstream after injection provides a direct measurement of the total blood volume by dilution principles.
The smaller marker, FD001, was also designed not to be subject to rapid metabolism, but is freely filtered by the kidneys. The decline in the concentration of the small FD001 marker is consistent with the renal clearance rate which, when scaled to a patient's actual vascular volume, provides an assessment of measured GFR. Accurate and timely measurements of vascular volume and GFR are critically important metrics in patients with acute congestive heart failure and cardiorenal syndrome, where assessing patients' volume status are key to the clinical management of the patient.[1,2,3]

Dextran Safety
As noted by the reviewer, dextran anaphylactoid reactions are well documented. For this reason, in development of its markers, FAST BioMedical has taken great care to understand and incorporate the body of knowledge known about dextrans and to enlist the assistance of the foremost experts in the area of dextran safety. Specifically, in the 1970s and 1980s, Pharmacia AB in Uppsala, Sweden conducted extensive research to understand dextran-induced anaphylactic reactions (DIAR) and demonstrate different approaches to managing the immunogenic potential of these molecules. The FAST BioMedical VFI was developed in conjunction with many of the former Pharmacia chemists and immunologists. These key leaders in the field of dextran biology demonstrated that the immunogenicity of dextrans could be significantly attenuated by either: (1) using a substituted dextran such as a carboxymethyl dextran or (2) pre- or co-administering a low molecular weight dextran (1 to 5 kD) to serve as a "hapten".[4,5] Specifically, in 1988, K.G. Lungstrom published hapten inhibition with pre-inoculation of dextran 1 kD to block antigen combining sites led to a 35-fold reduction in dextran-induced anaphylactic reactions, and a 90-fold reduction in fatal dextran reactions.[4] The vast majority of reported reactions were reported as mild (skin reactions, bradycardia, hypotension, etc.), and Lungstrom reported only 2 fatal reactions in over 5.1 million doses, making the use of dextran with a 1 kD hapten inhibitor safer than other commercially available colloids as well as many other injectable products . [1]

In 1977, Johannes Ring published a prospective multicenter study looking at the safety of various colloids including plasma protein fractions, dextran, gelatin solutions and hydroxyethylstarch (HES).[5] Ring's study reported the following incidence of anaphylactoid reactions:

[1] Katz, S.D.: In Search of Euvolemia in Heart Failure. *Journal of the American College of Cardiology*. 2014; 2: 2213-1779.
[2] Ronco, C., Kaushik, M., et al.: Diagnosis and Management of Fluid Overload in Heart Failure and Cardio-Renal Syndrome: The "5B" Approach. *Seminars in Nephrology*. 2012; 32: 129-141.
[3] Miller, W.L., Mullan, B.P.: Understanding the Heterogeneity in Volume Overload and Fluid Distribution in Decompensated Heart Failure is Key to Optimal Volume Management: Role for Blood Volume Quantitation. Journal of the American College of Cardiology. 2014; 2: 2213-1779.
[4] Lungström, K.G.: Safety of Dextran in Relation to Other Colloids – Ten Years' Experience with Hapten Inhibition. *Infusionsther Transfusionsmed.* 1993; 20: 2006-10
[5] Ring J, Messmer K.: Incidence and severity of anaphylactoid reactions to colloid volume substitutes, *Lancet* 1977; 1; 466-469.

Colloid	Number of infusions registered	Number of anaphylactoid reactions	Fractional incidence of anaphylactoid reactions
Plasma proteins	85,630	12	0.014 %
Dextrans	85,882	28	0.032 %
Gelatin	12,989	15	0.115 %
HES	16,405	15	0.085 %

Additional studies by Caroline Mendler showed that concomitant administration of dextran 1 kD is as effective as pre-inoculation in reducing dextran reactivity.[6] This is a significant finding for products such as the FAST BioMedical VFI where the additional time required to pre-inoculate the patient may impede timely patient care.

Development of the FAST VFI

FAST BioMedical has taken great care to develop a product that minimizes the potential for intolerance reactions. This begins with the procurement of raw pharmaceutical-grade dextran which is derived from the B512 Leuconostoc mesenteroids strain, which yields a highly homogenous dextran with very limited branching. A 1957 paper by Elvin Kabat described the relationship between the linearity of the dextran molecule and its immunogenic potential, demonstrating that the limited branching of the B512 strain led to lower reactivity when compared to dextrans derived by other strains.[7] Today, the B512 strain is still considered the safest of all dextran strains, and this dextran strain is exclusively used to produce the dextran employed in the synthesis of the FAST BioMedical VFI.

In effort to further incorporate the historical learnings from past dextran development efforts, FAST BioMedical convened an advisory committee of former Pharmacia dextran researchers, chemists and clinical consultants including Karl-Gostä Ljungstrom, Harriet Hedin, Tony de Belder, and Peter Buckley. Many of these experts are cited in the seminal literature on dextran anaphylaxis. The committee provided input on the composition of the FAST BioMedical VFI. Specifically, the committee indicated that the low molecular weight marker, FD001, at approximately 5 kD in the FAST BioMedical VFI is very likely to act as a hapten inhibitor, having the same ability to bind antibodies without causing a precipitating immune response. Further, it was concluded that FD001 at approximately 5 kD dextran could be co-administered with FD003 at approximately 150 kD based upon the aforementioned studies performed by Caroline Mendler.

The advisory committee also favored use of a modified carboxymethyl dextran to further reduce the immunogenic potential of the FAST BioMedical VFI. This assertion was based on the 1975 paper by Wolfgang Richter where he demonstrated that the use of substituted dextrans, such as carboxymethyl dextran, significantly reduced the reactivity of the dextran molecule.[8] The process for synthesizing the

[6] Mendler, C.: Hapten Inhibition of Dextran-Induced Anaphylactic Reactions in Dogs. The University of Munich Institute of Surgical Research Ph.D., dissertation. 1980.

[7] Kabat, E.A., Turino, A.B.: Studies on the Immunochemical Basis of Allergic Reactions to Dextran in Man. *Journal of Clinical Investigation*. 1957; 36: 1160-1170.

[8] Richter, W.: Effect of Substitution on Reactivity of B 512 Dextran Fractions with Anti-B 512 Dextran in Heterologous *Passive Cutaneous Anaphylaxis. International Archives of Allergy and Applied Immunology*. 1975; 48: 505-512.

carboxymethyl dextran used in the FAST BioMedical VFI was then developed in conjugation with Tony de Belder, a former colleague of Wolfgang Richter's at Pharmacia and world-renowned expert in the field of dextran and dextran derivatives. In considering the dextran strain, the role of FD001 as a likely hapten inhibitor, and the use of substituted (carboxymethyl) dextran, FAST BioMedical has made substantial efforts to learn from and utilize established dextran science. We believe that these efforts have greatly reduced the immunogenic potential of the FAST BioMedical VFI as compared to unmodified dextran products.

Patient Monitoring and Treatment

All patients enrolled in the EMPAKT CHF study will be actively monitored for potential intolerance reactions to the FAST VFI. The FAST VFI has been administered 97 times in 89 patients through the course of the preceding 3 clinical studies. Patient populations in those studies included healthy subjects and patients with advanced chronic diseases such as CKD, COPD, hypertension, diabetes, and polycystic kidney disease. No SAEs were reported in the prior studies, though AEs commensurate with a mild to moderate immunological response were noted including pruritus (5), nausea (4), vomiting (3), diarrhea (2), abdominal pain (1), and urticaria (1). In all cases, the symptoms resolved without sequalae. It is also noteworthy that an intolerance reaction may be more likely in a healthy subject with a fully functional immune system than in the typical heart failure patient whose immune system may be somewhat compromised.

The EMPAKT CHF study will be conducted on hospitalized patients who will have regular access to nurses and physicians through the course of normal clinical care. Because dextran-related anaphylaxis is almost exclusively an acute onset event, the physician or nurse 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-minute blood draws specified in the protocol. In periods where a nurse or physician are not present at the patient's bedside, the patient will have access to a call button to immediately notify the clinical staff should they experience symptoms of concern or a medical emergency. The following classification system will be used to assess the severity of the patient's condition should the patient report any symptoms consistent with dextran-related anaphylaxis.

Classification of Dextran Anaphylaxis by Symptom

Type	Grade/ Severity	Skin	Gastrointestinal system	Respiratory system	Cardiovascular system
Anaphylactoid	I	Flush, erythema, urticaria	--	--	--
Anaphylactoid/anaphylactic	II	Flush, erythema, urticaria	Nausea	Dyspnea	Tachycaria, hypotension
Anaphylactic	III	Flush, erythema, urticaria	Vomiting, defecation	Bronchospasm, cyanosis	Shock
Anaphylactic	IV	Flush, erythema, urticaria	Vomiting, defecation	Respiratory arrest	Cardiac arrest

All patients enrolled in the EMPAKT CHF study will be hospitalized, and access to appropriate supportive therapy will be quickly administered should signs of an intolerance reaction be observed. Any adverse or severe adverse events will be reported as specified in the clinical protocol.

6.1.1.3 Treatment of Overdose

The FAST VFI is contained in single-dose vials, making significant overdose unlikely. Though the vials are overfilled with 4mL/62.6 mg in order to ensure that a full 3mL/47mg dose can be drawn, previous studies have evaluated the safety of the VFI using a 3mL/150mg dose, over two times the amount of material contained in the single-dose vials for this study. There is no known antidote for overdose of VFI. In the event of a suspected overdose, the patient should be monitored appropriately and should receive supportive therapy, as necessary.

6.1.2 Treatment schedule

	Day 1	**Day 3**
Dose / Units	47mg/ 3mL	47mg/ 3mL
Pharmaceutical Form	Liquid VFI	Liquid VFI
Route of Application	IV Bolus	IV Bolus

Instructions for dose preparation:

1. Add 4mL of sterile water to the vial of VFI over 4 seconds.
2. Gently swirl the vial for 3 minutes, avoiding allowing the liquid to reach the rubber stopper. Product can be held up to light to observe the reconstitution process.
3. Place vial on a roller/rocker table to mix for 30 minutes.
4. Product is ready for drawing up of 3 mL dose into syringe.

Instructions for application:

Ensure the IV line is clear of and free flowing (can be done with a saline flush). Attached the VFI syringe and inject steadily within 30 seconds. Follow the dose with a 5mL flush of normal saline.

6.1.3 Storage requirements and administration (distribution and return of study drug)

The VFI will be stored in a refrigerator at 2 to 8°C (36 to 46°F).

The VFI will be prepared within 60 minutes of dosing, and the prepared product will be stored at room temperature (25°C). The product will be protected from sunlight and ultraviolet light until dosing.

6.1.4 Treatment compliance

Training:

A minimum of two hours of training with a FAST BioMedical employee is required to review and discuss the FAST PV and mGFR Technology. This includes training on the use of the FAST VFI and the risks involved in each step of performing the measurements.

6.2 Placebo / reference medication

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

6.3 Concomitant medication / concomitant therapy

Patients may continue/use medications/therapies approved and/or prescribed by their treating physician.

6.4 Procedures in case of emergency

The FAST VFI (FD004) was well tolerated in the nonclinical studies conducted to date. Anaphylactic responses to dextrans, while rare, must be considered as a possibility for the high molecular weight dextrans being evaluated by FAST. As such, all testing facilities should have immediately available procedures to administer treatment for an anaphylactic response should it occur. We will ensure that each testing facility is prepared prior to the start of dosing.

7 Study procedures

All procedures specified in the study protocol should be performed for every single subject enrolled in the trial. In the case, trial participants miss a study visit every attempt should be made to regain contact by telephone, written communication or record review to determine if adverse events have occurred.

7.1 Recruitment procedures and informed consent process

The Principal Investigator, his or her proxy / designee and the member of the trial team will have the full responsibility to screen for suitable patients.

The study group consists of nephrologists (KSO, JS, TS, KUE) and cardiologists (FE, BP). In our hospital (Charité - Universitätsmedizin) patients with acute decompensated heart failure (HF) are usually admitted to the cardiology departments or to the nephrology departments (if renal dysfunction is the leading cause for HF decompensation). Admission to the hospital may occur by direct referral from an outpatient physician or after initial evaluation and treatment at the emergency room. Since we plan to enroll patients with acute decompensated heart failure across a wide spectrum of renal function, we will enroll patients from both the cardiology and nephrology departments.

To facilitate effective screening, a designated study physician will work with treating physicians at the the nephrology and the cardiology departments on a daily basis to identify patients with acute decompensated heart failure. All patients with a diagnosis of acute decompensated heart failure or "hydropic decompensation" (a frequently used clinical designation for acute decompensated heart failure in Germany) will be identified by the study physician and an initial discussion regarding study participation will take place between the study physician and the treating physicians at the departments of cardiology or nephrology. The study physician will then approach these patients and inform them about the study, ask for their participation and provide them with patient information material regarding the study. On the next morning, having allowed the patient a sufficient time period to reflect on study

participation, written informed consent will be obtained. This day will be designated "Day 1" of the study and patients will be evaluated and treated according to study protocol.

A screening log for documentation of these patients will be used. All pre-screened patients will be documented in the screening log and assigned a screening number.

Patients who do not refuse the trial will be asked to participate and the written informed consent could be given by the patient.

During the initial consultation, the following will be performed:

1. Inform the patient about the study. Provide patient information in writing. If the patient does not a priori refuse participation, inform him/her about the further diagnostic procedures to check eligibility. Inform that he/she has still time to reconsider the matter and the opportunity to ask questions. Inform the patient that informed consent in writing must be signed before performing any of the following procedures during this visit. Obtain the patient's informed consent in writing on two copies of the form. Sign both forms. Provide one copy to the patient. Store the other copy in the Investigator Site File.
2. After getting the signed informed consent, the study related activities as outlined in this protocol will be performed.

We expect to see approximately 250 patients during the recruitment time with a diagnosis of acute decompensated heart failure. As a consequence, the required number of 50 patients seems feasible.

7.2 Methods of avoiding simultaneous enrolment in other trials

During the process of getting an informed consent, it will be mentioned by the study physician / study investigator that there is no possibility of participating in other clinical trials at the same time. The patient will be informed about this issue in the interview with the investigator and this information is part of the informed consent form.

7.3 Enrolment

Patients, who meet all inclusion criteria and who have given their written informed consent will be enrolled in the trial.

7.4 Clinical examinations and trial-related deviations from clinical practice

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 [a]		X						
Demographics [b]		X						
Comorbidities [c]		X						
Full medication list n enrollment into the study and during follow-up [d]		X	X	X	X	X		
Patient Symptom Assessment Survey [e]		X	X	X	X	X		
Clinical Presentation/ Exam [f]		X	X	X	X	X		
Treating Physician Survey [g]		X		X				
Study-Related Laboratory Measurements [h]		X	X	X	X	X		
Routine Clinical Parameters [i]	X*	X	X	X	X	X		
Clinical Data [j]	X*	X	X	X	X	X		
Adverse events		X	X	X	X	X	X	X
Blood specimen for marker analysis [k]		X		X				
Discharge from study								X
Adjudication Committee Evaluation[l]								X
WOCBP Pregnancy	X			X				X

test [m]								

[a] See Table 2 for Structural Heart Disease Criteria

[b] See Table 3 for specific demographic parameters

[c] See Table 4 for specific instructions on collecting comorbidities

[d] See Table 5 for specific instructions on collecting medications

[e] See Annex D for the Patients Symptom Assessment Survey

[f] See Table 6 for Clinical presentation/exam parameters

[g] See Annex E for the Treating Physician Survey

[h] See Table 7 for Study Related Laboratory Measurements

[i] See Table 8 for Routine Clinical Parameters

[j] See Table 9 for Clinical Data collected

[k] 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.

[l] See Table 10 for the Adjudication Committee Evaluation Questions; Adjudication Committee meets after all collected data available.

[m] See Table 11 for Details of Pregnancy test

*will be collected retrospectively after study inclusion

7.4.1 Pre-trial examinations (screening / inclusion examination)

The following screening examinations will be performed:

- Medical history and Comorbidities (Table 4)
- Clinical examination (Table 6) Including vital signs, renal function assessment
- Concominent Medications (Table 5)
- Demographics (Table 3)
- Serum Laboratory testing (Table 7 and Table 8)

7.4.2 Examinations during trial

The following examinations will be performed:

Table 6: Clinical presentation/Exam Parameters
*Height (cm)
Weight (kg)
*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 (0-4)
Ankle/lower extremity edema (grade 0-4; according to Guelph General Hospital Congestive Heart Failure Pathway)
Pulmonary rales
Systolic blood pressure (mmHg) (morning, supine)
Diastolic blood pressure (mmHg) (morning, supine)

**Systolic blood pressure (mmHg) (morning, 30 sec after standing up)
**Diastolic blood pressure (mmHg) (morning, 30 sec after standing up)
Heart rate (beats per minute)(morning, supine)
Heart rate (beats per minute)(morning, 30 sec after standing up)
SpO2 (%) (morning)
Jugular venous distension (>4 cm above sternal angle with 45° upper body elevation) (morning)
Respiratory rate (breaths per minute) (morning)
O2 supply requirement (in L/min) (morning)

*Only assessed Day 1

*Only assessed Day 1 and Day 3

All examinations during the trial are listed in Tables 1-9, in section 4.5.

7.4.3 Final examination

- On the final follow-up visit (phone call) any adverse events will be recorded.

7.4.4 Laboratory assessments

Table 7: Study-related laboratory measurements
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)
Biobanking of EDTA Plasma, Heparin-Plasma, Serum, whole blood, centrifuged urine (10 aliquots a 200 µl each)

*Only Collected Day 1 and Day 3, for use in the calculation of ePC by Nadler's formula

** Only Collected Day 1, 3 and 5

Table 8: 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

*Only on Day 1

Table 11: Pregnancy Tests in WOCBP
Medically monitored pregnancy tests with a minimum sensitivity 25mU/ml will be assessed within 24 hours prior first study drug administration, on Day 3 and 30 in WOCBP

All examinations during the trial are listed in Tables 1-9, in section 4.5.

7.5 Individual trial duration

- From enrolment in the study until discharge is 30 ± 5 days.

8 Risk-benefit-assessment

A rapid and accurate measurement of plasma volume (PV) and glomerular filtration rate (GFR) is important in chronic heart failure (CHF), acute kidney injury (AKI) and chronic kidney disease (CKD) for assessment of impairment, diagnosis, and prompt treatment. This study primarily serves the purpose of obtaining information about the tolerability and safety of the FAST PV and mGFR Technology in CHF patients. The FAST Technology has been previously used in three clinical studies. 87 patients have been dosed with the FAST VFI. There have been no reported SAEs and few minor AEs across all studies (please refr to the Investigator´s Broschure, Version 6.0, dated July 2018 (FAST-IDE-IB_51378), section 4 and 5).

Based on the results of prior clinical and non-clinical assessments of the FAST PV and mGFR Technology the risks in participating in the trial are considered acceptable. However, they include the usual risks of participating in clinical trials, which are related to possible allergic reactions and blood drawing via venipuncture. There are no perceived direct benefits to patients participating in this trial. However, thorough medical check-ups may be seen as an advantage. The safety of the patients will be observed during all study phases.

Medical progress is based on research which ultimately must rest in part on experimentation involving humans. Eligible patients may consider participation in this clinical trial because they want to contribute to the advancement of medical knowledge. Still, considerations related to the well-being of the individual patients enrolled into this clinical study must take precedence over the interests of science and society. Based on available information and the design of the study, the sponsor and the investigator consider the trial to be ethically acceptable. The duration of hospitalization and the medical surveillance are considered adequate to ensure safety of the patients.

9 Termination and subsequent treatment

9.1 Premature termination of the individual participant

Patients are free to discontinue the study at any time, for any reason, and without prejudice to further treatment. The investigator may remove a patient if, in the investigator's judgment, continued participation would pose unacceptable risk to the patient or to the integrity of the study data. All procedures for early termination must be completed. Reasons for removal or withdrawal may include:

- Admission to the ICU prior to either the first or second dose
- The patient develops a stated exclusion criterion during the course of study
- If the patient experiences an acute anaphylactoid reaction during the course of administering the FAST VFI, administration will stop immediately
- Evidence of an anaphylactoid reaction (dyspnea, urticaria, flush, bronchospasm, or severe systemic hypotension)
- Administrative decision by the investigator or sponsor
- Significant protocol deviation
- Patient noncompliance
- Safety concern by the investigator or sponsor
- Lost to follow-up
- Pregnancy

Patients who are withdrawn for reasons other than safety issues may be replaced at the discretion of the sponsor and investigator.
In the event of a patient's withdrawal, the investigator will promptly notify the medical monitor and will make every effort to complete the EOS assessments. All withdrawn patients with ongoing clinically significant clinical or laboratory findings will be followed until the finding is resolved or medically stable; reasonable attempts will be made to follow-up with patients.

9.2 Premature termination of the clinical study

The study will be discontinued if the Sponsor or its designee judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP guidelines.
The following events, cause premature termination of the clinical study:

- Unjustifiable risk and toxicity in risk-benefit analysis (decision taken by principal Investigator)
- New scientific evidence provided during the study that could affect the patient's safety (benefit-risk analysis no longer positive)
- Decision of the Sponsor or the Competent Authority that the study should be discontinued.

9.3 Follow-up and continuing treatment after regular / premature termination

After completing all the protocol treatment and visits, patients will continue with regular visits according to usual practice of the center.

In the case of premature termination, the reason for withdrawal must be entered on the appropriate case report form (CRF) page and must be followed for safety and efficacy until 5 days after discontinuation.

10 Safety monitoring

10.1 Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign [(i.e including an abnormal laboratory findings, symptom or disease) in a subject or clinical investigation subject after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

An untoward medical occurrence may be a study endpoint as well as meeting the definition for an AE.

The occurrence of AEs should be sought by non-directive questioning of the patient at each visit throughout the study. AEs also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments.

Abnormal laboratory values or test results constitute AEs if they are in line with the definition above (ICH E2A, section 1) and fulfill at least one of the following criteria:

- they induce clinical signs or symptoms,
- they are considered clinically significant,
- they require therapy.

Clinically significant abnormal laboratory values or test results should be identified through a review of values outside of normal ranges/clinically notable ranges, significant changes from baseline or the previous visit, or values which are considered to be non-typical in patient with underlying disease. Investigators have the responsibility for managing the safety of individual patient and identifying AEs.

10.2 Serious Adverse Event (SAE)

An SAE is any AE (appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s) or medical conditions(s) which meets any one of the following criteria:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent

  - social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant, i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

10.3 Adverse Reactions

Adverse reactions are all untoward and unintended responses to an investigational medicinal product related to any dose administered.

10.4 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any suspected adverse reaction related to the study treatment that is both serious and unexpected.

"Unexpected" means that the nature and severity of the adverse reaction are not consistent with the information about the study medication in question set out in the reference safety information (investigator's brochure).

10.5 Evaluation of Expectedness

The evaluation of expectedness will be done by the pharmacovigilance and the sponsor representative. The expected (S)AEs could be found in the investigator's brochure (section 4). The investigator's brochure contains also a section "reference safety information" (RSI) (section 5.6).

10.6 Treatment of (S)AEs

All AEs should be treated appropriately. Treatment may include one or more of the following:

- no action taken (i.e. further observation only)
- concomitant medication given
- non-drug therapy given.

The action taken to treat the AE should be recorded on the AE CRF.

10.7 Assessment of SAEs

As far as possible, each AE should be evaluated to determine:

- the severity grade (grade 1 to 5 according CTCAE v5.0)
- its relationship to the study drug (assessment of causality)
- its duration (start and end dates or if continuing at final exam)
- action taken (no action taken; what kind of action taken, e.g. hospitalization)
- whether or not it constitutes a serious adverse event (SAE).

10.8 Severity grade (according CTCAE v5.0)

- Grade 1: Mild: The Adverse Event is transient and can be tolerated easily
- Grade 2: Moderate: The Adverse Event causes discomfort and impedes normal activities
- Grade 3: Severe: The Adverse Event causes severe impairment of normal activities
- Grade 4: Life-threatening or disabling

- Grade 5: Death related to AE.

10.8.1 Assessment of causality

To assess causality between administration of the investigational product and the Adverse Event the following definitions apply:

- Sure: The reaction comprehensively follows the administration of the investigational product in the right timeframe or can be measured in body tissues or fluids or represents a known or expected response to the study medication or disappears after discontinuation or dose reduction and reoccurs after re-exposure.

- Probable: The reaction comprehensively follows the application of the investigational product in the right timeframe or represents a known or expected response to the study medication or disappears after discontinuation or dose reduction and cannot be explained by known characteristics of the patient's disease.

- Possible: The reaction comprehensively follows the application of the investigational product in the right timeframe or represents a known or expected response to the study medication, but could easily be caused by other factors.

- Unlikely: Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible); disease or other drugs provide plausible explanations

- Not related: Adequate Information supporting the assumption that there is no causality

- Cannot be evaluated: The causality cannot be determined.

10.9 Documentation of AEs and SAEs

All Serious Adverse Events (SAEs) and all Adverse Events (AEs) need to be documented in the patients' clinical file and in the respective CRF pages by the investigator, no matter, whether the investigator suspects a causal relationship to the investigational product or not. The documentation needs to include:

- Duration (start and end dates) or if the event is ongoing an outcome of not recovered/not resolved should be reported
- Action taken regarding study treatment
- Whether other medication or therapies have been taken (concomitant medication/non-drug therapy)
- Its outcome (not recovered/not resolved; recovered/resolved; recovering/resolving, recovered/resolved with sequelae; fatal; or unknown).

Related signs symptoms and laboratory changes should be summarized to a specific disease. The event will be recorded in the CRF. SAEs need to be documented on a separate SAE form.

Out of normal range laboratory data need to be analysed concerning their clinical relevance by the Investigator – and if relevant documented as an AE itself.

All adverse events need to be followed until they subside or stabilize.

The Sponsor will carefully document all SAEs reported by the Investigator. His documentation will be sent to the relevant regulatory authorities and to relevant authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory and if they so request.

10.10 Follow-up of Adverse Events

Once an AE is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

The Sponsor may request additional information on specific AEs of interest and may make requests to perform additional diagnostic tests to further assess the safety profile of the study drugs. Such information may include diagnostic procedure reports, discharge summaries, autopsy reports, and other relevant information that may help in assessing the reported AE. All additional information will be de-identified prior to collection by the Sponsor or its partners.

The investigator should also instruct each patient to report any new AE (beyond the protocol observation period) that the patient, or the patient's personal physician, believes might reasonably be related to study drug. This information should be recorded in the investigator's source documents; however, if the AE meets the criteria of an SAE, it must be reported to the Sponsor.

10.11 Reporting of SAEs

The Investigator will report any Serious Adverse Event within 24 hours after becoming aware to the Sponsor and will afterwards send an extended written record

This announcement will be done via fax or email to:

KKS Charité
Fax: +49 (0)30 – 450 7553-856
or email to
pharmacovigilance-kks@charite.de

Every event will be documented on a record form and will immediately be sent to the above given address. If at that point all required information is not available, succeeding records will be sent. In the event of death a copy of the autopsy record could be added.

Exceptional rules:
In this clinical trial the following SAEs are excluded from the reporting requirement:

- Serious or unexpected events which occur after enrolment, but before treatment was initiated
- Hospitalization or extension of hospitalization required for therapeutic procedures of the protocol
- Other events: For example events that cause hospitalization but were planned before enrolment (e.g. (planned surgery)

10.12 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Sponsor will report all suspicious cases of Suspected Unexpected Serious Adverse Reactions (SUSARs) which had been occurred in one of clinical trials conducted by the same

sponsor with the same drug substance/IMP to the relevant Ethics Committee, the relevant regulatory authorities and to relevant regulatory authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory immediately, at the latest 15 days after it becomes known. He will also inform all Investigators involved in the trial.

In case of a fatal or life threatening SUSAR the Sponsor will report all information relevant for judging the event immediately, at the latest 7 days after the event becomes known to the relevant Ethics Committee, the relevant regulatory authorities and to relevant regulatory authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory as well as to all Investigators involved in the trial. After a further 8 days all further relevant information must be available.

10.13 Other safety issues requiring expedited reporting

The Sponsor will immediately, at the latest 15 days after it becomes known report all circumstances that require a revision of the risk-benefit analysis to the relevant Ethics Committee, the relevant regulatory authorities and to relevant regulatory authorities of other European member states and other contracting states of the EWR agreement, if the study is run in their territory. This especially includes:

- Singular cases of expected severe adverse events with an unexpected outcome.
- Increased incidence of expected severe adverse events that are judged as being clinically relevant.
- SUSARs which occur after termination of the clinical trial Events related to study procedures or development of the study medication, which could affect a patient's safety.

All person-related data will always be transmitted pseudonymised.

10.14 Development Safety Update Report

The sponsor submits a list of all SUSAR/SARs yearly or on request, together with an extensive safety report on the investigational products to the respective competent authorities and the concerned Ethics Committees.

11 Documentation

11.1 Case Report Forms (CRF)

Data collected on each patient will be recorded on an eCRF (electronically). This trial will be performed using an electronic case report form (eCRF) (SecuTrial). The investigator and the trial site staff will receive system documentation, training and support for the use of the eCRF. In case of new trial site staff the training can be performed by experienced personnel of the respective trial site.

All protocol-required information collected during the trial must be documented in the eCRF by the investigator, or a designated representative. All data entry, modification or deletion will be recorded automatically in an electronic audit trail indicating the individual subject, the original value, the new value, the reason for change, who made the change and the time and date of the change. All data changes will be clearly indicated. Former values can be viewed in the audit trail. All electronic data will be entered by the site (including an electronic audit trail) in compliance with applicable record retention regulations in a web- based data capturing system known as secuTrial.

Investigators or their designee must enter the information required by the protocol onto the electronic case report forms (eCRF). Data items from the eCRF are entered into the study database.
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The investigator must complete the eCRF and must print the patient identification list and store it with other study documents, *e.g.* the protocol, the investigators' brochure and any protocol amendments, in a secure place. All entries to the eCRF must be made as described in the electronic case report form manual.

Data on subjects collected on eCRF during the trial will be documented in a pseudonymous fashion; the subject will only be identified by the pseudonym. The investigator must maintain source documents for each patient in the study, consisting of all demographic and medical information, including laboratory data, electrocardiograms, etc., and keep the signed informed consent form. All information on eCRF must be traceable to these source documents in the patient's file. Data without a written or electronic record will be defined before trial start and will be recorded directly on the eCRF, which will be documented as being the source data. The definition can be found in the investigator site file.

Essential documents, as listed below, must be retained by the investigator for as long as needed to comply with national and international regulations. The investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents include:

1. IRB/IEC approvals for the study protocol and all amendments
2. All source documents and laboratory records
3. CRF copies (paper copies or electronic copies on a CD-ROM)
4. Patients' informed consent forms (with study number and title of trial)
5. Any other pertinent study document.

A clinical study monitor will review the eCRF and compare the content to the source data. In addition, the investigator or designated colleague will provide access to designated sponsor representative(s) for the periodic review of source documents (*e.g.* hospital and clinic records) to assure accuracy and completeness of the eCRF.

All clinical work conducted under this protocol is subject to GCP rules. This includes an inspection by the sponsor and/or health authority representatives at any time. The investigator will agree to the inspection of study-related records by health authority representatives and/or the sponsor.

11.2 Investigator Site File (ISF)

All essential documents will be kept in the Investigator Site File which will be stored at the study site in accordance with ICH GCP chapter 8. The sponsor will provide the investigator with an investigator's file. This file should be used for all trial-related documents. The investigator will be responsible for keeping the investigator's file updated and ensuring that all required documents, as specified in ICH-GCP guidelines, are filed. The file will be made available for monitoring and/or auditing by the sponsor or its representative and regulatory agencies.

11.3 Drug accountability / flow of study medication

All drug accountability records must be kept current, and the Investigator must be able to account for all used and unused vials of study medication. These records should contain the dates, quantity, and study medication:

- Received at site
- Administered to each subject,
- Disposed of at the site or returned to the Sponsor or designee

The clinical monitor responsible for the study site will provide written approval for the destruction or return of unused study medication following reconciliation of all clinical supplies.

12 Quality management

12.1 Control of trial progress and data quality

12.1.1 Monitoring

Monitoring as the act of overseeing the conduct and progress of clinical trials needs to be performed by consistent standards and qualified personnel. Therefore the coordination and realization of the monitoring will be performed by the Coordinating Center for Clinical Studies (KKS Charité) Berlin.

The monitoring personnel will visit the clinical study sites on a regular basis and at least once before the first subject has been enrolled, during the course of the study, and at study completion. The monitor is responsible for the implementation of the study procedures at the sites.

The objectives of the monitoring procedures are to ensure that the trial subject's safety and rights as a study participant are respected, that accurate, valid and complete data are collected, and that the trial is conducted in accordance with the trial protocol, the principles of ICH-GCP and local legislation.

Study data will be verified in accordance to the original medical records (Source Data Verification). The sponsor/investigators allow the monitor to have access to any or all the study materials needed for source data verification and proper review of the study progress. At all times, the sponsor/investigators/monitors will maintain the confidentiality of the study documents. Furthermore, problems with inconsistent and incomplete data will be discussed.

All investigators agree that the monitor regularly visits the trial site and assure that the monitor will receive appropriate support in his activities at the trial site. The declaration of informed consent includes a statement to the effect that the monitor has the right – while observing the provisions of data protection legislation – to compare the case report forms (p/eCRFs) with the trial subject's medical records (doctor's notes, ECGs, laboratory printouts etc.). The investigator will secure access for the monitor to all necessary documentation for trial-related monitoring. The role allocation in secuTrial provides the monitor with access possibilities to all data captured electronically. Furthermore the investigators will prepare the consent forms as PDF files and submit them to the Trust agency, which then verifies the data and drafts regular reports.

The aims of the monitoring visits are as follows:

- To check the declarations of informed consent.
- To monitor trial subject safety (occurrence and documentation/reporting of AEs and SAEs).
- To check the completeness and accuracy of entries on the p/eCRF.
- To validate the entries on the p/eCRF against those in the source documents (source data verification, SDV).
- To evaluate the progress of the trial.
- To evaluate compliance with the trial protocol.
- To assess whether the trial is being performed according to GCP at the trial site.
- To discuss with the investigator aspects of trial conduct and any deficiencies found.

A monitoring visit report is prepared for each visit describing the progress of the clinical trial and any problems (e.g. refusal to give access to documentation).
By signing the declaration of informed consent the participants allow access to their documents. With the signature in the protocol, the investigators confirm that auditors and health authority inspectors may have access to the study documentation and accordant medical records. Auditors and inspectors are bound by professional confidentiality and may not pass on any personal information that comes to their knowledge. In the course of audits or inspections, data in the case report forms will be compared with the data from medical records. All the documentation held by the investigators within the scope of the clinical trial, as well as the drug logs of the study medications will be verified.

12.1.2 Data Quality Assurance

The sponsor assumes responsibility for implementing and maintaining quality assurance and quality control systems with written Standard Operation Procedures (SOPs) to ensure that the study is conducted and data are generated, documented and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. The sponsor also takes responsibility for securing agreement from all involved parties to ensure direct access to the trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor and inspection by authorities. Quality control will be applied to each stage of the study to ensure that all data are reliable and have been processed correctly.

12.1.3 Audits / inspections

Authorized representatives of the Sponsor, a regulatory authority, or an Independent Ethics Committee (IEC) may visit the center to perform audits or inspections, including source data verification. The purpose of a Sponsor audit or inspection is to systematically and independently examine all study related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

12.2 Reference institutions

1. The KKS will conduct the regulatory affairs, data quality management, monitoring, SAE- Management.
   Coordinating Center for Clinical Studies Berlin- Koordinierungszentrum für Klinische Studien (KKS), Campus Virchow-Klinikum, Augustenburger Platz 1,13353 Berlin, Germany.

2. The Institute of Biometry and Clinical Epidemiology, Charité Campus Mitte is responsible for the statistical planning. Furthermore it will conduct statistical data analyses and biometric reporting.

13 Data entry and data management

All patient related data will be recorded under a pseudonym. Every patient will receive a patient number / pseudonym which will be unique for this individual patient. The Investigator will compile a confidential list, which relates these patient numbers to the patient's full name. This list will only be accessible to the study team and the monitor. Original patient files may be viewed by monitors, auditors and inspectors.

13.1 electronic Case Report Form (eCRF) Data Entry

Investigators or their designee must enter the information required by the protocol onto the electronic case report forms (eCRF). Data items from the CRF are entered into the study database.

The investigator must complete the eCRF and must print the patient identification list and store it with other study documents, *e.g.* the protocol, the investigators' brochure and any protocol amendments, in a secure place. All entries to the eCRF must be made as described in the electronic case report form manual.

Please see section 11.1 of this protocol for information regarding the eCRF and data entry.

13.2 Data management

Data required for the analysis will be acquired and transferred electronically to a central database at the KKS Charité by means of an electronic data capture system (EDC).

The secuTrial® Software solution of interActive Systems (iAS) is a worldwide reachable web-based system (remote data entry [RDE]). The system operates according to the principle of online data capture and is in compliance with the FDA 21 CFR Part 11 and ICH-GCP. In addition, it contains further functions to perform plausibility, consistency and range checks of the study data.

The log-in in the system requires a previous authentication for access, and runs by a secure data transfer-protocol (SSL) to exclude any improper manipulation of data by unauthorized parties. The system will automatically keep an audit trail of all entries and corrections to the eCRF.

The secuTrial® Software solution is based on an Oracle database that does not allow direct access. All access rights of trial participants will be defined according to their trial functions. The defined access rights will e.g. allow to read or to enter study data depending on their functions (Monitor, clinical investigator, etc.).

Query management is an integrated part of this RDE system. This function allows the communication between the Monitor and the trial participants. In case of errors or improper data, the responsible trial participant will get queries (messages) to correct the errors or to check improper data on the eCRF.

The trial data will be stored digitally on a remote server with daily backups. After end of trial all data will be exported for checks of consistency and plausibility. After performing the checks, the data matrix will be transferred for the statistical evaluation.

13.3 Source Data and Patient Files

The information in original documents and records (e. g. patient files, laboratory notes) are defined as Source Data and will be reviewed by the Monitor for Source Data Verification. All data that will be recorded directly in the CRF/eCRF without prior written or electronic record will be described in the protocol and considered to be Source Data.

14 Statistical Analysis

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 are purely descriptive. The aim is to generate new hypotheses to be tested in subsequent phase III trials.

All analyses will be performed using validated statistical software. A statistical analysis plan included detailed instructions for the analysis will be finalized before start of any analysis.

Size Estimation
We plan to enrol 50 eligible patients. Due to the explorative nature of this phase II study a formal sample size calculation is not possible. However, the sample size of 50 patients is sufficient to guarantee reasonable accuracy with respect to the main objectives of the trial as specified below.

Primary objectives:

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

- Safety will be assessed by determination of the absolute and relative frequencies of AEs and SAEs related to 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:

- To evaluate how estimated PV (ePV) and estimated total blood volume (TBV) assessments on days 1 and 3 (by established measures such as Nadler's formula or Metropolitan Life Tables) predict measured PV (mPV; as assessed by FAST methodology) and measured TBV (mTBV; calculated from mPV and measured hematocrit) at these time points. Simple linear regression models will be generated and potentially adjusted for relevant confounders.

- 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, NYHA stage, respiratory rate) on days 1 and 3 predicts mPV and mTBV at these time points. Optimal linear models will be chosen by comparing the results of different variable selection models for the corresponding linear model (backward selection based on p-Values and Akaike Information Criterion, best subset selection etc.).

- To evaluate whether eGFR calculation by the CKD-EPI formula on days 1 and 3 provides an accurate estimate of measured GFR (mGFR; by assessed by FAST

methodology) in heart failure patients undergoing active fluid management. Simple linear regression models will be generated and potentially adjusted for relevant confounders.

- 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 48-72 hours. Logistic regression models will be generated and potentially adjusted for other relevant confounders.

- 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. Logistic regression models will be generated and potentially adjusted for other relevant confounders. Associations will be assessed by chi-square interaction tests and by Spearman's correlation coefficient.

- To evaluate whether patients with low mPV or low mTBV (cutoffs potentially adjusted for some of the following variables age, gender, body height, body weight, optimal body weight (dry weight), hematocrit) on day 1 or on day 3 are at risk of developing low-output complications within the next 24-48 hours (e. g. subjectively reported dizziness/nausea, PGA, hypotension, need for iv-fluid therapy). Optimal logistic regression models will be selected by comparing the results of different variable selection models for the corresponding linear model (backward selection based on p-Values and Akaike Information Criterion, best subset selection, etc.).

- To evaluate whether patients with a high mPV or a high mTBV (cutoffs potentially adjusted for some of the following variables age, gender, body height, body weight, optimal body weight (dry weight), hematocrit) 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). Optimal logistic regression models will be selected by comparing the results of different variable selection models for the corresponding linear model (backward selection based on p-Values and Akaike Information Criterion, best subset selection, etc.).

- To evaluate whether the time course of ePV and estimated TBV (eTBV) from day 1 to day 3 adequately reflects the time course of mPV and mTBV at these time points. Mean ePV and mPV and mean eTBV and mTBV will be compared for each time point separately (using paired t-tests) and by investigating the whole time curve (comparing the times when maximal and minimal volumes are reached and the areas under the curve)).

- 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. Linear, logistic or Cox-regression models will be utilized as appropriate.

- 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.

eGFR and mGFR will be compared for each time point separately (using paired t-tests) and by investigating the whole time curve (comparing the times when maximal and minimal GFRs are reached and the areas under the curve).

- 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?

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 based 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. We will calculate absolute and relative frequencies of the “yes”-answers to the questions specified above together with 95% confidence intervals.

14.1.1 Definition of population for analysis

The primary analysis population will be the intention-to-treat population including all patients receiving the study medication at least once.

A sensitivity analysis will be performed based on the per-protocol-population including all patients without major protocol violations.

The safety analysis will be based on the intention to treat population.

15 Reporting

15.1 Statistical report

The statistical analysis report will be written by the Institute of Biometry and Clinical Epidemiology in supervision of [redacted] in cooperation with the Sponsor and the Principal Investigator. All data in this report is confidential.

15.2 Study report

The composition of a final integrated report will be conducted in accordance with ICH E3: Structure and Contents of Clinical Study Reports.The study report will be submitted to IRB and Competent Authority within 12 months after the end of the study (LVLP), according to § 13 subsection 9 of the GCP-ordinance.

15.3 Publication (policy)

The study results will be published irrespective of the study outcome.

16 Ethical, legal and regulatory aspects

16.1 ICH-GCP-guidelines

This trial will be conducted in accordance with the current ICH-GCP-guidelines, especially ICH-GCP [E6(R2)]. Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki (1996), and that the clinical trial data are credible.

Information regarding the testing history of the FAST VFI and its safety information is located in Section 8 of this protocol and in section 5 of the investigators brochure (IB).

16.2 Legal requirements of the study

- Approval of Ethics Committee
- Approval of competent authority
- Notification to regional authorities
- Informed Consent
- Insurance
- Data privacy and confidentiality

16.2.1 Approval of Ethics Committee

Study protocol, patient information and consent form will be presented to the responsible Ethics Committee for survey (here: Ethics Committee of the local state Berlin). The study will only start after ethics approval has been granted. The Ethics Committee will immediately be informed (by the Sponsor) of all changes to the protocol (according to applicable local law and regulation) and of all events that could affect a patients safety. The Ethics Committee will also be informed of all suspected SUSARs and of regular or premature termination of the study.

The Investigators have to register with the Ethics Committee (enter proof of qualification) before they enroll any patients.

16.2.2 Approval of competent authority

The trial will be submitted to the responsible Competent Authority for authorization (here: BfArM, Federal Institute for drugs and Medical Device, Bonn, Germany). The trial will only be initiated after authorization has been granted.

16.2.3 Notification to local authorities (LaGeSo)

Prior to enrolment of the first patients into the trial the sponsor, his legal representatives/ contractors and all investigators are responsible for notification of his/her participation in the trial to the local regulatory authority, (according to § 67 subsection 1 of the Medicinal Products).

According to applicable local law and regulation the sponsor, his legal representatives/ contractors and all investigators are also responsible to notify the local regulatory authority of amendments, premature terminations of the study and the regular trial termination.

16.2.4 PROTOCOL AMENDMENTS

In order to guarantee most comparable conditions during all intervals of the trial and in the interests of valid statistical analysis, the investigators, the coordinating investigator or any other person involved in the trial conduct may not alter the study conditions agreed upon and set out in this protocol. Amendments should be made only in exceptional cases and by mutual agreement within the steering committee. Any amendment must be set out in writing, at the same time giving the reasons, and signed by all parties concerned. The amendment then becomes part of the study protocol.
Amendments which might have an substantial impact on the clinical trial (according to section 10, subsection 1 of the GCP-ordinance) including on the well-being of the subject (substantial amendments), require an additional approval by the Ethics Committee and by the Competent Authority.
In addition, a further informed consent form is to be signed by all trial subjects enrolled in the trial, who are affected by the amendment. Minor changes will only be submitted to the Ethics Committee and the competent federal authority in a written form.
The investigator may implement a deviation from, or a change of the protocol to eliminate an immediate hazard to trial subjects without prior EC approval opinion. As soon as possible, the implemented deviation or change, the reason for it, and if appropriate, the proposed protocol amendment should be submitted to the coordinating investigator for agreement.

16.2.5 Patient information and informed consent

Patient Information

Before enrolment every patient will receive full oral and written information about the nature, purpose, expected advantages and possible risks of the trial.

Consent to participation in the trial:

The patient will agree to participation in the trial by signing the informed consent form. Patients must be given an opportunity to enquire about details of the study. After a sufficient period of time for the individual's consideration and decision, comprehension and consent shall be documented on the consent form by the dated signature of the patient and the Investigator/ treating doctor. If a patient is able to consent but cannot sign himself/herself, oral information and written consent need to be testified and signed by a witness.

Design and language will be adjusted to the study site's needs. The final versions of patient information and consent will be submitted to the Ethics Committee during the process of

getting an IRB opinion for the trial. Both the patient information and the patient consent form are prepared in duplicate. One of each form for the Investigator, a duplicate will be handed to the patient.

16.2.6 Patient insurance

For participating patients, an insurance (according to applicable local law and regulation) is provided according to the German drug law by “HDI Global SE”, 14052 Berlin. The insurance number is 5701032603017. The sum insured is 50.000.000 € for the study EMPAKT-CHF and is 500.000 € per person.

The insurance has been finalized over:

16.2.7 Data Privacy and confidentiality

The participants’ data will be saved in a pseudonymous form, which will neither contain initials nor full date of birth, in accordnance to Regulation 2016/679 (General Data Protection Regulation)). All regulative requirements applying to data protection will be met. Re-identification of a participant subject’s name is possible from the patient identification log or via the independent trust agency. The identification log will be kept in a locked research office at the Trial site where access is only possible by the principal Investigator or persons authorized by the principal Investigator.

Patients will be informed that their disease-related data will be saved for scientific purpose (Publication, etc.) using a pseudonym. Consenting patients have got the right to be informed about the data recorded. Patients will also be informed that their pseudonymized data will be forwarded to the Competent Authority and to the Ethics Committee responsible, in accordance with legal notification obligation for drug safety. Patients, who disagree with this process of data transfer, are not allowed to participate in this study.

16.3 Archiving of data / access to records

Originals of all study-related report forms will be stored in the study headquarters at the trial site for at least 10 years after completion of the trial (according to applicable local law and regulation).

The Investigator / principle Investigator stores all administrative documents (correspondence with the Ethics Committee, the Supervising Authority, trial center,

study site), patient identification log, the signed patient consent forms, copies of the data documentation form and common study documentation (protocol, amendments).

16.4 Financing

The study will be supported by a research grant from FAST BioMedical.

17 References

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18 Appendices

Annex D: Patient Symptom Assessment

Patient Symptom Assessment Sheet (FAST Study)

Patient ID: emp-☐☐☐

Date: ☐☐ ☐☐ ☐☐☐☐ (DD MM YYYY)

Zeichnen Sie **auf der Linie ein Kreuz** ein. Versuchen Sie durch die Position des Kreuzes, Ihre Beschwerden/Ihren Gesundheitszustand möglichst genau zu beschreiben:

1. Bitte beschreiben Sie ihren Zustand **jetzt gerade:**

Ich fühle mich so schlecht wie nie zuvor — Ich fühle mich so gut wie nie zuvor

2. Bitte beschreiben Sie Ihren Zustand **in den letzten 24 Stunden:**

Ich fühle mich so schlecht wie nie zuvor — Ich fühle mich so gut wie nie zuvor

3. Bitte beschreiben Sie ihren Zustand **jetzt gerade:**

Ich habe überhaupt keine Luftnot — Ich bin habe mehr Luftnot als je zuvor

4. Bitte beschreiben Sie Ihren Zustand **in den letzten 24 Stunden:**

Ich habe überhaupt keine Luftnot — Ich bin habe mehr Luftnot als je zuvor

5. Bitte beschreiben Sie ihren Zustand **jetzt gerade:**

Ich habe keinerlei Übelkeit — Ich bin habe mehr Übelkeit als je zuvor

6. Bitte beschreiben Sie Ihren Zustand **in den letzten 24 Stunden:**

Ich habe keinerlei Übelkeit — Ich bin habe mehr Übelkeit als je zuvor

7. Bitte beschreiben Sie ihren Zustand **jetzt gerade:**

Ich habe keinerlei Schwindel — Ich bin habe mehr Schwindel als je zuvor

8. Bitte beschreiben Sie Ihren Zustand **in den letzten 24 Stunden:**

Ich habe keinerlei Schwindel — Ich bin habe mehr Schwindel als je zuvor

Annex E: Treating Physician Survey

Physician Assessment Sheet
(FAST VFI Study)

Patient ID: emp-☐☐☐

Frage 1 ist durch den betreuenden Arzt am **Tag 1** (erste Gabe FAST VFI) auszufüllen:

Datum Tag 1: ☐☐ ☐☐ ☐☐☐☐ (DD MM YYYY)

Frage 1: Bitte beschreiben Sie den aktuellen **intravasalen Volumenstatus** (nicht Gesamtkörpervolumenstatus!) des Patienten. Zeichnen Sie **auf der Linie ein Kreuz** ein. Versuchen Sie durch die Position des Kreuzes, den Zustand möglichst genau abzubilden.

Schwerst intravasal hypovoläm — Intravasal euvoläm — Schwerst intravasal hypervoläm

Fragen 2, 3 und 4 sind durch den betreuenden Arzt am **Tag 3** (zweite Gabe FAST VFI) nach Eintreffen der Laborwerte auszufüllen:

Datum Tag 3: ☐☐ ☐☐ ☐☐☐☐ (DD MM YYYY)

Frage 2: Bitte beschreiben Sie den aktuellen **intravasalen Volumenstatus** (nicht Gesamtkörpervolumenstatus!) des Patienten. Zeichnen Sie **auf der Linie ein Kreuz** ein. Versuchen Sie durch die Position des Kreuzes, den Zustand möglichst genau abzubilden.

Schwerst intravasal hypovoläm — Intravasal euvoläm — Schwerst intravasal hypervoläm

Frage 3: Bitte geben Sie Ihre Einschätzung: In den letzten 48 Stunden hat sich das intravasale Blutvolumen des Patienten …

- ◯ vergrößert.
- ◯ nicht verändert.
- ◯ verkleinert.

Frage 4: Bitte geben Sie Ihre Einschätzung: In den letzten 48 Stunden hat sich die Nierenfunktion (glomeruläre Filtrationsrate) des Patienten …

- ◯ verbessert.
- ◯ nicht verändert.
- ◯ verschlechtert.