Clinical Study Protocol

A Pilot, Open-Label Study to Evaluate the Safety, Tolerability, and Performance of the FAST PV Technology™ in Chronic Dialysis Patients with Extremely Reduced or No Kidney Function

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Confidentiality Statement

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1. SYNOPSIS

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<tr><td>Protocol Title:</td><td>A Pilot, Open-Label Study to Evaluate the Safety, Tolerability, and Performance of the FAST PV Technology in Chronic Dialysis Patients with Extremely reduced or no Kidney Function</td></tr>
<tr><td>Indication:</td><td>Quantitative determination of plasma volume (PV) and interstitial volume (ISV)</td></tr>
<tr><td>Investigators and Clinical Research Units:</td><td>Patients will be recruited and evaluated at a single site located in the United States.</td></tr>
<tr><td>Objectives:</td><td><u>Primary:</u>
• To evaluate PV and ISV using the FAST PV Technology and iohexol in patients on chronic dialysis predialysis.
• To evaluate the interaction between PV and ISV measured after a dialysis with a repeat injection of VFI using the FAST PV Technology.
• To assess the safety and tolerability of visible fluorescent injectate (VFI)™ (employing the FAST PV Technology) in chronic dialysis patients with extremely reduced or no renal function.</td></tr>
<tr><td>Endpoints:</td><td><u>Primary:</u>
• To measure quantitatively the ISV and PV of patients pre and post dialysis using the FAST PV Technology and Iohexol measurement.
• Directly compare quantitative difference of ISV and PV measured by the FAST PV Technology and Iohexol measurement to the volume removed during dialysis.
• To assess safety through adverse events (AEs) and serious adverse events (SAEs), clinical laboratory tests, physical examination findings and vital signs.</td></tr>
<tr><td>Study Design:</td><td>This is a pilot, single-center, open-label study designed to evaluate the safety, tolerability, and performance of the FAST PV Technology in patients on chronic dialysis.
The planned dose cohorts are presented in the table below:
<table>
<tr><th>Cohort</th><th>Population</th><th>eGFR[a]</th><th>Base VFI Dose[b]</th><th>Number of Doses</th><th>Comparator Dose</th><th>Number of Doses</th><th>Number of Subjects</th></tr>
<tr><td>1</td><td>Dialysis Patients</td><td>≤ 2 mL/min/ 1.73 m^2</td><td>3 mL</td><td>2</td><td>Iohexol 5 mL</td><td>2</td><td>10</td></tr>
</table>
Abbreviations: eGFR = estimated glomerular filtration rate; N/A = not applicable.
[a] Patients must be on chronic hemodialysis for ≥ 3 months and oliguric defined as ≤2 urinary voids per day.
[b] 3 mL for subjects/patients ≥ 40 and ≤ 100 kg + 1 mL for every 40 kg increase in body weight

Administration of VFI will occur within 28 days of screening.
Patients will receive 1 dose of VFI and 1 dose of iohexol approximately 4 hours prior to undergoing dialysis followed by a second dose of VFI and second dose of iohexol approximately 1 hour after completing dialysis. Patients will be discharged following completion of Day 1 activities. A follow-up visit will occur at the patient's next 2 dialysis appointments (approximately 2-3 days after Day 1) and 1 week after Day 1. A follow-up phone call will be performed on Day 31 (± 1 day).</td></tr>
<tr><td>Subject Selection Criteria:</td><td>Males and females ≥ 18 will be enrolled in the study. Patients must be oliguric (have an estimated glomerular filtration rate (eGFR) ≤ 2 mL/min/1.73 m2) and must be receiving maintenance chronic dialysis.</td></tr>
<tr><td>Investigational Product, Dose, and Route of</td><td>VFI, 3 mL for subjects/patients ≥ 40 and ≤ 100 kg + 1 mL for every 40 kg increase in body weight, bolus injection</td></tr>
</table>

Administration:	
Comparator, Dose, and Route of Administration:	Omnipaque™ 300 (iohexol), 647 mg of iohexol equivalent to 300 mg of organic iodine/1 mL (each mL of iohexol solution contains 1.21 mg tromethamine and 0.1 mg edetate calcium disodium), 5 mL, bolus injection
Criteria for Evaluation:	Safety: Adverse events will be collected and evaluated as they occur throughout the study. Safety assessments, including physical examinations, vital signs, and clinical laboratory tests, will be performed at specified time points. Performance: Measurements of PV and ISV will be determined using the FAST BioMedical Fluorescent Instrument (FBFI) and iohexol. Plasma Volume: Plasma volume will be determined using FAST PV Technology. Interstitial Volume: Interstitial Volume will be determined using the FAST PV Technology based on both FAST VFI and Iohexol plasma concentrations.
Planned Sample Size:	The anticipated sample size of approximately 10 is appropriate for pilot studies of similar design.
Statistical Analysis:	Analysis Populations: Safety Population: All subjects who receive any amount of study product. Performance Population: All subjects who have either a PV or ISV measurement using any of the techniques under study. Safety Analysis: The number and percentage of subjects reporting any treatment-emergent AE will be tabulated by system organ class, preferred term (coded using Medical Dictionary for Regulatory Activities), and cohort. Treatment-emergent AEs will be further classified by severity and relationship to study product. Vital signs and clinical laboratory test data (observed and change from baseline) will be summarized by cohort and time point using appropriate descriptive statistics. Physical examination findings results will only be listed. Performance Analysis: PV and ISV Measurements determined by FAST PV Technology will be compared to measurements determined by iohexol for both Dose 1 and Dose 2. Basic statistical analyses including correlations and Bland-Altman plots will be performed. Changes in PV and ISV measurements between Dose 1 and Dose 2 determined by the FAST PV Technology and iohexol will be compared to the known volume of fluid removed during dialysis treatment. Basic statistical analyses including correlations and Bland-Altman plots will be performed. Residual amounts of FAST VFI will be assayed on the Day 3 and Day 8 follow-up visits to assess the continued removal of the FAST VFI in this patient population.

Table 1. Schedule of Assessments and Procedures

Study Procedure	Screening[a]	Day 1		Follow-Up		Discharge[b]
	Days -28 to -2	Dose 1	Dose 2	Day 3	Day 8	Day 31
Informed consent	X					
Inclusion/exclusion criteria[a]	X	X				
Demographics	X					
Height	X					
Body weight	X	X	X			
BMI[c]	X	X				
Medical and surgical history	X	X[d]				
Physical examination	X	X				
Vital signs[e]	X	X[f]	X[f]			
Clinical laboratory tests[g]		X[gh]	X[h]			
Hematocrit		X[i]	X[i]			
nT pro-BNP		X				
Creatinine phosphokinase		X[h]		X	X	
Liver function tests		X		X	X	
Timed Urine Collection[j]		X	X			
Pregnancy test (nonmenopausal females only)	X	X				
Outpatient visit	X	X	X	X	X	X
FAST VFI administration		X	X[k]			
Iohexol administration		X	X			
FD001 and FD003 plasma samples		Refer to Table 2		X[l]	X[l]	
Iohexol plasma samples		Refer to Table 2				
Adverse events		X	X	X	X	X
Serious adverse events		X	X	X	X	X
Concomitant medications	X	X	X			

Abbreviations: BMI = body mass index; FD001 = 5 kD carboxymethyl dextran; FD003 = 150 kD carboxymethyl dextran; FSH = follicle-stimulating hormone; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; VFI = visible fluorescent injectate.

a. Assessments conducted upon enrollment will be used to reconfirm a subject's eligibility for enrollment into the study.
b. Discharge procedures will be performed following completion of all Day 31 assessments. Subjects who terminate from the study early will have discharge procedures performed at the time of discontinuation.
c. BMI will be calculated using the height obtained at screening.
d. Medical and surgical history will be collected and documented to determine if any changes have occurred since screening.
e. Vital signs will be measured after the subject has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes.
f. Vital signs will be measured within approximately 30 minutes prior to VFI and iohexol administrations and approximately 60-, 120- and 180-minutes post VFI and iohexol administrations.
g. Refer to Table 6 for a detailed list of clinical laboratory test parameters, which will be collected from the medical

Table 1. Schedule of Assessments and Procedures						
	Screening[a]	**Day 1**		**Follow-Up**		**Discharge**[b]
Study Procedure	**Days -28 to -2**	**Dose 1**	**Dose 2**	**Day 3**	**Day 8**	**Day 31**

record.

h. BUN and serum creatinine will be drawn pre-dose dose 1 and post the dose 2 180 min VFI blood draw.

i. Predose

j. Pooled urine collection (from patient arrival to departure) to measure total volume, creatinine and urea nitrogen. No catheter.

k. VFI administration of Dose 2 will occur approximately 8 hours following VFI administration of Dose 1

l. Blood samples to be drawn after dialysis session to allow for determination of any remaining FAST VFI.

NOTE: In the event multiple postdose procedures are required to be conducted at the same nominal time point, the timing of performance blood sample collections will take priority over all other scheduled activities. In practice, the following order is recommended: (1) vital signs assessments; (2) performance blood sampling; (3) clinical laboratory tests sampling; (4) physical examination. Vital signs may be conducted up to 10 minutes prior to the nominal time to minimize the potential autonomic effects of blood draws on these measurements.

Table 2. Performance Sampling Schedule – (Two Performance Periods)				
Nominal Time Relative to Start of VFI Bolus Injection	**FD001 and FD003 Plasma Sample**	**Iohexol Plasma Sample**	**Volume of Blood drawn[b]**	**Timed Urine Collection**
Predose (within 30 minutes)	X	X	6 mL	Pool urine from patient arrival to departure.
0 minutes	VFI and Iohexol Administration			
15 minutes	X	X	7 mL	
60 minutes	X	X	7 mL	
80 minutes	X		4 mL	
120 minutes	X	X	7 mL	
180 minutes	X	X	7 mL	
Dialysis Treatment (4 hours)				
Pre- Rinseback	X		4 mL	
Equilibrium time Post-Dialysis (1 hour)				
Predose (within 5 minutes of drug administration)	X	X	7 mL	
0 minutes	VFI and Iohexol Administration[a]			
15 minutes	X	X	7 mL	
60 minutes	X	X	7 mL	
80 minutes	X		4 mL	
120 minutes	X	X	7mL	
180 minutes	X	X	7 mL	

Blood Samples will be drawn at approximately the times listed in Table 2.2; however due to the nature of the study some variation in timing may occur. Regardless of the time and number of blood draws, no subject will have more than 80 mLs blood drawn for this study within a 24-hour period.
Abbreviations: FD001 = 5 kD carboxymethyl dextran; FD003 = 150 kD carboxymethyl dextran; VFI = visible fluorescent injectate.

[a.] Second VFI and iohexol administration will occur approximately 8 hours after the initial administrations.

[b.] Each blood draw will be taken as follows: 1 mL waste draw, 3mL FAST blood sample, 3 mL Iohexol Sample
Note: Blood samples will be collected from the venous side of the arteriovenous fistula/graft. Performance blood samples will be collected as close to the nominal time point as possible. The allowable window for postdose blood sample collection relative to the start of VFI/iohexol bolus injection will be ±5 minute.

2. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
ADL	activities of daily living
AE	adverse event
Ae_{0-24}	amount of FD001 excreted in urine from time 0 to 24 hours
AIDS	acquired immunodeficiency syndrome
AKI	acute kidney injury
BP	blood pressure
bpm	beats per minute
BUN	blood urea nitrogen
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL_r	renal clearance
CL_{r0-24}	renal clearance from time 0 to 24 hours
CRU	clinical research unit
CYP	cytochrome P450
DILI	drug-induced liver injury
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EOS	end-of-study
FD001	5 kD carboxymethyl dextran
FD003	150 kD carboxymethyl dextran
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GFR	glomerular filtration rate
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HR	heart rate
ICH	International Council for Harmonisation
IEC	independent ethics committee
INR	international normalized ratio
IRB	institutional review board

Abbreviation	Definition
IV	intravenous(ly)
ISV	interstitial volume
IVV	intravascular volume
LOQ	limit of quantitation
MDRD	Modification of Diet in Renal Disease
mGFR	measured glomerular filtration rate
OTC	over-the-counter
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PR (interval)	interval measured from the beginning of the P wave to the beginning of the QRS complex in the heart's electrical cycle as measured by electrocardiogram
PV	plasma volume
QRS (interval)	the interval between the Q wave and the S wave in the heart's electrical cycle as measured by electrocardiogram; principal deflection in the electrocardiogram
QT (interval)	a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle as measured by electrocardiogram
QTc (interval)	QT interval corrected for heart rate
QTcF (interval)	QT interval corrected for heart rate using Fridericia's formula
RR (interval)	the time elapsed between 2 consecutive R waves as measured by electrocardiogram
SAE	serious adverse event
S_{cr}	serum creatinine
SRC	safety review committee
SRM	study reference manual
TEAE	treatment-emergent AEs
UADE	unanticipated adverse device effect
ULN	upper limit of normal
VFI	visible fluorescent injectate

3. STUDY ADMINISTRATIVE STRUCTURE

Sponsor:	FAST BioMedical 9075 N Meridian Street, Suite 200 Indianapolis, IN 46260, USA Telephone: (866) 700-7310 ext. 701
Sponsor's Responsible Medical Officer:	Bruce Molitoris, MD Chief Medical Officer FAST BioMedical
Principle Investigator:	Arjun D. Sinha, MD, MS Renal Section Chief Richard L. Roudebush VA Medical Center 1481 W. 10th St. 111N Indianapolis, IN 46202
Sponsor's Contact:	James Strickland President FAST BioMedical
Analytical Laboratories:	Iohexol Plasma Concentration Determination: Jesse Seegmiller, PhD Assistant Professor Dept. Lab. Med. and Pathology University of Minnesota Advanced Research & Diagnostic Laboratory Room D185 Mayo Bldg. 420 Delaware St. SE Minneapolis, MN 55455, USA Telephone: (612) 625-6198 FD001 and FD003 Concentration Determination and PV and ISV Determinations: Dan Meier, PhD Vice President of Product Development FAST BioMedical Telephone: (866) 700-7310 VA Central Lab 1481 W. 10th Street Indianapolis, IN 46202 Telephone: 317 554 0000

Patients will be recruited and evaluated at a single site located in the United States.

4. INTRODUCTION AND BACKGROUND

4.1. Introduction

The mortality of patients on chronic hemodialysis (HD) remains high at 17% per year and similarly the 5-year survival rate is low at only 42% (United Stated Renal Data System, 2018), which is worse than for advanced colon cancer (O'Connell et al, 2004). Cardiovascular events are the leading cause of death (United Stated Renal Data System, 2018), and an important but often unrecognized cause for excess cardiovascular mortality on HD is volume overload. Volume overload contributes to hypertension (Agarwal et al, 2009) and left ventricular hypertrophy (Hur et al, 2013), both of which are associated with cardiovascular events and mortality (Sarnak et al, 2003). Experimental objective measures of volume status have directly associated volume overload with mortality (Agarwal 2010; Wizemann et al, 2009).

Despite classical recognition for the importance of removing adequate volume with HD, volume overload remains common in the HD population. HD patients are hospitalized frequently, with an average of 1.7 hospitalizations per year with rehospitalization within 30 days of discharge occurring in 38% of discharges (United Stated Renal Data System, 2018), which is far in excess of the general population. Volume overload conservatively contributes to over 10% of both initial hospitalizations and readmissions within 30 days(United Stated Renal Data System, 2018), with 1 analysis finding a 14% yearly rate for acute dialysis performed for volume overload at a cost to Medicare of $266 million in 2010 dollars (Arneson et al, 2010).

The persistent morbidity and mortality attributable to volume overload is in part due to the lack of a validated objective measure of volume status for the dialysis population (Sinha 2011). In the absence of an objective method, the subjective dry weight method is used to manage volume status on HD. The dry weight method remains essentially unchanged since it was first described over 60 years ago and it relies on the clinical interview and exam to detect volume overload (Thompson et al, 1967). Unfortunately, the clinical exam is not sensitive for detecting small but clinically relevant volume overload (Sinha and Agarwal, 2009), and changes in dry weight of only 0.6 to 1 kilogram have been shown to be clinically significant (Agarwal et al 2009; Hur et al, 2013).

There is a clear and longstanding need for a reliable objective measure of volume status for use in guiding volume removal in the chronic HD population.

4.2. Investigational Device Background

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

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 PV and GFR.

In cases where there near zero or zero kidney function, the FAST PV and mGFR Technology can be used to determine the interstitial volume of a patient. The distribution of the FD001, measured by timed blood samples, combined with the measured PV, allows for the ISV determination.

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. This technology could also be used to determine the effect of a clinical maneuver on GFR such as volume resuscitation.

4.3. Study Product Background

The bolus 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 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.

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 subject 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.

In previous studies, the FD001 component of the FAST VFI was used to determine mGFR. In this study, the FD001 marker will be used to measure the volume of distribution or interstitial volume (ISV). Its distribution into the interstitial space, combined with the measurement of PV by FD003 will allow for the calculation of a true measured volume of distribution.

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, subjects will be closely monitored for signs of allergy and anaphylaxis, and an emergency resuscitation kit and team will be available throughout the performance period.

4.4. Summary of Findings to Date

4.4.1. Nonclinical Studies

Nonclinical studies are summarized in Section 3 of the current FAST PV and mGFR Technology Investigator's Brochure.

4.4.2. Effects in Humans

4.4.2.1. Summary of Clinical Findings

FAST BioMedical has conducted 4 clinical studies evaluating the FAST PV and mGFR Technology in healthy subjects (Study FAST_GFR-01.1), in healthy subjects and subjects with compromised renal function (Study Pilot B/Phase 2a), in healthy subjects and patients with varying degrees of renal impairment (Study Phase 2b) and in hospitalized ADHF patients (Study Phase 2c).

The Phase 1 single-dose, dose-ranging clinical study was conducted in Germany with the FAST PV and mGFR Technology (Study FAST_GFR-01.1). In this study, 32 subjects divided into 4 cohorts of 8 subjects (6 subjects receiving the VFI and 2 subjects receiving placebo) between the ages of 21 and 45 years completed the study. Doses of the VFI evaluated in this study were 5, 15, 75, and 150 mg administered by slow bolus injection. The placebo subjects received 0.9% normal saline for injection. The components of the device used in the study included a catheter--based fiber optic probe placed in the subject's peripheral vein to allow the concentration of fluorescent markers to be monitored and an optical device to excite and detect the markers.

Single-dose administration of VFI in a range of 5 to 150 mg was safe and well tolerated. Overall, 9 subjects (28.1%) experienced 15 treatment-emergent adverse events (TEAEs), which were considered reasonably related to the VFI administration or study procedures by the investigator. Most related TEAEs were observed after administration of 15 mg VFI (9 TEAEs reported by 3 subjects). Two subjects (both receiving 15 mg VFI) reported 6 TEAEs (pruritus and gastrointestinal disorders), which were considered related to VFI (mainly injection site pain and hematoma at the blood sampling site). The most frequently reported TEAEs were nervous system disorders (10 subjects [31.3%], 10 TEAEs), which mainly included headache considered unrelated to the VFI or study procedures. All TEAEs were of mild to moderate intensity and there were no serious adverse events (SAEs); no subject discontinued the study due to an adverse event (AE). No treatment-related TEAEs were observed after the highest dose. There were no adverse device effects. Safety laboratory parameters, vital signs, and electrocardiogram (ECG) parameters showed no clinically relevant time- or treatment-related differences.

The Phase 2a clinical study was a prospective study evaluating the FAST PV and mGFR Technology in subjects with preserved and impaired kidney function. In this study, 4 cohorts of 8 subjects and 1 cohort of 6 subjects were planned to receive 75 mg of the VFI (50 mg FD001 and 25 mg FD003). A total of 33 subjects were enrolled with 1 of 6 planned subjects enrolled in the AKI group. Cohorts 1 and 5 had normal kidney function with GFR of ≥ 60 mL/min, Cohort 2 GFR was estimated at 30 to 59 mL/min, Cohort 3 GFR was 15 to 29 mL/min, and Cohort 4 was planned to be AKI subjects with S_{cr} ≥ 2-fold increase or eGFR > 50% decrease compared to baseline. Of the 33 subjects enrolled, 19 (57.6%) subjects experienced 37 TEAEs during the study. There were no treatment-emergent SAEs during the study. The majority of laboratory parameters were within normal ranges or considered not clinically significant. No clinically significant changes in vital signs were noted during the study. The majority of subjects had ECGs that were normal or had abnormalities that were considered not clinically significant. Two subjects, both with medical histories significant for cardiac disorders, had abnormalities that were considered to be clinically significant AEs.

The Phase 2b study was a prospective, open-label study designed to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of FAST PV and mGFR Technology in healthy subjects and patients with varying degrees of renal impairment. A total of 32 subjects were planned to be enrolled in 4 cohorts of up to 8 subjects/patients each. Healthy subjects 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. Subjects 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. Subjects in Cohort 2 received a dose of VFI followed 160 minutes later by a single dose of iohexol (refer to Section 4.5 for additional information on 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 FAST VFI was safe and well tolerated in healthy subjects 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.

The Phase 2c study was an investigator-initiated, human clinical trial designed to evaluate the safety and functionality of the FAST PV and mGFR Technology in hospitalized CHF patients. Patients enrolled in the study were administered VFI (Day 1) with a second dose occurring 48h (+/- 5h) after the initial dose (Day 3). A total of 37/50 (74%) patients had a treatment-emergent AE (TEAE) while 21/30 (42%) had a treatment-emergent SAE (TESAE). One patient died during the study, but the death was deemed to be not related to the intervention. Further, there were no treatment related SAEs and 10/50 (20%) patients had a treatment related AE with maximal mild severity. In total, 4 patients (4/50, 8%) suffered from treatment related diarrhoea including 1 with a moderate severity while for all other related TEAES only 1 patient was affected each time (1/50, 2%). Of all 50 patients, 3 patients (6%) were affected by pneumonia, cardiac failure, and acute kidney injury each while 2 patients had endocarditis. All other SAEs occurred only in 1 patient each (1/50, 2%).

4.4.2.2. Pharmacokinetics and Pharmacodynamics in Human Subjects

In the first-in-human clinical study (Study FAST_GFR-01.1), mean plasma concentrations of FD001 increased dose dependently with peak concentrations observed at 0.25 hours after administration (first blood sample). Thereafter, mean concentration decreased rapidly with no concentration above the limit of quantitation (LOQ) observed at 48 hours postdose. Mean plasma concentrations of FD003 increased dose dependently with peak concentrations observed at 0.25 (5 mg VFI) to 2 hours (75 and 150 mg VFI) after injection. Thereafter, mean concentrations decreased with a constant rate until 312 hours postdose. A slower elimination rate was observed until 480 hours after administration (end of the observation period). At 480 hours, all subjects in the 75- and 150-mg groups had concentrations of FD003 above the LOQ.

In the Phase 2a clinical study, following IV bolus injection of the VFI to patients with varying degrees of kidney function, FD001 generally reached maximum observed concentration following the end of the infusion and decreased in a multiexponential, first order elimination manner with early-phase distribution apparently complete at 8 to 12 hours. The mean time at which the maximum plasma concentration was observed was approximately 0.25 hours. Mean plasma elimination half-life estimates increased with decreasing renal function (5.64 hours in Cohort 5, 9.48 hours in Cohort 2, and 18.3 hours in Cohort 3). In contrast, mean plasma FD003 elimination half-life estimates were generally similar regardless of renal function (90.9 hours in Cohort 5, 123 hours in Cohort 2, and 125 hours in Cohort 3). Mean FD003 volume estimates indicated that FD003 appeared to occupy a volume space that corresponds to PV. Mean FD003 clearance did not change with decreasing renal function.

The Phase 2b clinical study showed that FD001 does not appear to accumulate in most healthy subjects with normal renal function when dosed at a 24-hour interval. The PK of the FAST VFI in patients with varying degrees of kidney function indicated the renal clearance of FD001 from 0 to 3 hours after dosing is indicative of GFR. The exposure to and elimination of FD003 was independent of renal function. FD003 distributed in a manner consistent with vascular volume. Additionally, FD003 remained stable in the vasculature for a period of at least 6 hours, allowing repeat measurements of PV during that time period without redosing.

The PV data showed good correlation and no bias when compared to Nadler's estimate (Nadler et al, 1962). A comparison of iohexol GFR to the FAST VFI mGFR showed excellent correlation through the entire range of GFRs measured. The FAST VFI method was able to accurately measure the change in PV within subjects due to the administration of 350 mL of 5% albumin. The FAST mGFR technology delivered clinically meaningful results as demonstrated by its ability to stratify patients by kidney function.

Pharmacokinetics were not studied in the Phase 2c study.

4.5. Iohexol Background

OMNIPAQUE 300 (hereafter referred to as iohexol) is indicated in adults for aortography including studies of the aortic arch, abdominal aorta and its branches, contrast enhancement for computed tomographic head and body imaging, cerebral arteriography, peripheral venography (phlebography), and excretory urography.

Following intravascular injection, iohexol is distributed in the extracellular fluid compartment and is excreted unchanged by glomerular filtration. It will opacify those vessels in the path of flow of the contrast medium permitting radiographic visualization of the internal structures until significant hemodilution occurs.

Approximately 90% or more of the injected dose is excreted within the first 24 hours, with the peak urine concentrations occurring in the first hour after administration. Plasma and urine iohexol levels indicate that the iohexol body clearance is due primarily to renal clearance (CL_r). An increase in the dose from 500 to 1500 mgI/kg does not significantly alter the clearance of the drug. The following PK values were observed following the IV administration of iohexol (between 500 to 1500 mgI/kg) to 16 adult human subjects: CL_r = 120 (86 to 162) mL/min; total body clearance = 131 (98 to 165) mL/min; and volume of distribution = 165 (108 to 219) mL/kg.

Renal accumulation is sufficiently rapid that the period of maximal opacification of the renal passages may begin as early as 1 minute after IV injection. Urograms become apparent in about 1 to 3 minutes with optimal contrast occurring between 5 to 15 minutes. In nephropathic conditions, particularly when excretory capacity has been altered, the rate of excretion may vary unpredictably, and opacification may be delayed after injection. Severe renal impairment may result in a lack of diagnostic opacification of the collecting system and, depending on the degree of renal impairment, prolonged plasma iohexol levels may be anticipated. In these patients, the route of excretion through the gallbladder and into the small intestine may increase.

Iohexol displays a low affinity for serum or plasma proteins and is poorly bound to serum albumin.

No significant metabolism, deiodination, or biotransformation occurs.

Iohexol enhances computed tomographic imaging through augmentation of radiographic efficiency. The degree of density enhancement is directly related to the iodine content in an administered dose; peak iodine blood levels occur immediately following rapid IV injection. Blood levels fall rapidly within 5 to 10 minutes and the vascular compartment half-life is approximately 20 minutes. This can be accounted for by the dilution in the vascular and extravascular fluid compartments which causes an initial sharp fall in plasma concentration. Equilibration with the extracellular compartments is reached in about 10 minutes; thereafter, the fall becomes exponential.

Refer to the OMNIPAQUE (iohexol) injection prescribing information (2010) for additional details.

4.6. Study Rationale

4.6.1. Rationale for Dose Selection

Each anticipated single dose for a 70-kg human will consist of 35 mg of FD001 and 12 mg of FD003, diluted at 15.7 mg/mL, for a total injection of 3.0 mL VFI, equivalent to 0.78 mg/kg or 28.9 mg/m^2 for a 60-kg human.

4.6.2. Rationale for Study Design

This is a pilot, single-center, open-label study designed to evaluate the safety, tolerability, and performance of the FAST PV Technology in chronic dialysis patients. The dosing schedule was determined based on anticipated clinical use.

Male and female subjects will be enrolled in the study. As no sufficient data are available on a potential influence of the VFI on embryo-fetal development, female subjects must be of non-childbearing potential or must provide confirmation of a known and medically acceptable contraception method. Reproductively active men must agree to either practice abstinence or utilize adequate contraception.

Subject safety will determine the progression or discontinuation of the study. In rare cases, dextran products have been documented to produce anaphylactic reactions in humans, and the onset of symptoms is documented to be acute. The VFI is a substituted dextran product that has shown little evidence of antigenicity in nonclinical studies.

The duration of confinement and medical surveillance are considered adequate to ensure safety of the subjects.

The FAST VFI has not been given to dialysis patients before. In the Phase 2c study, a patient with a measured GFR of 16.7 mL/min/1.73m^2 was dosed with VFI with no AE or SAEs related to the study drug observed. Although the patients in this study will receive 2 doses of FAST VFI, the initial doses of VFI and iohexol will be removed during the patient's dialysis session. The second dose of VFI and Iohexol will be removed between 36 and 48 hours later at the first follow-up dialysis session. The concentration the patients in this study will be exposed to is higher for longer than previous studies due to the lack of kidney function; however, the FD001 and iohexol, will continue to distribute into the extracellular fluid of the patient. The FD003 exposure is unchanged from prior studies.

5. OBJECTIVES

5.1. Primary Objectives

- To evaluate PV and ISV using the FAST PV Technology and iohexol in patients on chronic dialysis predialysis.

- To evaluate the interaction between PV and ISV measured after a dialysis with a repeat injection of VFI using the FAST PV Technology.

- To assess the safety and tolerability of visible fluorescent injectate (VFI) (employing the FAST PV Technology) in chronic dialysis patients with extremely reduced or no renal function.

6. STUDY DESIGN

6.1. Study Design and Overview

This is a pilot, single site, open-label study designed to evaluate the safety, tolerability, and performance of the FAST PV Technology in dialysis patients.

The planned dose cohorts are presented in Table 3.

Table 3. Planned Dose Cohorts

Cohort	Population	eGFR[a]	Base VFI Dose[b]	Number of Doses	Comparator Dose	Number of Doses	Number of Subjects
1	Dialysis Subjects	≤ 2 mL/min/1.73m^2	3 mL	2	Iohexol 5 mL	2	Up to 10

Abbreviation: N/A = not applicable
[a.] Patients must be on chronic hemodialysis for ≥ 3 months and oliguric defined as ≤ 2 urinary voids per day.
[b.] 3 mL for subjects/patients ≥ 40 and ≤ 100 kg + 1 mL for every 40 kg increase in body weight

Administration of VFI will occur within 28 days of screening.

Patients will receive 1 dose of VFI and 1 dose of iohexol approximately 4 hours prior to undergoing dialysis followed by a second dose of VFI and iohexol approximately 1 hour after completing dialysis. Patients will be discharged following completion of Day 1 activities. Patients will be seen and evaluated on their next 2 dialysis sessions for any adverse reaction by answering questions about their health, approximately on Day 3 and Day 8. A follow-up phone call will be performed on Day 31 (± 1 day).

Refer to Table 1 for the Schedules of Assessments and Procedures.

6.1.1. Duration of Study

Participation is expected to last up to 59 days, including a screening period of up to 28 days and an on-study period of up to 31 days (consisting of a single performance period and a follow-up phone call).

6.1.2. Definition of Study Completion

Discharge/EOS procedures will be performed as specified in the Schedules of Assessments and Procedures (Table 1); subjects/patients who terminate from the study early will have discharge/EOS procedures performed at the time of discontinuation. Subjects/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 subjects/patients. The subject's/patient's participation in the study will end once all study assessments and follow-up have been completed.

6.1.3. End of Study

The end of the study is defined as the date when the last subject/patient has completed all study procedures up to and including the follow-up phone call/EOS visit as outlined in the Schedules of Assessments and Procedures (Table 1).

7. SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects/patients must meet all the following criteria in order to be enrolled in the study.

7.1. Inclusion Criteria

Subjects/patients must meet all inclusion criteria to be eligible for study participation.

1. Males or females ≥ 18 years of age.
2. Females must be of non-childbearing potential (eg, postmenopausal [defined cessation of regular menstrual periods for at least 1 year confirmed by age ≥ 60 or surgically sterile by hysterectomy, bilateral oophorectomy, or bilateral tubal ligation [documentation required]) or be using a medically acceptable form of birth control (a barrier method, intrauterine device, or hormonal contraception) from screening through 30 days after administration of the last dose of VFI.
3. Males who are sexually active and whose partners are females of childbearing potential must agree to practice abstinence or use condoms from screening through 90 days after administration of the last dose of VFI, and their partners must be willing to use a medically acceptable method of contraception (a barrier method, intrauterine device, or hormonal contraception) from screening through 90 days after administration of the last dose of VFI.
4. Males must agree to not donate sperm from screening through 90 days after administration of the last dose of VFI.
5. Subjects/patients must be able to communicate effectively with the study personnel.
6. Patients must be on chronic hemodialysis for ≥ 3 months and oliguric defined as ≤ 2 urinary voids per day.
7. Patients must have an average interdialytic weight gain of at least 2 kg.
8. Patients must have an A-V dialysis shunt.
9. Patients must have a functioning A-V dialysis shunt, either fistula or graft.

7.2. Exclusion Criteria

Subjects/patients will not be eligible for study participation if they meet any of the exclusion criteria, or will be discontinued at the discretion of the investigator if they develop any of the exclusion criteria during the study.

1. Subject is a pregnant or nursing (lactating) woman, 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.
2. History or presence of conditions which, in the judgment of the investigator, are known to interfere with the distribution, metabolism, or excretion of drugs.
3. History or presence of conditions that may place the subject/patient at increased risk as determined by the investigator.

4. History of surgery or major trauma within 12 weeks of screening or surgery planned within 4 weeks of the study including during study participation.
5. Has taken other investigational drugs within 30 days or 5 half-lives of the investigational drug's PK, PD, or biological activity, whichever is longer, prior to first dose of VFI in this study.
6. Prior exposure to VFI or known allergy or hypersensitivity to dextran, 5-aminofluorescein dye, or 2-sulfohexamine rhodamine dye.
7. History of any clinically significant allergic or negative reactions, side effects, or anaphylaxis to iodine, dyes, shellfish, isotopes, or dextran molecules.
8. Significant blood loss (> 450 mL) or has donated 1 or more units of blood or plasma within 4 weeks prior to study participation.
9. Presence of significant hemodynamic instabilities defined as systolic BP <100 on dialysis requiring saline infusion in the past 4 weeks.
10. Presence of ascites and/or 4+ anasarca.
11. Any other condition or prior therapy that, in the investigator's opinion, would make the subject unsuitable for the study, or unable or unwilling to comply with the study procedures.
12. Involved in the planning or conduct of this study.
13. Unwilling or unlikely to comply with the requirements of the study.
14. Clinically significant ongoing bleeding, changing hemoglobin, or experienced significant blood loss within last 4 weeks.
15. Had a PRBC transfusion in the prior 2 weeks
16. Use of midodrine.
17. Any other condition or prior therapy that, in the investigator's opinion, is likely to deteriorate during study participation
18. Subjects suffering from significant non-cardiac diseases of other organ systems (eg, malignancies, significant neurological diseases).
19. Subject has a psychiatric disease or a history of illicit drug use that would prohibit them from complying with study requirements.
20. Use of hemodialysis catheter as primary vascular access for hemodialysis

7.3. Subject Withdrawal

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

- Withdrawal of consent
- Administrative decision by the investigator or sponsor
- Ineligibility
- Significant protocol deviation
- Subject/patient noncompliance
- Safety concern by the investigator or sponsor
- Lost to follow-up

Subjects/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 subject's/patient's withdrawal, the investigator will promptly notify the sponsor and will make every effort to complete discharge procedures. All withdrawn subjects/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 subjects/patients.

7.4. Early Termination of Study

The study may be terminated at any time by the sponsor if serious side effects occur, if potential risks to study participants are identified, if the investigator does not adhere to the protocol, or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In the event that the clinical development of the study product is discontinued, the sponsor shall inform all investigators/institutions and regulatory authorities.

8. TREATMENT OF SUBJECTS

8.1. Identity of Study Product and Comparator

The identity of the study product and comparator are presented in Table 4.

Table 4. Study Product and Comparator

Study Product	Dosage Form	Strength	Dose
Visible Fluorescent Injectate	Solution for injection	35 mg FD001/12 mg FD003	3 mL + 1 mL for every 40 kg increase in body weight (refer to Table 7.2)
Comparator			
Omnipaque 300 (iohexol)	Solution for injection	647 mg of iohexol equivalent to 300 mg of organic iodine/1 mL	5 mL

VFI will be sourced by FAST BioMedical. The VFI is packaged in a 10-mL, 20-mm clear glass vial with a 20-mm gray stopper and blue cap, and the product is a deeply colored lyophilized cake.

Iohexol will be sourced by the VA. Iohexol is packaged in a clear 10 mL bottle with a brown cap, and the product is a colorless to pale-yellow liquid. Each mL of iohexol solution contains 1.21 mg tromethamine and 0.1 mg edetate calcium disodium.

8.2. Treatments Administered

Each subject/patient will receive 2 doses of VFI and 2 doses of iohexol, which will be administered IV through a bolus injection. An IV catheter will be inserted into the subject's arteriovenous fistula or graft by an experienced dialysis nurse.

Patients will receive 1 dose of VFI and 1 dose of iohexol approximately 4 hours prior to undergoing dialysis followed by a second dose of VFI and second dose of iohexol approximately 1 hour after completing dialysis.

The dose of VFI will be 3 mL for subjects/patients ≥ 40 and ≤ 100 kg and will increase by 1 mL for every 40 kg increase in body weight as outlined in Table 5.

Table 5. Visible Fluorescent Injectate Doses								
Body weight		**Dose**	**VFI**	**FD001**	**FD003**	**VFI**	**FD001**	**FD003**
(lbs)	**(kg)**	**(mL)**	**(mg)**	**(mg)**	**(mg)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**
88	40	3	47.01	35.01	12	1.18	0.88	0.300
154	70	3	47.01	35.01	12	0.67	0.50	0.171
220	100	3	47.01	35.01	12	0.47	0.35	0.120
223	101	4	62.68	46.68	16	0.62	0.46	0.158
309	140	4	62.68	46.68	16	0.45	0.33	0.114
311	141	5	78.35	58.35	20	0.56	0.41	0.142
397	180	5	78.35	58.35	20	0.44	0.32	0.111
399	181	6	94.02	70.02	24	0.52	0.39	0.133
485	220	6	94.02	70.02	24	0.43	0.32	0.109
487	221	7	109.69	81.69	28	0.50	0.37	0.127
573	260	7	109.69	81.69	28	0.42	0.31	0.108
575	261	8	125.36	93.36	32	0.48	0.36	0.123
661	300	8	125.36	93.36	32	0.42	0.31	0.107
664	301	9	141.03	105.03	36	0.47	0.35	0.120
750	340	9	141.03	105.03	36	0.41	0.31	0.106

A low-protein snack will be served prior to administration of VFI (eg, toast, bagel, Graham crackers, or primarily carbohydrate-based small portion). Water will be allowed as desired up to 1.5 liters, but must be tracked on the CRF. Meals appropriate for the patient's medical condition will be served.

8.3. Method of Assigning Subjects to Cohorts

Eligible subjects/patients will be enrolled in a single cohort.

8.4. Measurements of Compliance

Study product and comparator will be administered by qualified healthcare professionals at the study site as designated by the investigator. Details regarding dosing, including the dose administered and the date and time of dosing, will be recorded.

8.5. Storage, Accountability, and Preparation

8.5.1. Storage Conditions

The VFI will be stored in a refrigerator set to maintain 2°C to 8°C (36°F to 46°F). Vials and glass or polymer bottles of iohexol will be protected from strong daylight and direct exposure to sunlight and will be stored at controlled room temperature at 20°C to 25°C (68°F to 77°F); excursions are permitted to 15°C to 30°C (59°F to 86°F).

The VFI will be prepared within 60 minutes prior to dosing, and the prepared product will be stored at room temperature (approximately 20°C to 25°C). The product will be protected from sunlight and ultraviolet light until dosing.

Iohexol will be drawn into a syringe for administration and stored at room temperature (approximately 20°C to 25°C).

8.5.2. Preparation

The procedure for reconstitution of the FAST VFI is located in Appendix A.

8.6. Packaging and Labeling

8.6.1. Study Product

The study product will be provided with appropriate labeling, including: sponsor name, address, and phone number, product name, active constituents, content by volume and number of units, batch number, expiry date, storage instructions, and the term “Caution: Investigational Device. Limited by US Federal Law for Investigational Use Only.”

8.6.2. Blinding of Treatment Assignment

Not applicable. This is an open-label study.

8.7. Concomitant Medications and Other Restrictions

8.7.1. Concomitant Medications

Maintenance medications as prescribed by their physicians are permitted. All medications (prescription and OTC), vitamin and mineral supplements, and herbs taken during the study will be documented on the concomitant medication case report form (CRF). Information recorded will include: start and stop dates and times, dose and route of administration, and indication. Medications taken for a procedure should also be included.

8.7.2. Other Restrictions

Subjects/patients will be instructed to adhere to the following restrictions:

- Strenuous activity (as assessed by the investigator) is prohibited from 72 hours prior to admission until completion of dosing.
- Subjects/patients are not permitted to consume alcohol from 24 hours prior to admission until completion of dosing.
- Subjects/patients are not permitted to consume any food and drink from outside of the VA during the testing period.
- Subjects/patients must comply with the VA smoking policy, if applicable.
- Subjects/patients are not permitted to participate in any other clinical trial or donate blood or plasma while participating in this clinical trial.

9. STUDY ASSESSMENTS AND PROCEDURES

Subjects/patients will undergo study procedures and assessments at time points specified in the Schedules of Assessments and Procedures (Table 1)

9.1. Medical and Surgical History

The investigator or designee will collect and document a complete medical and surgical history at screening. Medical and surgical history will be collected and documented at test day admission to determine if any changes have occurred since screening. The medical history should include all active medical problems regardless of time of onset.

9.2. Demographic Characteristics

Demographic characteristics including sex, age, race, and ethnicity will be recorded.

9.3. Physical Measurements

Height (cm) and body weight (kg) without shoes will be recorded. Body mass index will be calculated using the height obtained at screening.

9.4. Performance Assessments

Plasma performance samples for determination of PV and GFR will be collected at time points specified in the Performance Sampling Schedules (Table 2). Concentration of FAST VFI will be determined on the FAST BioMedical Fluorescent Instrument (FBFI). Concentrations of iohexol will be determined via the validated University of Minnesota LC-MS/MS method.

Measurements of PV and ISV will be determined using the FAST BioMedical Technology and mathematical equations.

9.5. Safety Assessments

9.5.1. Adverse Events

Subjects will be monitored for AEs from administration of the first dose of VFI through the follow-up phone call/EOS visit. Refer to Section 10 for additional details.

9.5.2. Clinical Laboratory Tests

The electronic medical record will be used to find the most recent values for the clinical laboratory tests listed in Table 6, all of which are checked with a frequency ranging from twice a month to once a quarter during the routine course of hemodialysis. The baseline laboratory test results for clinical assessment for a particular test will be defined as the last measurement prior to the first dose of VFI.

During the screening period, if a subject/patient has an out-of-range value for a clinical laboratory parameter which the investigator believes is not clinically significant, but the investigator wants to confirm with a repeat laboratory test, a single repeat is allowed to confirm the initial result.

Table 6. Clinical Laboratory Tests

Hematology	Serum Chemistry
Hematocrit	Sodium
Hemoglobin	Potassium
Red blood cell count	Chloride
Mean corpuscular volume	Albumin
White blood cell count	Alkaline phosphatase
Platelet count (estimate not acceptable)	Glucose
	Total cholesterol
	BUN
	Creatinine
	Total bilirubin
	Calcium
	Total protein
	Phosphorous

For any laboratory test value outside the reference range that the investigator considers clinically significant during the on-study period (ie, following dose administration), the investigator will:

- Repeat the test to verify the out-of-range value and clinical significance.
- Follow the out-of-range value to a satisfactory clinical resolution.
- Record as an AE any laboratory test value that (1) is confirmed by repeat and the investigator considers clinically significant, (2) requires a subject/patient to be discontinued from the study, (3) requires a subject/patient to receive treatment, or (4) requires a change or discontinuation of the study product (if applicable).

9.5.2.1. Kidney Function

- Patients must be on chronic hemodialysis for ≥ 3 months and oliguric defined as ≤ 2 urinary voids per day.

9.5.2.2. Other Tests

A certified laboratory will be utilized to process and provide results for the additional lab tests listed below, to be drawn prospectively for the study unlike the baseline tests listed in Table 6.

- Pregnancy test (nonmenopausal females only)
- nT Pro-BNP

- Creatinine phosphokinase
- Liver function tests: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin
- Timed Urine Collection (patient arrival to departure on visit 2) for:
  - Total Volume
  - Creatinine
  - Urea Nitrogen

9.5.3. Vital Signs

Vital signs assessments will include oral temperature (°C), respiratory rate (breaths per minute), systolic and diastolic BP (mmHg), and pulse rate (bpm). Blood pressure and pulse rate will be measured after the subject/patient has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes.

Vital signs abnormalities that (1) are considered clinically significant initially and on confirmation, (2) require a subject/patient to be discontinued from the study, (3) require a subject/patient to receive treatment, or (4) require a change or discontinuation from the study product (if applicable) will be recorded as AEs.

9.5.4. Physical Examination

Complete physical examinations (including eyes, ears, nose and throat, cardiac, peripheral vascular, pulmonary, musculoskeletal, neurologic, abdominal, lymphatic, and dermatologic systems) will be performed, and abnormal findings will be documented in the subject's/patient's CRF.

An abnormal physical examination finding that is considered clinically significant and (1) requires the subject/patient to be discontinued from the study, (2) requires the subject/patient to receive treatment, or (3) requires a change or discontinuation of the study product (if applicable) will be recorded as an AE.

9.5.5. Appropriateness of Safety Assessments

Safety evaluations selected for this study are typical of those for this subject/patient population and utilize widely accepted measures.

10. ADVERSE EVENTS

An AE is defined as any untoward medical occurrence in a subject administered a pharmaceutical product during the course of a clinical investigation. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational product, whether or not thought to be related to the investigational product. Any worsening of the patient's disease under study or other medical conditions will also be considered to be an adverse event, unless it is within the normal range of disease fluctuation for that patient.

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Subjects will be monitored throughout the study for AEs, from administration of the first dose of VFI through the follow-up phone call/EOS visit. Adverse events that are identified at the last assessment visit (or the early termination visit) as specified in the protocol must be recorded on the AE CRF with the status of the AE noted. All events that are ongoing at this time will be recorded as ongoing on the CRF. All (both serious and nonserious) AEs must be followed until they are resolved or stabilized, or until attempts to determine resolution of the event are exhausted. The investigator should use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events.

The procedures specified in Section 10.4 are to be followed for reporting SAEs.

10.1. Recording Adverse Events

Adverse events are to be recorded on the AE page of the CRF. The following information will be recorded:

- Assessment of whether or not the AE is an SAE (Section 10.2.1)
- Assessment of AE intensity (Section 10.2.2)
- Assessment of AE relationship to study product (Section 10.2.3)
- Action taken - categorized as none, study drug discontinued, dose modified, required concomitant medication, required procedure, or other.
- Outcome - recorded as event resolved, resolved with sequelae, ongoing, or death.

10.2. Assessment of Adverse Events

The investigator will assess each AE for seriousness, intensity, and relationship to study product.

10.2.1.Serious Adverse Events

The investigator is responsible for determining whether an AE meets the definition of an SAE. An SAE is any AE occurring from ICF signing through the follow-up phone call/EOS visit that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Note: SAEs require immediate reporting to the sponsor. Refer to “Reporting Serious Adverse Events” (Section 10.4) for details.

10.2.2.Intensity

The intensity of an AE will be graded according to the following definitions:

- Mild: Any symptom of which the subject is aware, but which is easily tolerated; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Moderate: Any symptom which is discomforting enough to cause interference with a subject's usual activity; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- Severe: Any symptom which causes a subject's inability to perform usual activity; severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

It should be noted that a severe adverse event need not be serious in nature and that an SAE need not, by definition, be severe.

10.2.3. Relationship to Study Product

The relationship of an AE to the study product should be determined by the investigator according to the following criteria:

- Not related: The event is most likely produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs, and does not follow a known response pattern to the study product, or the temporal relationship of the event to study product administration makes a causal relationship unlikely
- Possibly related: The event follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study product, but could have been produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs
- Related: The event follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study product, and cannot be reasonably explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs

10.3. Discontinuation due to Adverse Events

Any subject who experiences an AE may be withdrawn at any time from the study at the discretion of the investigator. Subjects withdrawn from the study due to an AE, whether serious or nonserious, may be followed by the investigator until the clinical outcome of the AE is determined. The AE(s) should be noted on the appropriate CRFs and the subject's progress should be followed until the AE is resolved or stabilized as determined by the investigator. The sponsor must be notified. If the AE relates to overdose of study treatment, the Investigator's Brochure should be consulted for details of any specific actions to be taken.

10.4. Reporting Serious Adverse Events

In the event of any SAE reported or observed during the study, whether or not attributable to the study drug, site personnel will report it immediately by telephone to the medical monitor. Any SAE attributable to study participation will be reported promptly to the IRB as required by the Richard L. Roudebush Veterans Affairs Medical Center.

10.5. Documentation and Reporting of Immediately Reportable Adverse Events

Any unexpected adverse event that could adversely affect the safety of the subjects or the conduct of the study and any serious adverse event, which occurs during the course of this study, will be reported to the sponsor immediately (ie, within 24 hours).

The information will comprise at least the following data:

- Name, address, and telephone number of the reporting investigator
- Investigational product(s)
- Study code
- Subject identification number, initials, sex, and date of birth
- Description of the adverse event, measures taken and outcome (if resolved)
- Likelihood of drug causation of the adverse event assessed by the investigator

The sponsor ensures that all relevant information about suspected serious unexpected adverse reactions that are fatal or life threatening is recorded and reported as soon as possible to the FDA, and in any case no later than 7 days after knowledge by the sponsor of such a case, and that relevant follow up information is subsequently communicated within additional 7 days. The study team will be responsible for informing the IRB of any promptly reportable events within 5 days of discovery, in accordance with IU policy.
All other suspected serious unexpected adverse reactions will be reported to the FDA as soon as possible but with a maximum of 15 days of first knowledge by the sponsor. The sponsor will inform all investigators. In accordance with IU policy, the study team will be responsible for informing the IRB of any promptly reportable events within 5 days of discovery including those listed below.
The sponsor will inform as soon as possible, but no later than 15 days after knowledge, the FDA and the IRB about any event which necessitates reconsideration of the benefit-risk-ratio of the investigational drug. These events are in particular:

- Single cases of expected serious adverse reactions with an unexpected outcome.
- An increased incidence of expected serious adverse reactions which is considered clinically relevant.
- Suspected serious unexpected adverse reactions occurring after a concerned person has completed the study.
- Events related to the conduct of the study or the development of the drugs possibly affecting the safety of the concerned persons.

All additional measures deemed necessary through new findings and taken by the sponsor or the investigator to protect the safety of the persons concerned and their triggering circumstances will be reported as soon as possible to the FDA and the IRB.

10.6. Pregnancy

The investigator must report any pregnancy to the medical monitor and to the Richard L. Roudebush Veterans Affairs Medical Center within 1 business day of becoming aware of the pregnancy. An uncomplicated pregnancy will not be considered an AE or SAE; however, all pregnancies will be followed through birth and 3 months postdelivery.

Pregnancies in the sexual partners of male subjects will be captured from the time the subject is first exposed to the investigational product until 90 days after last exposure to the investigational product.

Any congenital abnormalities in the offspring of a subject who received study drug will be reported as a SAE. The outcome of any pregnancy and the presence or absence of any congenital abnormality will be recorded in the source documentation and reported to the medical monitor and sponsor.

10.7. Drug-induced Liver Injury

Subjects will be monitored for signs of drug-induced liver injury (DILI). Study product will be withheld in the event of potential DILI.

Potential events of DILI will be defined as meeting all of the following criteria (as specified in the FDA Guidance for Industry Drug-induced Liver Injury: Premarketing Clinical Evaluation, 2009):

- Alanine aminotransferase or aspartate aminotransferase > 3 × ULN
- Total bilirubin > 2 × ULN without initial findings of cholestasis (elevated serum alkaline phosphatase)
- No other reason can be found to explain the combination of laboratory value increases (eg, acute viral hepatitis; alcoholic and autoimmune hepatitis; hepatobiliary disorders; nonalcoholic steatohepatitis; cardiovascular causes; concomitant treatments)

Potential events of DILI will be reported as SAEs (Section 10.4). All subjects with potential DILI will be closely followed until abnormalities return to normal or baseline or until all attempts to determine resolution of the event are exhausted.

11. STATISTICAL CONSIDERATIONS

11.1. Sample Size Calculation

The anticipated sample size of approximately 10 subjects is not powered.

11.2. Analysis Populations

Safety Population: All subjects who receive any amount of study product.

Performance Population: All subjects who have either a PV or GFR measurement using any of the techniques under study.

11.3. Endpoints

11.3.1. Primary Endpoints

- To measure quantitatively the ISV and PV of patients pre and post dialysis using the FAST PV Technology and Iohexol measurement.
- Directly compare quantitative difference of ISV and PV measured by FAST PV Technology and Iohexol measurement to the volume removed during dialysis.
- To assess safety through adverse events (AEs) and serious adverse events (SAEs), clinical laboratory tests, physical examination findings and vital signs.

11.4. Safety Analysis

The number and percentage of subjects reporting any TEAE will be tabulated by system organ class, preferred term (coded using Medical Dictionary for Regulatory Activities), and cohort. Treatment-emergent AEs will be further classified by severity and relationship to study product.

Vital signs and clinical laboratory test data (observed and change from baseline) will be summarized by cohort and time point using appropriate descriptive statistics. Physical examination findings will only be listed.

11.5. Performance Analysis

11.5.1. Plasma Volume

Plasma volume will be determined using the FBFI measured plasma concentrations of FD003 and the FAST PV Technology.

11.5.2. Interstitial Volume

Interstitial volume will be determined using the FBFI measured plasma concentrations of FD001 and the FAST PV Technology as well as by HPLC analysis of iohexol concentrations.

12. ACCESS TO SOURCE DATA/DOCUMENTS

The investigator will provide direct access to source data and documents for individuals conducting study-related monitoring, audits, Institutional Review Board/Independent Ethics Committee (IRB/IEC) review, and regulatory review. The investigator must inform the study subject that his/her study-related records may be reviewed by the above individuals without violating the subject's privacy of personal health information in compliance with Health Insurance Portability and Accountability Act of 1996 (HIPAA) regulations.

Attention is drawn to the regulations promulgated by the FDA under the Freedom of Information Act providing, in part, that information furnished to clinical investigators and IRBs will be kept confidential by the FDA only if maintained in confidence by the clinical investigator and IRB. By signing this protocol, the investigator affirms to the sponsor that the investigator will maintain, in confidence, information furnished to him or her by the sponsor and will divulge such information to the IRB under an appropriate understanding of confidentiality with such board.

13. QUALITY CONTROL AND QUALITY ASSURANCE

FAST BioMedical will implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure the study is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements.

13.1. Conduct of Study

This study will be conducted in accordance with the ICH E6 Guidelines on good clinical practice (CPMP/ICH/135/95). Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by an IRB or IEC; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each subject will give his or her written, informed consent before any protocol-driven tests or evaluations are performed.

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate IRB/IEC, except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study and are approved by FAST BioMedical. Any deviation may result in the subject having to be withdrawn from the study, and may render that subject non-evaluable.

13.1.1. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, investigator, or site staff.

At the outset of the study, a process for defining and handling protocol deviations will be established. This will include determining which violations will be designated “key,” requiring immediate notification to FAST BioMedical. The investigator is responsible for seeing that any known protocol deviations are recorded and handled as agreed.

13.2. Protocol Amendments

Only the sponsor may modify the protocol. Amendments to the protocol will be made only after consultation and agreement between the sponsor and the investigator. All amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/IEC prior to their implementation.

13.3. Monitoring of Study

The investigator will permit the site monitor to review study data as frequently as is deemed necessary to ensure data are being recorded in an adequate manner and protocol adherence is satisfactory.

The investigator will provide supervised access to medical records for the monitor to verify CRF entries. Only de-identified data will leave the Richard L. Roudebush Veterans Administration Medical Center. The investigator is expected to cooperate with FAST BioMedical or a designee in ensuring the study adheres to GCP requirements. Only de-identified data will leave the Richard L. Roudebush Veterans Administration Medical Center.

The investigator may not recruit subjects into the study until FAST BioMedical or a designee has conducted a site initiation visit (in-person or via telephone) to review the protocol and CRF in detail.

14. ETHICS

14.1. Institutional Review Board/Independent Ethics Committee Approval

14.1.1. Ethics Review Prior to Study

The investigator will ensure that the protocol and consent form are reviewed and approved by the appropriate IRB/IEC prior to the start of any study procedures. The IRB/IEC will be appropriately constituted and will perform its functions in accordance with FDA regulations, ICH GCP guidelines, and local requirements as applicable.

14.1.2. Ethics Review of Other Documents

The IRB will approve all protocol amendments (except for sponsor-approved logistical or administrative changes), written informed consent documents and document updates, subject recruitment procedures, written information to be provided to the subjects, available safety information, information about payment and compensation available to subjects, the investigator's curriculum vitae and/or other evidence of qualifications, and any other documents requested by the IRB/IEC and regulatory authority as applicable.

14.2. Written Informed Consent

The nature and purpose of the study will be fully explained to each subject. The subjects must be given ample time and opportunity to inquire about details of the study, to have questions answered to their satisfaction, and to decide whether to participate. Written informed consent must be obtained from each subject prior to any study procedures being performed.

15. DATA HANDLING AND RECORD KEEPING

15.1. Data Reporting and Case Report Forms

15.1.1. Case Report Forms

The investigator will be provided with CRFs and will ensure all data from subject visits are promptly entered into the CRFs in accordance with the specific instructions given. The investigator must sign the CRFs to verify the integrity of the data recorded.

15.1.2. Laboratory Data

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. If a central laboratory has been selected to conduct any or all tests, it is essential all samples be analyzed at that laboratory. The investigator must maintain source documents such as laboratory reports and complete history and physical examination reports.

15.1.3. Retention of Source Documents

The investigator must maintain source documents such as laboratory reports, x-rays, ECGs, consultation reports, and complete history and physical examination reports.

15.2. Retention of Essential Documents

The study essential documents must be maintained as specified in the ICH guidelines for GCP and the applicable regulatory requirements. The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational device. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with sponsor. It is the responsibility of FAST BioMedical to inform the investigator/institution as to when these documents no longer need to be retained.

VA research records will be maintained by the investigator for 6 (six) years following the federal fiscal year end (September 30) after the study has been closed by the VA in accordance with the VHA Records Control Schedule or longer if required by other Federal regulations.

As described in the Informed Consent and HIPAA authorization, data collected during the study may be used and analyzed after study expiration, however data will be de-identified prior to sharing outside the VA study team.

15.3. Sample Retention

Samples will be labeled with a unique code study identifier, the collection date, and collection time; there will be no protected health information (PHI) on the samples.

Unless otherwise required by the U.S. Food and Drug Administration or other laws or regulations, samples will be stored for a maximum of 18 months after collection and then destroyed.

15.4. Privacy

Access to all VA files will be made by study staff who are required to complete and maintain training on privacy, HIPAA, and human subjects' protection. All data files will be maintained on a secure drive on the research server at RLR VAMC and will be accessible only to the PI, project coordinator, and data manager. Any identifiable data will be kept in restricted folders on the research secured server at RLR VAMC with access restricted to authorized members of the study team. Paper documents will be kept in a locked cabinet in PI's locked office inside a locked office suite on the 7th floor of the RLR VAMC.

Study numbers will be assigned to each subject such that lab samples will be labeled with a unique code study identifier, the collection date, and collection time; there will be no protected health information (PHI) on the samples.

Any publications will not have subject names or identifying information.

Additionally, all PHI will be de-identified prior to leaving the VA and will be checked by the privacy officer before release.

16. ADMINISTRATIVE INFORMATION

16.1. Financing and Insurance

Financing and insurance will be addressed in a separate agreement between FAST BioMedical and the clinical study site.

16.2. Publication Policy

FAST BioMedical will retain ownership of all data. All proposed publications based on this study will be subject to sponsor's approval requirements.

17. REFERENCES

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APPENDIX A PREPARATION OF FAST VISIBLE FLUORESCENT INJECTATE

1. Add 4 mL of sterile water to the vial of VFI over 4 seconds.
2. Gently swirl the vial for 3 minutes; avoid allowing the liquid to reach the rubber stopper.
3. Place vial on a roller/rocker table to mix for 30 minutes.
4. Product is ready for drawing up of dose.

SIGNATURES

Protocol Title: A Pilot Open-Label Study to Evaluate the Safety, Tolerability, and Performance of FAST PV Technology in Chronic Dialysis Patients with Extremely Reduced or No Kidney Function

FAST BioMedical Signature

This clinical study protocol has been reviewed and approved by FAST BioMedical.

James Strickland President	Date

Protocol Title: A Pilot Open-Label Study to Evaluate the Safety, Tolerability, and Performance of FAST PV Technology in Chronic Dialysis Patients with Extremely Reduced or No Kidney Function

FAST BioMedical Signature

This clinical study protocol has been reviewed and approved by FAST BioMedical.

Bruce Molitoris, MD Medical Director	Date

Protocol Title: A Pilot Open-Label Study to Evaluate the Safety, Tolerability, and Performance of FAST PV Technology in Chronic Dialysis Patients with Extremely Reduced or No Kidney Function

Investigator Signature

I agree to conduct the aforementioned study according to the terms and conditions of the protocol, Good Clinical Practice (GCP) guidelines, and all other applicable local and regulatory requirements. All information pertaining to the study will be treated in a confidential manner.

Site Name

Print Name

Signature

Date