Clinical Study Protocol

A Phase 2b Open-Label Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of FAST PV and mGFR Technology™ in Healthy Subjects and Patients with Varying Degrees of Renal Impairment

Protocol Number: 36770001

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Protocol Number: 36770001

Protocol Title: A Phase 2b Open-Label Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of FAST PV and mGFR Technology™ in Healthy Subjects and Patients with Varying Degrees of Renal Impairment

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.

Emmanuel DeNoia, MD	Date

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.

Dana V. Rizk, MD	Date

FAST BioMedical Signatures

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

James Strickland President	Date
Bruce Molitoris, MD Medical Director	Date

2 SYNOPSIS

Protocol Title: A Phase 2b Open-Label Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of FAST PV and mGFR Technology™ in Healthy Subjects and Patients with Varying Degrees of Renal Impairment

Study Phase: 2b

Indication: Quantitative determination of plasma volume (PV) and measured glomerular filtration rate (mGFR)

Investigators and Clinical Research Units:

Cohorts 1 and 2:
Emanuel DeNoia, MD
ICON Early Phase Services, LLC

Cohort 3:
Dana V. Rizk, MD
University of Alabama Birmingham
Emanuel DeNoia, MD
ICON Early Phase Services, LLC

Cohort 4:
Dana V. Rizk, MD
University of Alabama Birmingham

Objectives:

Primary:

- To assess the safety and tolerability of visible fluorescent injectate (VFI)™ (employing the FAST PV and mGFR Technology™) compared to Iohexol (employing Iohexol clearance methods) in healthy subjects and patients with impaired renal function
- To evaluate the pharmacokinetics (PK) of FD001 and FD003 in healthy subjects and patients with impaired renal function
- To evaluate the PK of FD001 and FD003 in healthy subjects receiving 2 doses of VFI separated by 24 hours to evaluate the reproducibility of the FAST mGFR Technology.

Secondary:

- To evaluate the relationship between FAST PV and mGFR Technology and standard clinical formulaic estimates of PV in healthy subjects
- To evaluate and compare glomerular filtration rates (GFRs) determined from FAST PV and mGFR Technology and Iohexol clearance in healthy subjects and patients with impaired renal function
- To evaluate the GFR measurements in relation to current clinical standards in healthy subjects and patients with impaired renal function
- To evaluate the FAST PV Technology's ability to measure changes in vascular plasma volume resulting from a fluid challenge after the baseline PV measurement

Hypotheses: This is an exploratory pilot investigation without any formal statistical hypotheses. All results will only be interpreted descriptively. Correlation analysis and Bland-Altman plots will be used to compare the FAST mGFR with Iohexol clearance.

Endpoints:

Primary:
Safety:

- Adverse events (AEs) and serious AEs (SAEs)
- Clinical laboratory tests, physical examination findings, 12-lead electrocardiograms (ECGs), and vital signs including pulse oximetry

Pharmacokinetics:

- Maximum observed plasma concentration (C_{max})
- Time that C_{max} was observed (t_{max})
- Area under the plasma concentration-time curve (AUC) from time 0 to 12 hours (AUC_{0-12}: FD001 only)
- AUC from time 0 to the time of the last quantifiable concentration (AUC_{last})
- AUC from time 0 extrapolated to infinity (AUC_{inf})
- Terminal elimination half-life ($t_{½}$)
- Terminal elimination rate constant (λ_z)
- Total body clearance (CL)
- Renal clearance from time 0 to 2 hours (CL_{r0-2}; FD001 only)
- Renal clearance from time 2 to 12 hours (CL_{r2-12}; FD001 only)
- Renal clearance from time 0 to 12 hours ($CL_{r0\ 12}$; FD001 only)
- Volume of distribution during the terminal phase (V_z)
- Volume of distribution at steady state (V_{ss})
- Amount of FD001 excreted unchanged in the urine from time 0 to 2 hour (Ae_{0-2})
- Amount of FD001 excreted unchanged in the urine from time 2 to 12 hour (Ae_{2-12})
- Amount of FD001 excreted unchanged in the urine from time 0 to 12 hour (Ae_{0-12})

Secondary:
Pharmacodynamic:

- PV as assessed by FAST PV and mGFR Technology
- PV as assessed by Nadler's formula
- Assess changes in PV after fluid challenge
- Assess repeatability of GFR measurements over 24 hours
- mGFR as assessed by FAST VFI and Iohexol clearance methods
- Estimated GFR (eGFR) (calculated by the Modification of Diet in Renal Disease [MDRD], Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] Creatinine, and Cockcroft-Gault equations)

Study Design:

This is a Phase 2b, prospective, open-label study designed to evaluate the safety, tolerability, PK, and pharmacodynamics (PD) of FAST PV and mGFR Technology in healthy subjects and patients with varying degrees of renal impairment.

For Cohorts 1 and 2, administration of the study drug will occur within 21 days of screening. Eligible subjects will be admitted to the clinical research unit (CRU) on Day -1, at which time assessments will be performed, and results from both screening and Day -1 (inclusion/exclusion criteria, body weight, height [screening only], body mass index [BMI], medical and surgical history, physical examination, vital signs and pulse oximetry, 12-lead ECG, clinical laboratory tests, renal function assessment, follicle-stimulating hormone [FSH; screening only] and pregnancy test [females only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

Study Design: continued

For Cohorts 3 and 4, administration of the study drug will occur within 21 days of screening.

Eligible patients enrolled at UAB will be admitted to the CRU on Day 1, at which time assessments will be performed and results from both screening and predose administration testing (inclusion/exclusion criteria, body weight, height [screening only], BMI, medical and surgical history, physical examination, vital signs and pulse oximetry, 12-lead ECG, clinical laboratory tests, renal function assessment, FSH [screening only] and pregnancy test [females only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

Eligible patients enrolled into Cohort 3 at ICON Early Phase Services, LLC will be admitted to the CRU on Day -1 and will remain resident in the CRU through discharge on Day 2. Eligible subjects will be admitted to the clinical research unit (CRU) on Day -1, at which time assessments will be performed, and results from both screening and Day -1 (inclusion/exclusion criteria, body weight, height [screening only], body mass index [BMI], medical and surgical history, physical examination, vital signs and pulse oximetry, 12-lead ECG, clinical laboratory tests, renal function assessment, follicle-stimulating hormone [FSH; screening only] and pregnancy test [females only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

The planned dose cohorts are presented in the table below:

Cohort	eGFR	VFI Dose	Number of Doses	Comparator Dose	Number of Doses	Number of Subjects
1*	≥ 60 mL/min/1.73 m^2	47 mg/3 mL	1	---	---	Up to 8
2	≥ 60 mL/min/1.73 m^2	47 mg/3 mL	2	Iohexol 5 mL	1	Up to 8
3	≥ 30 and < 60 mL/min/1.73 m^2	47 mg/3 mL	1	Iohexol 5 mL	1	Up to 8
4	≥ 15 and < 30 mL/min/1.73 m^2	47 mg/3 mL	1	Iohexol 5 mL	1	Up to 8

*Subjects in Cohort 1 will receive a volume challenge

Cohort 1:
Eligible subjects ≥ 50 kg in weight will receive a single dose of VFI followed 130 minutes later by a 350 mL infusion of 5% albumin over 30 minutes on Day 1. Eligible subjects < 50 kg in weight will receive a single dose of VFI followed 130 minutes later by a maximum infusion of 5% albumin of 7 mL/kg over 30 minutes on Day 1. Subjects will remain resident in the CRU for at least 24 hours after VFI administration for safety assessment. Subjects will return to the CRU for an end-of-study (EOS) visit on Day 21 (± 1 day).

Cohort 2:
Eligible subjects will receive 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 (on Day 2) after the initial dose of VFI. Subjects will remain resident in the CRU for at least 24 hours after the second VFI administration for safety, PK, and PD assessments. Subjects will return to the CRU for follow-up visits on Days 5 (± 1 day), 9 (± 1 day), and 16 (± 1 day), and an EOS visit on Day 22 (± 1 day).

Cohorts 3 and 4:
Eligible patients will receive a single dose of VFI followed 160 minutes later by a single dose of Iohexol on Day 1.

Study Design: continued	Patients enrolled into Cohort 3 at ICON Early Phase Services will remain resident in the CRU for at least 24 hours after VFI administration for safety, PK, and PD assessments. Patients will return to the CRU for follow-up visits on Days 4 (± 1 day), 8 (± 1 day), and 15 (± 1 day), and an EOS visit on Day 21 (± 1 day). Patients enrolled into Cohorts 3 or 4 at UAB will remain in the CRU through the 12-hour sample collection after VFI administration for safety, PK, and PD assessments. Patients will stay at a local hotel near the CRU and will return to the CRU on Day 2 for 24-hour sample collection. Patients will return to the CRU for follow-up visits on Days 4 (± 1 day), 8 (± 1 day), and 15 (± 1 day), and an EOS visit on Day 21 (± 1 day).
Subject Selection Criteria:	Males and females (women must be post-menopausal or be using a medically acceptable form of birth control) 18 to 75 years of age, inclusive, with a body weight of at least 40 kg will be enrolled in the study. Reproductively active men must agree to either practice abstinence or utilize adequate contraception. Healthy subjects in Cohorts 1 and 2 must have an eGFR calculated using the CKD-EPI Creatinine Equation (2009) $\geq$ 60 mL/min/1.73 m^2 and a BMI of 18.0 to 40.0 kg/m^2, inclusive. Patients with impaired renal function in Cohort 3 must have an eGFR $\geq$ 30 and < 60 mL/min/1.73 m^2 and patients with impaired renal function in Cohort 4 must have an eGFR $\geq$ 15 and < 30 mL/min/1.73 m^2. Patients in Cohorts 3 and 4 must have a BMI of 18.0 to 40.0 kg/m^2, inclusive.
Investigational Product, Dose, and Route of Administration:	VFI, 47 mg/3 mL, bolus injection (35mg of FD001 and 12mg of FD003)
Comparator Products, Dose, and Route of Administration:	Omnipaque™ 300 (Iohexol), 5 mL, bolus injection (Cohorts 2 through 4)
Criteria for Evaluation:	Safety: Adverse events (AEs) will be collected and evaluated as they occur throughout the study. Safety assessments, including physical examinations, vital signs including pulse oximetry assessments, 12-lead ECGs, and clinical laboratory tests, will be performed at specified time points. Pharmacokinetics: Pharmacokinetic samples for plasma FD001 and FD003 concentration determination in Cohort 1 will be collected predose and 15, 30, 60, 120, 165, 195, 205, 310, 370, and 480 minutes and 12 and 24 hours post VFI dose. Pharmacokinetic samples for plasma FD001 and FD003 concentration determination in Cohort 2 will be collected predose and 15, 30, 60, 120, 170, 310, 370, and 480 minutes, 12 and 24 hours post VFI dose on Days 1 and 2, and on Days 5 (± 1 day), 9 (± 1 day), 16 (± 1 day), and 22 (± 1 day). Pharmacokinetic samples for plasma FD001 and FD003 concentration determination in Cohorts 3 and 4 will be collected predose and 15, 30, 60, 120, 170, 310, 370, and 480 minutes, 12, 24, and 32 hours post VFI dose, and on Days 4 (± 1 day), 8 (± 1 day), 15 (± 1 day), and 21 (± 1 day). Pharmacokinetic samples for urine FD001 concentration determination in Cohorts 2 through 4 will be collected predose, from dosing (0 hour) to 2 hours postdose, and from 2 to 12 hours postdose. Subjects in Cohort 2 receiving a second VFI dose on Day 2 will follow the same urine collection schedule as Day 1. Plasma concentrations of FD001 and FD003 will be determined using a validated fluorometric assay.

Criteria for Evaluation: continued

Urine concentrations of FD001 will be determined using a validated method and concentration of FD001 will be multiplied by urine volume to determine the amount of excreted FD001.

The following PK parameters will be calculated from the plasma and urine concentration/volume data using noncompartmental methods and actual sampling times: C_{max}, t_{max}, AUC_{0-12} (FD001 only), AUC_{last}, AUC_{inf}, $t_{½}$, λ_z, CL, CL_{r0-2} (FD001 only), CL_{r2-12} (FD001 only), $CL_{r0\ 12}$ (FD001 only),V_z, V_{ss}, Ae_{0-2}, Ae_{0-2}, and Ae_{0-12}.

Pharmacodynamics:
Plasma Volume (Cohorts 1 through 4): Plasma volume will be determined using FAST PV Technology.

Additionally, PV will be estimated using Nadler's formula.

Glomerular Filtration Rate (Cohort 1): Measured GFR will be determined using FAST mGFR Technology.

Glomerular Filtration Rate (Cohorts 2 through 4): Measured GFR will be determined using FAST mGFR Technology and Iohexol clearance methods. Correlation analysis and Bland-Altman plots will be used to compare the FAST mGFR with Iohexol clearance.

Additionally, eGFR will be calculated primarily using the CKD-EPI equations, with additional calculations being performed using the MDRD and Cockcroft-Gault equations (Cohorts 1 through 4).

Planned Sample Size:

Approximately 32 subjects/patients will be enrolled in 4 cohorts consisting of up to 8 subjects/patients each. Only subjects in Cohorts 2 through 4 will contribute to the plasma concentration PK statistical analysis; plasma concentrations for subjects in Cohort 1 will be analyzed for CL_r, C_{max}, and using the FAST mGFR and PV measurement calculations only.

The sample size in this study is representative of Phase 2b studies of this type. Power calculations were not used to derive the sample size.

Statistical Analysis:

Analysis Populations:
Pharmacokinetic Full Population: All subjects in Cohorts 2 through 4 who receive a known amount of study drug and have at least one quantifiable concentration of FD001 or FD003 in plasma or FD001 in urine and all subjects in Cohort 1 who receive a known amount of study drug. Plasma concentrations for subjects in Cohort 1 will not contribute to the overall PK statistical analysis and will be analyzed for CL_r, C_{max}, and using the FAST mGFR and PV measurement calculations only.

Pharmacokinetic Evaluable Population: All subjects in Cohorts 1 through 4 who receive a known amount of study drug and have at least 1 estimable PK parameter.

Safety Population: All subjects who received any amount of study drug.

Pharmacodynamic Evaluable Population: All subjects in Cohorts 1 through 4 who have either a PV or GFR estimate using any of the techniques under study. Subjects in Cohort 1 will not contribute to the overall PD statistical analysis.

Statistical Analysis: continued

Pharmacokinetic Analysis: For Cohorts 2 through 4, subject plasma concentration-time profiles will be listed and summarized for each analyte by nominal sampling time. Summary statistics will be listed and summarized for each PK parameter by cohort and analyte. Plasma concentrations for subjects in Cohort 1 will not contribute to the overall PK statistical analysis and will be analyzed for CL_r, C_{max}, and using the FAST mGFR and PV measurement calculations only.

Pharmacodynamic Analysis:
Plasma Volume (Cohorts 2 through 4):
The FAST Technology for determining PV will be compared to PV estimated using Nadler's formula. Plasma volume data will be listed and summarized by presenting descriptive statistics of raw data. The correlation as well as the associated plot will be presented for comparing PV between methods. Cosgriff et al. 1999 shows that a combination of the correlation and a histogram of the magnitude of difference is needed when no measured comparator is available. Subjects in Cohort 1 will not contribute to the overall PD statistical analysis.

Glomerular Filtration Rate (Cohorts 2 through 4):
Measured GFR determined using the FAST VFI Technology will be compared to the mGFR determined using the Iohexol clearance test. The results will be listed by subject and summarized by presenting descriptive statistics for each cohort. Correlation analysis and Bland-Altman plots will be used to compare the FAST mGFR with Iohexol clearance. Subjects in Cohort 1 will not receive Iohexol and will not contribute to the overall PD statistical analysis.

Estimated GFR calculated using primarily the CKD-Epi equation in addition to the MDRD, and Cockcroft-Gault equations will be listed by subject and summarized by presenting descriptive statistics for each cohort.

Safety Analysis:
12-lead ECG, vital signs, pulse oximetry, and clinical laboratory test data (observed and change from baseline) will be summarized by cohort, time point, and treatment using appropriate descriptive statistics. Physical examination findings will be listed only.

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

Table 2.1 Schedule of Assessments and Procedures – Cohort 1 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Admission[a]**	**Treatment Period**														**Discharge**	**EOS[b]**
Study Day	**-21 to -2**	**-1**	**1**														**2**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection	**-**	**-**	**Pre**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**130 m**	**165 m**	**195 m**	**205 m**	**310 m**	**370 m**	**480 m**	**12 h**	**24 h**	**480 h**
Informed consent	X																	
Inclusion/exclusion criteria	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															X	X
Vital signs and pulse oximetry[e]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[e]	X	X	X			X											X	
Clinical laboratory tests[f]	X	X													X[g]		X	X
Renal function assessment[h]	X	X																
Urine protein to creatinine ratio[i]			X														X	
Serology (HIV, HBsAg, HCV)	X																	
FSH (females only)	X																	
Pregnancy Test (females only)	X	X																

Table 2.1 Schedule of Assessments and Procedures – Cohort 1 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Admission[a]**	**Treatment Period**														**Discharge**	**EOS[b]**
Study Day	**-21 to -2**	**-1**	**1**														**2**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection	**-**	**-**	**Pre**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**130 m**	**165 m**	**195 m**	**205 m**	**310 m**	**370 m**	**480 m**	**12 h**	**24 h**	**480 h**
Urine drugs of abuse and serum alcohol screen	X	X																
In-house residency		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
FAST VFI administration				X														
5% Albumin Infusion									X									
FD001 and FD003 PK/PD plasma and urine samples			Refer to Table 2.4 for the PK and PD sampling schedule.															
AEs				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BMI = body mass index; ECG = electrocardiogram; EOS = end-of-study; FSH = follicle-stimulating hormone; h = hours; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; m = minutes; PD = pharmacodynamic; PK = pharmacokinetic; Pre = predose; VFI = visible fluorescent injectate

a. Assessments conducted upon clinical research unit admission will be used to reconfirm a subject's eligibility for enrollment into the study.
b. Subjects who terminate from the study early will have EOS procedures performed at the time of discontinuation.
c. BMI will be calculated using the height obtained at screening.
d. Interim medical and surgical history will be collected to determine if any changes have occurred since screening.
e. Vital signs and ECGs 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. Refer to Table 8.3 for a detailed list of clinical laboratory test parameters.
g. Hematology and serum chemistry only. Glucose and total cholesterol measurements will not be reported at 480 minutes as the subject will not have been fasting for 8 hours prior.
h. Measurement of serum blood urea nitrogen, serum creatinine, and calculation of estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; 2009), and Cockcroft-Gault equations.
i. Subjects should be fasting prior to urine protein to creatinine ratio measurement.

NOTE: In the event that multiple postdose procedures are required to be conducted at the same nominal time point, the timing of PD blood sample collections will take priority over all other scheduled activities except for dosing. In practice, the following order is recommended: (1) ECG recording; (2) vital signs assessments; (3) PD blood sampling; (4) clinical laboratory tests sampling; (5) physical examination. Electrocardiograms and vital signs may be conducted up to 15 and 10 minutes, respectively, prior to the nominal time to minimize the potential autonomic effects of blood draws on these measurements.

Table 2.2 Schedule of Assessments and Procedures – Cohort 2 (Repeat-dose Administration of VFI)

Study Procedure	**Screening**	**Admission[a]**	**Treatment Period**														**Discharge**	**Follow-up**			**EOS[b]**
Study Day	**-21 to -2**	**-1**	**1 and 2**														**3**	**5 (± 1)**	**9 (± 1)**	**16 (± 1)**	**22 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection	**-**	**-**	**Pre**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**72 h**	**168 h**	**336 h**	**480 h**
Informed consent	X																				
Inclusion/ exclusion criteria	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															X				X
Vital signs and pulse oximetry[e]	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[e]	X	X	X			X											X				
Clinical laboratory tests[f]	X	X													X[g]		X	X[g]	X[g]	X[g]	X
Renal function assessment[h]	X	X																			
Urine protein to creatinine ratio[i]			X														X				
Serology (HIV, HBsAg, HCV)	X																				
FSH (females only)	X																				
Pregnancy Test (females only)	X	X																			

Table 2.2 Schedule of Assessments and Procedures – Cohort 2 (Repeat-dose Administration of VFI)

Study Procedure	Screening	Admission[a]	Treatment Period														Discharge	Follow-up			EOS[b]
Study Day	-21 to -2	-1	1 and 2														3	5 (± 1)	9 (± 1)	16 (± 1)	22 (± 1)
Nominal Time Relative to Start of VFI Bolus Injection	-	-	Pre	0 m	15 m	30 m	60 m	120 m	160 m	170 m	280 m	310 m	340 m	370 m	480 m	12 h	24 h	72 h	168 h	336 h	480 h
Urine drugs of abuse and serum alcohol screen	X	X																			
In-house residency		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
FAST VFI administration[j]				X													X				
Iohexol administration[k]									X												
FD001 and FD003 PK/PD plasma and urine samples			Refer to Table 2.5 for the PK and PD sampling schedule.																		
Iohexol PD plasma samples			Refer to Table 2.5 for the PK and PD sampling schedule.																		
AEs				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BMI = body mass index; ECG = electrocardiogram; EOS = end-of-study; FSH = follicle-stimulating hormone; h = hours; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; m = minutes; PD = pharmacodynamic; PK = pharmacokinetic; Pre = predose; VFI = visible fluorescent injectate

a. Assessments conducted upon clinical research unit admission will be used to reconfirm a subject's eligibility for enrollment into the study.
b. Subjects who terminate from the study early will have EOS procedures performed at the time of discontinuation.
c. BMI will be calculated using the height obtained at screening.
d. Interim medical and surgical history will be collected to determine if any changes have occurred since screening.
e. Vital signs and ECGs 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. Refer to Table 8.3 for a detailed list of clinical laboratory test parameters.

g. Hematology and serum chemistry only. Glucose and total cholesterol measurements will not be reported at 480 minutes as the subject will not have been fasting for 8 hours prior.
h. Measurement of serum blood urea nitrogen, serum creatinine, and calculation of estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; 2009), and Cockcroft-Gault equations.
i. Subjects should be fasting prior urine protein to creatinine ratio measurement. The 24-hour measurement on Day 2 will also act as the predose measurement for VFI dose 2. An additional ratio will be calculated 24 hours post VFI dose 2.
j. VFI administration on Day 2 will occur 24 hours following VFI administration on Day 1.
k. Iohexol administration will only occur on Day 1.

NOTE: In the event that multiple postdose procedures are required to be conducted at the same nominal time point, the timing of PK and PD blood sample collections will take priority over all other scheduled activities except for dosing. In practice, the following order is recommended: (1) ECG recording; (2) vital signs assessments; (3) PK and PD blood sampling; (4) clinical laboratory tests sampling; (5) physical examination. Electrocardiograms and vital signs may be conducted up to 15 and 10 minutes, respectively, prior to the nominal time to minimize the potential autonomic effects of blood draws on these measurements.

Table 2.3.1 UAB Schedule of Assessments and Procedures – Cohorts 3 and 4 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Treatment Period**														**Discharge**		**Follow-up**			**EOS[b]**
Study Day	**-21 to -1**	**1**														**2**		**4 (± 1)**	**8 (± 1)**	**15 (± 1)**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection		**Pre (UAB)**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**32 h**	**72 h**	**168 h**	**336 h**	**480 h**
Informed consent	X																				
Inclusion/ exclusion criteria	X	X[a]																			
In House Residency		X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Demographics	X																				
Height	X																				
Body weight	X	X[a]																			X
BMI [c]	X	X[a]																			
Medical and surgical history	X	X[a,d]																			
Physical examination	X	X[a]														X					X
Vital signs and pulse oximetry[e]	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[e]	X	X[a]			X											X					
Clinical laboratory tests[f]	X	X[a]												X[g]		X		X[g]	X[g]	X[g]	X
Renal function assessment[h]	X	X[a]																			

Table 2.3.1 UAB Schedule of Assessments and Procedures – Cohorts 3 and 4 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Treatment Period**													**Discharge**		**Follow-up**			**EOS[b]**
Study Day	**-21 to -1**	**1**													**2**		**4 (± 1)**	**8 (± 1)**	**15 (± 1)**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection		**Pre (UAB)**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**32 h**	**72 h**	**168 h**	**336 h**	**480 h**
Urine protein to creatinine ratio[i]		X														X					
Serology (HIV, HBsAg, HCV)	X																				
FSH (females only)	X																				
Pregnancy Test (females only)	X	X																			
Urine drugs of abuse and serum alcohol screen	X	X[a]																			
In-house residency		X	X	X	X	X	X	X	X	X	X	X	X	X	X						
FAST VFI administration			X																		
Iohexol administration								X													
FD001 and FD003 PK/PD plasma samples		Refer to Table 2.6 for the PK and PD sampling schedule.																			

Table 2.3.1 UAB Schedule of Assessments and Procedures – Cohorts 3 and 4 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Treatment Period**													**Discharge**		**Follow-up**			**EOS[b]**
Study Day	**-21 to -1**	**1**													**2**		**4 (± 1)**	**8 (± 1)**	**15 (± 1)**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection		**Pre (UAB)**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**32 h**	**72 h**	**168 h**	**336 h**	**480 h**
Iohexol PD plasma samples		Refer to Table 2.6 for the PK and PD sampling schedule.																		
AEs			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BMI = body mass index; ECG = electrocardiogram; EOS = end-of-study; FSH = follicle-stimulating hormone; h = hours; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; m = minutes; PD = pharmacodynamic; PK = pharmacokinetic; Pre = predose; VFI = visible fluorescent injectate

a. Assessments conducted upon clinical research unit admission will be used to reconfirm a patient's eligibility for enrollment into the study. Coagulation (INR) not repeated at predose, only at screening.
b. Patients who terminate from the study early will have EOS procedures performed at the time of discontinuation.
c. BMI will be calculated using the height obtained at screening.
d. Interim medical and surgical history will be collected to determine if any changes have occurred since screening.
e. Vital signs and ECGs will be measured after the patient has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes.
f. Refer to Table 8.3 for a detailed list of clinical laboratory test parameters.
g. Hematology and serum chemistry only. Glucose and Total Cholesterol measurements will not be reported at 480 minutes as the patient will not have been fasting for 8 hours prior.
h. Measurement of serum blood urea nitrogen, serum creatinine, and calculation of estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; 2009), and Cockcroft-Gault equations.
i. Patients should be fasting prior to urine protein to creatinine ratio measurement.

NOTE: In the event that multiple postdose procedures are required to be conducted at the same nominal time point, the timing of PK and PD blood sample collections will take priority over all other scheduled activities except for dosing. In practice, the following order is recommended: (1) ECG recording; (2) vital signs assessments; (3) PK and PD blood sampling; (4) clinical laboratory tests sampling; (5) physical examination. Electrocardiograms and vital signs may be conducted up to 15 and 10 minutes, respectively, prior to the nominal time to minimize the potential autonomic effects of blood draws on these measurements.

Table 2.3.2 ICON Schedule of Assessments and Procedures – Cohorts 3 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Day -1 (ICON Cohort 3 Only)**	**Treatment Period**														**Discharge**		**Follow-up**			**EOSb**
Study Day	**-21 to -1**	**Day -1**	**1**														**2**		**4 (± 1)**	**8 (± 1)**	**15 (± 1)**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection			**Pre (ICON)**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**32 h**	**72 h**	**168 h**	**336 h**	**480 h**
Informed consent	X																					
Inclusion/ exclusion criteria	X	X[a]																				
In House Residency (ICON Only)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Demographics	X																					
Height	X																					
Body weight	X	X																				X
BMI [c]	X	X[a]																				
Medical and surgical history	X	X[a,d]																				
Physical examination	X	X[a]															X					X
Vital signs and pulse oximetry[e]	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[e]	X	X[a]	X[a]			X											X					
Clinical laboratory tests[f]	X	X[a]													X[g]		X		X[g]	X[g]	X[g]	X
Renal function assessment[h]	X	X[a]																				

Table 2.3.2 ICON Schedule of Assessments and Procedures – Cohorts 3 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Day -1 (ICON Cohort 3 Only)**	**Treatment Period**														**Discharge**		**Follow-up**			**EOSb**
Study Day	**-21 to -1**	**Day -1**	**1**														**2**		**4 (± 1)**	**8 (± 1)**	**15 (± 1)**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection			**Pre (ICON)**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**32 h**	**72 h**	**168 h**	**336 h**	**480 h**
Urine protein to creatinine ratio[i]			X														X					
Serology (HIV, HBsAg, HCV)	X																					
FSH (females only)	X																					
Pregnancy Test (females only)	X	X																				
Urine drugs of abuse and serum alcohol screen	X	X																				
In-house residency		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
FAST VFI administration				X																		
Iohexol administration									X													
FD001 and FD003 PK/PD plasma samples	Refer to Table 2.6 for the PK and PD sampling schedule.																					

Table 2.3.2 ICON Schedule of Assessments and Procedures – Cohorts 3 (Single-dose Administration of VFI)

Study Procedure	**Screening**	**Day -1 (ICON Cohort 3 Only)**	**Treatment Period**														**Discharge**		**Follow-up**			**EOSb**
Study Day	**-21 to -1**	**Day -1**	**1**														**2**		**4 (± 1)**	**8 (± 1)**	**15 (± 1)**	**21 (± 1)**
Nominal Time Relative to Start of VFI Bolus Injection			**Pre (ICON)**	**0 m**	**15 m**	**30 m**	**60 m**	**120 m**	**160 m**	**170 m**	**280 m**	**310 m**	**340 m**	**370 m**	**480 m**	**12 h**	**24 h**	**32 h**	**72 h**	**168 h**	**336 h**	**480 h**
Iohexol PD plasma samples	Refer to Table 2.6 for the PK and PD sampling schedule.																					
AEs					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BMI = body mass index; ECG = electrocardiogram; EOS = end-of-study; FSH = follicle-stimulating hormone; h = hours; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; m = minutes; PD = pharmacodynamic; PK = pharmacokinetic; Pre = predose; VFI = visible fluorescent injectate

a. Assessments conducted upon clinical research unit admission will be used to reconfirm a patient's eligibility for enrollment into the study. Coagulation (INR) not repeated at predose, only at screening.
b. Patients who terminate from the study early will have EOS procedures performed at the time of discontinuation.
c. BMI will be calculated using the height obtained at screening.
d. Interim medical and surgical history will be collected to determine if any changes have occurred since screening.
e. Vital signs and ECGs will be measured after the patient has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes.
f. Refer to Table 8.3 for a detailed list of clinical laboratory test parameters.
g. Hematology and serum chemistry only. Glucose and Total Cholesterol measurements will not be reported at 480 minutes as the patient will not have been fasting for 8 hours prior.
h. Measurement of serum blood urea nitrogen, serum creatinine, and calculation of estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; 2009), and Cockcroft-Gault equations.
i. Patients should be fasting prior to urine protein to creatinine ratio measurement.

NOTE: In the event that multiple postdose procedures are required to be conducted at the same nominal time point, the timing of PK and PD blood sample collections will take priority over all other scheduled activities except for dosing. In practice, the following order is recommended: (1) ECG recording; (2) vital signs assessments; (3) PK and PD blood sampling; (4) clinical laboratory tests sampling; (5) physical examination. Electrocardiograms and vital signs may be conducted up to 15 and 10 minutes, respectively, prior to the nominal time to minimize the potential autonomic effects of blood draws on these measurements.

Table 2.4 Pharmacokinetic and Pharmacodynamic Sampling Schedule – Cohort 1 (Single-dose Administration of VFI)

Study Day	Time (Relative to Start of VFI Bolus Injection)	FD001 and FD003 PK/PD Plasma Sample	Time (Relative to Start of 5% Albumin Infusion)
1	Predose	X	-
	0	VFI Administration	
	15 minutes	X	-
	30 minutes	X	-
	60 minutes	X	-
	120 minutes	X	-
	130 minutes	5% Albumin Infusion Start (over 30 min)	
	160 minutes	5% Albumin Infusion Ends	
	165 minutes	X	35 minutes
	195 minutes	X	65 minutes
	205 minutes	X	75 minutes
	310 minutes	X	180 minutes
	370 minutes	X	240 minutes
	480 minutes	X	350 minutes
	12 hours	X	-
2	24 hours	X	-

Abbreviations: PD = pharmacodynamic; PK = pharmacokinetic; VFI = visible fluorescent injectate

Note: Blood samples may not be collected from the same arm used for VFI administration. In the event that vascular access cannot be established in both arms, a single arm may be used with documentation in the subject's records. PK/PD blood samples will be collected as close to the nominal time point as possible. Allowable windows for PK/PD blood sample collection relative to dosing will be as follows: within 30 minutes predose, ± 1 minute through 205 minutes postdose , ± 2 minutes through 480 minutes postdose, and ± 10 minutes through 24 hours postdose. Subjects should evacuate their bladder prior to beginning 5% albumin infusion.

Table 2.5 Pharmacokinetic and Pharmacodynamic Sampling Schedule - Cohort 2 (Repeat-dose Administration of VFI)

Study Day	Time (Relative to Start of VFI Bolus Injection)	FD001 and FD003 PK/PD Plasma Sample	FD001 PK Urine Sample	Time (Relative to Start of Iohexol Bolus Injection)	Iohexol PD Plasma Sample
1	Predose	X	X[a]	Predose	X
	0 minutes	VFI Administration			
	15 minutes	X	0 to 2 hours[b]	-	-
	30 minutes	X		-	-
	60 minutes	X		-	-
	120 minutes	X		-	-
	160 minutes	Iohexol Administration			
	170 minutes	X	2 to 12 hours[b]	10 minutes	X
	280 minutes	-		120 minutes	X
	310 minutes	X		150 minutes	X
	340 minutes	-		180 minutes	X
	370 minutes	X		210 minutes	X
	480 minutes	X		320 minutes	X
	12 hours	X		560 minutes	X
2	Predose	X[e]	-	-	-
	0 minutes	VFI Administration[c]			
	15 minutes	X	0 to 2 hours[b]	-	-
	30 minutes	X		-	-
	60 minutes	X		-	-
	120 minutes	X		-	-
	170 minutes	X	2 to 12 hours[b]	-	-
	310 minutes	X		-	-
	370 minutes	X		-	-
	480 minutes	X		-	-
	12 hours	X		-	-
3	24 hours[d]	X	-	-	-
5	72 hours	X	-	-	-
9	168 hours	X	-	-	-
16	336 hours	X	-	-	-
22	480 hours	X	-	-	-

Abbreviations: PD = pharmacodynamic; PK = pharmacokinetic; VFI = visible fluorescent injectate

a. Subjects will be asked to void their bladder prior to VFI dosing; a small sample (~10mL) will be saved.
b. Urine will be collected and pooled from time 0 through 2 hours and from 2 hours through 12 hours. A total volume of urine in each pool will be measured. Subjects will be asked to void their bladder at the 2 hour and the 12 hour time point. Time of void should be recorded.
c. VFI administration on Day 2 should occur 24 hours ± 1 hour after VFI administration on Day 1.
d. Time relative to start of the second VFI administration.
e. Predose PK/PD sample must be taken within 5 minutes prior to VFI dosing on Day 2.

Note: Blood samples may not be collected from the same arm used for VFI administration. In the event that vascular access cannot be established in both arms, a single arm may be used with documentation in the subject's records. PK/PD blood samples will be collected as close to the nominal time point as possible. Allowable windows for PK/PD blood sample collection relative to dosing will be as follows: within 30 minutes predose, ± 1 minute through 170 minutes postdose, ± 2 minutes through 480 minutes postdose, ± 10 minutes through 24 hours postdose, and ± 1 day through Day 22. In the event samples for VFI and Iohexol are scheduled to be taken at the same time, VFI samples should be taken first, followed by Iohexol samples.

Table 2.6 Pharmacokinetic and Pharmacodynamic Sampling Schedule – Cohort 3 and 4 (Single-dose Administration of VFI)

Study Day	Time (Relative to Start of VFI Bolus Injection)	FD001 and FD003 PK/PD Plasma Sample	FD001 PK Urine Sample	Time (Relative to Start of Iohexol Bolus Injection)	Iohexol PD Plasma Sample
1	Predose	X	X[a]	Predose	X
	0 minutes	VFI Administration			
	15 minutes	X	0 to 2 hours[b]	-	-
	30 minutes	X		-	-
	60 minutes	X		-	-
	120 minutes	X		-	-
	160 minutes	Iohexol Administration			
	170 minutes	X	2 to 12 hours[b]	10 minutes	X
	280 minutes	-		120 minutes	X
	310 minutes	X		150 minutes	X
	340 minutes	-		180 minutes	X
	370 minutes	X		210 minutes	X
	480 minutes	X		320 minutes	X
	12 hours	X		560 minutes	X
2	24 hours	X	-		-
	32 hours	X	-		-
4	72 hours	X	-		-
8	168 hours	X	-		-
15	336 hours	X	-		-
21	480 hours	X	-		-

Abbreviations: PD = pharmacodynamic; PK = pharmacokinetic; VFI = visible fluorescent injectate

a. Patients will be asked to void their bladder prior to VFI dosing; a small sample (~10mL) will be saved.
b. Urine will be collected from time 0 through 2 hours and pooled and from 2 hours through 12 hours and pooled. A total volume of urine in each pool will be measured. Patients will be asked to void their bladder at the 2-hour and the 12-hour time point. Time of void should be recorded.

Note: Blood samples may not be collected from the same arm used for VFI administration. In the event that vascular access cannot be established in both arms, a single arm may be used with documentation in the patient records. PK/PD blood samples will be collected as close to the nominal time point as possible. Allowable windows for PK/PD blood sample collection relative to dosing will be as follows: within 30 minutes predose, ± 1 minute through 170 minutes postdose , ± 2 minutes through 480 minutes postdose, ± 10 minutes through 24 hours postdose, ± 1 hour through 32 hours postdose and ± 1 day through Day 21. In the event samples for VFI and Iohexol are scheduled to be taken at the same time, VFI samples should be taken first, followed by Iohexol samples.

LIST OF ABBREVIATIONS

Abbreviation	Definition
λ_z	terminal elimination rate constant
ADL	activities of daily living
AE	adverse event
Ae_{0-2}	amount of FD001 excreted unchanged in the urine from time 0 to 2 hours
Ae_{2-12}	amount of FD001 excreted unchanged in the urine from time 2 to 12 hours
Ae_{0-12}	amount of FD001 excreted unchanged in the urine from time 0 to 12 hours
AIDS	acquired immune deficiency syndrome
AKI	acute kidney injury
AUC_{0-12}	area under the plasma concentration-time curve from time 0 to 12 hours
AUC_{extrap}	area under the plasma concentration-time curve from the time of last observed concentration extrapolated to infinity expressed as a percent
AUC_{inf}	area under the plasma concentration-time curve from time 0 extrapolated to infinity
AUC_{last}	area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration
BP	blood pressure
bpm	beats per minute
BUN	blood urea nitrogen
BVA	Blood Volume Analyzer
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL	total body clearance
CL_{r0-2}	Renal clearance from time 0 to 2 hours
CL_{r2-12}	Renal clearance from time 2 to 12 hours
CL_{r0-12}	Renal clearance from time 0 to 12 hours
C_{last}	last measurable concentration
C_{max}	maximum observed concentration
CRU	clinical research unit
CV%	coefficient of variation
CYP	cytochrome P450
DILI	drug-induced liver injury
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate

Abbreviation	Definition
EOS	end-of-study
FD001	5 kD carboxymethyl dextran
FD003	150 kD carboxymethyl dextran
FD004	liquid visible fluorescent injectate agent, combination of FD001 and FD003 in a 3:1 ratio
FDA	Food and Drug Administration
FIH	first-in-human
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GM	geometric mean
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HR	heart rate
ICH	International Council for Harmonisation, formerly the International Conference on Harmonisation
IEC	independent ethics committee
INR	international normalized ratio
IRB	institutional review board
IV	intravenous(ly)
MDRD	Modification of Diet in Renal Disease
mGFR	measured glomerular filtration rate
MRT_{inf}	mean residence time extrapolated to infinity
NADPH	nicotinamide adenine dinucleotide phosphate
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 ECG
PV	plasma volume
QRS (interval)	the interval between the Q wave and the S wave in the heart's electrical cycle as measured by ECG; principal deflection in the ECG
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 ECG
QTc (interval)	QT interval corrected for heart rate
QTcF (interval)	QT interval corrected for heart rate using Fridericia's formula

Abbreviation	Definition
RR (interval)	the time elapsed between 2 consecutive R waves as measured by ECG
SAE	serious AE
SAP	statistical analysis plan
S_{cr}	serum creatinine
SRC	safety review committee
$t_{½}$	terminal elimination half-life
TEAE	treatment-emergent AEs
t_{max}	time at which the maximum concentration was observed
ULN	upper limit of normal
VFI	visible fluorescent injectate
V_z	volume of distribution during the terminal phase
V_{ss}	volume of distribution at steady state

STUDY ADMINISTRATIVE STRUCTURE

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Certified clinical laboratories will be used for this study. Contact information will be specified in a separate study reference manual.

3 INTRODUCTION AND BACKGROUND

3.1 Introduction

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.

3.2 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.

3.3 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 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 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.

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, subjects 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 the previous first-in-human (FIH) or phase 2a clinical study. Should this effect be observed, subjects will be provided with disposable protective sunglasses to wear until the effect is reversed.

3.4 Summary of Findings to Date

3.4.1 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.

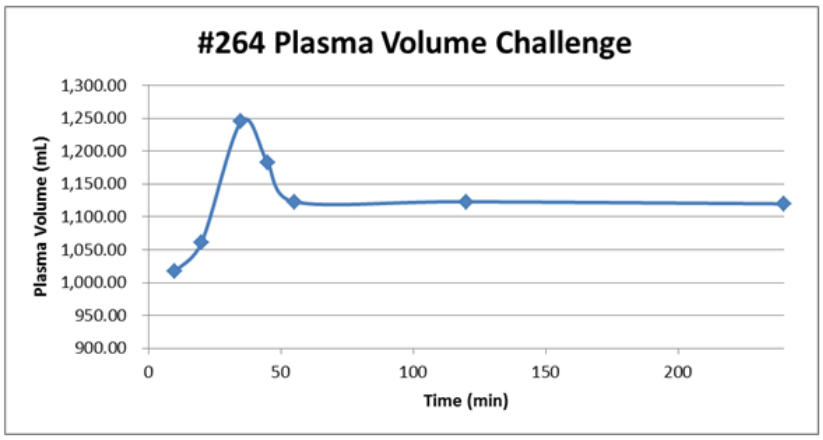
Results from the CYP inhibition studies suggest that:

- 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]).

FAST has completed pre-clinical work in dogs to assess the robustness and effectiveness of the PV measurement. Normal saline (350 mL) was infused over 10 minutes into a healthy, anesthetized, fasting dog. Multiple blood samples were drawn pre- and post-infusion to allow FAST to monitor the change in volume status in response to the volume challenge. The results (Figure 3.1) showed an immediate increase in volume, followed by a slower return to equilibrium.

Figure 3.1 The results of a FAST Plasma Volume Measurement Volume Challenge Test in a healthy dog model.



3.4.2 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 subjects were enrolled with 8 subjects (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 subjects completed the study as planned and were included in the safety analysis. Overall 9 subjects (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 subjects). No related TEAEs were observed after the highest dose. Only 2 subjects (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 subject 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 subjects 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.

3.4.3 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 Table 3.2.

Table 3.2 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).

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

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

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 subjects, 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 subjects.

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 subjects 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 subjects at 504 hours. The observed half-life of FD003 ranged from 90 hours for healthy subjects 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.

3.5 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 (CL), 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.

3.6 Study Rationale

3.6.1 Rationale for Dose Selection

Doses of VFI for this study were selected based on results of the Phase 1 FIH study in healthy subjects and in the Phase 2a study in subjects 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 subjects 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 planned Phase 2b study, a single administration of VFI 47 mg/3 mL is expected to be well tolerated and easily measured in all subjects. Additionally, for Cohort 2, where subjects will receive 2 doses of VFI, each dose being 47 mg/3mL it is expected that it will be well tolerated. As the FD001 marker is cleared and the doses are separated by 24 hours, the maximum level of FD001 in the subjects after the second dose is not anticipated to exceed the dose of 52.5 mg of FD001 given in Phase 2a. Also, as the amount of FD003 has been reduced in the new VFI formulation, a second dose of VFI will result in values close to the amount previously dosed to subjects. In the Phase 2a clinical study a dose of 22.5 mg of FD003 was given, for Phase 2b the maximum amount of FD003 to be given following repeat dosing is 24 mg. This repeat-dose strategy was agreed to by FDA in written correspondence dated February 24, 2016. The predose sample taken on Day 2 for Cohort 2 will be analyzed and provide the residual amount of both FD001 and FD003. The FD003 residuals can then be subtracted as would 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, these normal subjects are not anticipating a residual level of FD001 in subjects on Day 2 that would affect 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.

Similarly, in Cohort 1 a volume challenge is being administered to mimic the dynamic volume changes that most likely prompt the use of the FAST PV Technology. By administering a volume challenge to healthy individuals FAST will be able to observe the effectiveness of the FAST VFI with regards to dynamic changes in the plasma volume over time in a safe and controlled manner.

3.6.2 Rationale for Study Design

This is a Phase 2b prospective, open-label study designed to evaluate the safety, tolerability, PK, and pharmacodynamics (PD) of the FAST PV and mGFR Technology in healthy adult subjects and patients with renal impairment.

Male and female subjects will be enrolled in the study. As no sufficient data are available on a potential influence of the investigational medicinal products 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. Though the FAST VFI is a substituted dextran product, and has shown little evidence of antigenicity in nonclinical studies, a cautious approach to dosing sequence is proposed.

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

3.7 Hypotheses

This is an exploratory pilot investigation without any formal statistical hypotheses. All results will only be interpreted descriptively. Correlation, analysis and Bland-Altman plots will be used to compare the FAST mGFR with Iohexol clearance.

4 OBJECTIVES

4.1 Primary Objectives

- To assess the safety and tolerability of VFI (employing the FAST PV and mGFR Technology) compared to Iohexol (employing Iohexol clearance methods) in healthy subjects and patients with impaired renal function
- To evaluate the PK of FD001 and FD003 in healthy subjects and patients with impaired renal function
- To evaluate the PK of FD001 and FD003 in healthy subjects receiving 2 doses of VFI separated by 24 hours to evaluate the reproducibility of the FAST mGFR Technology

4.2 Secondary Objectives

- To evaluate the relationship between FAST PV and mGFR Technology and standard clinical formulaic estimates of PV in healthy subjects
- To evaluate and compare GFRs determined from FAST PV and mGFR Technology and Iohexol clearance methods in healthy subjects and patients with impaired renal function
- To evaluate the GFR measurements in relation to current clinical eGFR equations in healthy subjects and patients with impaired renal function
- To evaluate the FAST PV Technology's ability to measure changes in vascular plasma volume resulting from a fluid challenge after the baseline PV measurement

5 STUDY DESIGN

5.1 Study Design and Overview

This is a Phase 2b, prospective, open-label study designed to evaluate the safety, tolerability, PK, and PD of FAST PV and mGFR Technology in healthy subjects and patients with varying degrees of renal impairment.

For Cohorts 1 and 2, administration of the study drug will occur within 21 days of screening. Eligible subjects will be admitted to the CRU on Day -1, at which time assessments will be performed, and results from both screening and Day -1 (inclusion/exclusion criteria, body weight, height [screening only], body mass index [BMI], medical and surgical history, physical examination, vital signs and pulse oximetry, 12-lead ECG, clinical laboratory tests, renal function assessment, follicle-stimulating hormone [FSH; screening only] and pregnancy test [females only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

For Cohorts 3 and 4, administration of the study drug will occur within 21 days of screening.

Eligible patients enrolled at UAB will be admitted to the CRU on Day 1, at which time assessments will be performed and results from both screening and predose administration testing (inclusion/exclusion criteria, body weight, height [screening only], BMI, medical and surgical history, physical examination, vital signs and pulse oximetry, 12-lead ECG, clinical laboratory tests, renal function assessment, FSH [screening only] and pregnancy test [females only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

Eligible patients enrolled into Cohort 3 at ICON Early Phase Services, LLC will be admitted to the CRU on Day -1 and will remain resident in the CRU through discharge on Day 2. Eligible subjects will be admitted to the clinical research unit (CRU) on Day -1, at which time assessments will be performed, and results from both screening and Day -1 (inclusion/exclusion criteria, body weight, height [screening only], body mass index [BMI], medical and surgical history, physical examination, vital signs and pulse oximetry, 12-lead ECG, clinical laboratory tests, renal function assessment, follicle-stimulating hormone [FSH; screening only] and pregnancy test [females only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

The planned dose cohorts are presented in Table 5.1.

Table 5.1 Planned Dose Cohorts

Cohort	eGFR	VFI Dose	Number of Doses	Comparator Dose	Number of Doses	Number of Subjects
1*	≥ 60 mL/min/1.73 m^2	47 mg/3 mL	1	---	---	Up to 8
2	≥ 60 mL/min/1.73 m^2	47 mg/3 mL	2	Iohexol 5 mL	1	Up to 8
3	≥ 30 and < 60 mL/min/1.73 m^2	47 mg/3 mL	1	Iohexol 5 mL	1	Up to 8
4	≥ 15 and < 30 mL/min/1.73 m^2	47 mg/3 mL	1	Iohexol 5 mL	1	Up to 8

*Subjects in Cohort 1 will receive a volume challenge

Cohort 1:

Eligible subjects ≥ 50 kg in weight will receive a single dose of VFI followed 130 minutes later by a 350 mL infusion of 5% albumin over 30 minutes on Day 1. Eligible subjects < 50 kg in weight will receive a single dose of VFI followed 130 minutes later by a maximum infusion of 5% albumin of 7 mL/kg over 30 minutes on Day 1. Subjects will remain resident in the CRU for at least 24 hours after VFI administration for safety, PK, and PD assessments. Subjects will return to the CRU for an end-of-study (EOS) visit on Day 21 (± 1 day).

Cohort 2:

Eligible subjects will receive 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. Subjects will remain resident in the CRU for at least 24 hours after the second VFI administration for safety, PK, and PD assessments. Subjects will return to the CRU for follow-up visits on Days 5 (± 1 day), 9 (± 1 day), and 16 (± 1 day) and an EOS visit on Day 22 (± 1 day).

Cohorts 3 and 4:

Eligible patients will receive a single dose of VFI followed 160 minutes later by a single dose of Iohexol on Day 1.

Patients enrolled into Cohort 3 at ICON Early Phase Services will remain resident in the CRU for at least 24 hours after VFI administration for safety, PK, and PD assessments. Patients will return to the CRU for follow-up visits on Days 4 (± 1 day), 8 (± 1 day), and 15 (± 1 day), and an EOS visit on Day 21 (± 1 day).

Patients enrolled into Cohorts 3 or 4 at UAB will remain in the CRU through the 12-hour sample collection after VFI administration for safety, PK, and PD assessments. Patients will stay at a local hotel near the CRU and will return to the CRU on Day 2 for 24-hour sample collection. Patients will return to the CRU for follow-up visits on Days 4 (± 1 day), 8 (± 1 day), and 15 (± 1 day), and an EOS visit on Day 21 (± 1 day).

Refer to Table 2.1, Table 2.2, and Table 2.3 for the Schedules of Assessments and Procedures.

5.1.1 Interim Safety Data Review

A safety review committee (SRC) will be convened at predetermined interval(s) to review safety data from this study. The SRC will consist of, at minimum, the investigator, medical monitor, and sponsor medical representative. If warranted, the committee may expand to include PK, PD, and other relevant subject matter experts. The SRC may request additional data including relevant PK and PD data, if necessary, and may recommend adequate safety measures including early termination of the study based on the discretion of the investigator or the criteria set forth in Section 6.4. However, should there be no AEs of clinical significance and no evidence of signals upon review of the safety data, subsequent dosing of subjects and patients will be permitted.

5.1.2 Duration of Study

Subject participation for Cohort 1 is expected to last up to 43 days, including a screening period of up to 21 days and an on study period of up to 22 days (consisting of a single treatment period and an EOS visit).

Subject participation for Cohort 2 is expected to last up to 44 days, including a screening period of up to 21 days and an on study period of up to 23 days (consisting of a single treatment period, follow-up visits, and an EOS visit).

Patient participation for Cohorts 3 and 4 is expected to last up to 43 days, including a screening period of up to 21 days and an on study period of up to 22 days (consisting of a single treatment period, follow-up visits, and an EOS visit).

5.1.3 Definition of Study Completion

End-of-study procedures will be performed as specified in the Schedules of Assessments and Procedures (Table 2.1, Table 2.2, and Table 2.3); subjects who terminate from the study early will have EOS procedures performed at the time of discontinuation. Subjects 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. The subject's participation in the study will end once all study assessments and follow-up have been completed.

5.1.4 End of Study

The end of the study is defined as the date when the last subject has completed all study procedures up to and including the EOS/early termination visit as outlined in the Schedules of Assessments and Procedures (Table 2.1, Table 2.2, and Table 2.3).

6 SELECTION AND WITHDRAWAL OF SUBJECTS

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

6.1 Inclusion Criteria

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

All Subjects/Patients:

1. Males or females ≥ 18 and ≤ 75 years of age.
2. Females must be of non-childbearing potential (eg, postmenopausal [defined as cessation of regular menstrual periods for at least 1 year confirmed by follicle-stimulating hormone (FSH) test (21.7 to 153 units/mL)] or surgically sterile by hysterectomy, bilateral oophorectomy, or tubal ligation [documentation required] or be using a medically acceptable form of birth control [eg, a barrier method, intrauterine device, or hormonal contraception]) from screening until 30 days after last dose.
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 study drug, 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 study drug.
4. Males must agree to not donate sperm from screening through 90 days after administration of the last dose of study drug.
5. Subjects must be able to communicate effectively with the study personnel.
6. Subjects must be informed of the nature and risks of the study and give written informed consent prior to screening.
7. Body mass index ≥ 18.0 and ≤ 40.0 kg/m^2, and body weight ≥ 40 kg at screening and CRU admission.

$$BMI = \text{weight (kg)}/(\text{height [m]})^2$$

Subjects with Normal Renal Function (Cohorts 1 and 2):

8. Subjects must have an eGFR calculated using the CKD-EPI Equation (Levey et al 2009) ≥ 60 mL/min/1.73 m^2 consistent with the National Kidney Foundation stages 1 and 2 of CKD.
9. Subjects must be otherwise healthy and without clinically significant abnormalities as assessed by review of medical and surgical history, physical examination, vital signs measurement, pulse oximetry, ECG, and clinical laboratory evaluations conducted at

screening and CRU admission. A single repeat measurement/test may be performed, at the discretion of the physician, to confirm vital signs, pulse oximetry, ECG, and clinical laboratory tests abnormalities (ie, to confirm that a subject is eligible).

Patients with Renal Impairment (Cohorts 3 and 4):

10. Patients in Cohort 3 must have an eGFR calculated using the CKD-EPI Equation (Levey et al 2009) ≥ 30 and < 60 mL/min/1.73 m^2 consistent with the National Kidney Foundation stages 3a and 3b of CKD.
11. Patients in Cohort 4 must have an eGFR calculated using the CKD-EPI Equation (Levey et al 2009) ≥ 15 and < 30 mL/min/1.73 m^2 consistent with the National Kidney Foundation stage 4 of CKD.
12. Patients may have clinical, ECG, and clinical laboratory findings consistent with their degree of renal dysfunction as assessed by review of medical and surgical history, physical examination, vital signs measurement, pulse oximetry, ECG, and clinical laboratory evaluations conducted at screening and CRU admission. A single repeat measurement/test may be performed, at the discretion of the physician, to confirm vital signs, pulse oximetry, ECG, and clinical laboratory tests abnormalities (ie, to confirm that a patient is eligible).

6.2 Exclusion Criteria

Subjects 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 in consultation with the medical monitor if they develop any of the exclusion criteria during the study.

All Subjects/Patients:

1. Female subject/patient is pregnant or breastfeeding.
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 at increased risk as determined by the investigator.
4. History of surgery or major trauma within 12 weeks of screening, or surgery planned during the study.
5. History of alcohol abuse, illicit drug use, significant mental illness, physical dependence to any opioid, or any history of drug abuse or addiction within 6 months of screening.
6. Systolic blood pressure (BP) < 90 or > 160 mmHg or diastolic BP < 50 or > 100 mmHg measured after the subject has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes at screening or CRU admission. A single repeat measurement may be performed to confirm vital signs abnormalities (ie, to confirm that a subject is eligible).

7. Heart rate (HR) < 40 or > 105 beats per minute (bpm) measured on ECG after the subject has been resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes at screening or CRU admission. A single repeat measurement may be performed to confirm ECG abnormalities (ie, to confirm that a subject is eligible).
8. Has taken other investigational drugs or participated in any clinical study 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 study drug in this study.
9. Prior exposure to VFI or known allergy to dextrans.
10. History of any clinically significant allergic or negative reactions, side effects, or anaphylaxis to iodine, dyes, shellfish, isotopes, or dextran molecules.
11. Significant blood loss (> 450 mL) or has donated 1 or more units of blood or plasma within 6 weeks prior to study participation.
12. Positive screen for human immunodeficiency virus (HIV)-1 or HIV-2 antibodies, hepatitis B virus surface antigen (HBsAg), or hepatitis C virus (HCV) antibody.
13. Diagnosis of acquired immune deficiency syndrome (AIDS).
14. History of nephrectomy or kidney transplant.
15. History of liver disease or screening liver function tests which exceed 2 × the upper limit of normal (ULN) or an albumin value of < 2.5 mg/dL.
16. International normalized ratio (INR) > 1.5 × ULN or a platelet count < 50,000.
17. Presence of significant hemodynamic instabilities.
18. Loss of Limb- as this alters the validity of eGFR and estimated PV equations.
19. 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.
20. Involved in the planning or conduct of this study.
21. Inability to tolerate venipuncture or poor venous access.
22. Unwilling to abstain from alcohol from 24 hours prior to admission until discharge from the CRU.
23. Unwilling to abstain from strenuous activity (as assessed by the investigator) from 72 hours prior to admission until discharge from the CRU.
24. Unwilling or unlikely to comply with the requirements of the study.

25. Use of over the-counter (OTC) drugs (including herbal preparations) within 3 days or 5 half-lives, whichever is longer, prior to administration of the first dose of study drug.
26. Use of nonsteriodal anti-inflammatory drugs within 3 days or 5 half-lives prior to administration of the study drug.

Subjects with Normal Renal Function (Cohorts 1 and 2):

27. Positive urine drugs of abuse or serum alcohol screen.

Patients with Renal Impairment (Cohorts 3 and 4):

28. Clinically significant ongoing bleeding, changing hemoglobin, or experienced significant blood loss within 2 weeks prior to administration of the first dose of study drug.
29. Positive urine drugs of abuse (except for prescribed medications) or serum alcohol screen.
30. Use of inotropes or vasopressors.
31. Any other condition or prior therapy that, in the investigator's opinion, is likely to deteriorate.

6.3 Subject Withdrawal

Subjects are free to discontinue the study at any time, for any reason, and without prejudice to further treatment. The investigator may remove a subject if, in the investigator's judgment, continued participation would pose unacceptable risk to the subject 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 noncompliance
- Safety concern by the investigator or sponsor
- Lost to follow-up

Subjects 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 withdrawal, the investigator will promptly notify the medical monitor and will make every effort to complete the EOS assessments. All withdrawn subjects 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.

Subjects will be withdrawn from the study if they meet specific stopping criteria for the study (see Section 6.4).

6.4 Early Termination of Study

6.4.1 Cohort Stopping Criteria

Dosing in a cohort will be suspended if any of the following occur:

- 2 or more subjects in the dose cohort meet individual stopping criteria (Table 6.1)
- 2 or more subjects in the dose cohort experience treatment-related clinically relevant (ie, moderate and/or severe) AEs
- 1 or more subjects in the dose cohort experience a treatment-related SAE

6.4.2 Individual Subject Stopping Criteria

The individual subject stopping criteria are presented in Table 6.1.

Table 6.1 Individual Stopping Criteria

Clinical Laboratory Parameter	Stopping Criteria
AST	> 5 × ULN
ALT	> 5 × ULN
Increase of S_{cr}	> 1.5 × baseline; > 1.5 × ULN

Subjects who have met one of the stopping criteria will continue with follow-up visits and PK/PD sampling unless the investigator or subject decides not to proceed.

Dosing of a cohort may be suspended on lesser changes in these parameters or change in other laboratory parameters at the discretion of the SRC.

7 TREATMENT OF SUBJECTS

7.1 Identity of Investigational Product

The identity of the investigational product is presented in Table 7.1.

Table 7.1 Investigational Product

Investigational Product	Dosage Form	Strength	Dose
Visible Fluorescent Injectate	Solution for injection	35mg FD001/12mg FD003	47 mg/3 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.

7.2 Identity of Comparator Products

The identity of the comparator products is presented in Table 7.2.

Table 7.2 Comparator Products

Comparator Product	Dosage Form	Strength	Dose
Omnipaque 300 (Iohexol)	Solution for injection	647 mg of Iohexol equivalent to 300 mg of organic iodine per mL	5 mL

Comparator products will be sourced by the CRU.

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.

7.3 Treatments Administered

Each subject will receive at least a single dose of the VFI, which will be administered IV through a bolus injection. Subjects in Cohort 1 will receive an infusion of 5% albumin (up to a maximum volume of 7 mL/kg over 30 minutes) 130 minutes after the dose of VFI in order to measure the increase in the subject's plasma volume using the FAST PV Technology.

An IV catheter will be inserted into the subject's arm. A single dose of VFI 47 mg/3 mL will be administered as a bolus injection through the catheter. A single dose of Iohexol 5 mL will be administered as a bolus injection through the same catheter 160 minutes after administration of VFI in Cohorts 2 through 4. Subjects in Cohort 2 will receive a second dose of VFI 47 mg/3 mL bolus injection 24 hours after the initial dose of VFI in order to assess the repeatability of the FAST mGFR Technology.

For safety reasons, the first 2 subjects in Cohort 2 will receive their second dose of VFI at least 24 hours before VFI dosing in the remaining subjects. Administration of VFI will be performed in a sequential manner with at least 60 minutes between each subject.

A low-protein snack will be served prior to administration of the VFI (eg, toast, bagel or primarily carbohydrate based small portion). Water will be allowed as desired.

Standard meals will be served for Cohorts 1 and 2. Meals appropriate for the patient's medical condition will be served for Cohorts 3 and 4.

7.4 Method of Assigning Subjects to Treatment Groups

Eligible subjects/patients will be enrolled in a cohort based on renal function and will be assigned sequential subject numbers.

7.5 Measurements of Treatment Compliance

Investigational product will be administered by qualified healthcare professionals at the CRU as designated by the investigator. Details regarding dosing, including the dose administered and the date and time of dosing, will be recorded. The prepared syringes to be used for VFI and Iohexol injections will be weighed and documented before and after dosing in order to calculate the exact amount of product administered.

7.6 Investigational Product Storage, Accountability, and Retention

7.6.1 Storage Conditions

The VFI will be stored in a refrigerator at 2 to 8°C (36 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 to 25°C (68 to 77°F); excursions are permitted to 15 to 30°C (59 to 86°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. The procedure for VFI preparation is located in Appendix A.

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

The investigator will ensure that all the study drugs are stored and dispensed in accordance with Food and Drug Administration (FDA) regulations concerning the storage and administration of investigational drugs.

7.6.2 Drug Preparation

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

7.7 Packaging and Labeling

7.7.1 Study Drug

The study drugs 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 New Drug Limited by US Federal Law for Investigational Use Only."

7.7.2 Blinding of Treatment Assignment

Not applicable. This is an open-label study.

7.8 Concomitant Medications and Other Restrictions

7.8.1 Concomitant Medications

For subjects in Cohorts 1 and 2, no concomitant medications (prescription, OTC, and herbal) may be administered, other than hormonal birth control, during the study unless they are prescribed by the investigator for treatment of specific clinical events (ie, AEs). However, for patients in Cohorts 3 and 4, 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 electronic case report form (eCRF). 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.

7.8.2 Other Restrictions

Subjects 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 discharge from the CRU.
- Subjects are not permitted to consume alcohol from 24 hours prior to admission until discharge from the CRU.
- Subjects are not permitted to consume any food and drink from outside of the CRU while residing at the CRU.
- Subjects must comply with the CRU smoking policy, if applicable.
- Subjects are not permitted to participate in any other clinical trial or donate blood or plasma while participating in this clinical trial.

8 STUDY ASSESSMENTS AND PROCEDURES

Subjects will undergo study procedures and assessments at time points specified in the Schedules of Assessments and Procedures (Table 2.1, Table 2.2, and Table 2.3).

8.1 Medical and Surgical History

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

8.2 Demographic Characteristics

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

8.3 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.

8.4 Pharmacokinetic Assessments

8.4.1 Drug Concentration Measurements

Plasma PK samples will be collected at time points specified in the PK and PD Sampling Schedules (Table 2.4, Table 2.5, and Table 2.6). Blood sample collection, processing, and shipping details will be outlined in a separate study reference manual.

Plasma concentrations of FD001 and FD0003 will be determined using a validated fluorometric assay at MRIGlobal.

Urine concentrations of FD001 will be determined using a validated method and concentration of FD001 will be multiplied by urine volume to determine the amount of excreted FD001.

8.4.2 Pharmacokinetic Parameters

Pharmacokinetic parameters will be calculated from the plasma and urine concentration data using noncompartmental methods (Phoenix WinNonlin®, Version 6.3 or later, Pharsight Corporation, St. Louis, MO) and actual sampling times. The following PK parameters will be determined:

C_{max}	Maximum observed plasma concentration
t_{max}	The time that C_{max} was observed
AUC_{0-12}	Area under the plasma concentration-time curve from time 0 to 12 hours; calculated using the linear/log trapezoidal rule (FD001 only)

AUC_{last}	Area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration; calculated using the linear/log trapezoidal rule
AUC_{inf}	Area under the plasma concentration-time curve from time 0 extrapolated to infinity; calculated as $AUC_{last} + C_{last}/\lambda_z$, where C_{last} is the last measurable concentration
AUC_{extrap}	Area under the plasma concentration-time curve from the time of last observed concentration extrapolated to infinity expressed as a percent
$t_{½}$	Terminal elimination half-life; calculated as $\ln(2)/\lambda_z$
λ_z	Terminal elimination rate constant
CL	Total body clearance; calculated as $Dose/AUC_{inf}$
CL_{r0-2}	Renal clearance from time 0 to 2 hours; calculated as Ae_{0-2}/AUC_{0-2}, where Ae_{0-2} is the amount of FD001 excreted in the urine from time 0 to 2 hours (FD001 only)
CL_{r2-12}	Renal clearance from time 2 to 12 hours; calculated as Ae_{2-12}/AUC_{2-12}, where Ae_{2-12} is the amount of FD001 excreted in the urine from time 2 to 12 hours (FD001 only)
CL_{r0-12}	Renal clearance from time 0 to 12 hours; calculated as Ae_{0-12}/AUC_{0-12}, where Ae_{0-12} is the amount of FD001 excreted in the urine from time 0 to 12 hours (FD001 only)
V_z	Volume of distribution during the terminal phase; calculated as CL/λ_z
V_{ss}	Volume of distribution at steady state; calculated as $MRT_{inf} \times CL$, where MRT_{inf} is the mean residence time extrapolated to infinity
Ae_{0-2}	Amount of FD001 excreted unchanged in the urine from time 0 to 2 hours
Ae_{2-12}	Amount of FD001 excreted unchanged in the urine from time 2 to 12 hours
Ae_{0-12}	Amount of FD001 excreted unchanged in urine from time 0 to 12 hours

Extrapolation methods will be used in Cohort 2, as needed, to correct concentration data after a second dose of FAST VFI in order to calculate PK parameters. Alternative methods (ie, compartmental modeling) may be used if extrapolation methods are inadequate. Computational details will be provided in a separate statistical analysis plan (SAP).

For the calculation of the FAST mGFR and PV in Cohort 2, residual levels of FD003 will be measured in the predose sample and those values subtracted from post-second-administration sample values to allow for a clean calculation of PV from dose 2. As these are healthy subjects we are not anticipating any level of residual FD001 that would affect the calculation of GFR.

8.5 Pharmacodynamic Assessments

Plasma PD samples for determination of PV and GFR will be collected at time points specified in the PK and PD Sampling Schedules (Table 2.4, Table 2.5, and Table 2.6). Blood sample collection, processing, and shipping details will be outlined in a separate study reference manual.

Additional PD assessments will be performed at time points specified in the Schedules of Assessments and Procedures (Table 2.1, Table 2.2, and Table 2.3).

8.5.1 Pharmacodynamic Parameters

8.5.1.1 Plasma Volume

Plasma volume will be determined using FAST PV Technology for Cohorts 1 through 4.

Additionally, PV will be estimated using Nadler's formula (Nadler et al 1962) for Cohorts 1 through 4. Nadler's formula (Nadler et al 1962) is as follows:

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

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

8.5.1.2 Glomerular Filtration Rate

Measured GFR will be determined using FAST mGFR Technology for Cohorts 1 through 4.

Glomerular filtration rate will also be determined using the Iohexol clearance test, a widely accepted research gold standard for measuring GFR in patients with stable renal function, for Cohorts 2 through 4. Plasma concentrations of Iohexol will be determined using a validated high performance liquid chromatography assay.

Additionally, eGFR will be calculated using the MDRD (Levey et al 1999), CKD-EPI (Levey et al 2009) and Cockcroft-Gault (Cockcroft et al 1976) equations for Cohorts 1 through 4.

The MDRD equation (Levey et al 1999) is as follows:

GFR (mL/min/1.73 m^2) = 175 × $(S_{cr})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American)

The CKD-EPI equation (Levey et al 2009) for eGFR for specified race, sex, and S_{cr} in mg/dL and µmol/L is presented in Table 8.1 and Table 8.2, respectively.

The Cockcroft-Gault equation (Botev et al 2009) is as follows:

GFR = [(140-Age) x (Weight) x 0.85 if female]/72 x S_{Cr}

Table 8.1 Chronic Kidney Disease Epidemiology Collaboration Equation for Estimating Glomerular Filtration Rate Expressed for Specified Race, Sex, and Serum Creatinine (mg/dL)

Race	Sex	S_{cr} (mg/dL)	Equation (age in years for ≥ 18)
Black	Female	≤ 0.7	GFR = 166 × $(S_{cr}/0.7)^{-0.329}$ × $(0.993)^{Age}$
Black	Female	> 0.7	GFR = 166 × $(S_{cr}/0.7)^{-1.209}$ × $(0.993)^{Age}$
Black	Male	≤ 0.9	GFR = 163 × $(S_{cr}/0.9)^{-0.411}$ × $(0.993)^{Age}$
Black	Male	> 0.9	GFR = 163 × $(S_{cr}/0.9)^{-1.209}$ × $(0.993)^{Age}$
White or other	Female	≤ 0.7	GFR = 144 × $(S_{cr}/0.7)^{-0.329}$ × $(0.993)^{Age}$
White or other	Female	> 0.7	GFR = 144 × $(S_{cr}/0.7)^{-1.209}$ × $(0.993)^{Age}$
White or other	Male	≤ 0.9	GFR = 141 × $(S_{cr}/0.9)^{-0.411}$ × $(0.993)^{Age}$
White or other	Male	> 0.9	GFR = 141 × $(S_{cr}/0.9)^{-1.209}$ × $(0.993)^{Age}$

Source: National Institute of Diabetes and Digestive and Kidney Diseases, Estimating Glomerular Filtration Rate (2015)

Table 8.2 Chronic Kidney Disease Epidemiology Collaboration Equation for Estimating Glomerular Filtration Rate Expressed for Specified Race, Sex, and Serum Creatinine (µmol/L)

Race	Sex	S_{cr} (µmol/L)	Equation (age in years for ≥ 18)
Black	Female	≤ 61.9	GFR = 166 × $(S_{cr}/61.9)^{-0.329}$ × $(0.993)^{Age}$
Black	Female	> 61.9	GFR = 166 × $(S_{cr}/61.9)^{-1.209}$ × $(0.993)^{Age}$
Black	Male	≤ 79.6	GFR = 163 × $(S_{cr}/79.6)^{-0.411}$ × $(0.993)^{Age}$
Black	Male	> 79.6	GFR = 163 × $(S_{cr}/79.6)^{-1.209}$ × $(0.993)^{Age}$
White or other	Female	≤ 61.9	GFR = 144 × $(S_{cr}/61.9)^{-0.329}$ × $(0.993)^{Age}$
White or other	Female	> 61.9	GFR = 144 × $(S_{cr}/61.9)^{-1.209}$ × $(0.993)^{Age}$
White or other	Male	≤ 79.6	GFR = 141 × $(S_{cr}/79.6)^{-0.411}$ × $(0.993)^{Age}$
White or other	Male	> 79.6	GFR = 141 × $(S_{cr}/79.6)^{-1.209}$ × $(0.993)^{Age}$

Source: National Institute of Diabetes and Digestive and Kidney Diseases, Estimating Glomerular Filtration Rate (2015)

8.6 Safety Assessments

8.6.1 Adverse Events

Subjects will be monitored for AEs from administration of the first dose of study drug through the EOS visit. Refer to Section 9 for additional details.

8.6.2 Clinical Laboratory Tests

A certified laboratory will be utilized to process and provide results for the clinical laboratory tests listed in Table 8.3. The baseline laboratory test results for clinical assessment for a particular test will be defined as the last measurement prior to the initial dose of study drug.

During the screening period, if a subject 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 8.3 Clinical Laboratory Tests

Hematology	Coagulation	Serum Chemistry	Urinalysis[a,b,e]
Hematocrit	INR[c]	Sodium	Nitrites
Hemoglobin		Potassium	Leukocytes
Red blood cell count		Chloride	Protein
Mean corpuscular volume		Albumin	Glucose
White blood cell count		Aspartate aminotransferase	Ketones
Platelet count (estimate not acceptable)		Alanine aminotransferase	Urobilinogen
		Alkaline phosphatase	Bilirubin
Neutrophils (absolute)		Creatine phosphokinase	Blood
Eosinophils (absolute)		Glucose[d]	pH
Basophils (absolute)		Total cholesterol[d]	Specific gravity
Lymphocytes (absolute)		BUN	
Monocytes (absolute)		Creatinine	
		Total bilirubin	
		Calcium	
		Total protein	
		Phosphorous	

a. In case of clinically significant findings, a microscopic analysis will be performed.
b. If 1+ or greater protein is noted in the urinalysis, a calculated urine protein to creatinine ratio will be performed.
c. Cohorts 3 and 4 only.
d. Should be collected after fasting for a minimum of 8 hours.
e. A urine protein to creatinine ratio will be performed only at predose on testing day and 24 hours postdose. Subjects will be fasting prior to this measurement.

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 to be discontinued from the study, (3) requires a subject to receive treatment, or (4) requires a change or discontinuation of the study drug (if applicable).

8.6.3 Other Tests

The following tests will be performed:

- Urine drugs of abuse (at a minimum, cocaine, cannabinoids, amphetamines, opiates, barbiturates, benzodiazepines, and phencyclidine [ICON site only) and serum alcohol screens
- Serology tests (ie, HIV-1 and HIV-2 antibodies, HBsAg, and HCV antibody)
- Follicle-stimulating hormone (females only; as needed to confirm postmenopausal status)
- Pregnancy Test (females only)

During the screening period, urine drugs of abuse and serum alcohol screens may not be repeated for eligibility.

8.6.4 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 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 to be discontinued from the study, (3) require a subject to receive treatment, or (4) require a change or discontinuation from the study drug (if applicable) will be recorded as AEs.

8.6.5 Pulse Oximetry

Oxygen saturation will be measured by pulse oximetry.

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

8.6.6 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 carefully documented in the subject's eCRF.

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

8.6.7 Electrocardiograms

Subjects must be resting quietly in a supine position or in the most recumbent position possible for at least 5 minutes before the ECG is obtained. The following ECG parameters will be recorded: HR, RR, PR, QRS, and QT and QT interval corrected for HR using Fridericia's formula (QTcF) intervals.

Electrocardiogram assessment will include interpretation of the tracings, eg, rhythm, presence of arrhythmia or conduction defects, morphology, any evidence of myocardial infarction, or ST segment, T-wave, and U-wave abnormalities. The investigator or designee is responsible for reviewing and over-reading the ECG interpretation, for assessing whether the ECG machine interpretation findings are accurate, appropriate, normal or abnormal, and for providing corrected interpretations as appropriate. In addition, any abnormal ECGs will be assessed for clinical significance.

Additional ECGs may be obtained if clinically indicated. Any additional relevant data obtained by the investigator during the course of this study will be supplied to the sponsor.

For any ECG that the investigator considers clinically significant, the investigator will:

- Repeat the ECG.
- Follow-up ECG(s) will be obtained if any significant abnormalities are detected after dose administration to document resolution and as clinically indicated.
- Record as an AE any ECG that (1) is confirmed and the investigator considers clinically significant, (2) requires a subject to be discontinued from the study, (3) requires a subject to receive treatment, or (4) requires a change or discontinuation of the study drug (if applicable).

8.6.8 Appropriateness of Safety Assessments

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

A crash cart containing epinephrine will be available for each dosing event throughout the study.

9 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.

Subjects will be monitored throughout the study for AEs, from administration of the first dose of study drug through the 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 eCRF with the status of the AE noted. All events that are ongoing at this time will be recorded as ongoing on the eCRF. 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 9.4 are to be followed for reporting SAEs.

9.1 Recording Adverse Events

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

- Assessment of whether or not the AE is a SAE (Section 9.2.1)
- Assessment of AE intensity (Section 9.2.2)
- Assessment of AE relationship to study drug (Section 9.2.3)
- 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

9.2 Assessment of Adverse Events

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

9.2.1 Serious Adverse Events

The investigator is responsible for determining whether an AE meets the definition of a SAE. A SAE is any AE occurring from ICF signing through the 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 a 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 medical monitor. Refer to "Reporting Serious Adverse Events" (Section 9.4) for details.

9.2.2 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.

9.2.3 Relationship to Study Drug

The relationship of an AE to the study drug 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 drug, or the temporal relationship of the event to study drug 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 drug, but could have been produced by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs
- Probably related: The event follows a reasonable temporal sequence from the time of drug administration, and/or follows a known response pattern to the study drug, and cannot be reasonably explained by other factors such as the subject's clinical condition, intercurrent illness, or concomitant drugs

9.2.4 Significant Adverse Events

Significant AEs are defined as marked hematological and other laboratory abnormalities (other than those meeting the definition of serious) and any events that led to an intervention, including withdrawal of study drug, dose reduction, or significant additional concomitant therapy, other than those reported as SAEs.

9.3 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 eCRFs and the subject's progress should be followed until the AE is resolved or stabilized as determined by the investigator. The medical monitor 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.

9.4 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 AND to the SAE hotline at ICON in accordance with procedures described in the study reference manual and/or SAE study-specific procedure. Site personnel will follow up with a faxed written report on the next working day as follows.

ICON SAE Hotline Telephone: (888) 723-9952

This telephone report must be followed within 24 hours by a FAX transmission of a completed SAE Report Form to the number indicated below:

Fax: (215) 616-3096

SAE Report Forms will be provided to the investigational site to assist in collecting, organizing, and reporting SAEs and follow-up information.

All SAEs should be followed to their resolution, with documentation provided to ICON on a follow-up SAE Report Form.

9.5 Pregnancy

The investigator must report any pregnancy to the medical monitor and to the ICON Medical and Safety Service within 1 business day of becoming aware of the pregnancy per pregnancy reporting procedures described in the study reference manual. 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.

9.6 Drug-induced Liver Injury

Subjects will be monitored for signs of drug-induced liver injury (DILI). Study drug 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 9.4). 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.

10 STATISTICAL CONSIDERATIONS

The statistical analysis will be conducted following the principles as specified in International Council for Harmonisation (ICH) Topic E9 (CPMP/ICH/363/96).

All statistical analyses will be described in a separate SAP.

10.1 Sample Size Calculation

Approximately 32 subjects/patients will be enrolled in 4 cohorts consisting of up to 8 subjects/patients each.

The sample size in this study is representative of Phase 2b studies of this type. Power calculations were not used to derive the sample size.

10.2 Analysis Populations

Pharmacokinetic Full Population: All subjects in Cohorts 2 through 4 who receive a known amount of study drug and have at least one quantifiable concentration of FD001 or FD003 in plasma or FD001 in urine and subjects in Cohort 1 who receive a known amount of study drug. Plasma concentrations for subjects in Cohort 1 will not contribute to the overall PK statistical analysis and will be analyzed for CL_r, C_{max}, and using the FAST mGFR and PV measurement calculations only.

Pharmacokinetic Evaluable Population: All subjects in Cohorts 1 through 4 who receive a known amount of study drug and have at least 1 estimable PK parameter.

Safety Population: All subjects who received any amount of study drug.

Pharmacodynamic Evaluable Population: All subjects in Cohorts 1 through 4 who have either a PV or GFR estimate using any of the techniques under study. Subjects in Cohort 1 will not contribute to the overall PD statistical analysis.

10.3 Endpoints

10.3.1 Primary Endpoints

10.3.1.1 Safety

- AEs and SAEs
- Clinical laboratory tests, physical examination findings, 12-lead EGs, and vital signs including pulse oximetry

10.3.1.2 Pharmcokinetics

- C_{max}
- t_{max}
- AUC_{0-12} (FD001 only)
- AUC_{last}
- AUC_{inf}
- $t_{½}$
- λ_z
- CL
- CL_{r0-2} (FD001 only)
- CL_{r2-12} (FD001 only)
- $CL_{r0\ 12}$ (FD001 only)
- V_z
- V_{ss}
- Ae_{0-2}
- Ae_{2-12}
- Ae_{0-12}

10.3.2 Secondary Endpoints

10.3.2.1 Pharmacodynamic

- PV as assessed by FAST PV and mGFR Technology
- PV as assessed by Nadler's formula
- Assess changes in PV after fluid challenge
- Assess repeatability of GFR measurements over 24 hours
- mGFR as assessed by FAST VFI and Iohexol clearance methods

- eGFR (calculated by MDRD, CKD-EPI, and Cockcroft-Gault equations)

10.4 Pharmacokinetic Statistical Analysis

For Cohorts 2 through 4, subject plasma concentration-time profiles will be listed and summarized for each analyte by nominal sampling time. Summary statistics will be listed and summarized for each PK parameter by cohort and analyte. Plasma concentrations for subjects in Cohort 1 will not contribute to the overall PK statistical analysis and will be analyzed for CL_r, C_{max}, and using the FAST mGFR and PV measurement calculations only.

10.5 Pharmacodynamic Statistical Analysis

10.5.1 Plasma Volume

The FAST PV Technology for determining PV will be compared to PV estimated using Nadler's formula. Plasma volume data will be listed and summarized by presenting descriptive statistics of raw data. The correlation as well as the associated plot will be presented for comparing PV between methods. Cosgriff et al. 1999 shows that a combination of the correlation and a histogram of the magnitude of difference is needed when no measured comparator is available.

10.5.2 Glomerular Filtration Rate

Measured GFR determined using the FAST VFI Technology will be compared to the mGFR determined using the Iohexol clearance test for Cohorts 2 through 4 only. The results will be listed by subject and summarized by presenting descriptive statistics for each cohort. Correlation analysis and Bland-Altman plots will be used to compare the FAST mGFR with Iohexol clearance.

Estimated GFR calculated using the MDRD, CKD-EPI, and Cockcroft-Gault equations will be listed by subject and summarized by presenting descriptive statistics for each cohort (Cohorts 1 through 4).

10.6 Safety Analysis

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

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

11 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.

12 QUALITY CONTROL AND QUALITY ASSURANCE

FAST BioMedical/ICON 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.

12.1 Conduct of Study

This study will be conducted in accordance with the FDA Code of Federal Regulations (CFR §312.50 and §312.56) and 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 the medical monitor and/or FAST BioMedical. Any deviation may result in the subject having to be withdrawn from the study, and may render that subject nonevaluable.

12.1.1 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. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications, food, drink, herbal remedies, or supplements that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. – either tests not done, incorrect tests done, or not done within the time frame specified in the protocol

- Procedural deviations such as incorrect storage of study drug, failure to update the ICF when new risks become known, or failure to obtain IRB approvals for the protocol and ICF revisions

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 the medical monitor and FAST BioMedical. The investigator is responsible for seeing that any known protocol deviations are recorded and handled as agreed.

12.2 Protocol Amendments

Only the sponsor may modify the protocol. Amendments to the protocol will be made only after consultation and agreement between the sponsor, the medical monitor, 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.

12.3 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 access to medical records for the monitor to verify eCRF entries. The investigator is expected to cooperate with FAST BioMedical or a designee in ensuring the study adheres to GCP requirements.

The investigator may not recruit subjects into the study until FAST BioMedical or a designee has conducted a visit at the site to conduct a detailed review of the protocol and eCRF. With agreement of FAST BioMedical, attendance at an investigator meeting may fulfill this requirement.

13 ETHICS

13.1 Institutional Review Board/Independent Ethics Committee Approval

13.1.1 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.

13.1.2 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.

13.2 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.

14 DATA HANDLING AND RECORD KEEPING

14.1 Data Reporting and Case Report Forms

14.1.1 Case Report Forms

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

14.1.2 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.

14.1.3 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.

14.2 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 product. 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.

15 ADMINISTRATIVE INFORMATION

15.1 Financing and Insurance

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

15.2 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.

16 REFERENCES

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Wang E, Meier DJ, Sandoval RM, Von Hendy-Wilson VE, Pressler BM, Bunch RM. A portable fiberoptic ratiometric fluorescence analyzer provides rapid point-of-care determination of glomerular filtration rate in large animals. Kidney Int. 2011;81(1):112–7.

Appendix A Preparation of FAST Visible Fluorescent Injectate™

1. Weigh vial of VFI prior to reconstitution. Record the pre-weight.
2. Add 4mL of sterile water to the vial of VFI over 4 seconds.
3. Gently swirl the vial for 3 minutes, avoiding allowing the liquid to reach the rubber stopper.
4. Place vial on a roller/rocker table to mix for 30 minutes.
5. Re-weigh vial and record post-weight.
6. Product is ready for drawing up of dose.