STATISTICAL ANALYSIS PLAN

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

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for

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
λ_z	terminal elimination rate constant
µmol	micromole(s)
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
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
BMI	body mass index
BP	blood pressure
bpm	beats per minute
BUN	blood urea nitrogen
CI	confidence interval
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL	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

Abbreviation	Definition
C_{max}	maximum observed concentration
CRU	clinical research unit
CSR	Clinical Study Report
CV%	coefficient of variation
dL	deciliter(s)
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EOS	end-of-study
FD001	5 kD carboxymethyl dextran
FD003	150 kD carboxymethyl dextran
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
INR	international normalized ratio
IV	intravenous(ly)
kg	kilogram(s)
L	liter(s)
m	miter(s)
MDRD	Modification of Diet in Renal Disease
min	minute(s)
mg	milligram(s)

Abbreviation	Definition
mGFR	measured glomerular filtration rate
MRT_{inf}	mean residence time extrapolated to infinity
mL	milliliter(s)
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
PT	preferred term
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
RR (interval)	the time elapsed between 2 consecutive R waves as measured by ECG
SAE	serious AE
SAP	statistical analysis plan
SD	standard deviation
S_{cr}	serum creatinine
SOC	system organ class
$t_{½}$	terminal elimination half-life
TEAE	treatment-emergent AE
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

Abbreviation	Definition
V_{ss}	volume of distribution at steady state
yr	year(s)

4 INTRODUCTION

This statistical analysis plan (SAP) is consistent with the statistical methods section of the final study protocol (Version 1.0, dated 18 May 2017) and includes additional detail of pharmacokinetic (PK), pharmacodynamic (PD) and safety summaries to be included in the clinical study report (CSR).

5 STUDY OBJECTIVES AND ENDPOINTS

5.1 Primary Objective

- To assess the safety and tolerability of VFI (employing the FAST plasma volume [PV] and measured glomerular filtration rate [mGFR] Technology) compared to Iohexol (employing Iohexol clearance methods) in healthy subjects and patients with impaired renal function
- To evaluate the PK of 5 kD carboxymethyl dextran (FD001) and 150 kD carboxymethyl dextran (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 visible fluorescent injectate (VFI) separated by 24 hours to evaluate the reproducibility of the FAST mGFR Technology

5.2 Secondary Objective(s)

- 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 methods in healthy subjects and patients with impaired renal function
- To evaluate the GFR measurements in relation to current clinical estimated glomerular filtration rate (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

5.3 Primary Endpoint(s)

5.3.1 Safety

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

5.3.2 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 (Ae0-12)

5.4 Secondary Endpoints

5.4.1 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)

6 STUDY DESIGN

6.1 General

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/patients 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 subjects/patients 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 and pregnancy test [screening only], urine drugs of abuse, and serum alcohol screen) will be reviewed to confirm eligibility.

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 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 the 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).

6.1.1 Study Population

Thirty-two male and non-childbearing potential female subjects between 18 to 75 years of age (inclusive), with a body mass index (BMI) ≥ 18.0 and ≤ 40.0 kg/m^2, and body weight ≥ 40 kg, were planned for enrollment. Subjects in Cohorts 1 and 2 will have an eGFR ≥ 60 mL/min/1.73 m^2, patients with impaired renal function in Cohort 3 will have an eGFR ≥ 30 and < 60 mL/min/1.73 m^2, and patients with impaired renal function in Cohort 4 will have an eGFR of ≥ 15 and < 30 mL/min/1.73 m^2.

6.1.2 Evaluations at Screening and Check-in

Screening and Check-in evaluations are presented in below Table 1.1 (Cohort 1), Table 1.2 (Cohort 2), and Table 1.3 (Cohorts 3 and 4) and Table 1.4 (Cohort 1), Table 1.5 (Cohort 2) and Table 1.6 (Cohorts 3 and 4) for PK and PD sampling schedule.

Table 1.1 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 1.1 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 1.4 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 2 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 1.2 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																				

Table 1.2 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**
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				
FAST VFI administration[j]				X													X				
Iohexol administration[k]									X												
FD001 and FD003 PK/PD plasma and urine samples			Refer to Table 1.5 for the PK and PD sampling schedule.																		
Iohexol PD plasma samples			Refer to Table 1.5 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 2 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 1.3 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**	**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]																			
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
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]																			
Urine protein to creatinine ratio[i]		X														X					

Table 1.3 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**	**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**
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	X	X				
FAST VFI administration			X																		
Iohexol administration								X													
FD001 and FD003 PK/PD plasma samples		Refer to Table 1.6 for the PK and PD sampling schedule.																			
Iohexol PD plasma samples		Refer to Table 1.6 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	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[a]	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 2 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 1.4 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 1.5 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 1.6 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.

6.1.3 Randomization and Treatment Assignments

This is an open-label study. Eligible subjects/patients will be enrolled in a cohort based on renal function and will be assigned sequential subject numbers.

6.1.4 Study Drug Administration

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.

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

Iohexol 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.

6.1.5 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.

6.1.6 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.

6.1.7 Pharmacokinetic Sampling Schedule

Pharmacokinetic samples to be taken for FD001 and FD003 at time points presented in Table 1.4 (Cohort 1), Table 1.5 (Cohort 2) and Table 1.6 (Cohorts 3 and 4).

6.2 Evaluation of Treatment Safety

6.2.1 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 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 result 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.

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.

6.2.2 Clinical Laboratory Assessments

Samples for laboratory assessments will be collected at time points presented in Table 1.1 (Cohort 1), Table 1.2 (Cohort 2), and Table 1.3 (Cohorts 3 and 4). The safety laboratory parameters to be assessed are presented in below Table 2.

Table 2 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.

6.2.3 Vital Signs

Vital signs assessments will be collected at time points presented in Table 1.1 (Cohort 1), Table 1.2 (Cohort 2), and Table 1.3 (Cohorts 3 and 4).

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.

6.2.4 Pulse Oximetry

Oxygen saturation will be measured by pulse oximetry at time points presented in Table 1.1 (Cohort 1), Table 1.2 (Cohort 2), and Table 1.3 (Cohorts 3 and 4).

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.

6.2.5 Physical Examinations

Complete physical examinations (including eyes, ears, nose and throat, cardiac, peripheral vascular, pulmonary, musculoskeletal, neurologic, abdominal, lymphatic, and dermatologic systems) will be performed at time points presented in Table 1.1 (Cohort 1), Table 1.2 (Cohort 2), and Table 1.3 (Cohorts 3 and 4) in the protocol, 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.

6.2.6 Electrocardiograms

Electrocardiograms will be collected at time points presented in Table 1.1 (Cohort 1), Table 1.2 (Cohort 2), and Table 1.3 (Cohorts 3 and 4).

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).

6.2.7 Other Safety Assessments

The Investigator or designee will collect a complete medical history at screening. Any updates to medical history will be recorded on Day -1.

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

Height (cm) and weight (kg) without shoes will be recorded.

6.2.8 Protocol Deviation Reporting

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

Deviations from the protocol with regard to subject enrollment or study conduct will also be noted in the source documentation.

7 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSIS

Nadler's formula in the protocol was corrected to estimate PV rather than blood volume.

Except for Nadler's formula, the conduct of the study and planned analyses are consistent with the final study protocol (Version 1.0, dated 18 May 2017).

8 QUALITY CONTROL AND QUALITY ASSURANCE METHODS FOR DATA ANALYSIS

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.

9 PHARMACOKINETIC AND PHARMACODYNAMIC ASSESSMENTS

9.1 Pharmacokinetic Assessments

9.1.1 Pharmacokinetic Analysis

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 modeling and simulation analysis plan (MSAP).

9.1.2 Treatment of Outliers

Individual plasma concentrations, if deemed to be anomalous, may be excluded from the analysis at the discretion of the pharmacokineticist following a review of available documentation (eg, bioanalytical report, clinical report) and communication with the sponsor. Any such exclusion will be clearly listed in the study report along with justification for exclusion.

Entire plasma concentration-time profiles for a patient may be excluded following review of available documentation (eg, bioanalytical report, clinical report) and communication with the sponsor. Any such exclusion will be clearly listed in the study report along with justification for exclusion.

9.1.3 Non-Quantifiable Concentrations

All concentration values reported as no results (not collected or not determined) values will be treated as missing. For the calculation of concentration summaries and plotting mean and individual concentration time profiles, below the quantifiable limit (BLQ) values will be treated as 0 prior to the first measurable concentration. After the first measurable concentration, subsequent BLQ values are treated as missing.

9.2 Pharmacodynamic Parameters

9.2.1.1 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 as follows:

$$PV_{males}[L] = (0.3669 \times (Height[m])^3 + 0.03219 \times (Body\ Weight[kg]) + 0.6041) \times (1 - hematocrit)$$

$$PV_{females}[L] = (0.3561 \times (Height[m])^3 + 0.03308 \times (Body\ Weight[kg]) + 0.1833) \times (1 - hematocrit)$$

9.2.1.2 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}[mg/dL])$^{-1.154}$ × (Age[yr])$^{-0.203}$ × (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 below Table 3.1 and Table 3.2, respectively.

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

GFR [mL/min] = [(140-Age[yr]) x (Weight[kg]) x 0.85 if female]/(72 x S_{Cr}[mg/dL])

Table 3.1 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	$GFR = 166 \times (S_{cr}/0.7)^{-0.329} \times (0.993)^{Age}$
Black	Female	> 0.7	$GFR = 166 \times (S_{cr}/0.7)^{-1.209} \times (0.993)^{Age}$
Black	Male	≤ 0.9	$GFR = 163 \times (S_{cr}/0.9)^{-0.411} \times (0.993)^{Age}$
Black	Male	> 0.9	$GFR = 163 \times (S_{cr}/0.9)^{-1.209} \times (0.993)^{Age}$
White or other	Female	≤ 0.7	$GFR = 144 \times (S_{cr}/0.7)^{-0.329} \times (0.993)^{Age}$
White or other	Female	> 0.7	$GFR = 144 \times (S_{cr}/0.7)^{-1.209} \times (0.993)^{Age}$
White or other	Male	≤ 0.9	$GFR = 141 \times (S_{cr}/0.9)^{-0.411} \times (0.993)^{Age}$
White or other	Male	> 0.9	$GFR = 141 \times (S_{cr}/0.9)^{-1.209} \times (0.993)^{Age}$

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

Table 3.2 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	$GFR = 166 \times (S_{cr}/61.9)^{-0.329} \times (0.993)^{Age}$
Black	Female	> 61.9	$GFR = 166 \times (S_{cr}/61.9)^{-1.209} \times (0.993)^{Age}$
Black	Male	≤ 79.6	$GFR = 163 \times (S_{cr}/79.6)^{-0.411} \times (0.993)^{Age}$
Black	Male	> 79.6	$GFR = 163 \times (S_{cr}/79.6)^{-1.209} \times (0.993)^{Age}$
White or other	Female	≤ 61.9	$GFR = 144 \times (S_{cr}/61.9)^{-0.329} \times (0.993)^{Age}$
White or other	Female	> 61.9	$GFR = 144 \times (S_{cr}/61.9)^{-1.209} \times (0.993)^{Age}$
White or other	Male	≤ 79.6	$GFR = 141 \times (S_{cr}/79.6)^{-0.411} \times (0.993)^{Age}$
White or other	Male	> 79.6	$GFR = 141 \times (S_{cr}/79.6)^{-1.209} \times (0.993)^{Age}$

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

10 STATISTICAL METHODS

10.1 General

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

All statistical tabulations and analyses will be done using SAS®, Version 9.3 or higher.

Unless otherwise noted, continuous variables will be summarized using the number of non-missing observations (n), arithmetic mean (mean), standard deviation (SD), median, minimum, and maximum; categorical variables will be summarized using the frequency count and the percentage of subjects in each category.

Geometric mean and Geometric CV% will be included for PK parameters, where applicable.

In the data listings, study day relative to single dose of IMP will be presented. Study day relative to single dose will be calculated as: event date – single dose date (+ 1 if event date ≥ single dose date).

Unless otherwise specified, baseline will be defined as the last non-missing measurement obtained prior day 1 treatment administration.

For safety summaries, the unscheduled and repeat assessments will not be summarized; however, all results will be included in the data listings.

10.1.1 Handling of Dropouts or Missing Data

Missing data will not be imputed.

10.1.2 Multicenter Studies

This is a multi-center study. Cohorts 1 and 2: ICON Early Phase Services, LLC and Cohorts 3 and 4: University of Alabama Birmingham.

10.1.3 Examination of Subgroups

No subgroups planned for this study.

10.1.4 Analysis Populations

Enrolled subjects: Subjects who signed informed consent and met all the criteria as per the protocol.

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 and have at least one quantifiable concentration of FD001 in plasma or urine.

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.

10.1.5 Subject Accountability

Summaries of analysis populations and subject disposition will be presented by cohort and overall and will contain the following information:

- Number of subjects screened
- Number of enrolled subjects
- Number and percent of subjects who received VFI
- Number and percent of subjects who received Iohexol
- Number and percent of subjects who received Albumin infusion
- Reason for not receiving VFI/ Iohexol/Infusion
- Number and percent of subjects who completed the study
- Number and percent of subjects who discontinued early from the study and reason for early discontinuation
- Number and percent of subjects in each of the analysis population defined in Section 9.1.4

Subject disposition data and exclusions from the analysis populations will be presented in listings.

10.1.6 Protocol Deviation Reporting

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.

The impact of each protocol deviation will be assessed. All key protocol deviations that have an impact on the analysis populations defined in Section 9.1.4 will be listed by subject.

10.1.7 Subject Demographics and Baseline Characteristics

10.1.7.1 Subject Demographics Characteristics

Demographics and baseline characteristics summary will be summarized descriptively by cohort and overall based on the Safety Set.

Summary statistics (n, mean, SD, minimum, median, and maximum) will be tabulated for the observed values for all continuous demographic parameters. Frequencies and percentages will be tabulated for categorical data. In addition, listings will also be provided.

10.1.7.2 Medical and Surgical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 20.0) and listed by subject for the Safety Population.

Medical and surgical history will be listed.

10.1.8 Analysis of Efficacy Data

No efficacy analysis planned for this study.

10.1.9 Analysis of Pharmacokinetic Data

For Cohorts 1 through 4, subject plasma and urine concentration-time profiles will be listed and summarized for each analyte by cohort and nominal sampling time. Summary statistics will be listed and summarized for each PK parameter by cohort and analyte. Only CL_r, A_e, C_{max}, and t_{max} will be computed and summarized for Cohort 1.

The plasma and urine drug concentrations will be summarized descriptively by cohort and nominal time point in tabular. In addition, the plasma and urine concentration-time will be displayed graphically on linear and semi-log scales by cohorts.

Pharmacokinetic parameters for each analyte will be listed and summarized descriptively by treatment using the PK full Population, including n, arithmetic mean, SD, minimum, median, maximum, geometric mean (GM), geometric CV%; geometric CV% calculated as the square root of the exponentiated SD of the natural log transformed data (SQRT(exp(sln^2)-1), where appropriate. For T_{max}, only n, minimum, median, and maximum will be reported.

10.1.10 Analysis of Pharmacodynamic Data

10.1.10.1 Plasma Volume (PV)

The FAST PV Technology plasma volume (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 and for change in plasma volume.

Correlation analysis will be done for comparing the PV measured using FAST PV method and PV estimated using Nadler's Formula for each cohorts. Pearson product-moment correlation coefficient, standard error with 95% confidence interval of the coefficient will be provided.

In addition scatter plot will also be provided to support the correlation analysis. Also, histogram will be provided for the magnitude of difference (PV using FAST PV - PV estimated using Nadler's formula).

10.1.10.2 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 will be done for comparing the GFR measurement determined using FAST VFI and mGFR technology with Iohexol clearance. Pearson correlation coefficient, standard error and 95% confidence interval for coefficient will be provided. In addition scatter plot will be provided for the GFR and mGFR measurements for Cohorts 2 through 4.

In addition, Bland-Altman plots will be created to compare the FAST VFI results to the mGFR determined using the Iohexol clearance test in each of the cohorts.

Additionally, 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).

10.1.11 Analysis of Safety Data

10.1.11.1 General

All safety analyses will be performed using the Safety Population.

10.1.11.2 Adverse Events

All AEs will be coded to system organ class (SOC) and preferred term (PT) using Medical Dictionary for Regulatory Activities (MedDRA Version 20.0 or higher) and presented by subject in data listings.

Adverse events with onset on or after the first dose of study drug, or with an onset before the first dose of study drug that increase in severity on or after the first dose of study drug, will be considered treatment-emergent.

The overall incidence of TEAEs (number and percentage of subjects) as well as the number of events will be summarized by cohort (and overall), mild, moderate or severe TEAE, serious TEAE, possibly or probably related TEAE, TEAEs leading to study drug discontinuation, and serious TEAEs resulting in death.

The TEAEs will be summarized and tabulated at both the subject (number [%] of subjects) and event (number of events) level:

- By cohort, SOC, and PT
- By cohort, SOC, PT, and maximum reported severity
- By cohort, SOC, PT, and maximum relationship to study drug

For the incidence at the subject level by SOC and PT, if a subject experiences more than 1 event within the same SOC and PT, only one occurrence will be included in the incidence.

For the incidence at the subject level by SOC, PT, and severity, if a subject experiences more than one event within the same SOC and PT, only the most severe occurrence will be included in the incidence.

For the incidence at the subject level by SOC, PT and relationship to study drug; if a subject experiences more than one event within the same SOC and PT, only the most closely related occurrence will be included in the incidence.

Any SAEs, AEs with outcome of death, or AEs resulting in discontinuation of study or study drug will be listed separately.

10.1.11.3 Clinical Laboratory Assessments

Clinical laboratory results will be included in the reporting of this study for hematology, coagulation, serum chemistry and urinalysis.

Absolute and change from baseline for each parameter of continuous clinical laboratory values (chemistry, hematology and urinalysis) will be summarized by cohort at each visit.

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. Any unscheduled measurements will only be displayed in the listings

Other laboratory tests such as urine drugs of abuse, serology tests & pregnancy test and follicle-stimulating hormone (FSH) will also be displayed in listings.

Laboratory data will be listed by subject at each visit. Clinical laboratory values that are outside of the normal ranges values will be flagged in the data listing. Clinical significance will be indicated in the data listing.

10.1.11.4 Vital Signs

Vital signs data including pulse oximetry will be listed by subject at each visit and time point. Absolute and change from baseline vital signs values will be summarized by cohort at each visit and time point. Any unscheduled measurements will only be displayed in the listings.

Clinical significance will be indicated in the data listing. Out of range vital signs values will be flagged in the data listing.

10.1.11.5 Electrocardiograms

Electrocardiogram data will be listed by subject at each visit and time point.

Observed value and change from baseline of ECG parameters (HR, RR, PR, QRS, and QT and QT interval corrected for HR using Fridericia's formula (QTcF) intervals) will be summarized by cohort at each nominal timepoint. Any unscheduled measurements will only be displayed in the listings. Clinical significance will be indicated in the data listing.

10.1.11.6 Physical Examinations

Physical examination findings will be presented in a subject listing.

10.1.11.7 Concomitant Medications

Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODD, Version March 2016 or later) and classified according to anatomical therapeutic chemical code levels 2 (therapeutic sublevel) and 4 (chemical sublevel).

Concomitant medications: are defined as those medications with a start date on or after the start of the infusion of study drug or started prior to the start of the infusion of study drug and were continued after the start of the infusion of study drug.

Concomitant medications will be summarized descriptively using frequency tables by ATC class and preferred name by cohort and overall on the Safety Population. Concomitant medication data will be listed.

10.1.12 Sample Size

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.

10.1.13 Interim Analysis

No interim analyses are planned for this study. For details of Interim Safety Data Review, please refer to the protocol Section 5.1.1.

10.1.14 General Conventions for Tables, Listings and Figures

For summary tables, unless otherwise specified, the number of decimal places provided in the SAS output will be based on the accuracy of the least accurate value in the raw data as follows:

n	integer
Arithmetic mean	1 decimal place more than the least accurate number in the raw data
SD	2 decimal place more than the least accurate number in the raw data
CV (%)	2 decimal places
Geometric mean	1 decimal place more than the least accurate number in the raw data
Geometric CV (%)	2 decimal places
Median	1 decimal place more than the least accurate number in the raw data
Minimum	same number of decimal places as raw data
Maximum	same number of decimal places as raw data
Confidence interval	same number of decimals as the associated statistic
Geometric mean ratio	2 decimal places

11 TABLES, FIGURES, AND LISTINGS

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Table 14.2.1.2	Summary of plasma pharmacokinetic parameters of FD001 and FD003 (PK Evaluable Population)
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Table 14.2.3.1	Summary of Pharmacodynamic Parameter- Plasma volume (PD Evaluable Population)
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Section 14.3.5	**Additional Safety Data Summaries**
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Listing 16.2.5.2	Individual Plasma Concentrations (PK full Population)
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