NCT05425719

CLINICAL STUDY PROTOCOL

A Pivotal, Open Label, Multi-Center, Safety and Pharmacokinetic Study of MB-102 (Relmapirazin) and the Use of the MediBeacon® Transdermal GFR Measurement System in Normal and Renal Compromised Subjects for the Evaluation of Kidney Function

Protocol Number:	100-003
Investigational Product:	MB-102 (Relmapirazin) / MediBeacon® Transdermal GFR Measurement System
Phase:	Pivotal
Sponsor:	MediBeacon Inc. 425 N. New Ballas Road St. Louis, MO 63141 USA
Date of Protocol	20 September 2022
Version of Protocol:	Version 4.0

Statement of Compliance

The study will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 R2 (ICH-GCP), and the applicable regulatory requirements including the National Medical Products Administration Good Clinical Practice (NMPA GCP), The Code of Federal Regulations Title 21 CFR Parts 812, 11, 50, 54 and 56 and ISO 14155: 2020 (3rd Edition) Clinical Investigation of Medical Devices for Human Subjects – Good Clinical Practice

1 Synopsis

Protocol Number:	100-003
Title:	A Pivotal, Open Label, Multi-Center, Safety and Pharmacokinetic Study of MB-102 (Relmapirazin) and the Use of the MediBeacon® Transdermal GFR Measurement System in Normal and Renal Compromised Subjects for the Evaluation of Kidney Function
Investigational Products:	MB-102 (Relmapirazin) MediBeacon® Transdermal GFR Measurement System (TGFR)
Number of Study Sites:	This study will take place at up to 10 investigational sites in the US and China
Phase:	Pivotal
Objectives:	• To establish that the MB-102 transdermal fluorescence measured GFR using the MediBeacon® Transdermal GFR Measurement System is comparable to the measured MB-102 plasma GFR • To evaluate the safety and tolerability of a single dose of MB-102 in subjects • To evaluate the safety and effectiveness of the MediBeacon® Transdermal GFR Measurement System for the non-invasive transdermal fluorescence detection of MB-102 in subjects
Study Design:	Multi-center, pivotal open-label pharmacokinetic study comparing transdermal measured GFR (tGFR) to plasma-derived indexed GFR (nGFR).
Number of Treated Subjects:	Up to 250 subjects are planned for enrollment. The US sites will plan to enroll at least 140 evaluable subjects; Chinese sites will plan to enroll at least 48 evaluable subjects. The population will be split into stratums in accordance with screening eGFR measured by the CKD-EPI equation: • Stratum 1: 50% of evaluable subjects with an eGFR ≥ 70 mL/min/1.73 m^2 • Stratum 2: 50% of evaluable subjects with an eGFR < 70 mL/min/1.73 m^2 Approximately 50% of subjects within each Stratum enrolled in the US will be Fitzpatrick skin scale (FSS) I-III and 50% of subjects will have FSS IV-VI. Subjects in the US will be enrolled across a spectrum of both eGFR (<120 - >15 mL/min/1.73 m^2) and FSS. Subjects enrolled in China will also be represented across the same spectrum of eGFR (<120 - >15 mL/min/1.73 m^2) however the FSS will not apply. This is because the Chinese subject demographic is unlikely to represent the full FSS spectrum. Subjects with incomplete assessments or major protocol deviations may be replaced. Thus, a maximum of 250 subjects may be enrolled.
Treatment:	Eligible subjects in stratums 1 and 2 will receive a single 130 mg dose of MB-102. Blood draws will be taken at pre-defined timepoints and the MediBeacon® Transdermal GFR Measurement System fluorescent measurements will be collected over a 12 hour - 24 hour period.

Study Duration per Subject:	Screening period: up to 21 days prior to dosing; dosing day, and a follow-up visit within 7+/-3 days of dosing.
Study Population:	**Main Criteria for Inclusion:** 1. Age ≥ 18 years – male or female a. Eligible female non-pregnant subjects who are either not of child-bearing potential or willing to use adequate contraception during the trial b. Males must be willing to practice abstinence or utilize adequate contraception from dosing day to at least 7 days post-dose c. For women of child-bearing potential, the subject should have a negative serum pregnancy test at screening, and agrees to one of the following acceptable contraceptive methods used consistently and correctly i.e. abstinence, oral contraceptive either combined or progesterone alone; injectable progesterone, implants of levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, IUD device or system or male partner sterilization d. Men will not donate sperm during the study and for 1 month following the last dose of study drug 2. Subjects who are capable of directly providing informed consent and who can comply with the requirements and restrictions required by the protocol 3. Adequate venous access sufficient to allow blood sampling per protocol requirements **Main Criteria for Exclusion:** 1. Subjects positive via PCR testing for COVID-19 a. Vaccinated subjects without symptoms of COVID-19 are not required to undergo PCR testing but may be tested at the discretion of the study site 2. Recent donation or loss of blood or plasma: 100 mL to 499 mL within 30 days prior to the initial dose of the study medication; or more than 499 mL within 56 days prior to the initial dose of study medication 3. Non-steroidal anti-inflammatory (NSAID) use within 3 days of MB-102 dosing 4. The subject has participated in a clinical trial and has received an investigational product within the following time ranges: prior to the first dosing day in the current study: either 30 days or 5 half-lives of the investigational product (whichever duration is longer). 5. History of skin sensitivity to adhesives (e.g. Band-Aids, surgical tape) 6. History of severe allergic hypersensitivity reactions (unacceptable adverse events) or anaphylactoid reaction to any allergen including drugs, MB-102 or other related products (intolerance to a drug is not considered a drug allergy). 7. Any characteristics such as acute or chronic medical condition or cardiac or laboratory or physical examination abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of the

	study results in the judgement of the investigator and would make the subject inappropriate for entry into this study. 8. Significant scaring, tattoos or alterations in pigmentation on the sternum or other sensor location testing areas that would alter sensor readings versus other areas of the skin 9. Any serious or uncontrolled medical disorder, active infection, physical exam finding, laboratory finding, or psychiatric condition that in the opinion of the investigator would limit the subjects' ability to complete study requirements or may put the subject at increased risk or compromise the interpretability of study results. 10. Currently receiving dialysis 11. Currently anuric 12. Subjects with positive serum pregnancy test 13. Subjects with an eGFR > 120 mL/min/1.73m^2
Primary Endpoint:	The primary endpoint is the performance measure of P30 for transdermal-derived GFR with respect to the plasma-derived indexed GFR, with a 95% confidence interval. The performance goal is 85%. Success for the study will be that the lower limit of the 95% CI is greater than 85%.
Safety Evaluation	• Safety of MB-102 will be evaluated through treatment emergent adverse events (TEAEs), where treatment emergence is defined with respect to the first administration of MB-102. • Safety of the MediBeacon® Transdermal GFR Measurement System will be evaluated through TEAEs, where treatment emergence is defined with respect to the start time of device use.
Statistical Analyses	The primary endpoint is the performance measure of P30 for transdermal-derived GFR with respect to the plasma-derived indexed GFR, with a 95% confidence interval. The performance goal is 85%. Success for the study will be that the lower limit of the 95% CI is greater than 85%. Safety of MB-102 will be evaluated through treatment emergent adverse events (TEAEs), where treatment emergence is defined with respect to the administration of MB-102. Safety of the MediBeacon® Transdermal GFR Measurement System will be evaluated through TEAEs, where treatment emergence is defined with respect to the start time of Transdermal GFR Measurement System use. Additional safety variables include physical examinations, vital sign measurements, ECGs, clinical laboratory assessments, and medication use.
Sample Size Determination	For a P30 true value of 0.95, alpha of 0.025, performance goal of 85%, power of 90%, the one-sided exact binomial test yields a sample size of 102.
Time Schedule:	Planned Start of Study: Q2 2022 Planned End of Study: Q4 2022

DOCUMENT APPROVALS

Richard Dorshow, PhD President & Chief Scientific Officer	Date
Joseph Pierro, MD Acting Head: Medical Affairs	Date
Nancy Morrison Acting Head: Regulatory Affairs	Date
Elizabeth LaPointe, MA Acting Head: Clinical Operations	Date

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2 List of Abbreviations and Definition of Terms

AE	Adverse event
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
BMI	Body Mass Index
BP	Blood pressure
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease – epidemiology collaboration
CO_2	Carbon dioxide
COVID-19	Coronavirus disease 2019
CRF	Case report form
Cl	Chloride
Cr	Creatinine
CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HCG	Human chorionic gonadotropin
HCT	Hematocrit
HEENT	Head, Ears, Eyes, Neck, Throat
I	Iodine
IB	Investigator's Brochure
ICF	informed Consent Form
ICH	International Council on Harmonisation
ICU	Intensive Care Unit
IDE	Investigational Device Exemption
IEC	Independent Ethics Committee
INR	International normalized ratio
IP	Investigational Product
ISO	International Organization for Standardization
K	Potassium
K_2EDTA	Potassium ethylenediaminetetraacetic acid
MedDRA	Medical Dictionary for Regulatory Activities
nGFR	Indexed plasma glomerular filtration rate
NIH	National Institute of Health
NSAID	Non-steroidal anti-inflammatory drug
OM	Operational Manual
ORFM	Optical Renal Function Monitor
OTC	Over the counter
PA	Physical Assessment (limited)
PE	Physical exam (full)
PI	Principal Investigator

PK	Pharmacokinetic
POC	Point of care
PP	Per Protocol
RBC	Red blood cell
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SCr	Serum creatinine
SNR	Signal to Noise Ratio
Tc-DTPA	Technetium diethylene triamine - pentaacetate
TEAE	Treatment-emergent adverse event
tGFR	Transdermal Glomerular Filtration Rate
TGFR	MediBeacon® Transdermal GFR Measurement System
UADE	Unanticipated Adverse Device Effect
Vd	Volume of Distribution
WBC	White blood cell
WHO	World Health Organization
WOCBP	Women of Child-Bearing Potential

3 Introduction

3.1 Background

Therapeutic interventions for acute kidney injury (AKI) patients are delayed due to current clinical practice of reliance on a serum creatinine blood test (SCr) for an estimation of glomerular filtration rate (GFR). Typically, 24-72 hours elapse from onset of a kidney injury event to a SCr indicating a non-normal level (Stevens et al, 2006). Since AKI affects upwards of 20% of hospitalized patients and 70% of critically ill patients in the intensive care unit (ICU), this contributes to high mortality rates (approaching 60% for those requiring renal replacement therapy [Endre at al., 2011]), development of chronic kidney disease (affecting an estimated 27 million Americans, accounting for ~ 24% of Medicare costs [NIH 2008]), and consequent progression to end stage renal disease (with an estimated 400,000 Americans on dialysis [Kidney Disease Statistics for the US]).

Glomerular filtration rate (GFR) is widely accepted as the best indicator of kidney function and is a key component of the diagnosis and management of renal impairment. A simple, accurate, and point-of-care (POC) method for determining GFR is needed, as current methods for determining GFR are either: 1) practical but lack accuracy, or 2) accurate but prohibitively complex, especially for frequent and recurrent measurements.

Current medical practice for general renal function assessment relies on estimated GFR (eGFR). This involves estimation using equations based on a single serum creatinine (SCr) measurement, or a measurement of creatinine clearance (CrCl) based on a 24-hour collection of urine plus measurement of a single SCr concentration. Although readily available and convenient, measurement of SCr as an indicator of renal function is problematic because it is a lagging indicator of current renal function, lacks sensitivity, and may be misleading because it is affected by age, state of hydration, renal perfusion, muscle mass, dietary intake, and many other anthropometric and clinical variables (Agarwal, 2003 and Verhave et al., 2005). CrCl estimation using timed urine collections can be more accurate, but problems with completeness of 24-hour urine collection often result in under or over estimation of CrCl.

Measuring GFR using urinary or plasma clearance of exogenous filtration markers is the accepted standard for evaluation of kidney function. In the past three decades, renally filtered exogenous agents such as inulin, iohexol, ^{125}Iodine-iothalamate (^{125}I-iothalamate), and technetium-99m-diethylene triamine - pentaacetate (^{99m}Tc-DTPA) have all been used to determine GFR (Stevens et al., 2006). From both a safety and effectiveness standpoint, these agents are acceptable, although they contain iodine, which can cause anaphylactoid reactions, and they can be nephrotoxic when very high volumes are used. However, they are not used routinely to determine GFR because the protocols are time-consuming, complex, and expensive, which make their repeated use impractical in many clinical settings, and they may require imaging equipment and administration of radioactive agents (Stevens and Levey, 2009). Accurate measurement of GFR at the patient's bedside is highly desirable in order to assess the patient's renal function. There is a significant need to develop a simple, accurate, and POC method for measuring GFR (Erley et al., 2001).

To address this unmet clinical need, MediBeacon is developing a combination product that requires the administration of a fluorescent tracer agent designated relmapirazin (also known as MB-102 and formerly known as MP-3180) that will be detected transdermally by a sensor which is attached to a device monitor (called the MediBeacon® Transdermal GFR Measurement System [TGFR]).

MB-102 has been engineered to be excreted from the body by the renal system, and specifically by the GFR mechanism. Normal functioning kidneys will remove this agent from the body with an approximate 2 hour half-life. Impaired functioning kidneys will take longer to excrete the agent and hence result in a longer half-life.

An initial algorithm had been developed from the Pilot 2 study Group 1 data to convert measured fluorescence half-life data to GFR. Pilot 2 study Groups 2 and 3 refined this algorithm for efficacy and robustness, as well as adding to the algorithm database.

The sensor contains the light source for excitation of the tracer agent, the agent having a peak absorption wavelength of 440 nm. The detector is able to acquire the emission of the tracer agent, the agent having a peak emission wavelength of 560 nm. The sensor for the TGFR device is small and limited to the materials necessary for excitation and acquisition, and collection and processing of the data is conducted within the monitor. The device monitor displays the TGFR data.

3.2 Nonclinical Data

Available nonclinical study data on MB-102 support that the chemistry of the drug substance/drug product, and the manufacturing of the drug substance/drug product does not present a risk to human subjects. Additional information on the 24 nonclinical studies performed on MB-102 is found in the Investigator's Brochure.

3.3 Clinical Data

A total of 266 subjects have been treated with an IV administration of MB-102 across the three pilot studies (Pilot 1A, Pilot 1B and Pilot 2 Groups 1, 2 and 3). The Pilot 1 studies enrolled subjects with an eGFR indicating normal renal function. The Pilot 2 study enrolled subjects with normal renal function and those with renal function in the CKD stage 1-5 range. In addition, a total of 22 subjects (11 healthy volunteers and 11 Crohn's patients) have received a 4 or 8 μmol/kg oral dose of MB-102 in a study evaluating the use of MB-102 in the evaluation of gut permeability (MediBeacon Protocol 300-01). The total population exposure to MB-102 as of this protocol is 288 subjects. A total of 52 subjects (18.1% of the total population across 4 studies) were reported to have adverse events (AE). All reported events were mild or moderate in severity. No severe events have been reported, nor have any serious adverse events been reported or unanticipated adverse device effects.

There have been no clinically significant findings with regard to vital signs, physical exams or ECGs. Overall MB-102 administered intravenously at a dose of 4 μmol/kg whether or not followed by a 5 mL (milliliter) dose of iohexol (Omnipaque™ 300), or at a fixed dose of 130 mg

/ 7 mL (n=44) or consumed orally at a dose of 4 µmol/kg (n=8) or 8 µmol/kg (n=14) was well tolerated in the populations studied to date.

Adverse events related to the device itself were due to the adhesive materials used to hold the ORFM (Optical Renal Function Monitor) sensor in place. All device related events were considered mild or moderate in severity. For more information, please see the Investigator's Brochure.

MB-102 equilibrates between the vasculature and tissues post IV administration. The plasma pharmacokinetics show a peak concentration at the first sample time point. In Pilot 1A and Pilot 2, the first sample time point taken was approximately 5 minutes post administration; in Pilot 1B the first sample time point taken was approximately 30 minutes post administration. The fluorescence pharmacokinetics, reflecting the agent concentration in the tissue, has a peak fluorescence intensity in the range of 30 - 90 minutes, depending on the subject. MB-102 concentration equilibration between the vasculature and the tissue occurs approximately 30 – 60 minutes after the peak.

In the Pilot 2 Groups 1 and 2, the first blood draw was taken at 5 minutes post administration of MB-102, and the last at 12 hours post administration. The transdermal fluorescence was measured at the sternum and one other location for each subject using the prototype device QuantumLeap and Radiance. For all 120 subjects over the wide range of GFR values, the following were observed:

- A two-compartment plasma pharmacokinetic profile
- A high correlation between the MB-102 and iohexol plasma derived GFR values
- A high correlation between the MB-102 plasma and transdermal fluorescence pharmacokinetics

More information is available in the Investigator's Brochure and in Appendix I.

3.4 Study Rationale

This clinical study will evaluate the use of MB-102 and the MediBeacon® Transdermal GFR Measurement System for the purpose of marketing application with the final device design.

4 Study Objectives

The objectives of this pivotal study are:

- To establish that the MB-102 transdermal fluorescence measured GFR using the MediBeacon® Transdermal GFR Measurement System is comparable to the measured MB-102 plasma GFR
- To evaluate the safety and tolerability of a single dose of MB-102 in subjects
- To evaluate the safety and effectiveness of the MediBeacon® Transdermal GFR Measurement System for the non-invasive transdermal fluorescence detection of MB-102 in subjects

5 Investigational Plan

5.1 Overall Study Design and Plan

Protocol 100-003 is a multi-center, open-label, pivotal trial studying the safety and pharmacokinetics of MB-102 and the use of the MediBeacon® Transdermal GFR Measurement System in normal and compromised renal function subjects with different skin color types comparing tGFR to plasma derived indexed GFR (nGFR).

Approximately 250 subjects are planned for enrollment. The US sites will plan to enroll 140 evaluable subjects; China sites will enroll 48 evaluable subjects. The population will be split into stratums in accordance with eGFR by the CKD-EPI equation.

Subjects will be screened and if deemed eligible, will be enrolled. On treatment day, subjects will have a transdermal sensor placed on their chest, and the MediBeacon® Transdermal GFR Measurement System will be initiated to collect background fluorescence. When this is completed, subjects will then receive a single dose of MB-102. Serial PK draws will be collected over a 12 to 24 hour period. Fluorescent measurements will also be collected during this period. For subjects with significant renal compromise, fluorescent measurements will continue until the sensor no longer detects MB-102 in the body. Following completion of the treatment period, subjects will return to the study center approximately 1 week later for a safety follow-up visit.

Subjects who do not complete the study or who have major protocol deviations may be replaced. Up to 250 subjects may be enrolled.

The global study will include 188 evaluable subjects. The US enrolment will have 140 evaluable subjects. Once the US sites have enrolled the targeted number of evaluable subjects, the US clinical sites will be closed. The US data alone may be used to support the market authorization and approval in the US. The completed OUS dataset, if available will be included in the US application.

5.2 Overview of Study Procedures

Potential subjects will be screened for eligibility within 21 days prior to planned dosing. Based on their screening eGFR (measured using the CKD-EPI equation), they will be assigned to Stratum 1 or Stratum 2:

- Stratum 1: eGFR ≥ 70 mL/min/1.73 m^2 (50% of subjects)
- Stratum 2: eGFR < 70 mL/min/1.73 m^2 (50% of subjects)

Following pre-dose procedures to ensure eligibility, one sensor will be affixed to the subject on the upper chest in the area between the shoulders (including the pectoralis and sternum area). The subject will then receive the MB-102 injection. MB-102 will be administered by IV injection over 30 – 60 seconds, followed by a saline flush IV over 30 - 60 seconds.

Stratum 1: PK sampling will be collected pre-dose and at 5, 15, 30, 60, 90, 120, 180, 240, 300,

360, 480, 600 and 720 minutes post-dose (12 hour collection period).

Stratum 2: PK sampling will be collected pre-dose and at 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 480, 600, 720 960, 1200, and 1440, minutes post-dose (24 hour collection period).

Safety assessments in the form of vital signs, safety labs, ECGs, and adverse event collection will occur during the dosing day.

Within 7 +/- 3 days of dosing, subjects will return to the study center for additional safety follow-up assessments.

5.3 Discussion of Study Design

The study is designed to validate the performance of the TGFR Measurement System.

5.4 Selection of Study Population

5.4.1 Number of Planned Evaluable Subjects

A total of 188 evaluable subjects are planned for enrollment. The population will be split into stratums accordance with eGFR by the CKD-EPI equation and location of enrollment:

- Stratum 1: 50% of subjects with an eGFR ≥ 70 mL/min/1.73 m^2
- Stratum 2: 50% of subjects with an eGFR < 70 mL/min/1.73 m^2

Subjects will be targeted across the spectrum of eGFR from normal through Stage 4 CKD. Subjects with an eGFR greater than 120 mL/min/1.73 m^2 or less than 15 mL/min/1.73 m^2 will be excluded. Efforts will be made to balance enrollment across the following eGFR levels such that each subcategory has minimum of 10 subjects:

- ≥ 90 mL/min/1.73 m^2
- 60 – 89 mL/min/1.73 m^2
- 45 – 59 mL/min/1.73 m^2
- 30 – 44 mL/min/1.73 m^2
- 15 – 29 mL/min/1.73 m^2

Of the evaluable subjects, 140 will plan to be enrolled in the United States and 48 will plan to be enrolled at sites in China.

Approximately 50% of subjects within each stratum enrolled in the US will be Fitzpatrick skin scale (FSS) I-III and 50% of subjects will have FSS IV-VI. In the US, subjects will be targeted across the full spectrum of FSS (I – VI) such that FSS cohorts I-II, III-IV and V-VI will each enroll no less than 20 subjects.

The FSS will not apply to subjects enrolled in China because the population demographic is

unlikely to support the full FSS spectrum.

If subjects are not considered evaluable, they will be replaced in the study with a total potential enrollment up to 250 subjects.

5.4.2 Inclusion Criteria

To be eligible for study entry, all subjects must satisfy all of the following criteria:

Main Criteria for Inclusion:

1. Age ≥ 18 years – male or female
    a. Eligible female non-pregnant subjects who are either not of child-bearing potential or willing to use adequate contraception during the trial
    b. Males must be willing to practice abstinence or utilize adequate contraception from dosing day to at least 7 days post-dose
    c. For women of child-bearing potential, the subject should have a negative serum pregnancy test at screening, and agrees to one of the following acceptable contraceptive methods used consistently and correctly i.e. abstinence, oral contraceptive either combined or progesterone alone; injectable progesterone, implants of levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, IUD device or system or male partner sterilization
    d. Men will not donate sperm during the study and for 1 month following the last dose of study drug
2. Subjects who are capable of directly providing informed consent and who can comply with the requirements and restrictions required by the protocol
3. Adequate venous access sufficient to allow blood sampling per protocol requirements

5.4.3 Exclusion Criteria

To be eligible for the study, subjects must not meet any of the criteria noted below:

1. Subjects positive via PCR testing for COVID-19
    a. Note: Vaccinated subjects without symptoms of COVID-19 are not required to undergo PCR testing but may be tested at the discretion of the study site
2. Recent donation or loss of blood or plasma: 100 mL to 499 mL within 30 days prior to the initial dose of the study medication; or more than 499 mL within 56 days prior to the initial dose of study medication
3. Non-steroidal anti-inflammatory (NSAID) use within 3 days of MB-102 dosing
4. The subject has participated in a clinical trial and has received an investigational product within the following time ranges: prior to the first dosing day in the current study: either 30 days or 5 half-lives of the investigational product (whichever duration is longer).
5. History of skin sensitivity to adhesives (e.g. Band-Aids, surgical tape)
6. History of severe allergic hypersensitivity reactions (unacceptable adverse events) or anaphylactoid reaction to any allergen including drugs, MB-102 or other related products (*intolerance to a drug is not considered a drug allergy*).

7. Any characteristics which, in the opinion of the investigator, makes the subject a poor candidate for participation in the clinical trial
8. Significant scaring, tattoos or alterations in pigmentation on the sternum or other sensor location testing areas that would alter sensor readings versus other areas of the skin
9. Any serious or uncontrolled medical disorder, active infection, physical exam finding, laboratory finding, or psychiatric condition that in the opinion of the investigator would limit the subjects' ability to complete study requirements or may put the subject at increased risk or compromise the interpretability of study results.
10. Currently receiving dialysis
11. Currently anuric
12. Positive serum pregnancy test
13. Subjects with an eGFR > 120 mL/min/1.73m^2

5.4.4 Removal of Subjects from Therapy or Assessments

In accordance with applicable regulations, a subject has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the investigator or at the institution where the subject's care is provided.

Reasons for withdrawal from the study may include one or more of the following:

- Withdrawal of consent
- Administrative decision by the PI or MediBeacon
- Adverse event requiring removal from the study
- Physician prescribes a treatment that would conflict with the subject's continued participation in the study

Subjects withdrawing from the study will be encouraged to complete the follow-up study visit including all safety evaluations. The reason(s) for withdrawal will be documented.

Reasonable efforts will be made to contact subjects who are lost to follow-up. All attempts and contacts must be documented in the subject's file.

The sponsor has the right to terminate the study at any time due to a safety issue or if special circumstances arise making further treatment of subjects impossible. In this event, the investigator(s) will be informed of the reason for study termination.

5.4.5 Replacement of Subjects

Should a subject meet eligibility, but is unable to be scheduled for dosing, that subject will be considered a screen failure. In addition, subjects who are unable to have a tGFR measured by the MediBeacon® TGFR Measurement System (due to premature sensor removal, for example) or a major protocol deviation (such as a dosing extravasation) may be replaced to ensure the minimum evaluable sample size is enrolled. All subjects with exposure to MB-102 will be included in the safety analysis.

5.5 Investigational Device

MediBeacon® Transdermal GFR Measurement System (TGFR) is an investigational device that is intended to measure the Glomerular Filtration Rate (GFR) in patients with normal or impaired renal function by noninvasively monitoring fluorescent light emission from an exogenous tracer agent (MB-102) over time.

Site staff will place and affix the sensors for all enrolled subjects. One sensor will be attached via adhesive pad to the upper chest. An adhesive clip, surgical tape or other means may also be used to alleviate tension on the sensor cord. Additional instructions for sensor placement will be provided in the Study Procedure Manual.

The only parts of the MediBeacon® TGFR that come into contact with the subject are the single-use adhesive pad, and the optical window. The optical window protrudes very slightly from the adhesive, ensuring consistent contact with the subject's skin. The sensors remain in contact with the skin and are held in place by means of a self-adhesive pad, typical of those used for disposable ECG patches. The sensor remains affixed to the subject for at least 12.5 hours for Stratum 1 and up to 24 hours for Stratum 2.

5.5.1 Premature Dislodgment of a TGFR Sensor from the Skin

Should a sensor be prematurely dislodged from the subject's skin during the measurement period and after injection with MB-102, it will not be replaced and the subject will be discontinued from the measurement period of the study (but will remain in the trial to be followed for safety). End of treatment assessments should be collected (safety labs, vitals, and ECG). If a sensor dislodges from the skin during the baseline period (prior to MB-102 injection), a new sensor may be used and the study re-started.

The TGFR is designed to require a clean baseline (pre-injection of MB-102) in order to accurately measure GFR. If MB-102 is in the body at the time a sensor is attached, it will not allow measurement.

5.5.2 Sensor Detachment from the TGFR Monitor

If a sensor becomes detached even temporarily from the TGFR monitor, the data will be compromised and may not be used in the final analysis. However, the sensor should be re-plugged into the monitor and the prompts on the screen should be followed so that fluorescent measurements can continue. This occurrence should be recorded as a major protocol deviation if the tGFR is NOT reported, and a minor deviation if the monitor displays the tGFR.

5.5.3 Identity of Investigational Device

The MediBeacon® Transdermal GFR Measurement System is manufactured by MediBeacon Inc. (St. Louis, MO, USA). All packaging and labeling operations will be performed according to current Good Manufacturing Practice (GMP), ISO 13485 and the relevant regulatory requirements.

5.5.4 Labeling

Each MediBeacon® Transdermal GFR Measurement System monitor and each sensor will be clearly labelled with a Serial Number and REF MDB (Rev).

Each device will be labelled with the following information:

CAUTION Investigational Device. Limited by United States Law to Investigational Use.

Additional labels will include the following information:

REF MDB ####### Rev 0#
LOT MDBNNNNNN-##
SN. ########
Non Sterile

5.5.5 Storage, Supply and Return/Destruction

Multiple investigational devices (monitors and sensors) will be provided to the clinical sites. Disposable items (sensors and any adhesive material) will be single-subject use only. Devices and disposable materials must be stored in a secure location and are only for use during the clinical trial. All devices (including any unused single use sensors) will be returned to the Sponsor at the conclusion of the trial. Instructions for return will be included in the Study Procedure Manual.

5.5.6 Device Accountability

All inventory received for clinical trial use will be tracked. All inventory will be returned to MediBeacon or disposed of following instructions from the Sponsor. An investigational device accountability record will be kept current and will contain:

- Subject ID (screening number)
- Device(s) monitor serial number
- Sensor serial number
- Date of dosing
- Any observations noted during the use of the device

5.6 Investigational Drug

MB-102 (relmapirazin) is a bis-serine aminopyrazine that will be used as an exogenous fluorescent Glomerular Filtration Rate (GFR) agent. This agent is excited by blue light and

emits green light.

MB-102 has the following characteristics:

- Produces fluorescence in vivo when excited by blue light, which can be reliably detected by transdermal measurement when MB-102 is injected intravenously
- Has elimination kinetics that allows determination of GFR from fluorescence measurement within a clinically useful timeframe
- Is filtered by the kidney
- Is not protein bound
- Is not expected to undergo secretion or tubular reabsorption
- Is not expected to be metabolized by the liver or other organs
- Is not expected to affect kidney function
- Has been shown to be safe in animal models, in therapeutic dose range
- Is photostable in its administration form and during sample handling and analysis
- Has been shown to be a GFR tracer agent in nonclinical animal models and early clinical studies

5.6.1 Stability of Drug Product

5.6.1.1 Active Pharmaceutical Ingredient (API)

A sixty (60) month stability-testing program conducted under Good Manufacturing Practices (GMP) for the Active Pharmaceutical Ingredient (API) Lot # 07-130227-02/02-43-01 (140 grams manufactured April 2013 by Cambrex Inc., formerly PharmaCore) used in the Pilot 1 and 2 studies was completed. Stability conditions was 25°C ± 2°C, 60% ± 5% R.H. Results at 60 months show negligible degradation in active ingredient purity and essentially no change in the total impurity amount with respect to the zero time parameters.

A sixty (60) month stability-testing program conducted under GMP for a second API lot (1 kg manufactured in August 2017 by Cambrex), API Lot # 2337-1706-00256 is on-going. Stability conditions was 25°C ± 2°C, 60% ± 5% R.H. Results show negligible degradation in active ingredient purity and essentially no change in the total impurity amount with respect to the zero time parameters at the four year time point.

A third GMP API lot of 1 kg was manufactured in June 2020 by Cambrex, Lot # 2337-2004-00667. A sixty (60) month stability-testing program has been initiated and is ongoing at conditions of 25°C ± 2°C, 60% ± 5% R.H. Results show negligible degradation in active ingredient purity and essentially no change in the total impurity amount with respect to the zero time parameters at the one year time point.

5.6.1.2 Formulated Product

A 12 month stability-testing program for the GMP formulated product Batch P02213 (~1500 mL), manufactured in April 2013 by AMRI (formerly Aptuit) from API Lot # 07-130227-02/02-43-01, which was used for the Pilot 1A and 1B studies, has been completed. Results show

negligible degradation in active ingredient purity and essentially no change in the total impurity amount with respect to the zero time parameters for the 2 - 8°C condition.

A 24 month stability-testing program for the GMP formulated product Batch P03915 (~3200 mL), manufactured in May 2015 by AMRI from API Lot # 07-130227-02/02-43-01, and which was used for Groups 1 and 2 in Protocol ORFM Pilot 2 study, has been completed. Stability condition was 2 - 8°C. Results show negligible degradation in active ingredient purity and essentially no change in the total impurity amount with respect to the zero time parameters.

Formulated product with designation Batch P04517 (~9000 mL) was manufactured under GMP conditions by AMRI using API Lot # 2337-1706-00256. This batch was placed on stability at 2 - 8°C for a three year period. At the 36 month time point, there was negligible degradation in active ingredient purity and essentially no change in the total impurity amount. This batch was used in Group 3 of Protocol Pilot 2 ORFM.

Clinical lots of drug product for use in Protocol 100-003 are in the process of being manufactured. Stability studies will be conducted on these lots.

5.6.2 Identity of Investigational Drug

MB-102 is packaged in amber vials at a concentration of 18.6 mg/mL in a 7.4 mL volume. MB-102 in formulation includes the following: sodium dihydrogen phosphate monohydrate, sodium hydroxide, sodium chloride, water for injection. Instructions for preparation of MB-102 will be included in the Study Procedure Manual.

5.6.3 Investigational Drug Labeling

MB-102 drug labels for US distribution include the following information:

Relmapirazin
7 mL of MB-102 Solution for injection only 18.6 mg/mL
BMR No: MDBP05 Lot No: P03322/1
Mfg. Date: 03/10/2022 Store at 2°C - 8°C
Caution: New Drug – Limited by Federal (United States) law to Investigational use.
Manufactured for MediBeacon

MB-102 drug labels for China distribution includes the following information:

English	China Distribution
Protocol Number: 100-003 MB-102 (Relmapirazin) 18.6 mg/mL solution for injection Each single use vial contains 7.4 mL MB-102 RELMAPIRAZIN in formulation Product for IV use only. Please refer to the protocol. Store at 2°C- 8°C Lot No: xxxxx Expiration date: TBD For clinical trial use only Sponsor: MediBeacon Inc. Manufacturer: Curia (Scotland) Ltd.	研究方案号: 100-003 MB-102 (Relmapirazin) 注射液 18.6 mg/mL 溶液 每瓶含7.4 mL MB-102 (Relmapirazin)注射液 本品仅供静脉注射使用，按照研究方案使用 2 - 8°C储存 批号: XXXXX 使用期限：TBD 仅用于临床试验 申请人：MediBeacon Inc. 生产商：Curia (Scotland) Ltd.

5.6.4 Investigational Drug Storage, Supply, Return/Destruction

Relmapirazin vials should be stored at 2°C – 8°C in a secure location. Temperature records will be maintained by the clinical site.

All study medications will be stored, reconciled and either returned to the sponsor or destroyed according to applicable regulations following authorization by MediBeacon.

5.6.5 Investigational Drug Accountability

Drug accountability will be maintained by the clinical site and will be reviewed during the study and 100% reconciled at the conclusion of the trial.

Drug accountability documentation may include, but may not be limited to, the following information:

- Receipt date
- Lot
- Expiry and/or manufacture date
- Dispensing information

5.7 Contact Procedures for Medical Issues Requiring Immediate Attention

Any medical-related issues or questions requiring immediate resolution or direction should be directed to the Sponsor medical monitor using the contact information listed below:

Joseph Pierro, MD (United States)
Cell: [redacted]

Email: [redacted]

For China, the following individual should be contacted:

Dr. Huimin Ma (Beijing, China)
Phone: [redacted]
Email: [redacted]

For submission of Serious Adverse Event information or Unanticipated Adverse Device Effects, the following email maybe used:

- Email: Safety@medibeacon.com

5.8 Prior and Concomitant Therapy

Throughout the study, investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate care, with the exception of other investigational agents or drugs that may alter renal function. All attempts should be made to maintain the subjects on a stable dosing regimen of medications from screening through the duration of the study.

Concomitant medication information will be collected for all subjects within 3 days of dosing through the follow-up visit. Additionally, use of any non-drug procedural therapies will be recorded for each subject including the nature of the therapy, the date/time of the procedure, and the reason for the therapy. Concomitant medication information taken at the time of an SAE will also be collected during the safety data follow-up.

5.8.1 Prohibited Concomitant Medications

The use of NSAIDs are prohibited 3 days prior to dosing for all subjects.

5.8.2 Stable Use of Immunosuppressive Medications

The protocol currently allows for subjects on immunosuppressant medications. Those medications that fall into this category are found in **Table 1.** This table also outlines timelines around stability of those medications required at the time of enrolment.

Table 1 Dose Stability Timelines for Subjects on Immunosuppressive Agents

Immunosuppressive Agent	Dose Stability Timeframe
Antithymocyte Globulin (rabbit)	No changes within 90 days
Basiliximab	No changes within 90 days
Infliximab	No changes within 90 days
Antithymocyte Globulin (equine)	No changes within 90 days
Rituximab	No changes within 90 days
Cyclosporine	No changes within 30 days
Tacrolimus	No changes within 30 days

6 Study Procedures

6.1 Schedule of Assessments and Procedures

The schedule of planned study assessments is shown in **Table 2.**

Table 2 Study Schedule of Events

Period	Pre-Screen (optional)	Screening	Dosing Day	Dosing Day	Dosing Day	Dosing Day	Follow-up Visit
		21 days to day -2 prior to Dosing	*Pre-dose*	*Dosing (time 0)*	*During 12 – 24 hours post IP injection*	*Completion of 12 hours or up to 24 hours post IP injection*	*7 days +/- 3 days*
Informed Consent[a]	X	X					
Inclusion / Exclusion		X	X				
Demographics		X					
Medical History		X	X				
Pregnancy Test for WOCBP[b]		X	X				
PE or Limited PA[c]		X	X				X
Vital Signs[d]		X	X		X	X	X
Height and Weight[e]		X	X				
Clinical Labs[f]		X	X			X	X
Mexameter measurements			X				
Transdermal Data Collection[g]			X	X	X	X	
Drink water [h]			X	X			
Administration of MB-102[i]				X			
PK blood collection[j]			X		X	X	
ECG[k]		X	X			X	
Concomitant Therapies[l]		X	X		X	X	X
Adverse Events[m]			X	X	X	X	X
Document Fitzpatrick Skin Color Type[n]	X	X					

Table 2 Legend

a. Pre-screening consent may be used to evaluate skin color only. Full informed consent is required prior to any study related procedures being conducted.
b. Negative pregnancy serum human chorionic gonadotropin (hCG) test at screening for women of childbearing potential (WOCBP) is required for eligibility. On dosing day, a urine pregnancy test will be conducted for WOCBP who agree to use an acceptable method of birth control
c. A full physical examination (PE) is required at the screening visit. A full PE or limited physical assessment (PA) may be conducted prior to dosing. (Limited PA includes assessments of HEENT, respiratory, cardiovascular, abdominal systems). A full or limited exam may be conducted at the follow-up visit.
d. Vital signs include blood pressure, respiration rate, heart rate and temperature. Note: temperature is collected at screening, pre-dose, at approximately 12 hours post-dose and at the follow-up visit (and not during multiple timepoints on dosing day). Whenever possible, the subject should be resting for approximately 5 minutes prior to all measurements. Vital signs will be collected at screening, pre-dose and at the following post-dose timepoints: 90, 250 and 500, 710 minutes (±5 min). For subjects followed for 24 hours, an additional measurement will be taken at 1450 minutes.
e. Height and Weight should be measured at screening; weight will also be measured on the day of dosing.
f. Clinical laboratory assessments will be analyzed by a central laboratory and consist of standard chemistry, hematology and urinalysis parameters per **Table 3.** Coagulation panel will be conducted at Screening only.
g. MediBeacon® Transdermal GFR Measurement System data collection will commence prior to dose administration. At least 15 minutes prior to dosing, sensors should be placed on the subject and the MediBeacon® Transdermal GFR Measurement System started. Data collection will continue through 12 - 24 hours of PK sampling. Once an tGFR is measured, sensors may be removed while PK sampling continues.
h. The subject should be given 240 mL of ambient water to drink the night prior (if brought in the night before dosing), the morning of dosing, and just prior to MB-102 dose administration
i. MB-102 will be administered as a dose of 130 mg (7 mL) via IV injection over a 30 - 60 second injection period. This will be followed by a saline flush over 30 - 60 seconds. Time of administration will be recorded.
j. Stratum 1: PK sampling will be collected pre-dose and at 5, 15, 30, 60, 90 (±2 min), 120, 180, 240, 300, 360, 480, 600 (±5 min) and 720 minutes post-dose (12 hour collection period). Stratum 2: PK sampling will be collected pre-dose and at 5, 15, 30, 60, 90 (±2 min), 120, 180, 240, 300, 360, 480, 600, 720, 960, 1200, and 1440 (±5 min) minutes post-dose (24 hour collection period). Blood draws should be via a venous catheter and will be processed in accordance with instructions available in the Study Procedure Manual.
k. A 12 lead ECG will be performed at Screening, prior to dosing, and at 700 minutes post dosing (±5 min). Subjects should be resting quietly for 10-15 minutes prior to the ECG collection.
l. Concomitant medications administered within 3 days prior to baseline through the final follow-up visit will be recorded.
m. Adverse events are collected from the time of sensor placement (for baseline measurement) through the follow-up visit.
n. Fitzpatrick Skin Scale will be assessed by evaluating the skin on the upper chest (where sensors will be placed).

6.2 Pre-screening

US sites will have the option to pre-screen study subjects to evaluate them directly for skin color in order to meet Fitzpatrick Skin Scale stratification recruitment requirements.

6.3 Screening

Screening will occur within 21 days (but at least 2 days prior to) the baseline / dosing day and will comprise of the following:

- Obtain written informed consent before any study-specific assessments are performed
- Assess eligibility against the inclusion and exclusion criteria
- Collect demographic data and medical history; review concomitant medications
- Obtain screening non-fasting laboratory tests including chemistry, hematology, and coagulation profile (See **Section 7.1.2.6** and **Table 3**). Perform serum pregnancy test in women of child-bearing potential and collect a urine sample for urinalysis.
- Perform COVID-19 testing for unvaccinated subjects or per study center requirements
    - Vaccinated subjects without COVID-19 symptoms are not required to have a COVID-19 test performed
- Collect height and weight measurements
- Perform a full physical exam and collect vital signs including temperature (**Sections 7.1.2.7** and **7.1.2.8**)
- Perform a 12 lead ECG (**Section 7.1.2.9**)
- Document Fitzpatrick Skin Scale Color Type by examining the upper chest (locations for possible sensor placement) (see **Appendix A)**

6.4 Night before Dosing Day

For institutions that prefer to bring study subjects to the institution the night before dosing day, the following procedures may be performed:

- Review subject eligibility
- Update medical history with any new information or issues since screening
- Perform a limited physical assessment
- Record concomitant medications taken within 3 days prior to dosing day

- Perform a urine pregnancy test for WOCBP
- Have the subject consume 240 mL of ambient water

6.5 Dosing Day: Baseline Assessments

Baseline assessments will be performed prior to dosing (with the exception of those procedures noted in Section 6.3 that were performed the night before for those sites that bring subjects in the night prior to dosing).

- Update medical history with any new information or issues occurring since the initial screening visit (if not performed the night before).
- Collect the subject weight (BSA and BMI will be calculated based on screening height and baseline weight measurements)
- Review subject eligibility
- Confirm that the subject has consumed 240 mL of ambient temperature water at least 1 hour prior to dosing time.
- Perform a limited physical assessment and collect vital signs (including temperature) (**Sections 7.1.2.7** and **7.1.2.8**).
- Perform a baseline ECG (**Section 7.1.2.9**).
- Record concomitant medications taken within 3 days of the dosing visit
- For WOCBP, a urine pregnancy test will be performed
- Clean and prepare the body areas for sensor attachment using an alcohol pad. Excess hair should be clipped (not shaved) from the area for sensor.
- Obtain melanin (only) measurements with the Mexameter (**Section 7.1.2.11**)
- A venous catheter should be placed in one arm for PK blood draws. A pre-dose baseline draw should be collected.
- Obtain baseline laboratory tests including chemistry, hematology, and urinalysis (See **Section 7.1.2.6** and **Table 3**).
- Prior to dosing, the device sensor should be attached to the subject and the MediBeacon® Transdermal GFR Measurement System data acquisition initiated. During this data acquisition period, no other study procedures should be performed.
- Adverse event collection begins

- Prior to dosing, have the subject consume an additional 240 mL of ambient water

- Place a venous catheter for the purpose of MB-102 dosing in the opposite arm. An appropriately sized angio-catheter should be selected based on study subject (22 – 24 gauge).

6.6 MB-102 Dosing

The dose of 130 mg MB-102 (7.0 mL injection) should be prepared based on instructions provided in the Study Procedure Manual. Final dose calculation should be based on weight and not volume, therefore dosing syringes will be weighed prior to administration. The prepared syringe should be maintained at ambient temperature. MB-102 dosing will be performed in the opposite arm from PK draws whenever feasible.

Administration of MB-102 will be via direct intravenous (IV) injection following angio-catheter placement. Care should be taken to prevent extravasations during dosing. Ideally, a 22 – 24 gauge angio-catheter and 10 mL syringe should be used for administration. Catheter size will be collected.

- Ensure Universal Standard precautions are taken with handwashing and gloving procedures; maintain aseptic technique when handling the prepared syringe
- Check the patency of the placed catheter by injecting a small volume of saline prior to MB-102 dosing
- Administration of MB-102 should be via a steady IV injection (without hard pressure) over 30 - 60 seconds (smaller bore catheters [24 gauge] may require a slower injection)
- Following MB-102, a 10 mL saline flush should be administered over 30 - 60 seconds
- The injection site should be observed for swelling or induration or yellow discoloration indicative of a drug infiltration. Drug infiltration may also cause discomfort or pain to the study subject.
- The dosing catheter will be removed after the saline flush is completed.

To avoid extravasation/infiltration:

- Ensure no dislodgement of the needle cannula during venepuncture
- Ensure the back vein wall is not punctured during placement
- Avoid strong pressure on the syringe plunger during saline or MB-102 administration
- Perform a slow injection over the full 30 - 60 seconds.

If an extravasation occurs, record it as both an adverse event and a protocol deviation. Subjects with evidence of MB-102 or saline extravasations will be discontinued from PK collection and fluorescent measurements but followed for safety. Subjects may be replaced at the discretion of the Sponsor.

The time noted at the completion of MB-102 injection (but prior to the saline flush) is the start-time for the timing of the PK draws.

Following dosing, the syringe will be weighed in order to calculate an accurate dose of MB-102.

6.7 Post-dose Study Procedures

6.7.1 Stratum 1 (12 hour follow-up)

During the course of the study day, it is particularly important for site teams to check the patency of PK catheters. If a catheter is determined to be compromised in anyway, another catheter may be placed in the same arm as the original catheter utilizing a different vein.

PK draws will be drawn via the venous catheter at the following timepoints:

- PK draw in K_2 EDTA tube at 5 minutes (±2 minute)
- PK draw in K_2 EDTA tube at 15 minutes (±2 minute)
- PK draw in K_2 EDTA tube at 30 minutes (±2 minutes)
- PK draw in K_2 EDTA tube at 60 minutes (±2 minutes)
- PK draw in K_2 EDTA tube at 90 minutes (±2 minute)
- Vital sign collection at 90 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 120 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 180 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 240 minutes (±5 minutes)
- Vital sign collection at 250 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 300 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 360 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 480 minutes (±5 minutes)
- Vital sign collection at 500 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 600 minutes (±5 minutes)
- Perform a 12 lead ECG at 700 minutes (±5 minutes)
- Vital sign collection (includes temperature) at 710 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 720 minutes (±5 minutes)

- Obtain laboratory tests including chemistry, hematology, and urinalysis (See **Section 7.1.2.6** and **Table 3**) at 725 minutes (±5 minutes)
- At the conclusion of TGFR measurements (when tGFR is reported on the monitor), the sensor may be removed
- Adverse events will be collected based on subject reports and medical team observations

6.8 Stratum 2 (24 hour follow-up)

PK draws will be drawn via the venous catheter at the following timepoints:

- PK draw in K_2 EDTA tube at 5 minutes (±2 minute)
- PK draw in K_2 EDTA tube at 15 minutes (±2 minutes)
- PK draw in K_2 EDTA tube at 30 minutes (±2 minutes)
- PK draw in K_2 EDTA tube at 60 minutes (±2 minutes)
- PK draw in K_2 EDTA tube at 90 minutes (±2 minute)
- Vital sign collection at 90 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 120 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 180 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 240 minutes (±5 minutes)
- Vital sign collection at 250 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 300 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 360 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 480 minutes (±5 minutes)
- Vital sign collection at 500 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 600 minutes (±5 minutes)
- Perform a 12 lead ECG at 700 minutes (±5 minutes)
- Vital sign collection (includes temperature) at 710 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 720 minutes (±5 minutes)

- Obtain laboratory tests including chemistry, hematology, and urinalysis (See **Section 7.1.2.6** and **Table 3**) at 725 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 960 minutes (±5 minutes)
- PK draw in K_2 EDTA tube at 1200 minutes (±5 minute)
- PK draw in K_2 EDTA tube at 1440 minutes (±5 minutes)
- Vital sign collection at 1450 minutes (±5 minutes)
- At the conclusion of TGFR measurements (when an tGFR is reported on the monitor), the sensor may be removed.
- Adverse events will be collected based on subject reports and medical team observations
- Subjects may be discharged from the study center

6.9 Follow-up Visit

Subjects will return for a safety follow-up visit approximately 7 +/-3 days after the dosing visit. The following assessments will be performed

- Perform a full or limited physical exam and collect vital signs including temperature (**Sections 7.1.2.7** and **7.1.2.8**)
- Obtain laboratory tests including chemistry, hematology, and urinalysis (**Section 7.1.2.6** and **Table 3**).
- Record concomitant medications
- Collect adverse events; review ongoing AEs and document resolution (when applicable)

6.10 Early Withdrawal / Unscheduled Visits

Any subject that withdraws from the study early for any reason, or who needs to have an unscheduled visit performed to assess a safety issue, will have the following assessments performed:

- Obtain laboratory tests including chemistry, hematology, and urinalysis profile (**Section 7.1.2.6** and **Table 3**).
- Perform a full or limited physical exam and collect vital signs (**Sections 7.1.2.7** and **7.1.2.8**)
- Record concomitant medications

- Collect adverse events; review ongoing AEs and document resolution (when applicable)
- Record withdrawal date and reason for withdrawal, if appropriate

6.11 Repeat Subjects

Subjects may be re-enrolled in the protocol in the following situations (and if no documented safety concerns regarding exposure to the investigational device or drug):

- When a dose extravasation has occurred
- When there is a device malfunction (sensor falling off during the monitoring period and before a tGFR has been reported)
- Back-up subjects who were not dosed

Stratum 1 subjects must have a minimum of a 3-day wash-out from prior exposure of MB-102 before receiving another dose. Stratum 2 subjects must have a minimum of a 7-day wash-out from prior exposure of MB-102 before receiving another dose

In some cases, the screening visit (if outside the 21-day window) may need to be repeated. Repeat subjects will maintain the same subject ID.

Subjects can be in the study a maximum of two times and only when the above situations have occurred.

7 Efficacy and Safety Variables

7.1 Efficacy and Safety Measurements Assessed and Flow Chart

Table 2 shows the planned study assessments.

7.1.1 Efficacy Assessments

This pivotal study is not designed to address treatment efficacy rather it is to evaluate the use of MB-102 and the MediBeacon® Transdermal GFR Measurement System to provide a point of care (POC) means to collect GFR data. The specific endpoints related to calculation of GFR are discussed in **Section 9**.

7.1.2 Safety Assessments

Safety will be monitored by the assessments described below as well as the collection of AEs at every visit after screening. Planned safety assessments are detailed in the MediBeacon® Transdermal GFR Measurement System Study Schedule (**Table 2**). The investigators or site staff will be responsible for monitoring the safety of subjects who have entered this study and will be responsible for detecting, documenting and reporting events that meet the definition of an adverse event (AE) or serious adverse event (SAE). Significant findings that were noted to be

present at or prior to dosing must be included in the relevant medical history page of the eCRF. Significant new findings that begin or worsen after dosing must be recorded on the AE page of the eCRF. Subjects should be followed until the event is resolved or stabilized. AEs are collected from the time of initial sensor placement until the completion of the follow-up visit.

7.1.2.1 Adverse Events

Adverse Event Definition

An adverse event is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, temporally associated with the use of a medicinal product, whether or not related to the investigational medical device or drug.

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. For the purposes of this trial, the device is the MediBeacon® Transdermal GFR Measurement System and the sensor system including the adhesive pads for the attachment of the optical sensors to the body.

Documentation and Reporting of Adverse Events

AEs should be reported and documented in accordance with the procedures outlined below. The following data should be documented for each AE:

- Description/term of the symptom event (underlying diagnosis)
- Date of first occurrence and date of resolution (if applicable)
- Classification of “serious” or “not serious” (**Section 7.1.2.2**)
- Severity
- Causal relationship
- Action taken
- Outcome of event

For abnormal clinically significant laboratory findings, the clinical manifestation (diagnosis) should be noted as an adverse event (e.g. anemia instead of low hemoglobin). Should a clinically significant lab abnormality occur that is not part of a diagnosis, the abnormality itself may be reported as an AE. Laboratory abnormalities that meet the criteria for an AE should be followed until they have returned to normal or an adequate explanation of the abnormality is determined. When an abnormal laboratory result corresponds to a sign/symptom of an already reported AE it is not necessary to separately record the lab result as an additional AE.

Assessment of Severity

Each AE will be assigned a category by the investigator as follows:

- **Mild:** results in minimal transient impairment of a body function or damage to a body structure and/or does not require any intervention other than monitoring or OTC medication.

- **Moderate:** Results in moderate transient impairment of a body function or transient damage to a body structure and/or requires intervention, such as administration of medication or transfusion or laparoscopic or endoscopic procedure to prevent permanent impairment of a body function or damage to a body structure.

  Severe: An event which is life threatening, results in permanent impairment of a body function or permanent damage to a body structure or necessitates significant intervention, such as major surgery, to prevent permanent impairment of a body function or permanent damage to a body structure. The term severe is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as serious, which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient‘s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An event that changes in severity (gets worse or gets better) should be noted as its worst severity.

Assessment of Causality

Every effort will be made by the investigator to assess the relationship of the AE, if any, to the study device (and components), investigational drug. Causality should be assessed using the categories presented below:

Unrelated	Clinical event with an incompatible time relationship to study device or study drug, and that could be explained by underlying disease or other drugs or is incontrovertibly not related to the study device.
Unlikely	Clinical event whose time relationship to study device investigational drug makes a causal connection improbable, but that could plausibly be explained by underlying disease or other drugs.
Possible	Clinical event with a reasonable time relationship to study device or investigational drug, but that could also be explained by concurrent disease or other drugs.
Probable	Clinical event with a reasonable time relationship to study device or investigational drug and is unlikely to be attributed to concurrent disease or other drugs.

Very Likely/Certain	Clinical event with plausible time relationship to study device or investigational drug, and that cannot be explained by concurrent disease or other drugs.

AEs that are deemed related should be further assessed to determine if a relationship to the investigational drug, or device can be elucidated.

Action Taken

The investigator will describe the action taken in the appropriate section of the eCRF, as follows:

- None
- Concomitant interventional treatment(s) administered (includes medications or procedures)
- Other, specify

Outcome of the Event

The investigator will describe the outcome of the event in the appropriate section of the eCRF, as follows:

- Unknown
- Resolved
- Ongoing
- Resolved with sequelae
- Death (with date and cause reported)

Follow-up of Adverse Events

Subjects with AEs should be followed until the event is resolved or until, in the opinion of the investigator and medical monitor, the event is stabilized or determined to be chronic.

7.1.2.2 Serious Adverse Events

Serious Adverse Event Definition

An SAE is any AE from this study that results in one of the following outcomes:

An adverse event that:

a) led to death;
b) led to serious deterioration in the health or the subject, that either resulted in:
    1) a life threatening illness or injury or
    2) a permanent impairment of a body structure or a body function, or
    3) in-patient or prolonged hospitalization, or
    4) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function;
c) led to fetal distress, fetal death or a congenital abnormality or birth defect

Reporting requirements for all SAEs is detailed in **Section 7.1.2.3**.

Note: Planned hospitalization for a pre-existing condition, or a procedure required by the protocol without serious deterioration in health, is not considered a serious adverse event.

7.1.2.3 Reporting of Serious Adverse Events

Any SAE must be reported by the investigator if it occurs during the clinical study whether or not the event is considered to be related to the MediBeacon® Transdermal GFR Measurement System, or the investigational drug. All serious adverse events must be reported by the Investigator (or designee) by submitting the Serious Adverse Event Report Form (via the electronic data capture (EDC) system) or via email to MediBeacon within 24 hours of learning of the adverse event.

The Sponsor safety team, in cooperation with the Investigator, will assess all serious adverse events considered drug or device-related for potential reportability to regulatory authorities and ethical committees as an Unanticipated Adverse Device Effect (UADE) or as an expedited serious adverse event.

Note: It is also the responsibility of the Investigator to inform their Independent Ethics Committee (IEC) of other serious adverse events (i.e. non-UADEs) as required by their IEC procedures and in conformance with regulations.

SAEs must be reported within 24 hours of knowledge.

For submission of Serious Adverse Event information or Unanticipated Adverse Device Effects, the following email maybe used:

Email: Safety@medibeacon.com

The investigator should not wait to receive additional information to document fully the event before notification to the Sponsor of an SAE, though additional information may be requested. Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

Instances of death, congenital abnormality, or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the investigator at any time and linked by the investigator to this study, should be reported to MediBeacon.

The sponsor or designee will promptly notify all relevant investigators and the regulatory authorities of findings that could adversely affect the safety of subjects, impact on the conduct of the study or alter the IEC approval/favorable opinion of the study. In addition, the sponsor, will expedite the reporting to all concerned investigators, to the IECs, and to the regulatory authorities of all adverse device reactions that are both serious and unanticipated.

7.1.2.4 Pregnancies

Testing for pregnancy for a female study subject with childbearing potential must be conducted at screening (or within 7 days of dosing). In addition, a urine pregnancy test will be performed prior to dosing on treatment day. Subjects who are pregnant or intend to become pregnant during the study will be excluded. Should a pregnancy occur with a study subject, the pregnancy should be reported to MediBeacon within 24 hours of the site's knowledge. All pregnancies will be followed to term, delivery or premature termination.

7.1.2.5 Device Observations / Malfunctions

All device observations, malfunctions or failures of the MediBeacon® Transdermal GFR Measurement System will be documented in the eCRF. In addition, failures of the sensor adhesive will be specifically documented.

7.1.2.5.1 Sensor Displacement

Prior dosing with MB-102:
Should a sensor be accidently removed (or significantly bumped) prior to receipt of MB-102, it should be removed from the subject, a new sensor placed on the subject, and baseline measurements re-started.

After dosing with MB-102:
If a sensor is accidently removed (or significantly bumped) following receipt of MB-102 but before a tGFR is calculated, the subject will remain in the study for safety follow-ups only for the full duration required by their stratum (12 hours or 24 hours). PK sample collection and fluorescent measurements will be terminated, however safety assessments (vital signs, ECGs and clinical safety labs) will be performed.

7.1.2.6 Clinical Laboratory Evaluation

Clinical laboratory assessments (**Table 3**) will be performed by a central lab in the US. A central lab will be used in China, if available. If the central lab is unavailable due to COVID-19, Chinese sites may utilize their local hospital laboratory if appropriately accredited. Reference ranges will be used by the investigator to assess the laboratory data for clinical significance and changes relative to baseline measurements. Clinically significant changes in laboratory values will be documented as adverse events.

Table 3 Clinical Laboratory Analytes

<table>
<tr><th>Chemistry Panel</th><th>Hematology</th><th>Other required lab tests</th></tr>
<tr>
<td>• Alkaline Phosphatase
• Alanine Aminotransferase (ALT)
• Albumin
• Aspartate Aminotransferase (AST)
• Bilirubin (total and direct)
• Blood Urea Nitrogen (BUN)
• Calcium (Ca)
• Carbon dioxide (CO2)
• Chloride (C)
• Creatinine (SCr)
• Glucose
• Phosphorus
• Potassium
• Sodium
• Total Protein
• Uric acid
• BUN/Creatinine ratio

All lab tests are non-fasting</td>
<td>• Hematocrit
• Hemoglobin
• WBC
• RBC
• Platelets
Differentials
• Neutrophils
• Lymphocytes
• Monocytes
• Eosinophils
• Basophils

Coagulation Parameters
Only performed at screening
• PT
• INR
• aPTT</td>
<td>For WOCBP
• Serum pregnancy test (screening and a urine dipstick on day of dosing
• Urinalysis
• Protein
• Glucose
• Ketones
• Hemoglobin
• Bilirubin
• Urobilinogen
• Acetone
• Nitrite
• Leucocytes
• pH
• Specific gravity
• Color
• Bacteria
If abnormalities are seen, then microscopic examination will be performed</td>
</tr>
<tr><td colspan="3">COVID-19 Test: nasopharyngeal swab (only performed at screening for unvaccinated subjects or per study center requirements)</td></tr>
</table>

7.1.2.7 Vital Signs

Vital signs (including blood pressure, heart rate, respiration, and temperature) will be measured at screening, at multiple timepoints on dosing day(s), and at the follow-up visit. Vital signs should be collected while the subject is in a seated position and after resting for approximately 5 minutes for all measurements. Temperature is only collected at screening, pre-dose, and the completion of the dosing day, and at the follow-up visit.

7.1.2.8 Physical Examinations or Limited Physical Assessments

Physical examinations (when conducted by a physician, or other medically qualified investigator) or limited physical assessments (when performed by a nurse or other qualified individual) should include at a minimum an assessment of the head, ears, eyes, nose, throat (HEENT), respiratory, cardiovascular, and gastrointestinal systems. It is preferred that the same medical professional perform the baseline and post dosing assessments. Significant findings that were present prior to dosing must be included in the Medical History eCRF page. Significant new findings that begin or worsen after dosing must be recorded on the AE page of the eCRF.

Clinically significant abnormalities occurring post-dose should be evaluated by a physician and noted as AEs.

The study investigator will assess the subject for the occurrence of clinically significant new findings that begin or worsen after dosing which must be recorded as an AE on the eCRF page. A full physical exam should be conducted at screening and limited physical assessments may be conducted at other time points and must also be inclusive of the relevant organ or body system of interest for assessing the AE

7.1.2.9 ECGs

Standard 12 lead ECGs will be collected at screening, prior to dosing and at 700 minutes (±5 min). The following parameters will be assessed: interpretation, ventricular rate, PR interval, QRS duration, QT, and QTcF.

Subjects should be allowed to rest quietly approximately 10-15 minutes prior to their ECG. Additional ECGs may be collected if an issue or safety concern is noted.

An appropriately trained and experienced investigator will interpret the ECGs. A subject with an abnormal clinically significant ECG during screening does not qualify for the study. A subject with an abnormal not clinically significant ECG does not qualify for dosing without investigator comment on the ECG source document/eCRF.

7.1.2.10 PK Sample Collection

PK samples will be collected at the timepoints noted in **Table 2.** Recommendations for venous access and sample processing information are available in the Study Procedure Manual. PK samples should be collected in a manner to prevent haemolysis and placed on ice until processed and aliquoted into cryovials. PK samples must be stored frozen at -70 to -80ºC until shipped for PK analysis.

7.1.2.11 Mexameter® Measurements

A commercially available Mexameter® device will be provided to the sites for the measurement of skin pigment (melanin) and erythema. The device should be used in accordance with the instructions provided in the Study Procedure Manual (including pre-use calibration). Measurements should be performed on subjects when they are not in close proximity to a window and should be made on the approximate location of sensor placement. It is advisable, when required, to clip excess hair as early in the dosing day as possible to allow transient redness due to the clipping process to fade. The site should note any skin irritation due to clipping as medical history. Mexameter® measurements of melanin will be collected prior to sensor placement.

7.2 Safety Review Committee

A Safety Review Committee (SRC) will be convened and will include the medical monitor, the safety manager(s), clinical monitor(s) and clinical manager. The SRC will oversee safety data

(AEs, vitals, clinical labs, ECG data, etc.) generated by enrolled study subjects on the trial. In addition, they will review all SAEs and UADEs and clinically significant safety observations noted during the study. Oversight work and study related decisions will be performed in accordance with a written Safety Review Committee Plan.

8 Information for the Investigator: Potential Risks of the Device

Adverse events that may be anticipated in this clinical study are outlined for the investigational drug and the MediBeacon® Transdermal GFR Measurement System.

8.1 Investigational Drug

Clinical data from the Pilot 1A and 1B studies (total n = 32 subjects) included the following adverse events:

- Pilot 1A (n = 16)
    - Vasovagal reaction (1 subject)
    - Dizziness (1 subject, 2 events)

These events occurred in fasting subjects shortly after dosing and resolved on that day. No other AEs, injection site reactions, clinically significant laboratory values or significant changes in vital signs were noted during this study.

- Pilot 1B (n = 16)
    - Headaches (56.3% of subjects)
    - Erythema (at the site of the adhesive) (100% of subjects)
    - Diarrhoea (1 subject)
    - Dizziness (occurring in 1 subject following release from the study center)

Erythema was thought to be related to the adhesive used to adhere the sensor to the skin. Headaches were deemed related to the head strap required to hold the forehead sensor in place. Sensor placement on forehead was discontinued post Pilot 1B.

MB-102 is excreted in the urine. Therefore, urine discoloration (chromaturia) is expected but has not been considered an adverse event in prior studies.

In the Pilot 2 study, a total of 234 subjects with normal to compromised renal function have been exposed to MB-102. A total of 120 subjects received a dose of 4 µmol/kg followed by a 5 mL injection of iohexol. Seventy (70) subjects received a single dose only (no iohexol) of 4 µmol/kg of MB-102) and 44 have received a fixed dose of 130 mg (7 mL). All reported AEs were mild or moderate in severity and resolved without sequalae. Overall, MB-102 given in conjunction with or without iohexol was well tolerated in this subject population.

A total of 40 adverse events were reported in 28 treated subjects in Groups 1, 2 and 3 for an AE rate of 12.0% in the treated population. A total of 31 events were considered mild and 9 moderate events occurred in 5 subjects (2.5% of the population). There were no severe events

reported. There were no Serious Adverse Events (SAEs) reported during the study. There were no Unanticipated Adverse Device Effects (UADEs) reported during the study.

Four AEs, dyspepsia, chromaturia, hot flush and (facial) rash were considered related to investigational product (MB-102); all events resolved by study completion. Dyspepsia and chromaturia were also considered to be related to iohexol. Of note, chromaturia is an expected effect of MB-102 (noted in the Investigator's Brochure) and expected in all subjects as the excretion of MB-102 will color the urine of subjects receiving the product. It is not a documented effect of iohexol (per the label).

Seven (7) AEs occurring in 7 subjects were considered related to the device (sensor attachment); these included application site erythema, dermatitis, and pruritus and were considered related to the adhesive material used to hold the sensor in place. Two events of application site discoloration (melanin spot at the sensor location) were noted in 2 subjects in the SOG cohort which resolved within 24 hours of removal of the sensor. Other reported AEs (conjunctivitis, nausea, vomiting, cardiac murmur, headache, anxiety, micturition urgency, pollakiuria, urine odor abnormal, peripheral oedema, fatigue, and a pruritis event [not at the sensor location] were considered not related to MB-102, the sensor device or iohexol. Injection site reactions (bruising, erythema, extravasation) were also not considered related to MB-102.

There may also be other risks that are unforeseen at this time. For additional information on the risk profile, please reference the current Investigator's Brochure.

8.2 MediBeacon® Transdermal GFR Measurement System

There are no direct risks associated with the MediBeacon® Transdermal GFR Measurement System (monitor portion) as it does not contact the study subject. Adverse events observed from the sensors are with regard to the methods used to affix it to the study subject. Risks may include a reaction to the sensor or adhesive material or the adhesive used on the skin clip.

8.2.1 Adhesive Clip

As with the adhesive used on the sensors, there is the chance of reaction to the adhesive used to attach the clip to the skin. Reactions could include redness, itchiness or other localized skin reactions. Clips will be provided, however other means (surgical tape, tegaderm, etc.) to secure the sensor cord may be used.

8.3 Trial Procedures

Blood collection may lead to light-headedness and dizziness in some study subjects. It is recommended to ensure subjects continue to hydrate and eat during the dosing day to mitigate the effect of blood loss.

Bruising and pain at the site of the venous catheters may also be expected.

8.3.1 Dose Extravasations

Should a dosing extravasation occur, a subject may report pain or burning during the injection. Subjects should be queried during the injection regarding any sensation. Subjects with MB-102 or saline extravasations will be discontinued from PK collection and fluorescent measurements but continued in the study for safety. All extravasations will be collected as adverse events and protocol deviations.

9 Statistical Methods

9.1 Overview

The transdermal-derived GFR data (tGFR) is obtained directly from the MediBeacon® Transdermal GFR Measurement System (TGFR) for each subject. This tGFR value is compared to the subject's $nGFR_{BSA}$ for the study endpoint determination.

9.2 Analysis Populations

The following analysis populations will be defined for the study:

- **Intent-to-Measure (ITM) Population** – The ITM population will consist of all subjects who are enrolled in the study in Stratum 1 & 2 and for whom an end of session tGFR has been calculated, and who have not had any major protocol deviations.
- **Modified Intent-to-Measure (mITM) Population** – The mITM population will consist of all subjects in the ITM population with no outlier PK parameters.
- **Safety Population** – The safety population will consist of all subjects who are enrolled in the study and have been dosed with MB-102.
- **Pharmacokinetic (PK) Population** – The pharmacokinetic population will consist of all subjects who are enrolled in the study and have any PK data.

The mITM population will be the primary analysis set for all effectiveness analyses. The Safety Population will be used for the analysis of all safety variables and baseline characteristics. The PK Population will be used for the analyses of PK data.

9.3 Subject Disposition

The number and percentage of subjects who complete the study or who terminate early from the study and the reasons for early termination will be collected and reported. Subjects will be considered to have completed the study if they complete the dosing day.

The number and percentage of subjects in each analysis population will be presented. Percentages will be based on the number of subjects enrolled.

9.4 Plasma Pharmacokinetic Analyses

The pharmacokinetic objective is to characterize the single dose pharmacokinetics of MB-102

following IV bolus dosing, specifically, to obtain the clearance and total volume of distribution of MB-102 using compartmental methods in Phoenix WinNonlin (Certara, Version 8.1 or higher).

Based on prior data, it is expected that a two-compartment model will adequately describe the pharmacokinetic profiles of MB-102. The following two-compartmental pharmacokinetic parameters will be determined:

- C_{max}: The maximum plasma concentration.
- AUC: Area under the plasma concentration-time curve
- $t_{1/2\alpha}$: Half-life associated with distribution phase
- $t_{1/2\beta}$: Half-life associated with elimination phase
- $t_{1/2}$: elimination half-life from central compartment.
- V: Total volume of distribution
- CL: Systemic clearance
- V1: Volume of distribution for central compartment
- V2: Volume of distribution for peripheral compartment

The two key PK parameters from compartmental analysis are CL and V. The clearance (CL) derived from the two-compartment model is the plasma-derived GFR used to calculate the BSA indexed nGFR that is the comparison to the tGFR for the P30 calculations. V is used in the calculation of GFR indexed to volume of distribution.

All PK concentrations and parameters will be listed. As each subject will have a unique GFR measurement, summary statistics are not applicable in this analysis.

Individual figures will be provided for plasma PK concentrations. All figures will be produced on both linear-linear and logarithmic- linear scales with data and the two-compartment fit.

9.5 Primary Endpoint Analysis

The primary endpoint is the performance measure of P30 for transdermal-derived GFR with respect to the plasma-derived indexed GFR, with a 95% confidence interval. The performance goal is 85%. Success for the study will be that the lower limit of the 95% CI is greater than 85%.

9.6 Safety Analyses

Safety of MB-102 will be evaluated through treatment emergent adverse events (TEAEs), where treatment emergence is defined with respect to the first dose of MB-102.

Safety of the MediBeacon® Transdermal GFR Measurement System will be evaluated through TEAEs, where treatment emergence is defined with respect to the start time of Transdermal GFR Measurement System use (placement of the sensor on the skin).

Additional safety variables include physical examinations, clinical laboratory assessments, ECGs, and concomitant medication use.

All safety analyses will be done for subjects receiving a single dose of MB-102.

9.6.1 Treatment-Emergent Adverse Events

AEs will be coded using the *Medical Dictionary for Regulatory Activities* (MedDRA). The system organ class and preferred term for each AE will be available, along with the verbatim reported term, event start and stop dates/times, seriousness, severity, relationship to treatment/device, action taken, and event outcome. SAEs will also be documented in a case narrative format.

The overall numbers and percentages of subjects who experienced any treatment-emergent AEs (TEAEs) will be presented. TEAEs will also be summarized at the subject level by system organ class and preferred term, and at the event level by system organ class/preferred term and severity and by system organ class/ preferred term and relationship to treatment.

9.6.2 Physical Examinations/Limited Physical Assessments

The results of physical examinations will be collected as adverse events if clinically significant or as medical history (if observations are noted prior to TGFR start). Physical exam data will not be listed.

9.6.3 Clinical Laboratory Assessments

Clinical laboratory tests include clinical chemistry, hematology, urinalysis and coagulation parameters. Each parameter will be summarized using the descriptive statistics. Details will be specified in the Statistical Analysis Plan (SAP). Also, individual subject results will be assessed against an appropriate reference range if available. The manifestation of any clinically significant abnormality that develops during the study will be reported as an AE.

9.6.4 ECGs

ECG data will be listed. Clinically significant findings will be noted as adverse events.

9.6.5 Prior and Concomitant Therapies

Concomitant medications administered within 3 days prior to MB-102 dose administration and through the follow-up study visit will be listed. Medications will be coded using the World Health Organization (WHO) drug dictionary. Data will be provided in one or more subject data listings. Concomitant non-drug therapies will be listed.

9.7 Determination of Sample Size

For a P30 true value of 0.95, alpha of 0.025, performance goal of 85%, power of 90%, the one-sided exact binomial test yields a sample size of 102.

9.8 Missing Data

Missing values will not be imputed.

9.9 Analysis Phases

The global study will include 188 evaluable subjects. The US enrolment will have 140 evaluable subjects. Once the US sites have enrolled the targeted number of evaluable subjects, the US clinical sites will be closed. The US data alone may be used to support the market authorization and approval in the US. The completed OUS dataset, if available will be included in the US application.

10 Quality Assurance and Quality Control

10.1 Training

The clinical site will be trained on the protocol and study procedures. In addition, Sponsor representatives may be onsite during dosing procedures for the initial subjects at each site to assist sites with the operation of the MediBeacon® Transdermal GFR Measurement System.

10.2 Audit and Inspection

The study site and study documentation may be subject to quality assurance audits during the course of the study by the sponsor, or its nominated representative. In addition, inspections may be conducted by regulatory authorities at their discretion.

10.3 Monitoring

Data for each subject will be recorded in an electronic data capture (EDC) system. Data collection must be completed for each subject enrolled in the study.

In accordance with GCP and International Conference on Harmonization (ICH) guidelines, the study monitor will conduct monitoring visits at regular intervals. During the visits, the study monitor will perform the source document verification and verification that investigator's obligations and all applicable regulatory requirements are being fulfilled. The frequency of monitoring visits will be determined by the rate of subject recruitment.

The investigator must permit the monitor, the IEC, the sponsor's internal auditors and representatives from regulatory authorities direct access to all study-related documents and pertinent hospital or medical records, including direct access to electronic medical records for confirmation of data contained within the EDC system. Subject confidentiality will be protected at all times.

10.4 Data Management and Coding

MediBeacon or designee will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant SOPs at MediBeacon or their designee.

Study sites will enter data into a 21 CFR Part 11 compliant electronic data capture (EDC) system via a secure internet connection. Data entered into the EDC system must be verifiable against source documents at the study site. Any changes to the data entered into EDC system will be recorded in the audit trail.

Missing or inconsistent data will be queried via the EDC system to the investigator for clarification. Subsequent modifications to the database will be documented.

Fluorescent data from the TGFR System will be downloaded to hard media then transferred electronically to the Data Management designee via a secure data transfer process per the Study Procedure Manual.

All medical terms (medical history, concomitant medications and adverse events) will be coded.

10.5 Protocol Deviations

The Investigator will not deviate from the clinical protocol without the prior written approval of MediBeacon except in medical emergencies or in unforeseen, isolated instances where minor changes are made that will not increase the subject's risk or affect the validity of the study. In medical emergencies, prior written approval for protocol deviations will not be required, but MediBeacon or their designee must be notified via telephone within 24 hours of occurrence. Prospective approval of protocol deviations to recruitment and enrolment criteria (also known as protocol waivers or exemptions) are not permitted.

Major Protocol Deviation is a significant deviation from the protocol that may impact subject safety and/or affect the integrity of clinical trial data. These may include, but are not limited to the following:

- Improper or inadequate informed consent procedures
- Subject did not meet eligibility criteria
- Subject received prohibited concomitant medication(s) at any time during the trial with the exception of low dose aspirin (which would be considered a minor deviation)
- A significant protocol required assessment or procedure was not performed per protocol requirements
- Subject received incorrect study medications at any time during the trial
- Significant departure from protocol-required dosing (dosing extravasation)
- Subject developed criteria that required withdrawal from study protocol but was not withdrawn
- The sensor was removed prior to measurement of a tGFR

A Minor Protocol Deviation is a deviation that does not impact subject safety and/or compromise the integrity of the clinical trial data.

Subjects who experience a sensor dislodgement, a dosing or device issue or an extravasation (which would be considered a major deviation) will have PK sample collection discontinued and the sensor removed, but will be asked to remain in the study for safety follow-up (including dosing day safety assessments and the follow-up visit). The event that triggered the discontinuation of PK / fluorescent data collection will be considered the protocol deviation, however missing PK sample collections will not need to be documented as individual protocol deviations.

Deviations will be documented at the site and submitted to the IEC (as required) and the sponsor.

10.6 Direct Data Collection

Fluorescent measurements will be collected directly by the MediBeacon® Transdermal GFR Measurement System and will not appear on any available source documentation with the exception of the tGFR which will be recorded onto the site source document and transcribed into the EDC. The MediBeacon® Transdermal GFR Measurement System will be a closed system.

11 Records / Retention

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the investigator in a secure study file. All study records will be available for inspection by the sponsor or its representatives, or a regulatory agency.

Investigator files containing all records and reports of the investigation should be retained for a minimum of two (2) years (US) and 5 years (China) after the completion/ termination of the investigational study or the date that the records are no longer required for purposed of supporting a premarket approval application. The Sponsor will determine with the investigational site as to this exact date so that the minimum two-year record retention can be accomplished. Records may be discarded upon written notification by the Sponsor. To avoid error, the Principal Investigator should contact MediBeacon, before the destruction of any records and reports pertaining to the study to ensure they no longer need to be retained.

In addition, in accordance with the Clinical Trial Agreement, the Sponsor should be contacted if the Principal Investigator plans to leave the investigational site so that appropriate arrangements for file custodianship can be made.

The following records must be maintained in designated study administrative files:

- Clinical protocol and all amendments
- Signed Investigator Agreement
- Independent Ethics Committee Approval Letter(s)documenting review and approval of all protocols, amendments, IBs, ICFs, subject materials, etc.

- IEC-approved informed consent(s) (including any revisions)
- Current CV for all Investigators and subinvestigators including evidence of current medical licensure
- Financial Disclosure Form for all Investigators
- Site Delegation of Authority Log
- Correspondence relating to this study
- Correspondence with the IEC
- IEC membership list and/or assurance
- Investigational site authorized study personnel signature list and delegation of authority
- Device Instructions for Use
- Lab certification, including a set of the lab's normal range for tests performed
- EDC Completion Guidelines
- Subject Screening & Enrolment Log
- Site Visit Log (e.g. for Monitor sign-in)
- Site Training records
- Investigational Device and Drug Accountability Logs
- Reports (includes Adverse Event reports and final reports from Investigator and Sponsor)
- Copy of all EC approved subject-related materials and/or study advertising materials

The following records must be maintained for each subject enrolled in the study:

- Signed subject consent forms
- Electronic copy of final completed case reports (to be provided by the sponsor at the end of the study)
- Jump drive with subject's fluorescent data from the TGFR system
- All lab work and testing results
- Record of any complications, adverse events, device problems and/or malfunctions, with supporting documentation
- Procedure reports, progress notes, physician and/or nursing notes, and subject office files
- Records pertaining to subject deaths

12 Ethics

12.1 Independent Ethics Committee (IEC)

Before initiation of the study at each study site, the protocol, all protocol amendments, Investigators Brochure, the ICF, the subject information sheet and any other relevant study documentation will be submitted to the appropriate IEC. Written approval of the study and all relevant study information must be obtained before the study site can be initiated or the study device is released to the investigator. Any necessary extensions or renewals of IEC approval must be obtained, in particular, for changes to the study such as modification of the protocol, the ICF, the written information provided to subjects, and/or other procedures.

The investigator will report promptly to the IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. The investigator will submit written

summaries of the study status to the IEC annually, or more frequently if requested by the IEC. On completion of the study, the site will notify the IEC that the study has ended.

12.2 Ethical Conduct of the Study

The ICH guidelines for current GCP (ICH R2, 2016), the National Medical Products Administration Good Clinical Practice (NMPA GCP), the Code of Federal Regulations Title 21 CFR Parts 812, 11, 50, 54 and 56 and ISO 14155: 2020(E) Clinical Investigation of Medical Devices for Human Subjects – Good Clinical Practice, as well as the ethical principles defined by the World Medical Association Declaration of Helsinki, and the requirements of national device and data protection laws and other applicable regulatory requirements will be followed.

12.3 Subject Information and Consent

The investigator is responsible for ensuring that no subject undergoes any study related examination or activity before that subject has given written informed consent to participate in the study. The written consent must be given by the subject, after detailed information about the study has been given and in accordance with any national provisions on the protection of clinical study subjects. The verbal explanation will cover all the elements specified in the written information provided for the subject.

The investigator or designated personnel will inform the subject of the objectives, methods, anticipated benefits, and potential risks and inconveniences of the study. The subject should be given every opportunity to ask for clarification of any points he/she does not understand and, if necessary, ask for more information. At the end of the interview, the subject will be given time to consider the study, if this is required, or if the subject requests more time. Subjects and/or legally authorized representatives will be required to sign and date the ICF. After signatures are obtained, a copy will be provided to the subject, and a signed ICF will be kept and archived by the investigator in the investigator's study file for possible inspection by regulatory authorities, the IEC, or sponsor.

It should be emphasized to the subject that he/she is at liberty to withdraw from the study at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give or who withdraw written informed consent should not be included or continue in the study.

12.4 Financing and Insurance

This clinical trial will be sponsored by MediBeacon and its conduct will be governed under an executed Clinical Trial Agreement that will define the coverage of costs associated with study implementation by the trial site. MediBeacon will maintain appropriate clinical trial insurance at limits agreed upon in the agreement.

12.5 Subject Confidentiality

All personal data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure confidentiality of those

data, in accordance with applicable national and/or local laws and regulations on personal data protection.

Monitors, auditors, and other authorized agents of the sponsor and/or its designee, the ethics committees approving this research, and any other applicable regulatory agency(ies), will be granted direct access to the study subjects' original medical records (including electronic medical records) for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

12.6 Reporting and Publication, Including Archiving

Publication of all study results in the form of abstracts, manuscripts, presentations, posters, etc. will be developed in accordance with the Clinical Trial Agreement.

13 References

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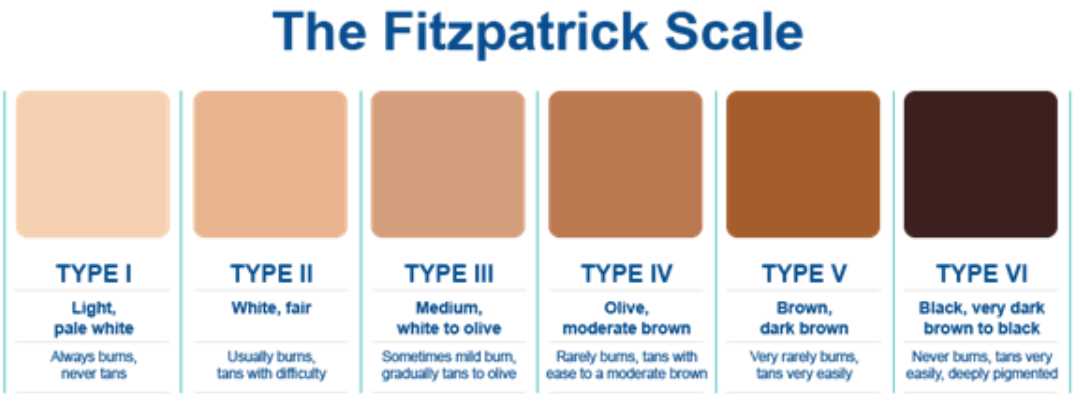
14 Appendix A Fitzpatrick Scale

The Fitzpatrick Scale should be used against the skin on the upper chest (on areas considered for sensor placement).



Source: Fitzpatrick, 1975

15 Appendix B Clinical Research Sites

Site Number	Institution	PI Name	Address	Country
401	Nucleus Network	George C. Canas, MD (Internal medicine / nephrology)	1000 Westgate Dr. Suite 149, St. Paul, MN 55114	USA
402	Research by Design, LLC	Paul W. Crawford, MD, FACP, FASN (Internal medicine / nephrology)	1080 South Western Avenue, Suite 201, Chicago, IL 60643	USA
403	Carolina Phase I Research	Matthew Hong, MD (Family medicine)	3100 Duraleigh Road, Suite 303 & 304, Raleigh, NC 27612	USA
404	Velocity Clinical Research	Margaret Chang, MD (Family medicine)	1410 S. Ridgewood Ave., Edgewater, FL 32132	USA
406	Endeavor Clinical Trials, LLC	Hernan Alfonso Salazar, DO (Family medicine)	9150 Huebner Road, Ste. 345, San Antonio, Tx 78240	USA
412	Peking University First Hospital	Ming-hui Zhao, MD (nephrology) Yimin Cui (Pharmacist)	No. 8 Xi Shi Ku Street, Xicheng District, Beijing, 100034	China
413	Affiliated Hospital of Xuzhou Medical University	Dong Sun, MD (nephrology)	No. 99, Huaihai West Road, Xuzhou City, Jiangsu Province, 2210029	China
414	Huashan Hospital Affiliated to Fudan University	Jun Xue, MD (nephrology) Jing Zhang	No. 12, Wulumugi Middle Road, Shanghai, 200040	China

Site Undergoing Qualification

Site Number	Institution	PI Name	Address	Country
415	West China Hospital of Sichuan University	Ping Feng MD (nephrology)	#37 Guoxue Alley, Wuhou District, Chengdu, Sichuan Province	China

Investigator Signature Page

Protocol Title: A Pivotal, Open Label, Multi-Center, Safety and Pharmacokinetic Study of MB-102 and the Use of the MediBeacon® Transdermal GFR Measurement System in Normal and Renal Compromised Subjects for the Evaluation of Kidney Function

Protocol Number: 100-003

Confidentiality and cGCP Compliance Statement

I, the undersigned, have reviewed this protocol, including appendices and other documents that may be considered part of the investigational plan, and I will conduct the study as described in compliance with this protocol, GCP, the ethical principles defined by the World Medical Association Declaration of Helsinki (2013), applicable regional regulations and relevant ICH guidelines.

Once the protocol has been approved by the IEC, I will not modify this protocol without obtaining prior approval of the MediBeacon Inc. and of the IEC. I will submit the protocol modifications and/or any ICF modifications to MediBeacon Inc. and IEC, and approval will be obtained before any modifications are implemented.

I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all eCRFs, laboratory samples or source documents forwarded to the sponsor. Clinical information may be reviewed by the sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

I will oversee all aspects of study conduct and will only appropriately delegate responsibilities to adequately qualified and trained staff. I will oversee the completion of source data collection such that all data collected for the study may be substantiated against accurate source medical records.

Information developed in this clinical study may be disclosed by MediBeacon Inc. to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

_____ _____

Investigator Signature Date

16 Signature of the Sponsor's Responsible Officers

16.1 Chief Scientific Officer

_____ _____
Richard Dorshow, PhD Date

MediBeacon Inc.
425 N. New Ballas Road
St. Louis, MO 63141
USA

16.2 Medical Officer

_____ _____
Dr. Joseph Pierro Date

MediBeacon Inc.
425 N. New Ballas Road
St. Louis, MO 63141
USA

Phone: [redacted] (Central Time; United States)

Appendix I Protocol 100-003 Supplemental Justifications