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MediBeacon Inc.

An Open Label, Multi-Center, Safety and Pharmacokinetic Bridging Study of MB-102 (Relmapirazin) and the Use of the MediBeacon® Transdermal GFR Measurement System using the TGFR Reusable Sensor with Disposable Adhesive Ring in Normal and Renal Compromised Subjects for the Evaluation of Kidney Function

Protocol No. 100-201, Version 4.0

Statistical Analysis Plan

Version 4.0

18 September 2024

Version History

Version	Version Date	Author/Title	Summary of Key Changes
1.0	5 January 2023	Nancy Morrison/Regulatory Consultant	Initial Release
1.1	20 April 2023	E. LaPointe Clinical Operations Consultant	Updated run-in cohort size (from 20 evaluable to 30 evaluable); clarified run-in cohort data as Tuning Data and not to be included in final analysis
2.0	23 August 2023	E. LaPointe Clinical Operations Consultant	Aligned SAP to Protocol version 3.0 (definition of study cohorts) and revised the definition of the ITM population
3.0	15 Nov 2023	E. LaPointe Clinical Operations Consultant	Revised the definition of the ITM population; added language regarding type I error during the interim analysis; removed geographic poolability analysis because use of Chinese sites was removed in an earlier version of the protocol
4.0	18 Sep 2024	E. LaPointe Clinical Operations Consultant	Updated the protocol version to the most up to date version of the protocol (version 3 to version 5.1); added language to sections 4: Primary Endpoint and 8.1 Analysis Sets to clarify that ITM and the mITM analysis would be based on Validation 2 and Validation 3 (if applicable) cohorts only. The Run-in cohort and the Validation 1 cohort contributed to tuning data on the study only as outlined in the protocol.

Document Approval

Approval	
Approved By	Liyang Xie, PhD
Signature	
Role	Biostatistician, LLX Solutions, LLC

Approval	
Approved By	Richard Dorshow, PhD
Signature	
Role	President and Chief Scientific Officer, MediBeacon

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1 Introduction

This statistical analysis plan (SAP) describes the planned statistical methods to be used during the reporting and analysis of data collected under the Clinical Investigation Protocol, "An Open Label, Multi-Center, Safety and Pharmacokinetic Bridging Study of MB-102 (Relmapirazin) and the Use of the MediBeacon® Transdermal GFR Measurement System using the TGFR Reusable Sensor with Disposable Adhesive Ring in Normal and Renal Compromised Subjects for the Evaluation of Kidney Function". This SAP should be read in conjunction with the study Clinical Investigation Plan and associated case report forms (CRFs). This version of the analysis plan has been developed with respect to the Study Protocol Version 5.1. Any revisions to the protocol or CRFs that impact the planned analyses may require updates to the SAP. If there are any discrepancies between the SAP and the protocol, the SAP will prevail.

2 Study Design

2.1 Overview

MediBeacon is expanding the MediBeacon® Transdermal GFR (TGFR) Measurement System with the addition of a reusable sensor for use in the system. The TGFR Measurement System is a combination product that requires the administration of a fluorescent tracer agent designated relmapirazin (MB-102) that will be detected transdermally by a sensor which is attached to the device console. The study is a bridging trial investigating effectiveness of the TGFR reusable sensor in the MediBeacon® Transdermal GFR Measurement System in normal and compromised renal function subjects with different skin color types. The drug portion of the combination product is unchanged from prior investigations.

The objectives of this study are:

- To establish that the MB-102 transdermal fluorescence measured GFR using the MediBeacon® Transdermal GFR Measurement System with the TGFR reusable sensor with disposable adhesive ring is comparable to the measured MB-102 plasma GFR
- To evaluate the safety and effectiveness of the MediBeacon® Transdermal GFR Measurement System and the TGFR reusable sensor with disposable adhesive ring for the non-invasive transdermal fluorescence detection of MB-102 in subjects

2.2 Number of Planned Sites and Subjects

This study will take place at up to 5 investigational sites in the US.

Up to 240 subjects are planned for enrollment. The population will be split into stratums in accordance with screening eGFR measured by the CKD-EPI equation (pre-2021):

- 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 will be Fitzpatrick skin scale (FSS) I-III and 50% of subjects will have FSS IV-VI. Subjects will be enrolled across a spectrum of both eGFR (15 mL/min/1.73 m2) and FSS.

Subjects will be screened and if deemed eligible, will be enrolled. Subjects will be considered enrolled in the study after

- Signing the study specific informed consent form
- Receiving the sensor, and
- Receiving the MB-102 injection

2.3 Replacement of Subjects

Should a subject not meet eligibility, or is unable to be scheduled for dosing, that subject will be considered a screen failure. In addition, subjects who are unable to have an "average session GFR" measured by the TGFR Measurement System (due to premature sensor removal, for example), a major protocol deviation (such as a dosing extravasation), or meet the agreed upon post-PK outlier exclusion criteria (defined in **Section 5.4**) may be replaced to ensure the minimum sample size is enrolled. If subjects are not considered evaluable, they will be replaced in the study with a total potential enrollment up to 240 subjects. All subjects with exposure to MB-102 will be included in the safety analysis.

2.4 Study Schedule

The study will consist of screening time period within 28 days (but at least 2 days prior to) the baseline / dosing day. Subjects will be screened and if deemed eligible, will be enrolled. Screening will compromise of the following:

- Obtain written informed consent form 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 (**Table 1**). 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 COVID-19 test performed
- Collect height and weight measurements
- Perform a full physical exam (by a physician) and collect vital signs. The full physical examination includes at a minimum an assessment of the head, ears, eyes, nose, throat (HEENT), respiratory, cardiovascular, and gastrointestinal systems. The vital signs include blood pressure, heart rate, respiration and temperature.
- Perform a 12 lead ECG. In addition to the interpretation, ventricular rate, PR interval, QRS duration, QT, and QTcF will be collected. Additional ECGs may be collected if an issue or safety concern is noted. 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.
- Document Fitzpatrick Skin Scale Color Type by examining the upper chest (locations for possible sensor placement) and collecting Mexameter data on possible sensor locations

For sites who elect to bring subjects into the clinic the night before the 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 (performed by a nurse or other qualified personnel)
- 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

On dosing day, subjects will have baseline assessments performed (with the exception of those procedures noted above if they were performed the night before):

- Review subject eligibility
- Update medical history with any new information or issues occurring since the initial screening visit
- Collect the subject weight; BSA (and BMI) will be calculated based on the Screening height and the day of dosing weight
- 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)

- Perform a baseline ECG
- Record concomitant medications taken within 3 days of the dosing visit
- Urine pregnancy test for WOCBP
- 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 Mexameter
- 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 (**Table 1**)
- 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 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). Note: the venous catheter may also be placed PRIOR to sensor attachment and baseline acquisition upon Sponsor direction.

When this is completed, subjects will then receive a single dose of 130 mg MB-102. Serial PK draws will be collected over a 12-hour or 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.

The population will be split into stratums accordance with baseline eGFR by the CKD-EPI equation and location of enrollment:

- Stratum 1: In this stratum, there will be 50% of subjects with an eGFR $\geq$ 70 mL/min/1.73 m^2. Eligible subjects will receive a single 130 mg dose of MB-102 and 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: In this stratum, there will be 50% of subjects with an eGFR <70 mL/min/1.73 m^2. Eligible subjects will receive a single 130 mg dose of MB-102 and 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).

- Subjects will return for a safety follow-up visit approximately 7 ± 3 days after the dosing visit. The following assessments will be performed:
  - Full or limited physical exam will be performed and vital signs (including temperature) will be collected
  - Laboratory tests including chemistry, hematology, and urinalysis will be obtained (**Table 1**)
  - Concomitant medications will be recorded
  - Adverse events will be recorded.

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:

- Full or limited physical exam will be performed and vital signs (including temperature) will be collected
- Laboratory tests including chemistry, hematology, and urinalysis will be obtained (**Table 1**)
- Concomitant medications will be recorded
- Adverse events will be recorded
- Withdrawal date and reason will be recorded (if applicable)

2.5 Randomization

This is a single-arm, multicenter, prospective clinical study where all eligible subjects will use the investigational device and be injected with MB-102. Subjects will not be randomized.

3 Study Objective and Endpoints

3.1 Primary Objective and Endpoint

The primary objective is to establish that MB-102 transdermal fluorescence measured GFR using the MediBeacon® Transdermal GFR Measurement System is comparable to the measured MB-102 indexed plasma GFR.

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%.

3.2 Pharmacokinetic Objective and Parameters

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.

3.3 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. Additional safety variables include physical examinations, clinical laboratory assessments, ECGs, and concomitant medication use. All clinically significant findings in physical examinations, clinical laboratory assessments and ECGs will be reported as an AE or as medical history (if observations are noted prior to dose administration).

4 Analysis of 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%.

The primary endpoint will be calculated for subjects enrolled into the Validation 2 and 3 (if applicable) cohorts only. The Run-in cohort and Validation 1 cohort enrollment were performed to provide final tuning data and used for software updates.

4.1 Subgroup and Stratified Analysis

For the primary effectiveness endpoints, all data will be included in the primary analysis. To examine the consistent device performance, two sub-group analyses will be performed. The first sub-group analysis will be nGFR vs tGFR for eGFR > 70 mL/min/1.73 m^2, and then for eGFR < 70 mL/min/1.73 m^2. The second sub-group analysis will be nGFR vs tGFR for FSS I-III, and then for FSS IV-VI.

FSS will be generated based on Mexameter melanin measurements collected on the dosing day instead of using the subjective screening categorization. The mean of the melanin will be calculated and the FSS applied per the Mexameter manual (below):

- **FSS I: Nordic/Celtic skin:** extremely white skin, often red hair and freckles, never tan, always sunburns, **average melanin content:** 0 - 100
- **FSS II: Light Caucasians:** very light skin, often blond, tans with difficulty and sunburns quickly, **average melanin content:** 100 - 150
- **FSS III: European mixed type/ very fair Asian skin:** light skin, dark blond to brown hair, tans sometimes, sunburns sometimes, **average melanin content:** 150 - 250
- **FSS IV: Mediterranean/fair Asian skin:** dark hair, tans well and sunburns rarely, **average melanin content:** 250 - 350
- **FSS V: Dark skin:** e.g. dark Asians, tans very well and sunburns almost never, **average melanin content:** 350- 450
- **FSS VI: Black skin:** Immediately tans and never sunburns, **average melanin content:** 450 – 999

Note that the 30 evaluable subjects used for the run-in cohort and the 20 subjects enrolled into Validation Cohort 1 will not be included in the primary endpoint analysis as these data are considered Tuning Data.

5 Pharmacokinetic Analysis

Pharmacokinetic blood samples will be collected at the time points noted in **Section 2.4.** Single-dose pharmacokinetic parameters for MB-102 will be calculated using compartmental analysis methods.

5.1 Compartmental Pharmacokinetic Data Analysis

A compartmental pharmacokinetic analysis will be performed separately using each subject's plasma concentration-time profile. Based on prior data, it is expected that a two compartmental model will provide an adequate fit using Phoenix WinNonlin (Certara, Version 8.1 or higher). Model PK parameters for each subject will be reported.

Parameter	Units[a]	Definition
AUC	h*ng/mL	area under the plasma concentration-time curve, calculated as AUC=A/α + B/β
C_{max}	ng/mL	maximum plasma concentration, calculated as Dose/V
$t_{1/2\alpha}$	h	half-life associated with distribution phase
$t_{1/2\beta}$	h	half-life associated with elimination phase
$t_{1/2}$	h	elimination half-life from central compartment
CL	L/h	systemic clearance
V	L	total volume of distribution
V1	L	volume of distribution for central compartment
V2	L	volume of distribution for peripheral compartment

5.2 Calculation of nGFR

$nGFR_{BSA}$ – calculation of indexing to body surface area (BSA):

The GFR for each subject is obtained from the two-compartment pharmacokinetic model.

BSA in units of m^2 is calculated for each subject from their height and weight using the formula[1]:

$$BSA = 0.007184 * height_{cm}^{0.725} * weight_{kg}^{0.425}$$

$$nGFR_{BSA} = \frac{GFR\ (mL/min)}{\frac{BSA\ (m^2)}{1.73}} = \frac{GFR\ (mL/min)}{BSA\ (m^2)} * 1.73 \quad \text{(Eqn. 1)}$$

Note that in clinical practice GFR indexed to BSA ($nGFR_{BSA}$) is reported as "mL/min/1.73 m^2".

$nGFR_{Vd}$ – calculation of indexing to volume of distribution (V_d):

The GFR and volume of distribution (V_d) for each subject is obtained from the two-compartment pharmacokinetic model.

$$nGFR_{Vd} = \frac{GFR\ (mL/min)}{\frac{V_d\ (L)}{13.3}} = \frac{GFR\ (mL/min)}{V_d\ (L)} * 13.3 \qquad \text{(Eqn. 2)}$$

The standard value of 13.3 L for V_d was derived from our earlier clinical studies by computing the mean BSA across all subjects and computing the ratio of 1.73 m^2 to the mean BSA. This ratio was then multiplied by the mean V_d across all subjects to obtain the standard value of 13.3 L.

Note: Dosing day height and dosing day weight will be used for BSA / BMI calculations, however in the event dosing day weight or height is unavailable, screening day weight or height may be used.

[1] *DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Medicine. 1916; 17:863-71.*

5.3 Criteria for Handling Concentration Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQ concentrations will be treated as missing. The following rules apply to the specific situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If a predose plasma concentration is missing, it will be set to 0 by default within Phoenix WinNonlin.

5.4 Treatment of Outliers in Pharmacokinetic Analysis

Four outlier exclusion criteria which will be identified following PK analysis are addressed below.

5.4.1 Exclusion and Remedy for Plasma Concentration at each Time Point

MediBeacon developed the analytical methodology for MB-102 concentration determination in plasma. This method was shown to be very robust and much simpler than the usual analytical methods employed for iohexol determination in plasma samples. In the Pilot 2 Group 1 study, a rigorous method transfer and technician training program resulted in exceptional agreement between agent concentration in plasma samples measured at the sponsor lab and the third-party bioanalytical lab. The data was published in the peer-reviewed journal "Analytical Methods" (Shieh et. al. 2018[2]). However, when processing the Pilot 2 Group 2 samples, obvious errors in sample concentration were randomly observed. All indications pointed to lack of carefulness on the technician doing the work at the third-party bioanalytical lab.

Thus to ensure accuracy, it is necessary to implement a check on the bioanalytical lab that is performing the plasma agent concentration measurements. The plasma concentration vs time data generated by the third party bioanalytical lab is crucial to the clinical study endpoint. We have instituted further training on

[2] *J. J. Shieh, I.R. Riley, R.B. Dorshow, Clinical analysis and quantitation of MB-102, a novel fluorescence tracer agent, in human plasma Royal Society of Chemistry, Anal. Methods, 2018, 10, 2376*

our analytical technique at the bioanalytical laboratory and have instituted written protocols covering the entire analysis procedure.

A review of all plots of such data both in linear space and semi-log space will be performed prior to the GFR calculation. An example of data in semi-log space is in **Figure 1**; red circles are the measured concentrations from a subject with normal kidney function, the blue line is the two-compartment pharmacokinetic fit.

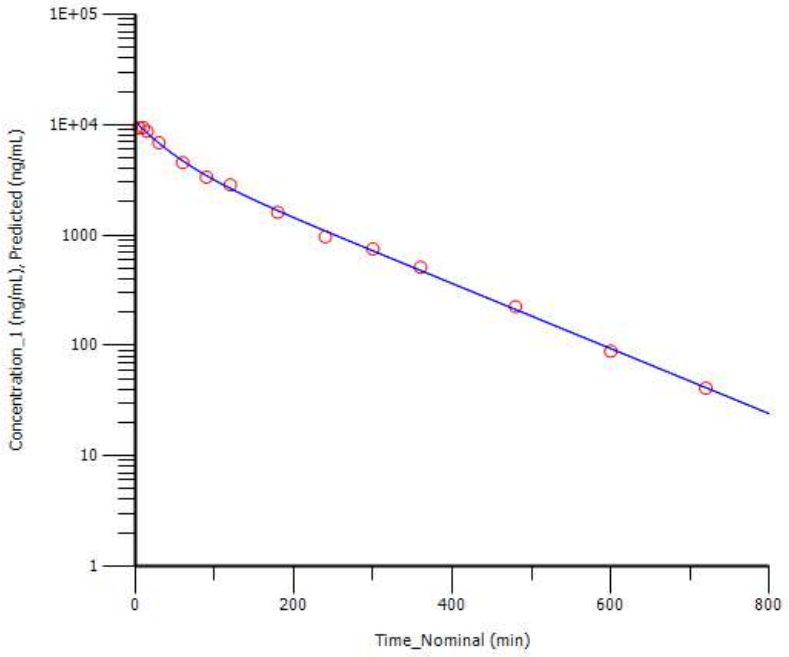

Figure 1 Plasma concentration as a function of time for a subject with normal renal function

Should a data point be observed that is 20% off the fit line, this may be an indication of an issue with the sample or with sample preparation. Thus, for any plasma sample value that is greater than 20% off the fit line, a re-analysis of agent concentration will be performed in that sample. The request will be for re-analysis in triplicate (3 new PK analyses of the timepoint), and the average value for concentration from the triplicate run will be used for that time point in the calculation of plasma GFR. If WinNonLin default parameters are not able to provide a pharmacokinetic result, then the full set of PK timepoints for that subject will be re-analyzed in triplicate and the means used in the final analysis.

Any quantifiable pre-dose concentration value will be considered anomalous and set to missing for the PK analysis, which will then be subsequently set to 0 by default in Phoenix WinNonlin.

It is anticipated that this exclusion criteria will seldom need to be employed due to our training program and written protocols at the third-party bioanalytical lab.

5.4.2 Exclusion Based on Subject Dosing Errors

In the initial implementation of the Pilot 2 Group 1, the site staff was not properly determining the amount dosed due to weighing errors of the agent-filled dosing syringe. The error in measured dose translated to error in GFR since:

GFR = Dose / AUC

where AUC is the area under the curve in the plasma concentration of MB-102 vs time plot. We subsequently implemented a strict weighing protocol to accurately determine the amount of administered dose, which has been followed for all subsequent studies, with the weighing protocol monitored by company staff at each study to prevent weighing errors of the dose.

In the 100-201 clinical study, which will be performed at up to 5 clinical sites, the clinical site staff will be expected to perform subject dosing error-free.. Written protocols will be provided to minimize dosing errors. However, as a check on dosing at the clinical sites, exclusion criteria based on the calculation of GFR from the standard two-compartment pharmacokinetic model and the indexed GFR will be implemented.

MB-102 plasma GFR is deduced using the MB-102 concentration vs time data and dose in the standard two-compartment pharmacokinetic model. We will use the most current version of WinNonlin for this task. The derived GFR is directly dependent on the input dose amount. This pharmacokinetic model also outputs many other parameters including the volume of distribution V_d. The derived V_d is also directly dependent on the input dose amount.

In the clinical setting, GFR is indexed to body surface area (BSA) using one of several formulas, all of which use the subject's height and weight. In fact, GFR indexed by BSA is how nephrologists stage kidney disease. The symbol $nGFR_{BSA}$ will be used for this indexing. Like non-indexed GFR, the GFR_{BSA} determination is also directly dependent on dose.

GFR can also be indexed by V_d. The symbol $nGFR_{Vd}$ will be used for this indexing. Because both the derived GFR and V_d are directly dependent on the input dose amount, indexing makes $nGFR_{Vd}$ independent of dose.

Due to the dependence of $nGFR_{BSA}$ on dose amount and independence of $nGFR_{Vd}$ on dose amount, comparison of these two values reveals errors in dosing. An example is in **Figure 2** shown below from some of our clinical data obtained in the device optimization studies.

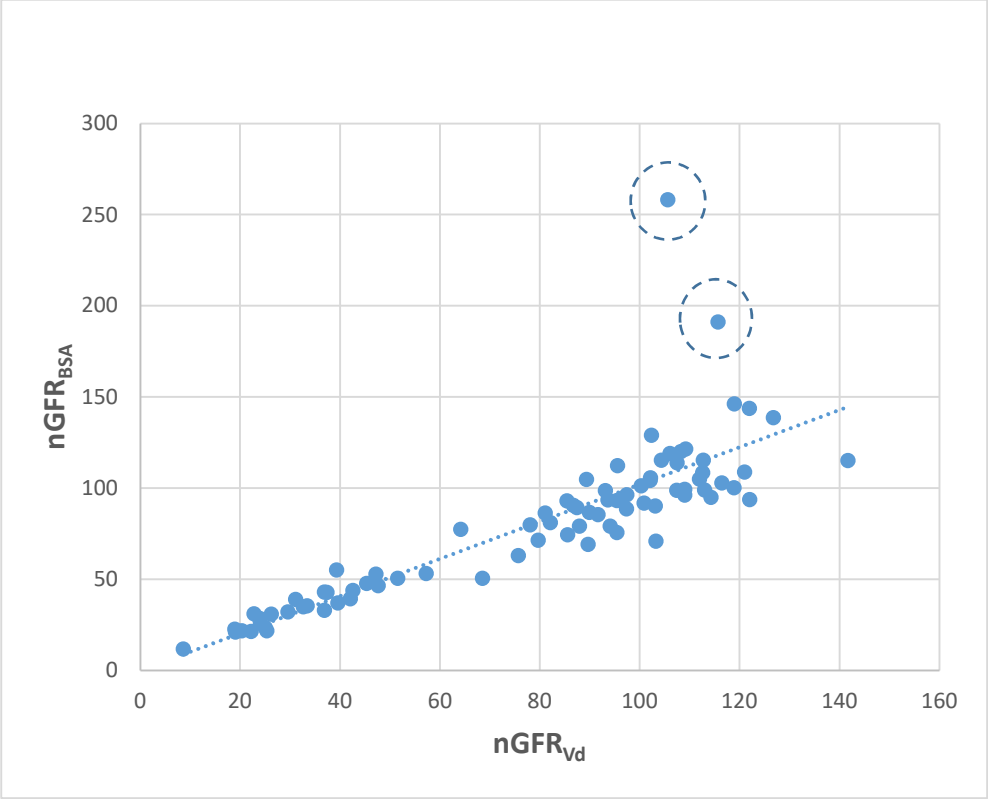

Figure 2: Plasma GFR indexing comparison

The y-axis is $nGFR_{BSA}$ and the x-axis is $nGFR_{Vd}$. Two of the 67 subjects are seen to be obvious outliers in a plot of $nGFR_{BSA}$ vs. $nGFR_{Vd}$. The conclusion is that a major dosing error has occurred for these two subjects.

It is anticipated that this exclusion criteria will seldom need to be employed due to our training program and written protocols at the clinical sites.

5.4.3 Exclusion Based on Small Dosing Errors and/or Extravasations

MediBeacon have found that plasma determined nGFR may also be affected by more subtle issues. These include small dosing errors and/or small extravasations of the MB-102 dose.

For example, after deleting the two extreme outliers in **Figure 2**, and correcting for a small linear trend in the absolute difference between $nGFR_{BSA}$ and $nGFR_{Vd}$ for the remaining subjects, the root mean square (RMS) percent error of the difference of the two indices was computed in 20 mL/min wide $nGFR_{Vd}$ subject bins, as shown in the **Figure 3**:

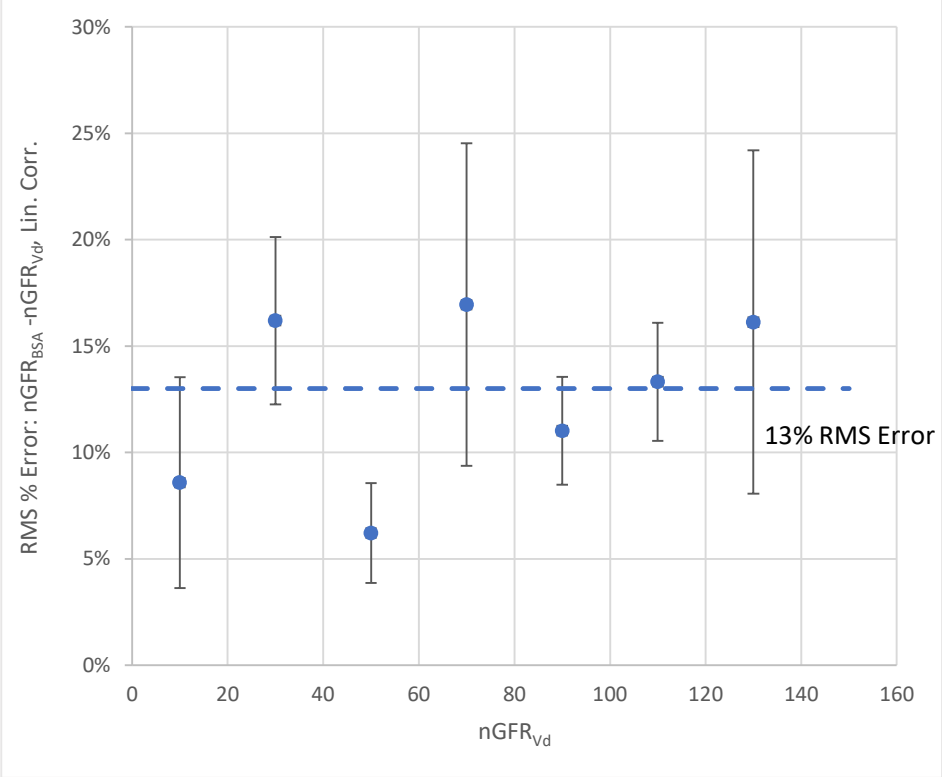

Figure 3: RMS percent error of $nGFR_{BSA}$ - $nGFR_{Vd}$ as a function of $nGFR_{Vd}$.

Subjects were grouped into 20 mL/min wide bins according to $nGFR_{Vd}$. Error bars represent ± the standard error within each binned group of subjects.

The resulting RMS percent error is seen to be independent of $nGFR_{Vd}$ with a combined value of 13% across all subjects.

Choosing two times the RMS percent error as a threshold limit for outlier detection (since it is similar to the standard deviation) results in identification of four additional outlier subjects, as shown in the **Figure 4** below.

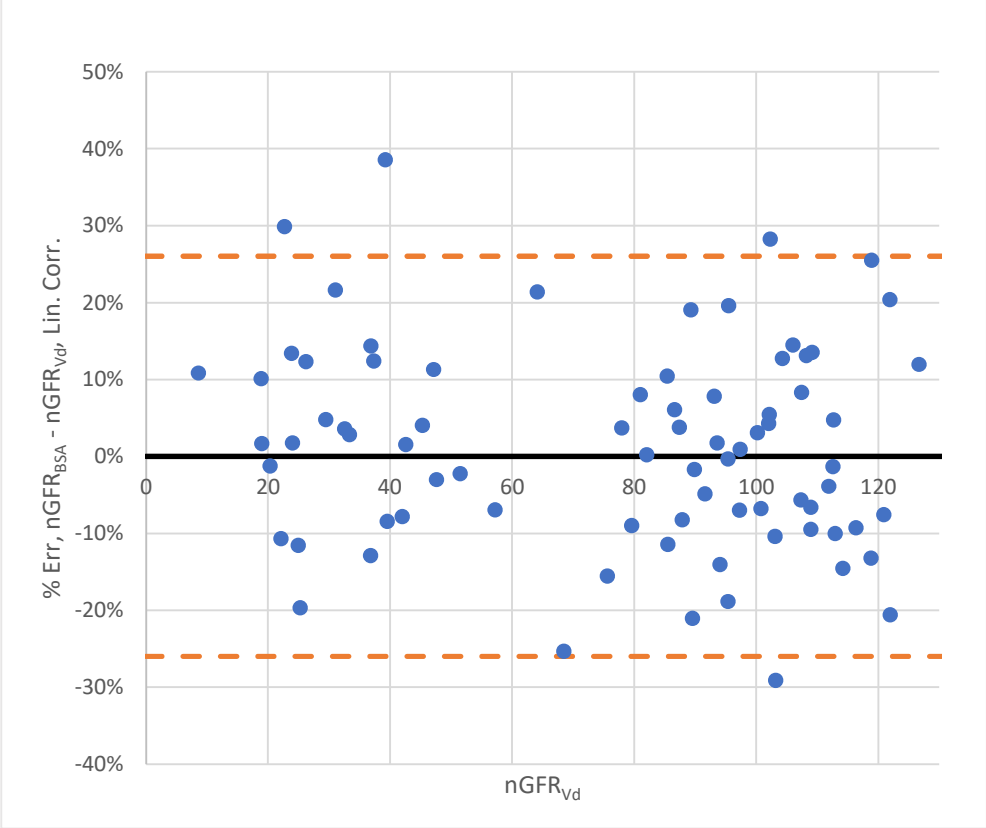

Figure 4: RMS percent error for $nGFR_{BSA}$ - $nGFR_{Vd}$ as a function of $nGFR_{Vd}$. Dashed lines are outlier thresholds

Summary: Two nGFR indexing calculations, $nGFR_{BSA}$ and $nGFR_{Vd}$, will be performed for all subjects in the 100-201 study. Any subject nGFR data that is an obvious outlier as described in section 2 above, and

any subject nGFR that is outside the threshold boundary as described in **Figure 4** will be eliminated before applying the remaining nGFR data to the evaluation of the tGFR endpoint criteria.

It is anticipated that this exclusion criteria will need to be seldom employed due to implementation of a rigorous training program and written protocols at the clinical sites. However, MediBeacon has historically seen evidence of these minor deviations and need to ensure this is not occurring in our 100-201 clinical study data set.

5.4.4 Exclusion Based on Body Size

Body surface area calculation for subjects at the extremes of body size are problematic. This issue has been widely discussed and a good summary is in Redlarski et al 2016[3]. Thus, any subjects with $nGFR_{BSA}$ > 120 mL/min/1.73m^2 will be excluded from the tGFR vs $nGFR_{BSA}$ dataset comparison. Note that clinically this is not relevant as these are highly normal subjects with respect to kidney function.

It is anticipated that this exclusion criteria will seldom be employed due to the enrollment exclusion criteria that excludes subjects with an eGFR > 120 mL/min/1.73m^2 per the CKD-EPI equation.

5.5 Presentation of Pharmacokinetic Data

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.

6 Transdermal Derived GFR Data

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.

[3] *G. Redlarski, A. Palkowski, M. Krawczuk, Body surface area formulae: an alarming ambiguity, Nature Scientific Reports 21Jun2016*

7 Analysis of Safety

All TEAEs reported during the study will be reviewed and adequately reported to comply with applicable regulations. All reportable TEAEs will be assessed by the investigator who will determine whether the event is related to MB-102 or related to the study device, and whether the event meets any of the criteria for seriousness. A serious adverse event (SAE) is any AE from this study that results in one of the following outcomes:

- led to death;
- 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;
- led to fetal distress, fetal death or congenital abnormality or birth defect.

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.

All reported TEAEs, including unanticipated adverse device effects (UADE), and serious adverse events (SAE) will be summarized for all enrolled subjects. The overall numbers and percentages of subjects who experienced any TEAEs will be presented. In addition to the TEAEs, description of all TEAEs, including description/term of the symptom event (underlying diagnosis), the date of first occurrence and date of resolution (if applicable), classification of serious or not serious, severity, causal relationship, action taken, and event outcome will be listed. 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.

In addition to the events reported as AEs, the parameters of clinical laboratory tests including clinical chemistry, hematology, urinalysis and coagulation parameters will be summarized using the descriptive

statistics. Clinical laboratory analytes are presented in **Table 1**. More information on descriptive statistics is presented in **Section 8.6**.

ECG data will be listed. Concomitant medications, coded using the World Health Organization (WHO) drug dictionary, administered within 3 days prior to MB-102 dose administration and through the follow-up study visit will be also listed. Furthermore, concomitant non-drug therapies will be listed.

7.1 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 will be performed in accordance with a written Safety Review Committee Plan.

7.2 Assessment of Causality

The casual relationship to the study procedures and the investigational device for each adverse event will be related as follows:

- **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.
- **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.

7.3 Assessment of Severity

The severity of the adverse events will be rated based upon the following grades:

- **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.

7.4 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

7.5 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)

7.6 Evaluation of Device Malfunctions

Device malfunctions will be tabulated and listed in a manner similar to the methods described for adverse events. Any device malfunction leading to an adverse event or study termination will be listed separately.

8 General Statistical Considerations

8.1 Analysis Sets

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 average session tGFR or an average snapshot tGFR* has been calculated, and who have not had any major protocol deviations that would impact TGFR system performance.
- **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.

* Note: this includes short gaps in data that are less than 30 minutes due to a resumption of the monitoring session where an "average snapshot GFR" is reported instead of an "average session GFR".

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.

The ITM and mITM populations will be analyzed from subjects enrolled in the Validation 2 and Validation 3 (if applicable) cohorts only.

8.2 Sample Size Determination

The One-Sample Proportion Test is used to calculate the sample size. The required sample size was calculated assuming the true proportion is 0.95, the test is one-sided exact binomial, $\alpha = 0.025$, and the power is $1 - \beta = 0.90$. The P30 sample size calculation yields 102 subjects. However, to remain consistent with the previous sample size from the pivotal study (protocol 100-003) for the TGFR, the study will enroll 140 evaluable subjects.

An adaptive trial design will be employed for the reusable sensor as follows:

1. Run-in cohort of up to 30 evaluable subjects will commence the study. Additional Run-in subjects may be enrolled at new clinical sites to ensure protocol compliance. Run-in subject data will be considered tuning data.
2. Validation Cohort 1 (approximately 20 evaluable subjects) will be used to confirm final algorithm selection. Validation Cohort 1 subject data will be considered tuning data.
3. Validation Cohort 2 (approximately 75 evaluable subjects) will be analyzed in an interim analysis to compare results against the primary endpoint specification. At that time, the primary endpoint null hypothesis will be tested using an exact binomial test at a one-sided nominal alpha level of 0.0150 using the method of Pocock for a two-stage design with the interim analysis performed based on the first 53.57% of the planned number of subjects. At the final analysis, the primary endpoint null hypothesis will also be tested at a one-sided nominal alpha level of 0.0150. This will control the overall Type I error rate at the one-sided 0.025 level.
4. If the primary endpoint has been achieved, the study will be terminated. If not, the trial will be continued.
5. Validation Cohort 3 (approximately 65 evaluable subjects) will be enrolled in the event the interim analysis did not meet the primary outcome measure. Final analysis will be of the 140 evaluable subjects.

The subjects will be chosen to span the range of renal function from normal to Stage 4 CKD impaired. The protocol will target enrolling 50% of subjects in the Fitzpatrick Skin Scale range of I-III and 50% within Fitzpatrick Skin Scale range of IV-VI.

8.3 Pooling Data Across Sites

Up to five US investigational sites will enroll subjects into the study. All investigational sites will follow the requirements of a common protocol and standardized data collection and validation procedures and forms. The sponsor will also adequately monitor the study to assure protocol compliance. The primary effectiveness and safety endpoints will be presented separately for each site using descriptive statistics. Poolability of the primary efficacy endpoint across investigational sites will be evaluated using a logistic regression model with a fixed effect for site at the 15% level of significance. If there is a significant effect of site (i.e., $p<0.15$ for the fixed effect for site), analysis will be repeated using site as a random effect. Sites enrolling fewer than 5 subjects will be combined to form one quasi site. Additional exploratory analyses may be performed to understand any variations in outcome by site.

8.4 Handling of Missing Data

All attempts will be made to limit the amount of missing data. No imputation will be utilized. If a data point is missing, that data point will not contribute to that portion of the analysis. The number of evaluable observations will be reported in the analysis so that extent of missing data can be assessed.

8.5 Statistical Software

All statistical analysis will be conducted using SAS version 9.4 or later (SAS Institute Inc., Cary, NC) or other widely accepted statistical or graphical software as required. The pharmacokinetic assessments will be performed using Phoenix WinNonlin (and possibly Phoenix NLME) Build 8.1 or higher.

8.6 Descriptive Statistics

Continuous data will be summarized with the number of evaluable observations, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized with frequency counts and percentages. Summaries will be based on observed data (i.e., no missing data will be imputed). Confidence intervals may be presented, where appropriate, using the t-distribution for continuous data and Clopper-Pearson Exact method for categorical variables.

8.7 Statistical Significance

Unless otherwise specified, hypothesis testing will be performed at the two-sided 0.05 significance level and one-sided 0.025 significance level. Nominal p-values will be rounded to four decimal places. If a nominal p-value is less than 0.0001 it will be reported as "<0.0001". In the event of multiple testing the overall type I error becomes inflated. There are several strategies to correct the alpha level when performing multiple tests. In this study, to control the inflation of overall type I error, the Benjamini-Hochberg method will be applied.

8.8 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.

8.9 Analysis

After the run-in cohort, the study will include up to 140 evaluable subjects. After 75 evaluable subjects have completed the study, an interim analysis will be conducted. If the primary endpoint has been reached, the study will be terminated. If the primary endpoint has not been completed, the study will continue until 140 evaluable subjects have completed the study.

9 Changes from Planned Analyses

Any changes to planned statistical analyses determined necessary prior to performing the analyses will be documented in an amended Statistical Analysis Plan and approved prior to the analysis when possible. Any other deviations or changes from the planned analyses deemed necessary due to violation of critical underlying statistical assumptions, data characteristics, or missing data will be clearly described in the clinical study report with justification and rationale.

10 Subject Listings

Subject listings will be provided for the primary endpoint. Listings will also be provided for adverse events.

All plasma concentrations will be provided in listings by subject and time point. Any blood measurements obtained outside of a ± 2 minute window around the scheduled time point for up through 90 minutes, and a ± 5 minute window for the remaining sampled collected will be identified as protocol deviations. Additionally, pharmacokinetic parameters will be provided in listings sorted by subject.

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. ECG data will be listed.

11 Tables

Table 1 Clinical Laboratory Analytes

Chemistry Panel	Hematology	Other required lab tests
• 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*	• Hematocrit • Hemoglobin • WBC • RBC • Platelets Differentials • Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils **Coagulation Parameters** *Only performed at screening* • PT • INR • aPTT	*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