STATISTICAL ANALYSIS PLAN

TITLE PAGE

An Open-Label Multiple-Dose Study of RZ358 in Patients with Congenital Hyperinsulinism

Final Version 2.0: AUG 17, 2022

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DECLARATION

I, the undersigned, declare that I have prepared the statistical analysis plan along with TLF mockups and that to the best of my knowledge this document is internally consistent with protocol and scientifically rational.

Prepared by:

(b) (4)

I, the undersigned declare that I have reviewed the statistical analysis plan along with TLF mockups and that to the best of my knowledge the document is internally consistent with protocol and scientifically rational.

Reviewed by:

(b) (4)

AUTHORIZATION: I, the undersigned, declare that I have reviewed the statistical analysis plan along with TLF mock-ups and that to the best of my knowledge the document accurately reflects the protocol objectives.

Authorized by:

(b) (4)

REVISION HISTORY

Version	Date	(b) (4)	Reasons
1.0	APR 20, 2022	(b) (4)	1. Changes in versions, MedDRA-25.0 and WHODD- WHODRUG B3 SEP2019. 2. Anticipated list of TLFs has been updated. 3. Added the two new listings for derived endpoint variables (CGM and SMBG). 4. Updated the section "Subgroup Analysis (7.1.11.)" due to the addition of one subgroup (Patients ages 2-6 years old) for two endpoints. 5. Updated the section "Safety Analysis (7.1.8.)" by adding a table of Vitals Abnormality. 6. Updated the section "Analysis Population (6.1)" with the modification of Per Protocol Population definition. 7. Updated the section "Efficacy Analysis" with addition of percent change summary and analysis for two efficacy endpoints.

LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
ADA	Anti-drug antibody
AE	Adverse event
ALT	Alanine aminotransferase
AM	Ante Meridiem
AOC	Area over the curve
AST	Aspartate aminotransferase
AUC	Area under the curve
CGM	Continuous glucose monitoring
CHI	Congenital hyperinsulinism
cm	Centimeters
CS	Clinically significant
CV	Coefficient of variation
DERC	Dose Escalation Review Committee
dL	Deciliter
ECG	Electrocardiogram
eCRF	Case report form
FFA	Free fatty acids
HbA1c	Hemoglobin A1c
Kg	Kilograms

Abbreviation or special term	Explanation
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
msec	Millisecond
NCS	Not clinically significant
PD	Pharmacodynamics
PK	Pharmacokinetics
TLF	Table, Listing and Figure
PM	Post Meridiem
POC	Point of care
RTSM	Randomization and Trail Supply Management
SAE	Serious adverse event
SD	Standard deviation
SE	Standard Error
SoC	Standard of care
SOC	System Organ Class
SMBG	Self-Monitoring of Blood Glucose
TEAE	Treatment emergent adverse event

1. INTRODUCTION

Insulin, secreted by the β cells of the Islets of Langerhans in the pancreas, is the major hormone for lowering blood glucose levels in the fasting and postprandial states. Abnormal elevations in insulin secretion can lead to profound hypoglycemia with cerebral damage and even death. Thus, under normal conditions, the control of insulin secretion is highly regulated to prevent hypoglycemia. Overproduction of insulin results in hyperinsulinemic hypoglycemia, characteristic of diseases such as CHI and post-gastric bypass hypoglycemia (PGBH). Congenital hyperinsulinism (CHI) is the most common cause of recurrent hypoglycemia in neonates and infants.

Diminution of insulin action at target tissues may represent a novel therapeutic approach for the treatment of conditions associated with hyperinsulinism. RZ358, previously referred to as XOMA-358 or XMetD, is a fully human immunoglobulin G, subclass 2 (IgG2) mAb that binds with high potency and selectivity to an allosteric site on the insulin receptor (INSR), acting as a negative modulator of insulin action on target cells by attenuating both the binding of insulin and its downstream signaling.

This statistical analysis plan (SAP) describes the statistical methods and data handling procedures to be followed during the final reporting and analyses of data collected for the study Protocol RZ358-606.

This SAP should be read in conjunction with the study protocol and case report form (CRF). This version of the plan has been developed using the protocol: RZ358-606, version amendment 5.0, dated 17MAR2021 and RZ358-606, version amendment 5.0 US, dated 15MAR2021 and eCRF: version 3.40, dated 04JUN2021.

This study aims to evaluate the safety, tolerability, pharmacokinetics, pharmacodynamics, and glycemic efficacy of multiple ascending doses of RZ358 over 8 weeks in patients with hyperinsulinemic hypoglycemia due to congenital hyperinsulinism (CHI).

2. STUDY DETAILS

2.1. Study Objectives

The objectives of this study are to evaluate the safety, tolerability, PK, PD, and glycemic efficacy of multiple ascending doses of RZ358 over 8 weeks in patients with hyperinsulinemic hypoglycemia due to CHI.

2.2. Study Design

This is a Phase 2, multi-center, open-label, multiple ascending dose study to evaluate the safety, tolerability, PK, PD, and glycemic efficacy of RZ358 in patients with hypoglycemia due to CHI. The

study will consist of up to 4 dose cohorts, which are to be enrolled independently and conducted sequentially, comprised of up to 6-8 patients per cohort to ensure each cohort includes at least 3 patients aged ≤10 years. Eligible patients will be successively enrolled into the cohort that is open for enrollment at that time, at the lowest dosing cohort that is yet to be filled.

In Cohorts 1, 2, and 3 the dose amount will be fixed during the entire 8-week Treatment Period, while in Cohort 4 there will be a scheduled bi-weekly dose-titration for the first 4 weeks, followed by a fixed dose amount thereafter for the remaining 4 weeks. The Sponsor may decide to add a cohort to repeat a dose level or to study an additional dose level, but the dose level for any cohort will not exceed the planned dose level for that cohort, as specified in this protocol.

Advancement to subsequent cohorts will not occur until at least 4 patients from the prior cohort have each received at least 4 weeks of study drug (2 bi-weekly doses), without safety concerns. A Dose Escalation Review Committee will assess all available and relevant interim data from each cohort to determine the appropriateness of dose escalation to a new cohort.

Table 1: Study Design/Cohort Dosing Regimens

	Dosing (mg/kg)			
Cohort	***Wk 1***	***Wk 3***	***Wk 5***	***Wk 7***
1	*3*	*3*	*3*	*3*
2	*6*	*6*	*6*	*6*
3	*9*	*9*	*9*	*9*
4	*3*	*6*	*9*	*9*

Following informed consent, patients will complete a Screening Period to determine eligibility. After the preliminary screening assessment, patients who are otherwise eligible will undergo scheduled self-monitoring of blood glucose (SMBG) by point of care (POC) glucometer as well as have a CGM sensor placed according to the instruction manual, for completion of a minimum10-day outpatient CGM evaluation to assess glycemic eligibility and establish a baseline. (b) (4) patients will use an SMBG for their POC throughout the trial. Patients taking existing and stable doses of standard of care (SoC) medications or nutritional supplementation for CHI at the time of screening should continue the same regimen and doses throughout this period.

(b) (4)

(b) (4) To meet glycemic qualification criteria, patients must have glucose values <70 mg/dL (3.9 mmol/L) for ≥4% of the overall monitored CGM time with at least 3 severe hypoglycemia events by CGM threshold of <50 mg/dL (2.78 mmol/L) during the last 7 days of a 10-day CGM evaluation period AND be experiencing ≥ 3 hypoglycemia events (<70 mg/dL) per week by SMBG and/or according to the Investigator's evaluation (b) (4)

During the Screening Period and throughout the study, patients will be requested to follow their usual diet (including enteral feeding, as applicable) and activity level, as evaluated and instructed by qualified study personnel.

At the conclusion of the CGM and glycemic eligibility evaluation, final eligibility will be determined, and fully eligible patients will check in to the inpatient facility on Day -1, to begin the Treatment Period. On Day -1, patients will begin a 12h baseline fasting challenge evaluation, which will conclude prior to Day 1 dosing. Re-screening may be permitted under certain extenuating circumstances (e.g. laboratory outlier suggesting result artifact, insufficient duration of CGM collection), only with pre-approval by the Sponsor.

Beginning at Day 1 of the Treatment Period, patients will receive study drug via IV infusion every 2 weeks over an 8 week total Treatment Period. Thus, patients who complete the entire Treatment Period will receive 4 doses of study drug over a total of 8 weeks. Patients will remain under inpatient observation for at least 48h following dosing on Week 1, and for at least 24h following dosing on Weeks 3, 5, and 7, for completion of PK blood sampling and safety assessments. Throughout the Treatment Period and through post-treatment follow-up Day 42 after the patient's final dose in the study (cumulative study Day 85), glucose levels will be assessed and recorded by CGM. (b) (4)

All patients will continue to use an SMBG as their POC throughout the trial.

Throughout the Treatment period and through the End of Treatment (EOT) visit on post-treatment follow-up Day 14 (cumulative study Day 57), SMBG by POC glucometer must be performed (b) (4)

In addition, SMBG by POC glucometer should be performed to confirm suspected hypoglycemia (by

CGM or symptoms) prior to implementing rescue measures. (b) (4) Thereafter, during the remainder of the Follow-up period, CGM and scheduled SMBG by POC glucometer will be stopped, but the latter may continue to be performed and recorded in the diary at the discretion of the patient and/or Investigator.

(b) (4)
(b) (4)

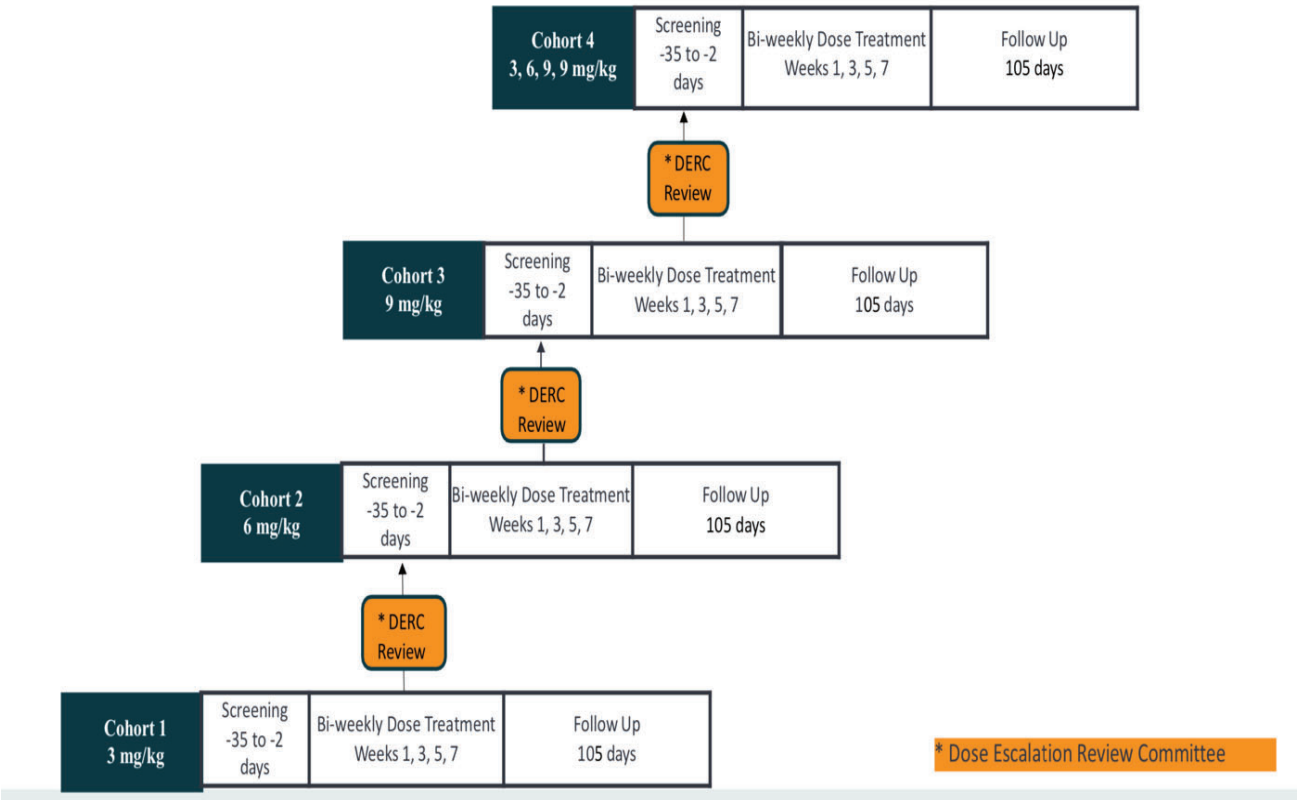
Otherwise, changes in the dose or regimen of background SoC therapies should not occur, except in the event that hypo- or hyperglycemic rescue criteria are met, or changes are deemed medically necessary by the Investigator. (b) (4)

At the conclusion of Week 7 dosing and discharge from the clinic, each patient will complete outpatient post-treatment follow-up visits at Days 14 (EOT), 28, and 42 (each ±3 days), and a final End of Study (EOS) follow-up visit on Day 105 (+3 days) for purposes of evaluating extended PK and safety over a period of 5 half-lives of the study drug.

Protocol versions 1.0, 2.0, and 3.0 were never implemented. Prior to implementing protocol version amendment 5.0, three patients in Cohort 1 were enrolled and treated under protocol version amendment 4.0, which differed from version 5.0 in dosing regimen (frequency) and the glucose entry criterion by CGM, as noted below:

1. To meet glycemic qualification criteria, patients must have glucose values <70 mg/dL (3.9 mmol/L) for ≥8% of the overall monitored time and for ≥60 min per day on at least 5 of the 10 CGM evaluation days AND be experiencing ≥ 3 hypoglycemia events (<70 mg/dL) per week by SMBG and/or according to the Investigator's evaluation.
2. Beginning at Day 1 of the Treatment Period, patients will receive study drug via IV infusion weekly for the first 4 weeks (induction dosing), followed by subsequent infusions every 2 weeks for an additional 4 weeks (maintenance dosing). Thus, patients who complete the entire Treatment Period will receive 6 doses of study drug over a total of 8 weeks.

Figure 1: Study Design



2.2.1. Schedule of Assessments

The schedules of study assessments and pharmacokinetics sampling schedule are presented in APPENDIX 1 and APPENDIX 2 of Protocol below

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2.3. Determination of Sample Size

The sample size chosen for this study was selected without statistical considerations. It has been determined adequate to meet the study objectives. Patients who withdraw from the study may be replaced on a case-by-case basis after discussion and agreement with the Sponsor's Medical Monitor.

Up to approximately 24 to 32 patients are planned to be enrolled into this study if all cohorts are completed. Each of the (up to) 4 cohorts will comprise up to 6-8 patients to ensure there are a minimum of 3 patients ≤10 years of age per cohort. Patients who discontinue the study before the Week 5 treatment and evaluation period may be replaced at the discretion of the Sponsor, if discontinuation was not due to a dose-limiting toxicity (DLT).

2.4. Randomization

This is an open-label study, but the study will utilize a randomization and trial supply management (RTSM) application built within the EDC platform to unify randomization, trial supply and EDC. Each patient will be provided a unique 6-digit patient ID number. The first three digits will represent the site number and the last three digits will represent the patient number at the site. The patient ID number will remain the same for both screening and randomization. In the event a cohort has not completed the enrollment criteria of at least 3 patients ≤10 years of age, patients >10 years of age can be stratified into a higher cohort as long as the dose escalation review has been completed and approved.

2.5. Blinding

This is an open-label study. Therefore, no blinding is applicable.

3. DATA ANALYSIS CONSIDERATIONS

The statistical analyses will be performed by Quartesian Clinical Research, using SAS Version 9.3 (or higher). All Tables, Listings and Figures (TLFs) will be produced in landscape format. In general, all data will be listed by the patient, cohort, and visit/timepoint, but not limited only to these variables. The number of variables presented in each listing can vary. Please refer to Mock tables, listings, and figures.

Data will be summarized by cohort and by RZ358 pooled treatment. The total number of patients in the study group (N) under the stated population will be displayed in the cohort header of summary tables.

Data will be summarized using descriptive statistics for continuous variables. Unless otherwise stated, descriptive statistics will include Number of patients (n), Mean, Standard Deviation (SD), Standard Error (SE), Minimum, Median, Maximum, and confidence interval (for efficacy parameters only). First Quartile (Q1) and Third Quartile (Q3) will also be included for duration (absolute time) parameters. Area over the curve (AOC) and area under the curve (AUC) of glucose will also include coefficient of variation (CV %), Geometric Mean, Geometric CV (%). In case of n=1, where n

indicates the number of evaluable patients at the particular time point, only n, mean, minimum and maximum will be displayed. The statistic "Missing" will also be evaluated by enumerating the number of missing entries/patients, if any at that visit, and presented as a summary statistic only for the available time points.

In summary tables of categorical variables, counts and percentages will be used. The count [n] indicates the actual number of patients with a particular value of a variable or event, which should always be less than or equal to the total number of patients in the respective cohort [N].

Decimal Precision Convention: The minimum and maximum statistics will be presented to the same number of decimal places as the raw data. The mean, median, Q1, Q3, Confidence interval (CI) for mean and proportion will be presented to one more decimal place than the raw data, whereas the standard deviation (SD), Standard Error (SE), coefficient of variation (CV %), Geometric Mean, and Geometric CV (%) will be presented to two more decimal places than the raw data.

The number and percentage of patients with missing values for a variable/category/event will also be presented for the resulting visits/time points under the "Missing" category. Percentage will be obtained by: % = (n/N) *100. Unless otherwise stated, all percentages will be expressed to one decimal place. P-value will be presented three decimal places or P-value closer to zero will be presented as <0.001 and p-value closer to one will be presented as >0.999.

The following conventions will be applied to analyses/data presentation:

All dates will be displayed in DDMMMYYYY format.

Unscheduled data for assessments that are specific to a discrete visit (rather than continuously measured e.g., SMBG or CGM) will be presented in Listings but not in Tables and Figures, unless missing data imputation rules (windows) are met, as described below.

Prior to implementing protocol version amendment 5.0, three patients in Cohort 1 were enrolled and treated under protocol version amendment 4.0, which differed from version 5.0 in dosing regimen (frequency) so, the following visit mapping will be followed for efficacy analysis, considering the dosing period:
"Subjects who were enrolled in Cohort 1 under protocol version amendment 4.0, their Week 1 and Week 2 data are aggregated and reported under '2 Weeks' timepoint. Similarly, Week 3, Week 4 and Week 5 data are aggregated and reported under '4 Weeks', their Week 6 and Week 7 are aggregated and reported under '6 Weeks', their Week 8 and Week 9 data are aggregated and reported under '8 Weeks/EOT' timepoint."

Handling of Missing Data

If the Adverse Event Relationship flag is missing, the relationship for adverse event will be imputed and will be considered as "Related". If the Adverse Event Severity grade is missing, the severity will be imputed and will be considered as "Severe". To handle missing or partial AEs and concomitant medication dates, the following rules will be applied.

For partial AE/CM Onset/Start Date:

1. If the year is unknown, then do not impute the date but assign a missing value.

Missing day and month

If the year is same as the year of first day on study medication, then the day and month of the start date of study medication will be assigned to the missing fields.

If the year is prior to the year of first day on study medication, then December 31 will be assigned to the missing fields.

If the year is after the year of first day on study medication, then January 1 will be assigned to the missing fields.

Missing month only

Treat day as missing and replace both month and day according to the above procedure.

Missing day only

i. If the month and year are same as the year and month of first dose date of study medication, then the start date of study medication will be assigned to the missing day.

ii. If either the year is before the year of the first dose date of study medication or if both years are the same, but the month is before the month of the first dose date of study medication, then the last day of the month will be assigned to the missing day.

iii. If either the year is after the year of the first dose date of study medication or if both years are the same, but the month is after the month of the first dose date of study medication, then the first day of the month will be assigned to the missing day.

For AE Stop Date:

If the stop date is complete and the imputed start date as above is after the stop date, the start date will be imputed by the stop date.

If the stop date is incomplete, no imputation needed.

For CM End Date:

CM end date will not be imputed if CM ongoing is checked.

1. If the year is unknown, then do not impute the date but assign a missing value.
2. If the month is unknown, then assign "December."
3. If the day is unknown, then assign the last day of the month.

If AE onset date is non-missing and is before the first dose date then the AE is considered to be prior treatment.

If AE onset date is non-missing and is occurred on the same date as the first dose with the event marked as start prior to first dose of study drug on the CRF then the AE is considered to be prior treatment.

If AE onset date is missing and is marked as start prior to the first dose of study drug on the CRF then the AE is considered to be prior treatment.

If CM start date is taken prior to the first dose of study drug regardless continuing to take the medication while on study treatment, then the CM is considered to be prior treatment.

Missing data imputation for Visit-Specific Assessments (e.g., safety labs, vital signs, hepatic ultrasound, 12-lead ECG):

An unscheduled assessment (full visit or partial assessment) that is discrete and associated with a specific visit will be imputed as the scheduled assessment for the nearest visit where the applicable assessment is missing. Unscheduled data collected prior to the first dose will be assigned to the nearest missing screening visit or assessment (instead of nearest post-treatment visit). Unscheduled data collected after the final dose will be assigned to the nearest missing treatment or post-treatment visit or assessment. When an unscheduled assessment represents a value that is repeated (to a scheduled value, i.e., no missing data), then these will be adjudicated by medical review prior to database lock, to determine the value for the scheduled summary. If the unscheduled/repeat value affirms the legitimacy of the original value, the original value will be used and the unscheduled/repeat value will be additionally included in listings. When the value refutes the legitimacy of the original value

(suggesting spurious original value), the unscheduled/repeat value will be imputed to that scheduled visit, and the original value will be excluded from the dataset and listings.

4. DEFINITIONS & DERIVATIONS

Baseline: Baseline is defined as the last non-missing value (or aggregate/integrated values in the case of CGM or SMBG) prior to the first administration of the study drug, unless otherwise stated. If the assessment occurs on the same day as the first study drug administration, then the time of assessment should be compared to the time of first dose administration.

Change from Baseline: The change from baseline values will be calculated as post-baseline value minus the baseline value.

Percent change from Baseline $= \frac{\boldsymbol{Post\ baseline\ value - Baseline\ value}}{\boldsymbol{Baseline\ value}} \times \mathbf{100}$

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(b) (4)

SMBG data entered by the patient into the electronic patient diary cannot be edited retroactively by the patient. It is both the source data and the data record, so correction of potentially spurious values cannot be performed (b) (4) listing of possible spurious values will be medically reviewed and adjudicated for exclusion from the analysis datasets prior to database lock.

Missing data for CGM or SMBG will otherwise not be imputed since it is collected continuously or semi-continuously.

Treatment-Emergent Adverse Event (TEAE): A TEAE is defined as any clinically significant event that starts or that worsens in severity (intensity or frequency) after the first dose of study drug. For purposes of this study, TEAEs for summary tabulation will be further defined as those events which occur within a period of time through the +42 days follow-up visit after the final dose of study drug. Adverse events with onset after ICF but before first dose of study drug or after last dose of study drug +42 days will be summarized and listed separately from TEAEs.

4.1. ENDPOINT DERIVATIONS

- **Daily percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM will be derived by,**

Daily percent time (%) = (Actual number of readings satisfying glucose target range of 70-180 mg/dL (3.9-10 mmol/L) / Total number of readings obtained in 24h period from 12 AM to 12 AM) × 100. *(Note: The day (24 hours) will be derived using date and time).*

- **8h overnight percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM will be derived by,**

8h overnight percent time (%) = (Actual number of readings satisfying glucose target range of 70-180 mg/dL (3.9-10 mmol/L) / Total number of readings obtained in 8h overnight period from 12 AM (midnight) to 8 AM) × 100.

- **Daily duration (min) and Daily percent time with hypoglycemia at each threshold of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM will be derived by,**

Daily percent time (%) with hypoglycemia <70 mg/dL = (Actual number of readings satisfying glucose level less than 70 mg/dL (3.9 mmol/L) / Total number of readings obtained in 24h period from 12 AM to 12 AM) × 100. *(Note: The day (24 hours) will be derived using date and time. Similarly, it will be derived for other glucose thresholds).*

Daily duration (min) with hypoglycemia <70 mg/dL: (Actual number of readings satisfying glucose level less than 70 mg/dL (3.9 mmol/L) in 24h period from 12 AM to 12 AM) × 5.
(Note: The day (24 hours) will be derived using date and time. Similarly, it will be derived for other glucose thresholds).

- **8h overnight duration (min) and 8h overnight percent time with hypoglycemia at each threshold of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM will be derived by,**

8h overnight percent time (%) with hypoglycemia <70 mg/dL = (Actual number of readings satisfying glucose level less than 70 mg/dL (3.9 mmol/L) / Total number of readings obtained in 8h overnight period from 12 AM (midnight) to 8 AM) × 100.
(Note: similarly, it will be derived for other glucose thresholds).

8h overnight duration (min) with hypoglycemia <70 mg/dL: (Actual number of readings satisfying glucose level is less than 70 mg/dL (3.9 mmol/L) in 8h overnight period from 12 AM (midnight) to 8 AM) × 5. *(Note: similarly, it will be derived for other thresholds).*

- **Daily hypoglycemia incidence (event rate) at each of the specified glucose thresholds by**

CGM will be derived by,

Daily hypoglycemia incidence (event rate) <70 mg/dL = (b) (4)

Daily hypoglycemia incidence (event rate) <60 mg/dL (b) (4)

Daily hypoglycemia incidence (event rate) <50 mg/dL = (b) (4)

- **8h overnight hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM will be derived by,**

8h overnight hypoglycemia incidence (event rate) <70 mg/dL (b) (4)

8h overnight hypoglycemia incidence (event rate) <60 mg/dL = (b) (4)

8h overnight hypoglycemia incidence (event rate) <50 mg/dL = (b) (4)

- **The average glucose level by CGM between midnight and 08:00 AM (or nearest pre-breakfast timepoint) will be derived by,**

Average glucose level by CGM between midnight and 08:00 AM = Sum of all actual glucose reading values in same units / Total number of glucose readings obtained between midnight (12 AM) and 8 AM.

- **Daily glucose $AOC_{0\text{-}24h,<70mg/dL}$ and $AUC_{0\text{-}24h,>70mg/dL}$ at each of the specified glucose thresholds will be derived by,**

The daily (24h period from 12 AM to 12 AM) glucose AOC (area over the curve) less than 70 mg/dL (3.9 mmol/L) and AUC (area under the curve) greater than 70 mg/dL (3.9 mmol/L) will be calculated using Trapezoidal rule,

$$AUC = \int_{a}^{b} f(x)dx \approx (b-a) * \frac{f(a)+f(b)}{2}$$

a and b are the fixed timepoints. f(a) and f(b) are the glucose concentration values of the respective timepoints. (Note: AOC will be calculated using the same formula. Similarly, it will be derived for other glucose thresholds). *For detailed example, see section 9.1.1 (Appendix)*

- **8h overnight glucose $AOC_{0\text{-}8h,<70mg/dL}$ and $AUC_{0\text{-}8h,>70mg/dL}$ at each of the specified glucose thresholds will be derived by,**

The 8h overnight (12 AM midnight to 8 AM) glucose AOC (area over the curve) less than 70 mg/dL (3.9 mmol/L) and AUC (area under the curve) greater than 70 mg/dL (3.9 mmol/L) will be calculated using Trapezoidal rule,

$$AUC = \int_{a}^{b} f(x)dx \approx (b-a) * \frac{f(a)+f(b)}{2}$$

a and b are the fixed timepoints. f(a) and f(b) are the glucose concentration values of the respective timepoints. (Note: AOC will be calculated using the same formula. Similarly, it will be derived for other glucose thresholds). *For detailed example, see section 9.1.1 (Appendix)*

- **Weekly incidence of hypoglycemia (event rate) at each of the specified glucose thresholds by SMBG will be derived by,**

Weekly incidence of hypoglycemia (event rate) <70 mg/dL = The number of unique, discrete, and non-continuous hypoglycemia events (<70 mg/dL threshold) per week over the evaluable period.

Weekly incidence of hypoglycemia (event rate) <60 mg/dL = The number of unique, discrete, and non-continuous moderate hypoglycemia events (<60 mg/dL threshold) per week over the evaluable period.

Weekly incidence of hypoglycemia (event rate) <50 mg/dL = The number of unique, discrete, and non-continuous severe hypoglycemia events (<50 mg/dL threshold) per week over the evaluable period.

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5. PRIMARY AND SECONDARY ENDPOINTS

5.1. Primary Endpoint

- Glycemic efficacy of RZ358 as evaluated by the average daily percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM
- Repeat-dose safety and tolerability of RZ358
- Repeat-dose pharmacokinetics of RZ358

5.2. Secondary Endpoints

- Average weekly incidence of hypoglycemia (< 70 mg/dL) by self-monitored blood glucose (SMBG)
- Average daily duration (min) and percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM
- Average daily hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM
- Average 8h overnight percent time in glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM
- Occurrence of hypoglycemia during a 12h fasting challenge.

5.3. Additional (Exploratory) Endpoints

- Average 8h overnight duration (min) and percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM
- Average 8h overnight hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM

- Average daily and 8h overnight area over and under the glucose curve (AUC, AOC) for each of the specified glucose thresholds by CGM
- Average glucose level between midnight and 8:00 AM (or nearest pre-breakfast time point) by CGM
- Average weekly hypoglycemia incidence (event rate) at each of the specified glucose thresholds by SMBG
- Intravenous or enteral nutritional (glucose/dextrose) supplementation
- Use of SoC or rescue medications for hypoglycemia (dextrose or other)
- Incidence of fasting blood glucose values >200, >250, and >300 mg/dL (>11.1, >13.9, and >16.6 mmol/L, respectively)
- PD Markers of RZ358 activity including fasting levels of glucose, insulin, c-peptide, glucagon, FFAs, and ketones (beta-hydroxybutarate).

6. ANALYSIS POPULATION AND TREATMENT GROUPS

6.1. Analysis Population

ITT Population: All patients who are enrolled into the study.

Safety Population: All patients enrolled in the study who receive at least one dose of study drug.

Per protocol Population: All patients who receive at least 4 weeks of study drug (2 bi-weekly doses), with no major protocol violations. A patient in the Per protocol population may be excluded from one or more specific efficacy analyses (i.e. SMBG-related endpoints or CGM-related endpoints), if they have a major protocol deviation affecting only that endpoint (e.g. non-compliance with SMBG only, or non-compliance with CGM only).

PK Population: All patients who receive study drug and have a sufficient number and quality (biologically plausible) of results.

6.2. Treatment Groups

The study will consist of up to 4 dose cohorts, which are to be enrolled independently and conducted sequentially, comprised of up to 6-8 patients per cohort to ensure each cohort includes at least 3 patients aged ≤10 years with hypoglycemia due to CHI.

In Cohorts 1, 2, and 3 the dose amount will be fixed during the entire 8-week Treatment Period, while in Cohort 4 there will be a scheduled bi-weekly dose-titration for the first 4 weeks, followed by a fixed dose amount thereafter for the remaining 4 weeks. *(Refer section 2.2, Table 1: Study Design/Cohort Dosing Regimens).*

The cohorts will be labelled in the TLFs as Cohort 1 (3 mg/kg), Cohort 2 (6 mg/kg), Cohort 3 (9 mg/kg), Cohort 4 (Dose Escalation mg/kg), and RZ358 Total.

7. ANALYSIS METHODS AND REPORTING DESCRIPTION

7.1.1. Subject Disposition

Subject disposition will be summarized using frequency count and percentage by cohort and RZ358 Total, for number of subjects screened, number of subjects enrolled, number of subjects in the ITT population, number of subjects in the Safety populations, number of subjects in the Per protocol (PP) population, number of subjects in the PK population, number of subjects who completed the study treatment, number of subjects who completed the study, number of subjects who discontinued the study but completed the treatment and number of patients who discontinued from the study along with the reasons for discontinuation from the study.

A listing of subject's disposition will be presented to describe dates of completion or early withdrawal for each patient with the reason for early discontinuation, if applicable.

7.1.2. Demography and Baseline Characteristics

The demographic and baseline characteristics will include age (years), age group (2-6, 7-11, 12-17 and ≥18 years), gender, ethnicity, race, height (cm), weight (kg), and BMI (kg/m^2). These will be summarized by cohort and RZ358 Total, for the ITT Population and Per protocol (PP) Population.

Baseline CHI characteristics will include genetic type, CHI medication history, pancreatectomy, exocrine pancreatic insufficiency, repeat surgeries, tube feeding catheter, and type of nutrition and will be summarized by cohort and RZ358 Total, for ITT Population.

Descriptive statistics (n, mean, SD, minimum, median, and maximum) will be presented for continuous variables. Count and percentage will be presented for each class of the categorical variables.

All individual patient demographic and baseline characteristic data will be listed.

7.1.3. Medical and Surgical History

Medical and Surgical history will be summarized by system organ class (SOC) and preferred term (PT) using count and percentage by cohort and RZ358 Total, for the Safety Population. Patients will be counted only once at the PT, only once at the SOC, and only once at patient level for the counting of total number of patients with a medical history term. Counts will be presented in descending frequency unless otherwise specified. Medical history will be coded using MedDRA version 25.0 Mar 2022.

Listing of medical and surgical history will also be presented for Safety Population.

7.1.4. Prior and Concomitant Medications

Any medication other than study drug, either prescription drug or over the counter (OTC) will be treated as concomitant medication.

Prior Medications

For a patient in the Safety Population, prior medications will include any medication taken within 30 days of signing ICF but stopped prior to patient's first dose of Study Medication.

Concomitant Medications

Concomitant medications are defined as prescribed medications and over the counter (OTC) preparations, including herbal preparations and vitamins, other than Study Medication taken within 30 days of ICF and during the treatment and follow-up phase.

Prior and concomitant medication will be categorized by preferred Term and ATC level X class per World Health Organization Drug Dictionary (WHODRUG; Version Global B3 SEP2019) will be summarized by cohort and RZ358 Total, for the Safety Population. The count and percentage of patients using each medication will be displayed. Patients who taken the same medication (in terms of PT) more than once will be counted only once for that medication.

All prior and concomitant medications will be presented in a listing for the Safety Population.

7.1.5. Protocol Deviation

Protocol deviations will be identified and classified by ICH category 1 through 5 and as minor or major and Important Protocol Deviations (as defined by ICH) will be summarized using count and percentage by cohort and RZ358 Total, for the Safety Population. A listing of patients with protocol deviations will be presented.

Compliance will be check for CGM & SMBG and below definitions will be used for the same.

CGM (Continuous glucose monitoring)

Percent compliance of CGM data will be defined as >=70% data capture during the overall monitored time during the treatment period, including >= 70% data capture during the efficacy evaluable period between last dose of study drug and the Day 14 EOT/Follow-up Visit.

$$CGM\ Compliance\ (\%) = \frac{\text{Actual number of 5 minutes interval of CGM readings}}{\text{Expected number of 5 minutes interval of CGM readings}} \times 100$$

Subjects will be considered compliant if the % compliance is calculated to be >=70% by each of the above criteria and compliance rate will be summarized using descriptive statistics. Overall compliance will also be summarized using counts and percentages by categorizing it into compliant and non-compliant.

Note: Data capture is defined as available recordings in the 10-day sensor period.

SMBG (Self-Monitoring of Blood Glucose)

Percent compliance of SMBG data will be defined as >=50% data capture during the overall monitored time during the treatment period, including >=75% data capture during the efficacy evaluable period between the last dose of study drug and the Day 14 EOT/Follow-up Visit.

$$SMBG\ Compliance\ (\%) = \frac{\text{Actual number of SMBG readings}}{\text{Expected number of SMBG readings}} \times 100$$

Subjects will be considered compliant if the % compliance meets each of the above criteria, and compliance rate will be summarized using descriptive statistics. Overall compliance rate will be summarized using counts and percentages by categorizing it into compliant and non-compliant.

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7.1.6. Study Drug Exposure and Treatment Compliance

Duration of study drug exposure will be summarized by cohort using descriptive statistics (n, mean, SD, minimum, median, and maximum) for the Safety Population. For each subject, duration of study drug exposure (days) will be calculated as below:

((Date of last administration of study drug+14 Days) - Date of first administration of study drug) + 1.

Compliance to the study drug will be calculated as:

$$\text{Treatment Compliance (\%)} = \frac{Actual\ number\ of\ doses\ taken}{Total\ number\ of\ scheduled\ doses} \times 100$$

Subjects will be considered compliant, if the % compliance is calculated at least 75% and have at least completed 4 weeks of study treatment. The compliance rate will be summarized by cohort using descriptive statistics (n, mean, SD, minimum, median, and maximum) for the Safety Population. Also,

overall compliance will be summarized by cohort using counts and percentages by categorizing it into compliant and non-compliant for the Safety Population. Separate listing will also be provided.

7.1.7. Efficacy Analysis

7.1.7.1. Primary Endpoint

A CGM system will be used to evaluate glycemic eligibility and efficacy endpoints for glycemic control. Several parameters to measure glycemic control by CGM will be evaluated over the 2-week End of Treatment period after the final dose, compared to baseline. The efficacy analysis will be conducted in the Intent–to–Treat (ITT), and Per-Protocol (PP) Populations.

The primary endpoint is the glycemic efficacy of RZ358 as evaluated by the average daily percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM. The derivation of daily percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) is presented in section 4.1. The primary endpoint of actual and change from baseline values will be summarized by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum, and 95% CI for Mean). Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations. Corresponding mean (SD) plot will also be produced to illustrate the descriptive statistics as appropriate.

7.1.7.2. Secondary Endpoints

The secondary endpoints to measure glycemic control by CGM and SMBG will be evaluated, at each of the specified timepoints, compared to baseline. The analysis of CGM parameters and SMBG parameters will be evaluated over the 2-week End of Treatment period after the final dose, compared to baseline. The secondary endpoints analysis will be conducted in the Intent–to–Treat (ITT), and PP Populations. The following secondary endpoints include:

- Average weekly incidence of hypoglycemia (< 70 mg/dL) by self-monitored blood glucose (SMBG)
- Average daily duration (min) and percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM
- Average daily hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM
- Average 8h overnight percent time in glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM

The derivations of above secondary endpoints are presented in section 4.1. The secondary endpoints of actual and change from baseline values will be summarized by visits, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI for Mean). Along with these, First Quartile (Q1) and Third Quartile (Q3) will also be presented for duration (min) parameters. Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations. Corresponding mean (SD) plots will also be produced for parameters to illustrate the descriptive statistics as appropriate.

The secondary endpoints "Average daily Percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM" and "Average weekly incidence of hypoglycemia (< 70, <60, <50 mg/dL) by self-monitored blood glucose" will also be summarized by the percent change from baseline by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI for Mean). Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations.

Occurrence of hypoglycemia during a 12h fasting challenge:

- Time to termination of fast
- Glucose nadir during fast
- Percentage of patients able to complete a fast
- Ketone levels at the conclusion of fast

Above mentioned parameters i.e., Time to termination of fast, Glucose nadir during fast, and Ketone levels at the conclusion of fast will be summarized by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI for Mean). Within cohort comparative analysis will be performed using paired t test by comparing week 7 visit with baseline visit, p-value from the test will be reported and analysis will be performed using Intent–to–Treat (ITT), and PP Populations.

Also, Percentage of patients able to complete a fast will be summarized using frequency count and percentage by visit, cohort, and RZ358 Total. Within cohort comparison of proportions will be performed using McNemar's test by comparing week 7 visit with baseline visit, p-value from the test will be reported and analysis will be performed using Intent–to–Treat (ITT), and PP Populations.

7.1.7.3. Exploratory Endpoints

The exploratory endpoints to measure glycemic control by CGM and SMBG will be evaluated, at each of the specified timepoints, compared to baseline. The analysis of CGM parameters and SMBG

parameters will be performed over the 2-week End of Treatment period after the final dose, compared to baseline. The exploratory endpoints analysis will be conducted in the PP and ITT Populations. The following exploratory endpoints include:

- Average 8h overnight duration (min) and percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM
- Average 8h overnight hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM
- Average daily and 8h overnight area over and under the glucose curve (AUC, AOC) for each of the specified glucose thresholds by CGM
- Average glucose level between midnight and 8:00 AM (or nearest pre-breakfast time point) by CGM
- Average weekly hypoglycemia incidence (event rate) at each of the specified glucose thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by SMBG

  Additionally, Average weekly hyperglycemia incidence (event rate) at each of the specified glucose thresholds of >180, >250, and >300 mg/dL (10.0, 13.9 and 16.6 mmol/L, respectively) by SMBG

The derivations of above exploratory endpoints are presented in section 4.1. The exploratory endpoints of actual and change from baseline values will be summarized by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI for Mean). Along with these, First Quartile (Q1) and Third Quartile (Q3) will also be presented for duration (min) parameters. AOC and AUC of above glucose parameters will also be summarized by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, CV (%), Geometric Mean, Geometric CV (%), Median, Minimum, Maximum and 95% CI for Geometric Mean). Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations. Corresponding mean (SD) plots will also be produced for above endpoints to illustrate the descriptive statistics as appropriate.

Comparison of glycemic efficacy of RZ358 by CGM/SMBG endpoints

The within cohort comparison of post baseline visits to baseline visit in glycemic efficacy of CGM/ SMBG endpoints over the 2-week End of Treatment period after the final dose will be analyzed using paired t-test and p-value from the test will be reported.

The null and alternative hypothesis are,

$H_0 : \mu_d = 0$ and $H_1 : \mu_d \neq 0$

Where, μ_d is the mean difference in glycemic efficacy of CGM/SMBG parameters within cohort.

The normality of the CGM/SMBG endpoints will be performed using Shapiro-Wilk Test. If the p-value from the test result is normal, that is, p-value ≥ 0.05, then paired t-test will be used; otherwise, a non-parametric Wilcoxon Sign Rank Test will be used.

Additional endpoints

Listings will be presented for the following additional endpoints.

- Intravenous or enteral nutritional (glucose/dextrose) supplementation
- Use of SoC or rescue medications for hypoglycemia (dextrose or other)

Pharmacodynamic Biomarker Analyses

Blood samples will be collected at scheduled timepoints, throughout the treatment periods of the study to evaluate the PD effects of the study drug. The PD of RZ358 compared to baseline will be assessed by:

- Average levels of glucose, insulin, c-peptide, glucagon, FFAs, and ketones (beta-hydroxybutarate), at each of the specified collection timepoints.

The actual and change from baseline values of the above parameters will be summarized using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI for Mean) at each of the specified collection timepoints by visit, cohort and RZ358 Total for the PP population.

- Incidence of safety fasting blood glucose values >200, >250, and >300 mg/dL (>11.1, >13.9, and >16.6 mmol/L, respectively).

Incidence of safety fasting blood glucose values >200, >250, and >300 mg/dL (>11.1, >13.9, and >16.6 mmol/L, respectively) will be summarized using frequency count and percentage at each of the specified collection timepoints by visit, cohort and RZ358 Total for the PP population.

7.1.7.4. Summary of Efficacy Endpoint Analysis Strategy

Endpoint	Statistical Method / Test	Analysis Population	Missing Data Imputation Approach	Parameters/Variables in the analysis (Stat. test/Model)	Analysis Time point
Primary Endpoint					
Glycemic efficacy of RZ358 as evaluated by the average daily percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average daily percent time, cohort	8 Weeks /EOT
Secondary Endpoint					
Average weekly incidence of hypoglycemia (< 70 mg/dL) by self-monitored blood glucose (SMBG)	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average weekly incidence, cohort	8 Weeks /EOT

Endpoint	Statistical Method / Test	Analysis Population	Missing Data Imputation Approach	Parameters/Variables in the analysis (Stat. test/Model)	Analysis Time point
Average daily duration (min) and percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average daily duration, Average daily percent time, cohort	8 Weeks /EOT
Average daily hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average daily hypoglycemia incidence, cohort	8 Weeks /EOT
Average 8h overnight percent time in glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average 8h overnight percent time, cohort	8 Weeks /EOT

Endpoint	Statistical Method / Test	Analysis Population	Missing Data Imputation Approach	Parameters/Variables in the analysis (Stat. test/Model)	Analysis Time point
Occurrence of hypoglycemia during a 12h fasting challenge. (Percentage of patients able to complete a fast)	Count and percentage, Comparison of proportions using McNemar Test.	PP Population and ITT Population	No imputation	eCRF	Week 7
Occurrence of hypoglycemia during a 12h fasting challenge. (Time to termination of fast, Glucose nadir during fast, Ketone levels at the conclusion of fast)	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	eCRF	Week 7
Additional Exploratory Endpoints					
Average 8h overnight duration (min) and percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average 8h overnight duration, Average 8h overnight percent time, cohort	8 Weeks /EOT

Endpoint	Statistical Method / Test	Analysis Population	Missing Data Imputation Approach	Parameters/Variables in the analysis (Stat. test/Model)	Analysis Time point
Average 8h overnight hypoglycemia incidence (event rate) at each of the specified glucose thresholds by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average 8h overnight hypoglycemia incidence, cohort	8 Weeks /EOT
Average daily and 8h overnight area over and under the glucose curve (AUC, AOC) for each of the specified glucose thresholds by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average daily and 8h overnight AUC & AOC, cohort	8 Weeks /EOT
Average glucose level between midnight and 8:00 AM (or nearest pre-breakfast time point) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average glucose level between midnight and 8:00 AM, Cohort	8 Weeks /EOT

Endpoint	Statistical Method / Test	Analysis Population	Missing Data Imputation Approach	Parameters/Variables in the analysis (Stat. test/Model)	Analysis Time point
Average weekly hypoglycemia incidence (event rate) at each of the specified glucose thresholds by SMBG	Descriptive statistics Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average weekly hypoglycemia incidence, cohort	8 Weeks /EOT
Glycemic efficacy of RZ358 as evaluated by the average daily percent time within a glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average daily percent time, cohort	8 Weeks /EOT
Average 8h overnight percent time in glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) by CGM	Descriptive statistics, Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average 8h overnight percent time, cohort	8 Weeks /EOT

Endpoint	Statistical Method / Test	Analysis Population	Missing Data Imputation Approach	Parameters/Variables in the analysis (Stat. test/Model)	Analysis Time point
Average weekly hyperglycemia incidence (event rate) at each of the specified glucose thresholds of >180, >250, and >300 mg/dL (10.0, 13.9 and 16.6 mmol/L, respectively) by SMBG	Descriptive statistics Paired T-test (analyzing post baseline visits with baseline visit)	PP Population and ITT Population	No imputation	Average weekly hyperglycemia incidence, cohort	8 Weeks /EOT
Incidence of fasting blood glucose values >200, >250, and >300 mg/dL (>11.1, >13.9, and >16.6 mmol/L, respectively)	Count and percentage	PP Population	No imputation	eCRF	8 Weeks /EOT
PD Markers of RZ358 activity including fasting levels of glucose, insulin, c-peptide, glucagon, FFAs, and ketones (beta-hydroxybutarate).	Descriptive statistics	PP Population	No imputation	eCRF	8 Weeks /EOT

7.1.8. Safety Analysis

The analysis of primary safety endpoints will include assessments of AEs, vital signs, 12-lead ECGs, infusion-site observations, physical examination, hepatic ultrasound results, and clinical laboratory evaluations including glucose values. All safety analyses will be performed using the Safety Population.

Adverse Events:

Adverse Events will be coded using (MedDRA version 25.0 Mar 2022) AE coding system for the purpose of summary tables. Treatment-emergent adverse events (TEAE) will be summarized by cohorts and RZ358 Total and AEs that are non-TEAE will be identified.

For each cohort, AEs will be summarized with frequency count and percentage by MedDRA SOC and PT, with all patients treated in that cohort as the denominator, unless otherwise specified. In addition, AEs will also be summarized by severity and relationship to study drug.

All AEs and TEAEs during the study will be summarized for the respective cohorts. A patient experiencing the same AEs and TEAEs multiple times will be counted only once for that PT. Similarly, if a patient experiences multiple AEs and TEAEs (preferred terms) within the same SOC then that patient will be counted only once for that SOC. When summarizing by severity, the event with highest severity will be counted. All AEs will be listed in chronological order of the events occurred.

For purposes of this study, TEAEs for summary tabulation will be further defined as those events which occur within a period of time through the +42 days follow-up visit after the final dose of study drug. Adverse events with onset after ICF but before first dose of study drug or after last dose of study drug +42 days will be identified separately from TEAEs.

Summary tables of the following AEs will be provided:

An overview of TEAE summary will be presented by cohorts, and it will include:

- ➢ Number of Subjects with any TEAEs
- ➢ Number of subjects with any TEAEs related to study drug
- ➢ Number of subjects with any TEAEs leading to treatment/study discontinuation
- ➢ Number of subjects with TEAEs by maximum severity grade
- ➢ Number of Subjects with any serious TEAEs
- ➢ Number of Subjects with any serious TEAEs related to study drug
- ➢ Number of Subjects with any serious TEAEs leading to treatment/study discontinuation
- ➢ Number of Subjects with any serious TEAEs leading to death

➢ Number of subjects with any non-TEAEs

A summary of the frequency (number and percentage of patients) of TEAEs and serious TEAEs by cohort will be presented by SOC, PT, and non-TEAEs will be separately identified.

Similarly, summary results will be generated for TEAEs by SOC, PT for severity grade>=3.

A summary of the frequency (number and percentage of patients) of TEAEs by cohort will be presented by SOC, PT and by related to study drug.

A summary of the frequency (number and percentage of patients) of TEAEs leading to treatment/study discontinuation by cohort will be presented by SOC and PT.

All AEs reported during the study will be present in a by-patient listing. Separate by-patient listings will be generated to present AEs, SAEs leading to discontinuation from treatment and the study and TEAEs leading to death.

Infusion Site Reactions:

A local infusion site reaction is any local reaction occurring at the site of injection following study drug administration. A local infusion site reaction, including, but not limited to, erythema, induration, and pain, should be reported as an adverse event.

Infusion site reactions will be summarized by cohort and RZ358 Total using frequency count and percentage for safety population.

All infusion site reactions will be presented in listing for safety population.

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Vital signs:

Vital signs measurement will include systolic and diastolic blood pressure (mmHg), temperature (degree Celsius), pulse rate (beats/minute) and respiratory rate (breaths/minute).
The actual and change from baseline values at each scheduled time-point will be summarized by visit, cohort and RZ358 Total (pediatric and adult patients) using descriptive statistics (n, Mean, SD, Median, Minimum, and Maximum) for Safety population. For categorical shift summaries, any available pre-treatment values (screening or pre-treatment baseline) may be used to define baseline, for the categorical assignment.

All vital signs data will be presented in listings for Safety population.

The below table will be referred to for the vital signs abnormalities.

Table 2: Vitals Abnormality

VS Ranges	1-3 Years	4-5 Years	6-8 Years	9-12 Years	13-16 Years	17-Onwards	≤12	≥13	Min	Max
Systolic BP							80-120	90-140		
Diastolic BP							50-80	55-90		
Temperature									36.0	38.0
Respiratory Rate							18-30	12-20		
Pulse Rate	94-179	72-195	61-116	54-111	47-108	60-100				

12-Lead ECG:

The 12-lead ECG measurements will include heart rate (bpm), QRS (msec), QT (msec), corrected QT (msec), RR (msec), and PR (msec) intervals, as well as an overall interpretation.

Actual and change from baseline value of quantitative ECG parameters will be summarized using descriptive statistics (n, Mean, SD, Median, Minimum, and Maximum) by visit, cohort and RZ358 Total (pediatric and adult patients) for safety population.

Overall assessment of safety ECGs (Normal, Abnormal - NCS, Abnormal – CS) will be summarized using frequency counts and percentage by visit, cohort and RZ358 Total for safety population. In addition, a shift table comparing overall interpretation (Normal, Abnormal - NCS, Abnormal - CS) of the ECG parameters at baseline vs treatment-emergent post-baseline (worst treatment-emergent category) will also be presented by cohort for safety population. For categorical shift summaries, any available pre-treatment values (screening or pre-treatment baseline) may be used to define baseline, for the categorical assignment.

Individual data listings of ECG results along with the Investigator-identified ECG abnormalities will be presented for each patient.

Clinical Laboratory Data

The Clinical Laboratory test category will include assessments of Hematology, Chemistry, Urinalysis, HbA1c, ADA Blood Collection.

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Quantitative parameters of hematology, chemistry, and urinalysis, actual and change from baseline values at each scheduled time-point will be summarized by visit, cohort and RZ358 Total (pediatric and adult patients) using descriptive statistics (n, Mean, SD, Median, Minimum, and Maximum) for safety population. All qualitative result parameters at each scheduled time-point will be summarized by visit, cohort and RZ358 Total using frequency counts and percentage for safety population.

Additionally, comparing the changes in categorical laboratory variables from baseline (Low, Normal, and High) / (Positive, Negative) vs treatment-emergent post-baseline (worst treatment-emergent category) will be presented by cohort using a shift table for safety population. For categorical shift summaries, any available pre-treatment values (screening or pre-treatment baseline) may be used to define baseline, for the categorical assignment.

A summary table will be presented for HbA1c by visit, cohort and RZ358 Total for safety population.

Anti-drug Antibody (ADA): Anti-drug Antibody (ADA) with categories ADA Negative, Pre-existing Immunoreactivity, Treatment Boosted, and Treatment-emergent. Positive will be summarized by cohort, and RZ358 Total using frequency count and percentage for safety population.

Definitions:

ADA Negative: defined as negative response in the ADA assay at all time points and those that exhibit a pre-existing response, regardless of any missing samples.

Pre-existing Immunoreactivity: defined as either a positive response in the ADA assay at baseline with all post first dose ADA results negative OR a positive response at baseline with all post dose ADA response less than 4-fold of baseline titer levels.

Treatment Boosted ADA: defined as a positive response post first dose that is greater than or equal to 4-fold over baseline titer level, when baseline results are positive.

Treatment-emergent Positive (D): defined as an ADA positive response post first dose when baseline results are negative or missing, OR ADA positive response more than 4-fold of a positive baseline titer.

Additionally, comparing the changes in categorical ADA Blood Collection from baseline (Positive, Negative) to post baseline visit will be presented by cohort using a shift table for safety population.

Individual data listings of laboratory results will be presented for hematology, chemistry, urinalysis HbA1c and ADA Blood Collection separately.

Physical examination:

The investigator or qualified designee will perform a complete physical examination (genitourinary examination not required) and targeted abbreviated physical examinations.

A full physical examination will include, at a minimum, assessment of the following systems: skin, head, ears, eyes, nose and throat, respiratory system, cardiovascular system, gastrointestinal system, neurological condition, blood and lymphatic systems, and the musculoskeletal system. An abbreviated physical examination is inclusive of neurological, cardiac, gastrointestinal, and pulmonary assessments.

The physical examination findings (Normal, Abnormal NCS, Abnormal CS) will be summarized using counts and percentages by visit, cohort and RZ358 Total for each body system using safety population.

Additionally, for each body system, changes in physical examination findings from baseline vs treatment-emergent post-baseline (worst treatment-emergent category) will be presented using a shift table for each cohort using safety population. For categorical shift summaries, any available pre-treatment values (screening or pre-treatment baseline) may be used to define baseline, for the categorical assignment.

All physical examination findings will also be presented in listing for safety population.

Hepatic Ultrasound

Hepatic ultrasound will be performed at the specified time points as listed in the Schedule of Assessments, and per the Investigator's institution's standard practice methodology. The results will be interpreted by qualified personnel.

The overall interpretation of hepatic ultrasound (Normal, Abnormal NCS, Abnormal CS) will be summarized using counts and percentages by visit, cohort and RZ358 Total for safety population.

Additionally, changes in overall interpretation of hepatic ultrasound from baseline vs treatment-emergent post-baseline (worst treatment-emergent category) will be presented using a shift table for each cohort using safety population. All hepatic ultrasound results will also be presented in listing for safety population.

7.1.9. PK Analysis

PK analysis plan development and performance of PK analysis will be managed by another vendor. Therefore, the reporting will be described in a supplementary PK analysis plan and report.

Following are the details of PK analysis:

The individual and mean RZ358 serum concentrations will be summarized by treatment cohort and planned sample times in tables and figures. Serum concentrations for samples collected within 30 min after the dosing infusion will be reported as the observed concentration at the end of infusion (CEOI). Concentrations will be summarized using descriptive statistics. The study design and planned PK sample collection are not suitable for the calculation of PK parameters by noncompartmental methods. Specifically, the number of PK samples within dosing intervals is insufficient to support noncompartmental estimation of AUC; and due to the long half-life of RZ358, steady state is not expected to be achieved by the end of the 8-week Treatment Period. Therefore, the observed serum RZ358 concentration-time data will be used to develop a population PK (popPK) model to describe the PK of RZ358 in patients with CHI. PopPK modeling will also investigate the effects of selected covariates on RZ358 PK such as age, body weight, gender and race, as data permit.

The popPK model will be used to estimate PK parameters for each treatment cohort, including but not limited to the following:
• AUCss: steady state area under the concentration-time curve.
• Cmaxss: steady state maximum concentration.
• Cavgss: steady state average concentration.
• t1/2: effective half-life.
• CL: total drug clearance.
• V: volume of distribution of the central compartment.

Descriptive statistics will be presented for PK parameters, including but not limited to Cmax, Tmax, t1/2, and AUC. The relationships between dose and Cmax and AUC will be analyzed for dose proportionality. The PK results of this study may be combined with those of other studies for analysis and modeling (e.g., population PK and PK-PD), and reported separately Detailed methods for the PK modeling will be provided in a separate PK modeling analysis plan.

7.1.10. Pooled Analyses

No pooled analyses will be conducted in this trial.

7.1.11. Subgroup Analyses

The subgroup analyses will be evaluated for the below mentioned primary, secondary, and exploratory endpoints of this study using Per-Protocol (PP) population.

- Glycemic efficacy of RZ358 as evaluated by the average daily percent time within a glucose target range of 70-180 mg/dL (3.9-10 mmol/L) by CGM
- Average weekly incidence of hypoglycemia (< 70 mg/dL) by self-monitored blood glucose (SMBG)
- Average daily percent time with hypoglycemia at thresholds of <70, <60, and <50 mg/dL (3.9, 3.3, and 2.8 mmol/L, respectively) by CGM
- Average weekly hypoglycemia incidence (event rate) at each of the specified glucose thresholds by SMBG
- Average weekly hyperglycemia incidence (event rate) at each of the specified glucose thresholds by SMBG

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specified glucose thresholds by SMBG".

7.1.12. Interim Analysis

No interim analyses planned for this trial.

7.1.13. Changes to Analyses Specified in Protocol

- Glycemic efficacy of RZ358 as evaluated by the average daily percent time within a glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) by CGM

Above mentioned endpoint will be derived as below:

Daily percent time within a glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) by CGM will be derived by,

Daily percent time (%) = (Actual number of readings satisfying glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) / Total number of readings obtained in 24h period from 12 AM to 12 AM) × 100. *(Note: The day (24 hours) will be derived using date and time).*

Above mentioned endpoint will be summarized and analyzed as below:

The endpoint of actual and change from baseline values will be summarized by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum, and 95% CI for Mean). Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations.

- Average 8h overnight percent time in glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) by CGM

Above mentioned endpoint will be derived as below:

8h overnight percent time within a glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) by CGM will be derived by,

8h overnight percent time (%) = (Actual number of readings satisfying glucose target range of 70-250 mg/dL (3.9-13.9 mmol/L) / Total number of readings obtained in 8h overnight period from 12 AM (midnight) to 8 AM) × 100.

Above mentioned endpoint will be summarized and analyzed as below:

The endpoint of actual and change from baseline values will be summarized by visits, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI

for Mean). Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations.

- Average weekly hyperglycemia incidence (event rate) at each of the specified glucose thresholds of >180, >250, and >300 mg/dL (10.0, 13.9 and 16.6 mmol/L, respectively) by SMBG

Above mentioned endpoint will be derived as below:

Weekly incidence of hyperglycemia (event rate) >180 mg/dL = The number of unique, discrete, and non-continuous hyperglycemia events (>180 mg/dL threshold) per week over the evaluable period.

Weekly incidence of hyperglycemia (event rate) >250 mg/dL = The number of unique, discrete, and non-continuous moderate hyperglycemia events (> 250 mg/dL threshold) per week over the evaluable period.

Weekly incidence of hyperglycemia (event rate) >300 mg/dL = The number of unique, discrete, and non-continuous severe hyperglycemia events (>300 mg/dL threshold) per week over the evaluable period.

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(Note: The week (7 days) will be derived using date and time.)

Above mentioned endpoint will be summarized and analyzed as below:

The endpoint of actual and change from baseline values will be summarized by visit, cohort and RZ358 Total using descriptive statistics (n, Mean, SD, SE, Median, Minimum, Maximum and 95% CI for Mean). Within cohort comparative analysis will be performed using paired t test by comparing each post baseline visits with baseline visit, p-value from the test will be reported and analysis will be performed using ITT and PP Populations.

REFERENCE

- ICH E3: Structure and Content of Clinical Study Reports.
- Clinical Study Protocol (RZ358-606 version amendment 5.0 dated 17MAY2021
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- Arnoux JB, Verkarre V, Saint-Martin C, et al. Congenital hyperinsulinism: current trends in diagnosis and therapy. Orphanet J Rare Dis 2011;6:63.
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9. APPENDIX

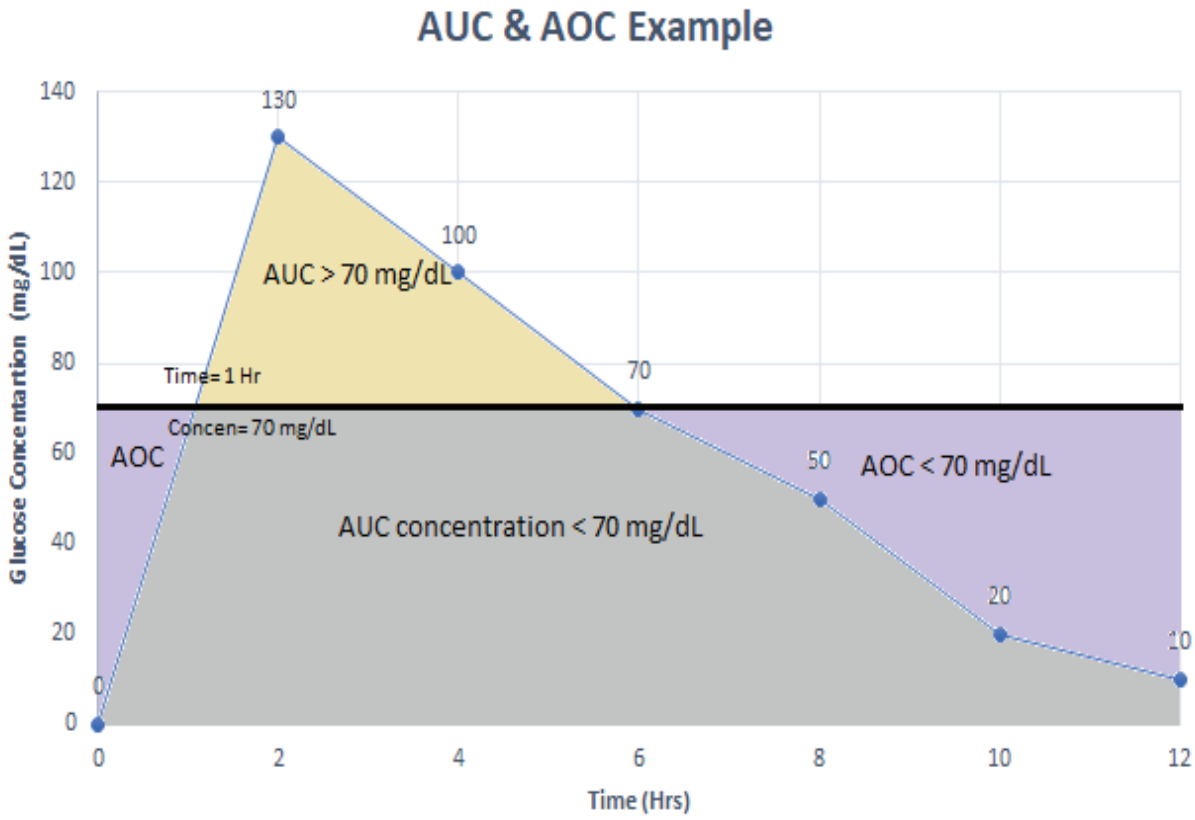
9.1.1. AUC and AOC Derivation

Daily glucose AOC and AUC at each of the specified glucose thresholds by CGM

The figure below is presented only to show the idea of the calculation of glucose AUC and AOC parameters and the shape of the curve is based on dummy data. The parameters will be calculated using Linear Trapezoidal Rule over the specified time range.

Daily glucose AOC (area over the curve) less than 70 mg/dL (3.9 mmol/L) and AUC (area under the curve) greater than 70 mg/dL (3.9 mmol/L) will be calculated using Trapezoidal rule,

$$AUC/AOC = \int_{a}^{b} f(x)dx \approx (b-a) * \frac{f(a)+f(b)}{2}$$



- From the above figure, the horizontal reference line is the glucose threshold of 70 mg/dL (3.9 mmol/L)
- Area over the curve (AOC) < 70 mg/dL will be calculated as sum of AOC parts (Lavender) using Linear Trapezoidal Rule
- Area under the curve (AUC) > 70 mg/dL will be calculated as sum of AUC parts (Yellow) using Linear Trapezoidal Rule

$AUC_{0\text{-}t}$ (mg/dL × Hours) for plasma glucose > 70 mg/dL

From the yellow region of the example figure, the glucose AUC (area under the curve) > 70 mg/dL is calculated as follows:

$$AUC_{0\text{-}12h>70mg/dL} = [(2-1)\ (0+60)/2] + [(4-2)\ (60+30)/2] + [(6-4)\ (30+0)/2]$$
$$= 30 + 90 + 30$$
$$= 150\frac{mg}{dL} \cdot hr$$

$AOC_{0\text{-}t}$ (mg/dL × Hours) for plasma glucose < 70 mg/dL

From the lavender region of the example figure, the glucose AOC (area over the curve) < 70 mg/dL is calculated as follows:

$AOC_{0\text{-}12h<70mg/dL}$ = [(1-0) (70+0)/2] + [(8-6) (0+20)/2] + [(10-8) (20+50)/2] + [(12-10) (50+60)/2]
= 35 + 20 + 70 + 110
$= 235\frac{mg}{dL} \cdot hr$

Similarly, these methods will be used to derive other endpoints, such as 8h overnight glucose AOC and AUC at each of the specified glucose thresholds <70, <60 and <50 mg/dL (3.9, 3.3 and 2.8 mmol/L, respectively) and >70, >60 and >50 mg/dL (3.9, 3.3 and 2.8 mmol/L, respectively) respectively by CGM.

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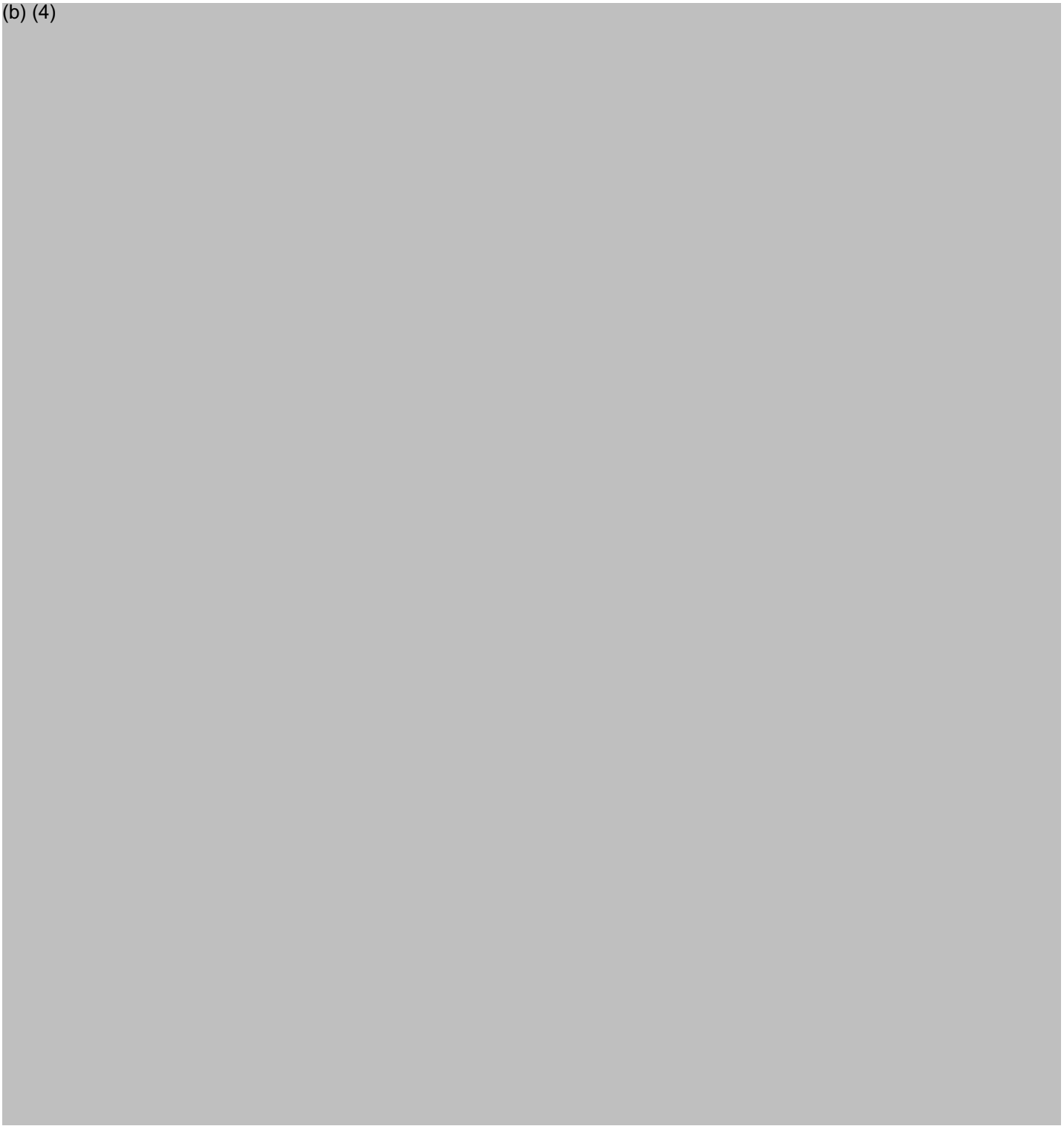

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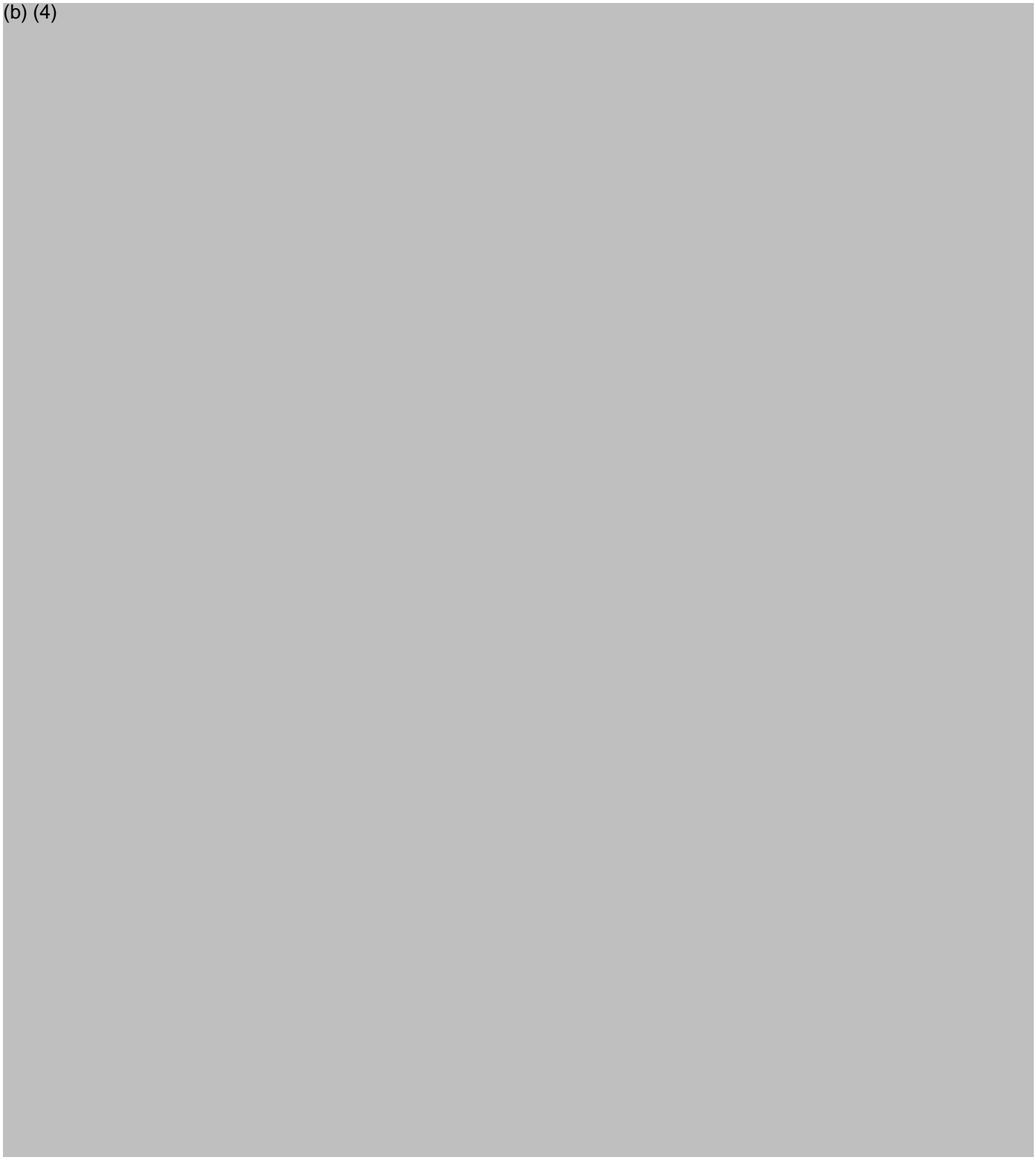

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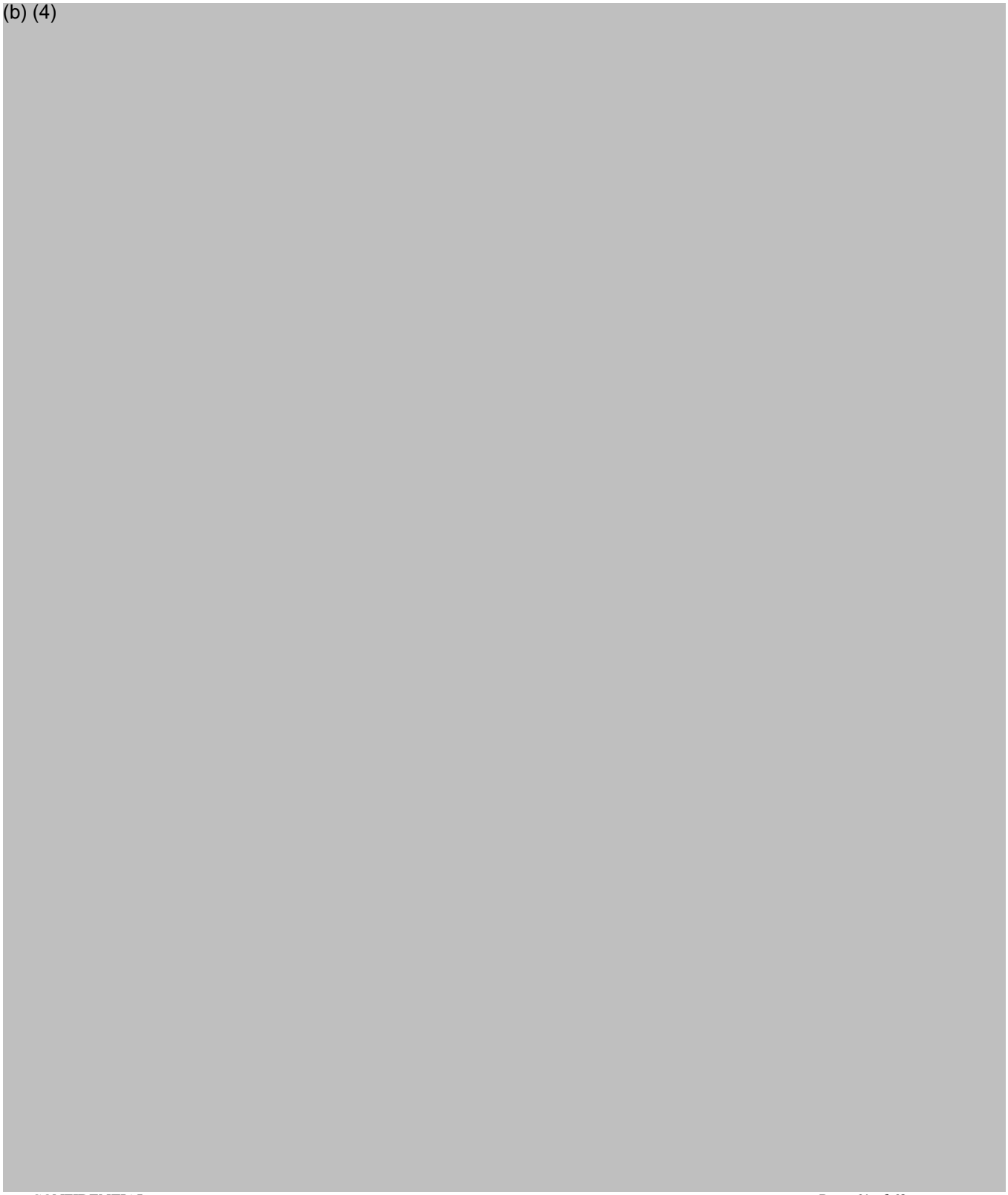

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