Cover Page for Statistical Analysis Plan

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NCT number	NCT05021640
Sponsor trial ID:	DCR-AUD-101
Official title of study:	A Phase 1, Double-blind, Placebo-controlled, Single-ascending-dose, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of DCR-AUD in Healthy Volunteers
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[redacted]

[redacted]

Dicerna Pharmaceuticals, Inc.

DCR-AUD-101

A Phase 1, Double-blind, Placebo-controlled, Single-ascending-dose, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of DCR-AUD in Healthy Volunteers

Statistical Analysis Plan

Version: 2.0

[redacted]

SPONSOR SIGNATURE PAGE

Approved by: [redacted] [redacted]

Dicerna Pharmaceuticals, Inc.

Approved by: [redacted] [redacted]

Dicerna Pharmaceuticals, Inc.

[redacted] SIGNATURE PAGE

Signatures below confirm that the Statistical Analysis Plan was developed in accordance with SOP-GDO-WW-019 and that it is approved for release.

This document has been approved and signed electronically on the final page by the following:

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REVISION HISTORY

Version No.	Effective Date	Summary of Change(s)
Final 1.0	14 Dec 2021	New Document
Final 2.0	Date of Last Signature	Update as per Protocol Amendment 5. Pharmacokinetic and Pharmacodynamic analysis section update.

LIST OF ABBREVIATIONS

Abbreviation / Acronym	Definition / Expansion
AE	Adverse event
AESI	Adverse event of special interest
ALDH2	Aldehyde dehydrogenase 2
ALDH2	Gene for aldehyde dehydrogenase 2
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ATC	Anatomical Therapeutic Chemical
AUC_{Above}	Area under the curve that is above the baseline
AUC_{Below}	Area under the curve that is below the baseline
BMI	Body Mass Index
BP	Blood pressure
Bpm	Beats per minute
BUN	Blood urea nitrogen
CCRS	Columbia-Suicide Severity Rating Scale
CL/F	Apparent clearance following extravascular (non-intravenous) administration
C_{last}	Last quantifiable concentration at t_{last}
CL_R	Renal clearance of drug from plasma
C_{max}	Maximum observed concentration
C_{min}	Minimum observed concentration at steady state
CPMS	Clinical Pharmacology, Modeling, and Simulation
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	Concentration at the end of a dosing interval
CV	Coefficient of variation
DBP	Diastolic blood pressure
DRM	Data Review Meeting
ECG	Electrocardiogram
EIA	Ethanol interaction assessment

Abbreviation / Acronym	Definition / Expansion
GGT	Gamma-glutamyl transferase
HR	Heart rate
HV	Healthy Volunteers
IA	Interim Analysis
IB	Investigator's Brochure
ICF	Informed consent form
IMP	Investigational Medicinal Product
INR	International normalized ratio
ISR	Injection site reaction
L	Linearity index
LDH	Lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MINI	Mini-International Neuropsychiatric Interview
NA	Not available
NCS	Not clinically significant
NK	Not known
PD	Pharmacodynamic
PK	Pharmacokinetic
PKP	Pharmacokinetic Population
PP	Pharmacodynamic Population
QT	The QT interval is measured from the beginning of the QRS complex to the end of the T wave
QTc	Corrected QT interval
QTcB	QT corrected using Bazett's formula
QTcF	QT corrected using Fridericia's formula
RNA	Ribonucleic acid
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SD	Standard deviation or single dose

Abbreviation / Acronym	Definition / Expansion
SEAS	Subjective Effects of Alcohol Scale
SOC	System Organ Class
SOP	Standard Operating Procedure
SP	Safety Population
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
TLFB	Timeline Follow Back
WHO-DD	World Health Organization - Drug Dictionary

1 INTRODUCTION

DCR-AUD is being developed for the treatment of alcohol use disorder (AUD) in adults using an RNA interference (RNAi) technology platform. DCR-A1203, the drug substance of DCR-AUD, is a synthetic double-stranded (hybridized duplex) RNA oligonucleotide conjugated to N-acetyl-D-galactosamine (GalNAc) ligands that enable subcutaneous (SC) delivery of stable, safe, highly specific, and long-acting reduction of mRNA in the liver via hepatic GalNAc carbohydrate receptors. DCR-AUD is designed to selectively reduce ALDH2 mRNA and ALDH2 levels in the liver, and thereby decrease the conversion of acetaldehyde to acetic acid after ethanol ingestion. The DCR-AUD-101 study is the first human study of DCR-AUD.

The overall goals of this study are:

1. To demonstrate that DCR-AUD single doses are safe and well-tolerated.
2. To characterize the PK of single doses of DCR-AUD
3. To characterize the PD of single doses of DCR-AUD and obtain safety data after alcohol exposure in adult healthy volunteers (HVs) as assessed by serial standardized Ethanol Interaction Assessments (EIAs)
    a. EIAs will be used to confirm target engagement. The degree of ALDH2 reduction is measurable only after ethanol administration, via levels of plasma acetaldehyde and acetate, quantitative assessment of 6 symptom responses during EIAs, and measurement of heart rate and facial skin temperature.
    b. The safety of ethanol ingestion in the presence of DCR-AUD will be assessed during EIAs, each of which will be followed by overnight admission and monitoring in a Phase 1 clinical unit. The Investigator will not discharge any participant experiencing ongoing effects of ethanol administration until it is deemed medically safe to do so.
    c. Serial EIAs will be used to determine the duration of action of DCR-AUD, so that an appropriate dose and dosing interval and safety monitoring plan for participants can be developed for later clinical trials.

The SAP describes the variables and populations, anticipated data transformations and manipulations and other details of the analyses not provided in the Clinical Study Protocol (CSP).

The analyses described in this SAP are based upon the following study documents:

- Study Protocol, Final, Amendment 5 (version 6.0) (Mar 14, 2022)
- Case Report Forms (Sep 08, 2022)

The SAP will be finalized prior to database lock or first unblinding of the study; whichever is earlier and describes the statistical analysis as it is foreseen when the study is being planned. If circumstances should arise during the study rendering this analysis inappropriate, or if improved methods of analysis should arise, updates to the analyses may be made and the SAP will be amended. Post database lock, modifications will be documented as Statistical Analysis Modification Requests and documented in Clinical Study Report (CSR).

2 STUDY OBJECTIVES

2.1 Primary Objective(s)

- To evaluate the safety and tolerability of single doses of DCR-AUD administered to adult HVs.

2.2 Secondary Objective(s)

- To characterize the PK of single doses of DCR-AUD in adult HVs
- To characterize the PD of single doses of DCR-AUD in adult HVs as assessed by serial standardized EIAs

2.3 Exploratory Objective(s)

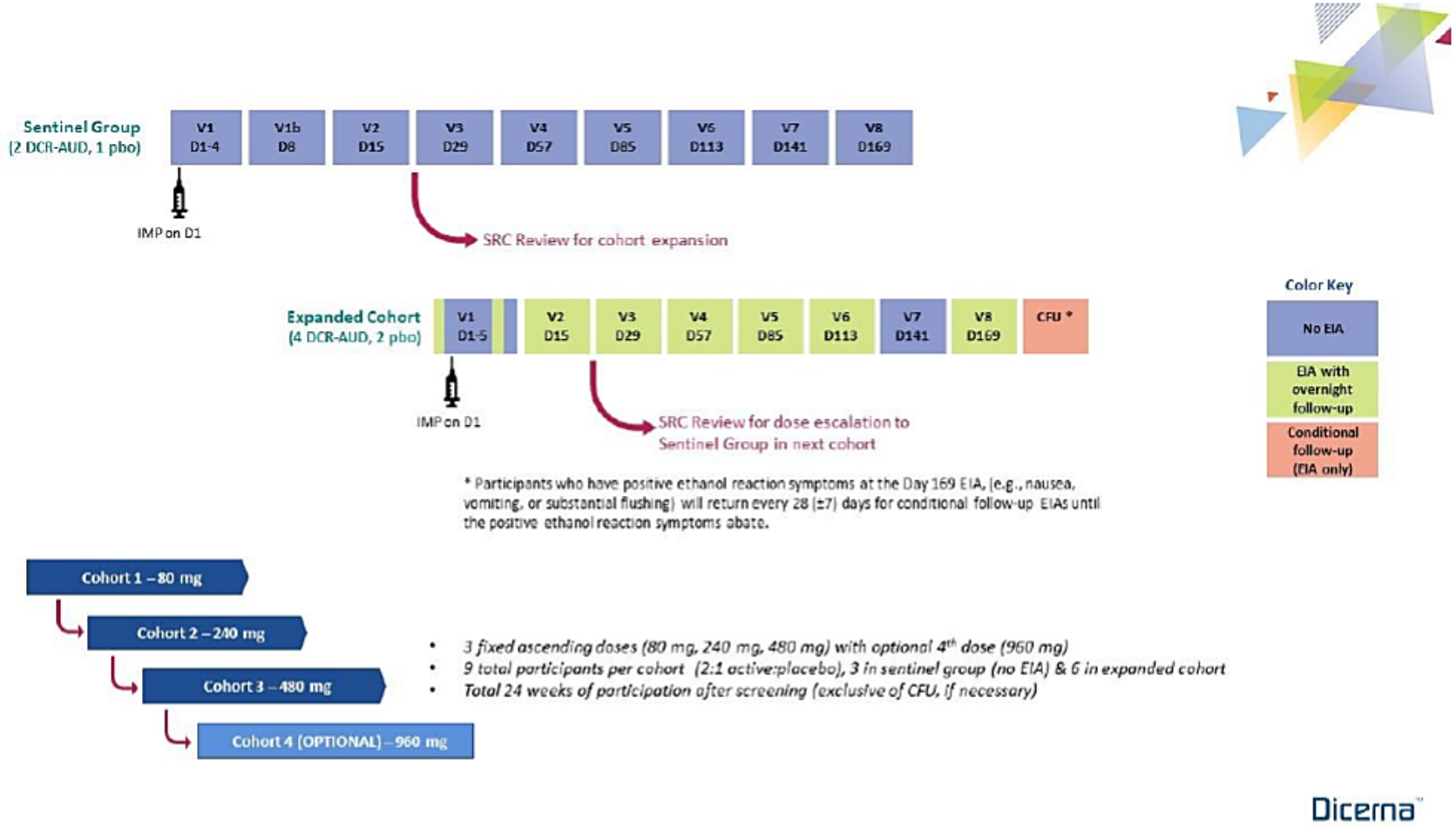
Not Applicable

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This phase I trial is a 24-week, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, PK, and PD of single-ascending doses (SAD) of DCR-AUD administered to adult HVs. The study schematic diagram is shown in **Figure 3-1**.

Figure 3-1 Study Schematic of DCR-AUD-101



Abbreviations: CFU: conditional follow-up; D: Day; EIA: ethanol interaction assessment; IMP: investigational medicinal product; pbo: placebo; SRC: Safety Review Committee; V: Visit

Individual participants will be screened for a period of up to 28 days. Participants may undergo rescreening one time at the discretion of the Investigator or Sponsor. The single doses of DCR-AUD will be administered to adult HVs across 3 fixed ascending-dose cohorts (80 mg, 240 mg, 480 mg) and 1 optional cohort (960 mg). Each cohort will comprise a sentinel group of 3 participants (2 active, 1 placebo) who will be followed for the assessment of safety and tolerability and characterization of PK but who will not undergo any EIAs. The sentinel group will remain under observation in clinic until Day 4 (after dosing on Day 1) and will return to clinic on Days 8 and 15 for clinical and laboratory test evaluations. The SRC will review available sentinel group safety data through the Day 15 assessments prior to authorizing cohort expansion within an individual cohort for the remaining 6 participants (4 active, 2 placebo). The 6 participants in the expanded cohort will be followed for the assessment of safety and tolerability, characterization of PK, and assessment of PD (via EIA). In addition to alcohol exposure on Day –1 (prior to administration of study intervention), these participants will undergo serial EIAs on Day 4 before being discharged from clinic on Day 5, and at 6 other times during the 24-week study period. All EIAs will be conducted as in-clinic assessments.

An SRC will be convened to review available safety data at a predefined decision point (Visit 2, Day 15) to ensure the safety of the participants in all cohorts. The scope of the review meetings will be defined in the SRC charter. In the event that the SRC decides to suspend or interrupt the study, an independent DSMC may be convened to review the decision by the SRC and recommend whether the study should be stopped, resumed with no changes, or resumed with changes.

3.2 Endpoints and Associated Variables

Primary Endpoint

- Incidence and severity of AEs, SAEs, and DLTs
- Changes from baseline in vital signs, 12-lead ECG, clinical laboratory tests, and physical examination findings

Secondary Endpoint

- Plasma PK parameters of DCR-AUD
- Urine PK parameters of DCR-AUD
- Change in PD biomarkers during standardized EIAs
    - Quantitative assessment of 6 symptom responses during EIAs
    - Plasma acetaldehyde, acetate, and EtOH
    - Heart rate and facial skin temperature
    - SEAS

3.2.1 Safety Variables

- Physical examinations
- Vital signs (Temperature (by skin refraction), pulse rate, respiratory rate, and blood pressure)
- 12-lead electrocardiograms (ECG): Heart rate, PR interval, QRS interval, QT interval and QT interval using Fridericia's correction [QTcF]
- Columbia-Suicide Severity Rating Scale
- Clinical laboratory tests (hematology, clinical chemistry, coagulation and routine urinalysis parameters)
- Adverse event (AE) assessments
- Dose Limiting Toxicity (DLT)

- Concomitant medication assessments
- Complement Panel
- SARS-CoV-2

3.2.2 Pharmacokinetic Variables

Pharmacokinetic concentration data will be obtained at time points described in the protocol version 6.0 as follows:

Plasma PK concentrations of DCR-AUD will be determined at the following nominal times: 0 (before study intervention administration) and at 15 and 30 minutes and 1, 2, 4, 6, 8, 24, 48, 72 hours and on day 15±2 after the SC injection.

Urine samples will be collected to measure urine concentrations of DCR-AUD at following nominal times: 4 hour (sample from pooled urine collection in period 0 - 4 hours after dosing), 8 hour (sample from pooled urine collection in period 4 - 8 hours after dosing), 12 hour (sample from pooled urine collection in period 8 -12 hours after dosing), 24 hour (sample from pooled urine collection in period 12 - 24 hours after dosing), 48 hour (sample from pooled urine collection in period 24 - 48 hours after dosing), and 72 hour (sample from pooled urine collection in period 48 - 72 hours after dosing).

Derivation of PK parameters will be the responsibility of Clinical Pharmacology, Modeling and Simulation (CPMS) group, [redacted] under supervision of Dicerna Pharmaceuticals, Inc. The details about PK parameter derivation and creation of Tables Listings and Figures related to PK will be described in this SAP.

If calculable, the following PK parameters listed in Table 3-1 will be determined for DCR-AUD in plasma following single subcutaneous dose administration.

Table 3-1 Plasma Pharmacokinetic Parameters of DCR-AUD After Single Subcutaneous Dose Administration

Parameter	WNL Name	CDISC Name	Definition
C_{max}	Cmax	CMAX	Maximum observed concentration
C_{last}	Clast	CLST	Last measurable concentration
C_{min}	Cmin	CMIN	Minimum observed concentration in a dosing interval after last dose administration
t_{max}	Tmax	TMAX	Time corresponding to occurrence of C_{max}
t_{last}	Tlast	TLST	
$t_{½}$	HL_Lambda_z	LAMZHL	Apparent terminal elimination half-life
λ_z	Lambda_z	LAMZ	Terminal elimination rate constant

Parameter	WNL Name	CDISC Name	Definition
$AUC_{0\text{-last}}$	AUClast	AUCLST	AUC from time zero to the last quantifiable concentration
$AUC_{0\text{-t}}$	AUCT	AUCINT(0-X)	AUC from time zero to some fixed time t (i.e., AUC_{24} or AUC_{48})
$AUC_{0\text{-inf}}$	AUCINF_obs	AUCIFO	AUC from time zero extrapolated to infinity
$\%AUC_{ex}$	AUC_%Extrap_obs	AUCPEO	Percentage of $AUC_{0\text{-inf}}$ obtained by extrapolation beyond t_{last}
CL/F	Cl_F_obs	CLFO	Apparent clearance following SC administration
V_z/F	Vz_F_obs	VZFO	Apparent volume of distribution during terminal phase

Source: NCI EVS Terminology Resources website:
http://www.cancer.gov/cancertopics/cancerlibrary/terminologyresources/cdisc.

All urine samples will be collected in the following intervals, 0-4, >4-8, >8-12, >12-24, >24-48, and >48-72 hours. Urine concentrations (C_{ur}0-4, C_{ur}4-8, C_{ur}8-12, C_{ur}12-24, C_{ur}24-48, C_{ur}48-72) and volume of urine collected (V_{ur}0-4, V_{ur}4-8, V_{ur}8-12, V_{ur}12-24, V_{ur}24-48, V_{ur}48-72) in each collection interval will be listed in the Urine PK Concentration Listing. The PK parameters listed in Table 3-2 will be calculated for DCR-AUD in urine following treatment administration.

Table 3-2 Urine Pharmacokinetic Parameters of DCR-AUD After Single Subcutaneous Dose Administration

Protocol Parameter	WNL Name	CDISC Name	Definition
Ae_{t1-t2}	Amount Recovered	RCAMINT	Amount of unchanged drug excreted in urine at each interval collection (t_1 to t_2), e.g. 0-4, >4-8, >8-12, >12-24, >24-48, and >48-72 (e.g. Ae_{0-4}, Ae_{4-8}, Ae_{8-12}, Ae_{12-24}, Ae_{24-48}, Ae_{48-72}).
Ae_{0-t}	Amount_Recovered	RCAMINT	Cumulative amount of unchanged drug excreted at a given time up to t hours, where t is 4, 8, 12 or 24, 48, 72 hours (e.g. Ae_{0-4}, Ae_{0-8}, Ae_{0-12}, Ae_{0-24}, Ae_{0-48}, Ae_{0-72})
$Fe\%_{0-t}$	Percent_Recovered	RCPCINT	Urinary cumulative excretion as % of unchanged drug up to t hours, where t is 4, 8, 12, 24, 48, or 72 hours, (e.g. Fe_{0-4}, Fe_{0-8}, Fe_{0-12}, Fe_{0-24}, Fe_{0-48}, Fe_{0-72})
CL_R	CLR@	RENALCL	Renal clearance of the drug from plasma

@Obtained through additional calculations in WNL outside of NCA model, as described in Table 4-1.

3.2.3 Pharmacodynamic Variables

To assess the effect of DCR-AUD on pharmacodynamic (PD) variables using blood biomarkers (alcohol, acetaldehyde, and acetate), blood samples will be collected at the times specified in the Schedule of Assessments. The PD biomarker variables to be estimated will be T_{max}, C_{max}, and $AUC_{0-2.5h}$ by dose cohorts and EIA days which can be calculated using the same methods as the correspondent PK parameters described in Table 3-1. Objective physiological biomarkers (heart rate and facial skin temperature) will also be assessed at the times specified in the Schedule of Assessments. Participants' subjective experience of the effects of alcohol will be assessed using the Subjective Effects of Alcohol Scale (SEAS).

Each cohort will have a sentinel group (n=3) that does not undergo any EIA assessment.
Note: In addition to standard vital sign measurements for safety, vital signs will be recorded as part of the EIA and results will be identified as such (e.g., HR for EIA) in the eCRF. In addition, a descriptive quantitative evaluation of 6 symptoms actively assessed at each of 4 time points during EIA challenges will be included.
Drug effect model will be used to derive the PD parameters listed in table below using Phoenix version 8.3 or higher. Derivation of these PD parameters will be the responsibility of CPMS group. The details of PD parameters are provided in Table 3-3.

Table 3-3 Plasma Pharmacodynamic Parameters of DCR-AUD After Single Subcutaneous Dose Administration

Protocol Parameter	WNL Name	Definition
t_{max}	Tmax	Time corresponding to occurrence of C_{max} (For acetaldehyde, acetate, and alcohol)
C_{max}	Cmax	Maximum observed concentration (in the unit of μM and ng/mL) (For acetaldehyde, acetate, and alcohol)
$C_{maxAbove}$	Cmax_Above_B	Maximum observed concentration that is above the baseline (in the unit of μM and ng/mL) (For acetaldehyde)
baseline	Baseline	Response value just prior to treatment administration
AUC_{Above}	AUC_Above_B	Area under the curve that is above the baseline (for acetaldehyde)
AUC_{Below}	AUC_Below_B	Area under the curve that is below the baseline (for acetate)
$AUC_{0\text{-}2.5h}$	AUCT	Area under the curve from time 0 to 2.5 hours (for acetaldehyde, acetate, and alcohol)

3.2.4 Immunogenicity Variables

Immunogenicity testing (anti-drug DCR-AUD antibodies) using designated serum samples from each participant is planned to be conducted.

The ADA samples collected in this study will be stored and analyzed in the future.

3.2.5 Efficacy Variables

Not Applicable.

3.2.6 Exploratory Variables

Not Applicable.

4 STATISTICAL METHODS

4.1 Data Quality Assurance

All tables, figures, and data listings (except PK data) to be included in the report will be independently checked for consistency, integrity, and in accordance with Standard Operating Procedures (SOPs) of [redacted].

4.2 General Presentation Considerations

The participants, who received "Placebo" in Cohort 1 to 4, will be pooled into a single placebo group (Pooled Placebo) for all summaries and presentation related to PD variables and safety for this study. The PK outputs will be generated for DCR-AUD by each dose cohort, as applicable.

4.2.1 Treatment

Cohort 1: 80 mg,
Cohort 2: 240 mg,
Cohort 3: 480 mg,
Cohort 4: 960 mg,
Pooled Placebo

4.2.2 Baseline

Baseline is defined as the last non-missing measurement prior to the date and time of the dose of study intervention (Day -1).

4.2.3 End of Study

'End of Study' for a participant is defined as the last available post-treatment assessment.

4.2.4 Study Day

Study days will be numbered relative to the first day of study drug administration.

- If the date of event is before the study drug administration, then:

Study day = (Date of measurement – Date of study drug administration [i.e. Day 1] in each period)

- If the date of event is on or after the study drug administration, then:

Study day = (Date of measurement – Date of study drug administration [i.e. Day 1] in each period) + 1

4.2.5 Controlled, Repeat, Retest, Scheduled and Unscheduled Assessment

This section is not applicable for PK data.

Repeat, retest, and unscheduled assessment will not be considered for the calculation of summary statistics and figures, unless assessment qualifies as baseline.

The average of controlled and planned (scheduled) assessments will be considered for the calculation of summary statistics and figures, if more than one controlled/planned assessment will be performed at a specific time point.

4.3 Software

All report outputs will be produced using SAS® version [9.4] or a later version in a secure and validated environment.

The PK and PD blood biomarker analyses will be conducted using Phoenix® WinNonlin (WNL) version 8.3 or later in a secure and validated environment.

All report outputs will be provided to the Sponsor in RTF/PDF format.

4.3.1 Summary and Representation of Data

All data will be listed according to the number of decimal places presented in the source data.

Continuous data will be summarized in terms of the mean, standard deviation (SD), median, inter quartile range (IQR), minimum, maximum, and number of observations, unless otherwise stated.

The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean, IQR, and median will be reported to one more decimal place

than the raw data recorded in the database. The SD will be reported to two more decimal places than the raw data recorded in the database. In general, the maximum number of decimal places reported shall be four for any summary statistic.

Departure from these general rules will be specified in the output shell document.

Categorical data will be summarized in terms of the number of participants providing data at the relevant time point (n), frequency counts and percentages.

Percentages will be presented to one decimal place. Percentages will not be presented for zero counts.

4.4 Analysis Population

For the purpose of analysis, the following population are defined.

Enrolled Population: All participants who sign the ICF.

Randomized Population: All participants who sign the ICF and who are randomly assigned to study intervention.

Safety Population (SP): All participants randomly assigned to study intervention and who receive the full dose of study intervention. Participants will be analyzed according to dose received.

Pharmacokinetic Population (PKP): All participants randomly assigned to study intervention and who receive a full dose of DCR-AUD and have sufficient data for at least 1 postdose PK assessment.

Pharmacodynamic Population (PP): All participants randomly assigned to study intervention and who receive a full dose of study intervention and have sufficient data for at least 1 postdose PD assessment.

4.5 Study Participants

4.5.1 Disposition of Participants

A clear accounting of the disposition of all participants who enter the study will be provided from screening (excluding screen failure data) to study completion.

A summary of participant study completion status and reason for study withdrawal will be provided for the enrolled population. This display will show the number and percentage of participants who withdrew from the study, including primary reasons for study withdrawal.

A by-participant listing of study discontinuation will be presented for the enrolled population. The listing will include dose reasons for study discontinuation.

A randomization listing will be presented and include the following: each participant's randomization number, the treatment to which the participant has been randomized and replacement randomization number (if any) for the randomized population.

4.5.2 Protocol Deviations

All protocol deviations are predefined in the separate document, Protocol Deviation Specification.

4.5.2.1 Protocol Deviations with PK Implications

Protocol deviations that may potentially impact PK parameter deviations include, but are not limited to:

- SC administration deviations – interruption of administration, etc.

- Missed PK samples that impact estimation of PK parameter(s)
- Concomitant medications not authorized by protocol
- PK samples obtained out of allowance window that may impact the estimation of PK parameter(s)

4.5.2.2 Protocol Deviations with Non-PK Implications

The defined protocol deviations will be collected during the study period by the site monitor/clinical team and programming team. All deviations related to study inclusion or exclusion criteria, conduct of the study, participant management or participant assessment, and handling of the participant's rights will be described.

Protocol deviations (mentioned in Sections 4.5.2.1 and 4.5.2.2) and analysis population will be reviewed in the data review report meeting to decide on the inclusion or exclusion of participant(s) from the analysis sets. Decisions regarding the exclusion of participants and/or participant data from analyses will be made prior to database lock and will be documented and approved.

Post database lock and based on PK and PD data availability all Analysis Datasets and TLFs related to PK and PD analysis set will be created and discussed with the sponsor in the data review meeting post DBL. Data review meeting minutes will be documented as an update to DRR minutes and approved by sponsor and [redacted] team.

A by-participant listing of major and minor protocol deviations will be provided including participant identifier, exclusion from specific analysis population, and protocol deviation classification and description.

All protocol deviations will be listed by participant for the randomized population. Protocol Deviations related to COVID-19 will be listed separately.

A summary table of major protocol deviations by cohort, treatment, pooled placebo, and overall will also be provided for randomized population.

4.6 Demographics and Baseline Characteristics

Demographic and anthropometric variables (age, sex, ethnicity, race, height, weight, and BMI) will be listed by participant. Demographic characteristics (age, sex, ethnicity, and race) and anthropometric characteristics (height, weight, and BMI) will be summarized by cohort, treatment, pooled placebo, and overall, for the SP.

4.7 Medical History and Concomitant Illnesses

Medical history data will be summarized by cohort, treatment, pooled placebo, and overall for the SP and listed by participant including visit, description of the disease/procedure, Medical Dictionary for Regulatory Activities (MedDRA Version 25.1 or latest) system organ class (SOC), MedDRA preferred term, start date, and stop date (or ongoing if applicable).

4.8 Prior and Concomitant Medications

Medication start and stop dates will be compared to the date of study drug administration to allow medications to be classified as either Prior only, both Prior and Concomitant, or Concomitant only.

Medications starting after the completion/withdrawal date will be listed but will not be classified or summarized.

Prior medications are those that started and stopped prior to the study drug administration.

Concomitant medications are those taken before or after study drug administration (including medications that started prior to dosing and continued after) and up to end of trial.

If a medication starts before the date of study drug administration and stops on or after the study drug administration, then the medication will be classified as both Prior and Concomitant.

If missing data prevents the medication being classified as prior or concomitant, the medication will be considered as concomitant for the data listings.

Prior and concomitant medication will be coded according to the World Health Organization Drug Dictionary (WHO-DD) (Version Sep, 2022 B3, or latest) and will be classified by Anatomical Therapeutic Chemical (ATC) categories.

Prior and concomitant medications will be summarized based on SP and will be listed by participant and will include the following information: reported name, preferred term, the route of administration, dose, frequency, start date/time, end date/time and indication.

4.9 Treatment Exposure

A listing of DCR-AUD administration will be created and will include the dose cohort, treatment received, date and time of administration, dose (unit), dose form, route of administration.
A summary table for DCR-AUD administration will also be provided based on SP.

4.10 Pharmacokinetics Analysis, Concentration, and Parameter TFLs, and Statistical Analysis of Pharmacokinetic Parameters for Final Analysis

4.10.1 Pharmacokinetic Concentrations

Concentration Listings:

Pharmacokinetic concentration data for DCR-AUD, will be listed by dose cohort (80 mg, 240 mg, 480 mg, and 960 mg), timepoint, and participant for the SP. Concentration listings will include nominal PK sampling time, actual sampling times relative to dose administration, deviation from nominal time, and percent deviation from nominal time, and concentrations. Plasma and urine concentrations below the lower limit of quantification (LLOQ) will be presented as below the limit of quantification (BLQ) in the listings and the LLOQ value presented as a footnote. Missing PK samples will be reported as no sample (NS) or not reportable (NR) as appropriate and considered excluded from PK analysis.

Concentration Summary Tables:

Source data as reported from the laboratory will be used for calculation of concentration summary statistics. Tabular summaries for concentration-time data will report N (number of subjects who received treatment), n (number of subjects with non-missing values), and n(BLQ) (the number of subjects with BLQ samples).

Concentration for DCR-AUD and will be summarized by dose cohort, and nominal timepoint with descriptive statistics for the PKP. The following descriptive statistics will be presented for plasma and/or urine concentrations obtained at each nominal time point: N, n, n(BLQ), arithmetic mean, SD, coefficient of variation (CV%), geometric mean, geometric CV% (calculated as: gCV% =SQRT(e^{s^2}-

1)*100; where s is the SD of the log-transformed values), median, IQR, minimum, and maximum values.

For summary tables, all BLQs will be considered zero, except for presentation of the geometric mean and geometric CV, which will use ½ the LLOQ. The number of BLQs and non-BLQs at each scheduled time point will be reported. Summary Statistics will not be calculated if non-BLQ concentrations at a scheduled time point is <3 and will be reported as NC.

The rules followed for calculation and presentation of concentration data with regards to the number of decimal places/significant digits for the listings of participant level concentrations and summary tables of concentration are as follows:

Concentration Listings and Tables	Rounding
Individual concentrations	*n* s.f. as supplied by bioanalytical laboratory
Minimum and Maximum	*n* s.f. capped at 3
Mean/SD/Median/Geomean	*n* s.f. capped at 3
CV%/gCV%	*n* s.f. capped at 3
N/n	Whole number

s.f = significant figures, d.p. = decimal place

Concentration Figures:

For arithmetic mean linear/linear graphs, all BLQ values will be substituted with zero for calculation of arithmetic mean and for log/linear graphs the log transformed arithmetic mean will be displayed (this should not include zero).

For geometric mean linear/linear graphs, all BLQ values will be substituted with ½ the LLOQ for calculation of geometric mean and for log/linear graphs the log transformed geometric mean will be displayed.

For individual linear/linear and log/linear graphs all BLQ values will be substituted as follows:

- BLQs at the beginning of a participant profile (i.e., before the first incidence of a measurable concentration) will be assigned to zero. When using log/linear scale, these timepoints will be considered missing.
- BLQs at the end of a participant profile (i.e., after the last incidence of a measurable concentration) will be set to missing.
- Single BLQs which fall between two measurable concentrations will be set to missing.
- Consecutive BLQs which fall between measurable concentrations will be set to missing. Measurable concentrations after consecutive BLQs will be set to missing.

To visualize participant-level concentrations and the comparison between dose cohorts, the descriptive PK graphs listed below will be generated

- Figure x.x.x: Individual participant profiles for DCR-AUD Plasma Concentration Time Data by dose cohort – (Linear Scale and Semi-Logarithmic Scale) (SP)
- Figure x.x.x: Mean (± SD) DCR-AUD Plasma Concentration Time Data – (Linear Scale and Semi-Logarithmic Scale) by (dose cohort) (PKP)
- Figure x.x.x: Overlaid individual participant profiles for DCR-AUD Plasma Concentration Time Data by dose cohort – (Linear Scale and Semi-Logarithmic Scale) (SP)

- Figure x.x.x: Mean (± SD) Cumulative Amount of DCR-AUD in Urine Versus Time by dose cohort (PKP)

Figures will be generated in color using unique line style and marker for each plot in the graph. For all PK concentration-time plots, linear scale will be used for x-axis (e.g., do not use an ordinal scale).

4.10.2 Pharmacokinetic Parameters

PK parameters will be provided by CPMS group at [redacted]. PK parameters of DCR-AUD, will be calculated by NCA methods from the concentration-time data using Phoenix® WinNonlin® Version 8.3 or higher following these guidelines: The Phoenix WinNonlin project file including all settings and analysis input file will be transferred to Dicerna when the PK analyses are completed.

- Actual time from single SC dose will be used in the calculation of all derived pharmacokinetic parameters, except when parameters are calculated for safety/dose escalation meetings when nominal times may be used to calculate PK parameters.
- There will be no imputation of missing data.
- Handling of BLQ samples for plasma PK parameters after single dose administration
    - BLQs at the beginning of a participant profile (i.e., before the first incidence of a measurable concentration) will be assigned to zero.
    - BLQs at the end of a participant profile (i.e., after the last incidence of a measurable concentration) will be set to missing.
    - Single BLQs which fall between two measurable concentrations will be set to missing.
    - Consecutive BLQs which fall between measurable concentrations will be set to missing. Measurable concentrations after consecutive BLQs will also be set to missing.
- BLQs in urine samples will be assigned to zero.

Pharmacokinetic parameters will be estimated according to the guidelines presented in Table 4-1.

Table 4-1 Pharmacokinetic Parameter and Estimation

Parameter	Guideline for Derivation
C_{max}, C_{min}, t_{max}, C_{last}	Obtained directly from the observed concentration-time data
$AUC_{0\text{-}last}$ (or $AUC_{0\text{-}t}$)	The AUC from zero time (pre-dose) to the time of last quantifiable concentration will be calculated by a combination of linear and logarithmic trapezoidal methods. Unless specifically requested and justified, the linear up/log down trapezoidal method will be employed. The $AUC_{0\text{-}t}$ is the sum of areas up to the time of the last quantifiable sample: $AUC_{0\text{-}t}=\int_0^t C_{last} * dt$
$AUC_{0\text{-}inf}$	The area from zero time extrapolated to infinite time will be calculated as follows: $AUC_{0\text{-}inf} = AUC_{0\text{-}t}+\frac{C_{last}}{\lambda_z}$ where C_{last} is the last observed quantifiable concentration.

Parameter	Guideline for Derivation
$\%AUC_{ex}$	The percentage of $AUC_{0\text{-inf}}$ obtained by extrapolation will be calculated as follows: $\%AUC_{ex} = \frac{AUC_{0\text{-inf}} - AUC_{0\text{-t}}}{AUC_{0\text{-inf}}} \times 100$. Unless otherwise determined by PK Scientist's best knowledge and judgment, if the $\%AUC_{ex}$ is greater than 20% the value, $\%AUC_{ex,}$ and all dependent parameters (ie, $AUC_{0\text{-inf,}}$ MRT, Vz and CL) will be flagged in listings and excluded from summary tables and statistical analysis of PK parameters, unless instructed otherwise by the Sponsor. The reason for exclusion will be listed/footnoted in parameter listings.
λz and t½	1. The apparent terminal phase rate-constant (λz) will be estimated by linear regression of concentration versus time data presented in a log-linear scale. 2. Data are primarily monotonically decreasing in magnitude and are representative of the actual decline in the log concentration-time curve. 3. Only those data points that are judged to describe the terminal log-linear decline will be used in the regression. 4. A minimum number of three data points in the terminal phase will be used in calculating λz with the line of regression starting at any post-C_{max} data point (C_{max} should not be part of the regression slope). Unless otherwise determined by PK Scientist's best knowledge and judgment or if instructed by the Sponsor, if the adjusted correlation coefficient (R^2 adjusted) is <0.85, then λz and all the λz dependent parameters (i.e. t½, $AUC_{0\text{-inf}}$, CL, MRT, and Vz) will be flagged in listings and excluded accordingly from summary tables and statistical analysis of PK parameters. The reason for exclusion will be listed/footnoted in parameter listings. 5. Unless otherwise determined by PK Scientist's best knowledge and judgment, the interval used to determine λz should be equal or greater than 1.5-fold the estimated t½, and if less than 1.5-fold, λz will be flagged in listings and might be excluded (based on sponsor specific requirements) from summary tables and statistical analysis of PK parameters. All the derived parameters (i.e. t½, $AUC_{0\text{-inf}}$, CL, MRT, and Vz) may also be flagged in listings and excluded from statistical analysis of PK parameters. The reason for exclusion will be listed/footnoted in parameter listings. 6. The t½ will be calculated as follows: $t½ = \ln 2/\lambda z = 0.693/\lambda z$ 7. Data points may be dropped from the linear regression if the PK Scientist considers the reported values to be anomalous. Any data points so designated should remain in the listings with a footnote and be identified in the study report with a rationale for exclusion.
CL/F	Following SC administration, systemic clearance of parent drug will be calculated from: $CL = \frac{Dose}{AUC_{0\text{-inf}}}$ Apparent clearance (CL/F) following SC dosing will be calculated.
V_z/F	Volume of distribution at terminal phase following SC dosing will be calculated from: $V_z/F = \frac{Dose}{\lambda_z \times AUC_{0\text{-inf}}} = (CL/F)/\lambda_z$
CL_R	Renal clearance will be calculated from the ratio of the appropriate values for urinary recovery and area under the concentration-time curve. Depending on the actual data, the "x" will be determined in the PK section of the CSR.

Parameter	Guideline for Derivation
	$CL_R = \frac{Ae_{(0-x)}}{AUC_{(0-x)}}$
A_e	Amount of unchanged drug recovered in urine is calculated as *A_e = Urine drug concentration * urine volume* This is calculated for each urine collection interval and then summed for the cumulative A_e.
F_e%	The percent of drug recovered in urine is calculated as *F_e = 100 * A_e/Dose* This is calculated for each urine collection interval and then summed for the cumulative F_e%.

PK Parameters Listings:

PK parameters will be listed by participant for the SP PK parameters that will be flagged and/or excluded from summary tables and statistical analyses of PK parameters will be flagged and footnoted with the reason for flagging/exclusion.

PK Parameter Summary Tables:

Biostatistics group will consider the derived PK parameters as source data and will use this data without rounding for calculation of PK parameters summary statistics tables.

PK parameters will be summarized by dose cohort with descriptive statistics for the PKP.

Tabular summaries for PK parameters will report N (number of subjects who received treatment) and n (number of subjects with non-missing values).

Descriptive statistics for calculated PK parameters will include N, n, arithmetic mean, SD, CV%, geometric mean, gCV%, median, IQR, minimum, and maximum values. For t_{max}, only N, n, median, minimum, maximum values will be presented. No descriptive statistics will be determined when fewer than three individual PK parameters are available.

The rules followed for presentation of PK parameters data with regards to the number of decimal places/significant digits for the listings of participant level PK parameters and summary tables of PK parameters are as follows:

PK Parameter Listings and Tables	Rounding
Derived Individual parameters	3 s.f.
Directly Derived Individual parameters (C_{max}, C_{12}, C_{24})	*n* s.f. as supplied by the analytical laboratory but not more than 3 s.f.
Minimum and Maximum	3 s.f.
Mean/SD/Median/Geomean	3 s.f.
CV%/gCV%	3 s.f.
Comparative estimates (e.g. ratios)	3 s.f.
CI and other percentages	3 s.f.
p-values	4 d.p.
N/n	Whole number
Exceptions for PK Tables	

t_{max} individuals and min/max	Determined based on the raw data for timing)
t_{max} median only	Determined based on the raw data for timing

s.f = significant figures, d.p. = decimal place

4.10.3 Pharmacokinetic Analysis

Assessment of Dose Proportionality

Dose proportionality will be assessed for C_{max}, and $AUC_{0\text{-last}}$ (or $AUC_{0\text{-t}}$), or $AUC_{0\text{-inf}}$ of DCR-AUD in the dose range of 80 mg to 960 mg using a power model. $AUC_{0\text{-inf}}$ will also be evaluated if $AUC_{0\text{-inf}}$ is evaluable for >80% of participants.

Individual PK parameter values will be used to perform a least-squares linear regression analysis, using the formula log_pkvar=A× log_dose + B, where 'log_pkvar' represents the natural log transformed C_{max}, and $AUC_{0\text{-last}}$ or $AUC_{0\text{-inf}}$ 'log_dose' represents the natural log transformed dose. An estimate of the slope of the regression line and corresponding 95% confidence interval (CI) will be obtained.

The following sample SAS code will be used:

```
PROC REG DATA=pkparam alpha=0.05;
        MODEL log_pkvar = log_dose / CLB;
RUN;
```

Where, CLB = 100(1 - α)% confidence limits for the parameter estimates.

For each of the parameters C_{max} and $AUC_{0\text{-last}}$, or $AUC_{0\text{-inf}}$ a plot of the log-transformed concentration against the log-transformed dose will be constructed including the fitted line from the linear regression and the line of unity.

Dose proportionality will be declared when the 90% CI for A lies completely within the critical region defined as:

$$1 + \frac{\ln(\theta_L)}{\ln(r)}, 1 + \frac{\ln(\theta_H)}{\ln(r)}$$

r: ratio of the highest to lowest administered doses
θ_L = the lowest critical limit of the ratio of dose-normalized mean values (Rdnm); and
θ_H = the upper critical limit of Rdnm.
PK dose proportionality will be concluded if the 90% C.I. of A for Cmax, and $AUC_{0\text{-last}}$ or $AUC_{0\text{-inf}}$ are entirely contained within the following bounds that depend on the dose range ratio (SmithBP, 2000) [2] (i.e., ln (960/80) = ln (12)):

- Lower bound = 1+ln (0.80)/ln (12) = 0.9102 and
- Upper bound = 1+ln (1.25)/ln (12) = 1.0898

If dose proportionality is not declared for any of the PK parameters by smith criteria (θ_L=0.8, θ_H=1.25), 90% CI for A would be compared to a less stringent critical region based on (θ_L=0.5, θ_H=2) (Hummel, 2006) [3].

- Lower bound = 1+ln (0.50)/ln (12) = 0.7211 and
- Upper bound = 1+ln (2)/ln (12) = 1.2789

4.11 Pharmacodynamics Analysis, Concentration, and Parameter TFLs, and Statistical Analysis of Pharmacodynamic Parameters

Individual participant's biomarker (acetaldehyde, acetate, and ethanol) plasma concentration values both unadjusted and adjusted for baseline, will be provided by Frontage Laboratories. Baseline unadjusted and adjusted acetaldehyde, acetate, and ethanol concentration values will be listed by dose cohort, timepoint, EIA days, and participant for the PP. Concentration listings will include nominal biomarker sampling time, actual sampling times relative to dose administration, and percentage change from baseline. The corresponding baseline for each timepoint will be taken on Day -1, see Table 6-1. A by participant listing of ALDH2 genetic mutation will also be provided. For the biomarker concentration summary tables, descriptive statistics (N, n, arithmetic mean, SD, CV%, geometric mean, gCV%, median, IQR, minimum, and maximum values) will be presented by dose cohort (placebo, 80 mg, 240 mg, 480 mg, and 960 mg), with and without the subjects with ALDH2 mutant(s), nominal timepoint, and EIA days. For time variables only median, T_{min} and T_{max} will be presented.

To visualize the comparison between dose cohorts the descriptive biomarker graphs will be generated.

- Figure x.x.x: Individual Participant Profiles for acetaldehyde, acetate, and ethanol, Plasma Concentration Time Data (Unadjusted and Adjusted for Baseline) by dose cohort and EIA day (SP)
- Figure x.x.x: Overlaid Individual Participant Profiles for acetaldehyde, acetate, and ethanol Plasma Concentration Time Data (Unadjusted and Adjusted for Baseline) by dose cohort and EIA day (SP)
- Figure x.x.x: Mean (± SD) baseline adjusted acetaldehyde, acetate, and ethanol Plasma Concentration Time Data by dose cohort and EIA day (PP)
- Figure x.x.x: Mean (± SD) baseline adjusted acetaldehyde, acetate, and ethanol Plasma Concentration Time Data by dose cohort and EIA day (PP) and with and without the subjects with ALDH2 Mutant
- Figure x.x.x: The Mean (± SD) baseline unadjusted acetaldehyde, acetate, and ethanol Plasma Concentration Time profiles by dose cohorts and EIA days (PP)
- Figure x.x.x: The Mean (± SD) baseline unadjusted acetaldehyde, acetate, and ethanol Plasma Concentration Time profiles by dose cohorts and EIA days (PP) with and without the subjects with ALDH2 Mutant
- Figure x.x.x: The mean (± SD) C_{max} (in the units of µM and ng/mL) and $AUC_{0\text{-}2.5h}$ (in the unit of ng*h/mL) of acetaldehyde, acetate, and ethanol following 4 Alcohol Drinks in 30 Minutes and Single Dose Administration of DCR AUD in Healthy Adults will be plotted with EIA days by dose cohorts (PP)
- Figure x.x.x: The mean (± SD) C_{max} (in the units of µM and ng/mL) and $AUC_{0\text{-}2.5h}$ (in the unit of ng*h/mL) of acetaldehyde, acetate, and ethanol following 4 Alcohol Drinks in 30 Minutes and

Single Dose Administration of DCR AUD in Healthy Adults with and without ALDH2 Mutant(s) will be plotted with EIA days by dose cohorts (PP)

For each figure, subjects belonging to each category will be shown on the same plot with separate figures for each level of dose. Participant level figures will use actual time, while mean plots will use planned time, except for predose time point(s), where time 0 will be used rather than actual time.

All placebo data will be put into one group in the figures regardless of dose cohort.

4.11.1 Pharmacodynamic Parameters

DCR-AUD is designed to selectively reduce ALDH2 levels in the liver and the conversion of acetaldehyde to acetic acid. The build-up of acetaldehyde causes unpleasant effects after drinking (e.g., headache, facial flushing, tachycardia, nausea, vomiting). As such, the PD activity of DCR-AUD will be assessed during EIAs using plasma biomarkers (alcohol, acetaldehyde, and acetate), assessment of 6 symptoms and their severity induced during EIAs, and objective physiological responses (heart rate and facial skin temperature). Participants' subjective experience of the effects of alcohol will be assessed using the SEAS. Instructions for the collection and handling of biological samples will be provided by the Sponsor in the Laboratory Manual and the Laboratory Specification document.

PD parameters will be calculated using drug effect model from the biomarker time data using Phoenix® WinNonlin® 8.3 or higher. The biomarker parameters described in Section 2.2.3 for alcohol, acetaldehyde, and acetate unadjusted and adjusted for baseline will be estimated for each cohort dose, with and without the subjects with ALDH2 mutant(s), and pooled placebo. The Phoenix® WinNonlin® project file including all settings and analysis input file will be transferred to Dicerna when the biomarker PD analyses are completed.

The unadjusted and adjusted biomarker parameters will all be listed. Descriptive statistics (number, arithmetic mean, SD, CV%, geometric mean, gCV%, median, IQR, minimum, and maximum) of adjusted and unadjusted parameters will be presented by dose cohort, with and without the subjects with ALDH2 mutant(s) and pooled placebo. For time variables only median, T_{min} and T_{max} will be presented.

To visualize the comparison between dose cohort following alcohol consumption the C_{max} and $AUC_{0\text{-}2.5h}$ parameters will be plotted as described in Section 4.11.

All placebo data will be put into one group in the figures regardless of dose cohort. Two-way ANOVA will be performed to compare the C_{max} and AUC of the biomarkers between the treatment cohorts and placebo group on the EIA days.

4.11.1.1 Ethanol Interaction Assessments

EIAs will include an active assessment of the six key symptoms (flushing, headache, palpitations, lightheadedness, nausea, and vomiting) that DCR-AUD may induce, in order to determine what dose of DCR-AUD gives the proper constellation of these symptoms.

These symptoms will be collected by active questioning of each participant at different time points during each EIA i.e. before alcohol administration, at 0 minutes, 15minutes, 30 minutes, 45 minutes, 60 minutes, 90minutes, 120 minutes and 150 minutes after study drug administration. Should an EIA

be missed, due to COVID-19 or other circumstances, the missed EIA may be conducted at the next scheduled visit or at an unscheduled visit at the discretion of the Investigator.
The following symptom scoring framework would be described as an arbitrary point system, where:

Symptom	No Symptom	Mild symptom	Moderate symptom	Severe symptom
Score	0	1	2	3

Each participant at each timepoint in an EIA after alcohol administration will be given a composite score for that timepoint as sum of all six symptom scores.

Baseline is defined as last non-missing value prior to alcohol administration.

Descriptive statistics of EIA composite score (n, mean, median, IQR, minimum and maximum) for absolute values and changes from baseline will be presented by dose cohort, Pooled DCR-AUD, pooled placebo, visit and EIA time point.

Descriptive statistics of 6 symptom EIA assessment (n, mean, median, IQR, minimum and maximum) for Total Scores, Peak post-alcohol administration Composite Score, C_{max} acetaldehyde, Delta 1: Peak post-dose score for a given visit's test minus the score at the pre-alcohol administration time on that day, Delta 2: Peak post alcohol administration score on the day of the test, minus the peak post-alcohol administration score for the same subject on day -1, Delta 3: The highest score attained by that subject in Delta 1 over all 6 tests after DCR-AUD was administered will be presented by dose cohort, pooled DCR-AUD, pooled placebo, and EIA Days/timepoint.

Peak EIA composite score for each subject at each EIA is defined as the highest post-dose composite score selected from the 3 post alcohol administration time points for that EIA.

To visualize subject-level composite score and the comparison between each cohort the EIA graphs listed below will be generated.

- Figure xx.1: Combined Individual DCR-AUD and placebo EIA composite score-Time Profiles (Linear Scale) by Treatment cohort, (SP)
- Figure xx.1: Combined Individual DCR-AUD and placebo Peak EIA composite score-Visit (Days) Profiles (Linear Scale) by Treatment cohort, (SP)

Serial EIAs will be performed in designated participants to assess the indirect PD effects of ALDH2 reduction (acetaldehyde increase), including plasma acetaldehyde, acetate, and ethanol levels, induced symptoms, heart rate, facial skin temperature, and subjective feelings of alcohol intoxication or intolerance using the SEAS. Adverse events, vital signs, and ECG will also be monitored and recorded for safety.

In addition to above plots, following plots will also be generated:

- At each EIA, change in peak symptom score from baseline after alcohol administration from pre-administration score on that day
- For each EIA change in the peak symptom score on that test day after alcohol administration from the peak score change on day –1 for that cohort (i.e. On day of EIA challenge before IMP was administered)

(It is expected that most subjects would have Day –1 pre-EIA challenge composite score of zero (0), i.e. Reporting none of 6 active surveillance symptoms before IMP and before any alcohol was

administered on day –1. However, to the extent that some or few patients might have some symptoms, the most informative change metric that would reflect what the subject experiences is the change in peak composite score at any given EIA after dosing from that pre-IMP and pre-alcohol, i.e. Baseline score at day –1.)

4.11.1.2 PD Biomarkers for EIA

4.11.1.2.1 EIA Blood Biomarkers

Plasma biomarkers to be assessed include measurement of acetaldehyde, acetate, and ethanol. Blood samples (maximum 32 mL/day) will be collected via a venous access device immediately prior to EtOH consumption (time 0) and at 15, 30, 45, 60, 90, 120, and 150 minutes post dose.
A by-patient listing of EIA blood biomarkers will be presented in SP. A summary table of T_{max}, C_{max}, and $AUC_{0\text{-}2.5h}$ of blood biomarkers will be presented by biomarker parameter, dose cohort, with and without the subjects with ALDH2 mutant(s) and pooled placebo in PD population. The same PK parameter analysis method described in Section 4.10 will be applied to the calculations of these biomarker parameters.
EIA related plots are described in Section 4.11.

To compare the C_{max} and $AUC_{0\text{-}2.5h}$ of the biomarkers between the active treatment doses and placebo on the EIA days, two-way ANOVA will be performed for participants with and without ALDH2 mutant(s):

- 80mg vs Placebo
- 240mg vs Placebo
- 480mg vs Placebo
- 960mg vs Placebo

Primary PK parameters will be separately analyzed (Two-way ANOVA) using a mixed effects model. The dependent variable will be the ln-transformed primary PK parameters (C_{max} and $AUC_{0\text{-}2.5h}$) and the independent variables will include fixed effects for treatment and participant as a random effect.

Geometric least-squares means for each treatment dose, point estimates and associated 90% CIs for the ratios for each primary PK parameter will be produced in tabular format. This will be anti-log transformed to obtain the point estimates and the 90% CI for the geometric mean ratio on the original scale.

Sample SAS Code:

```
ODS SELECT NOPRINT(NOWARN);
  ODS OUTPUT CovParms= Cv1 lsmeans=lsm estimates=est Diffs=diff1;
  PROC MIXED DATA=DATA1 NOITPRINT;
  BY COMPOUND ANALYTE PPSPEC PARAMN PARAMCD PARAM;
  CLASS SUBJID TRTA;
  MODEL LN_PK = TRTA/DDFM=KR;
  RANDOM SUBJID ;
  LSMEANS TRTA/PDIFF ALPHA=0.10 CL;

  ESTIMATE "DCR-AUD vs PLACEBO" TRTA 1 -1 /CL ALPHA=0.10;
  RUN;
```

where:

COMPOUND: compound used in the study

ANALYTE : analyte of the drug (can be parent or metabolite)

PPSPEC : Specification (e.g. Plasma/Urine/CSF)

PARAMN : numerical order of PK parameter

PARAMCD :short name of PK parameter

PARAM: PK parameter

LN_PK; natural log transformed value of PK parameter

SUBJID: Participant

TRTA: actual treatment

4.11.1.2.2 EIA Physiological Responses

Heart rate and facial Skin temperature:
EIA assessments will include measurement of heart rate and facial skin temperature. Facial skin temperature will be measured using a surface scanning thermometer. Heart rate will be monitored by telemetry during the EIAs. Site personnel will be provided training in the use of all study-specific instruments (see the Study Reference Manual).

Heart rate, using telemetry, and facial skin temperature will be collected immediately pre dose (time 0) and at 15, 30, 45, 60, 90, 120, and 150 minutes post dose.

A by-participant listing of EIA physiological responses will be presented.

Descriptive statistics of Peak Heart rate and Facial skin temperature (n, mean, SD, median, IQR, minimum and maximum) for peak absolute values and peak changes from baseline will be presented by active dose cohort, pooled DCR-AUD, pooled placebo, and visit/timepoint.

Peak EIA Heart rate/Facial skin temperature is defined as maximum recorded Heart rate/Facial ski temperature from pre-alcohol administration baseline for each visit/timepoint.

4.11.1.2.3 EIA Subjective Effects of Alcohol Scale

For each EIA, participants will complete the SEAS pre dose (during the Rest Period) and at 30, 60, 90, 120, and 150 minutes post dose. The SEAS is a 14-item tool that allows participants to rate the subjective effects of alcohol. Participants will rate the extent to which they are feeling each of the effects in the present time.

Table 4-2 Subjective Effects of Alcohol Scale (SEAS)

Factor	Feeling	Not at All						Moderately				Extremely
High Arousal Negative	Demanding	0	1	2	3	4	5	6	7	8	9	10
	Rude	0	1	2	3	4	5	6	7	8	9	10
	Aggressive	0	1	2	3	4	5	6	7	8	9	10
High Arousal Positive	Woozy	0	1	2	3	4	5	6	7	8	9	10
	Dizzy	0	1	2	3	4	5	6	7	8	9	10
	Wobbly	0	1	2	3	4	5	6	7	8	9	10
High Arousal Positive	Fun	0	1	2	3	4	5	6	7	8	9	10
	Lively	0	1	2	3	4	5	6	7	8	9	10
	Funny	0	1	2	3	4	5	6	7	8	9	10
	Talkative	0	1	2	3	4	5	6	7	8	9	10
High Arousal Positive	Mellow	0	1	2	3	4	5	6	7	8	9	10
	Secure	0	1	2	3	4	5	6	7	8	9	10
	Relaxed	0	1	2	3	4	5	6	7	8	9	10
	Calm	0	1	2	3	4	5	6	7	8	9	10

A by-participant listing of SEAS feeling categories will be presented.

A summary table will be presented by factored “feeling” category, visit, time point, active dose cohort, pooled DCR-AUD, and pooled placebo in PD population.

4.11.2 Immunogenicity

The ADA samples collected in this study will be stored and analyzed in the future.

4.12 Safety Evaluation

All safety summaries and analyses will be based upon the SP. All safety and tolerability parameters (AEs, laboratory data [hematology, coagulation, clinical chemistry, routine urinalysis, complement panel, and other screen test parameters], vital signs, physical exam findings, Alcohol Consumption via Timeline Follow Back, Columbia-Suicide Severity Rating Scale, DLT, and ECG parameters will be evaluated as following (mentioned in section 4.12.1 to section 4.12.9).

4.12.1 Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Concomitant illnesses, which existed before entry into the clinical study, will not be considered AEs unless they worsen during the treatment period. Pre-existing conditions will be recorded as part of the participant's medical history.

A treatment-emergent adverse event (TEAE) is defined as an AE that begins or that worsens in severity after the study drug has been administered.

Adverse Events of Special Interest

Injection Site Reaction
An ISR is characterized by an intense adverse reaction (usually immunologic) developing at the site of an injection after 4 or more hours post-injection. Subcutaneous administration of the study intervention may cause a specific local reaction originating around the injection site, such as erythema, itching, discomfort and pain, and could include more severe manifestations such as ulceration or necrosis.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 25.1 or higher and CTCAE Version 5.0.

Unless specified otherwise, all adverse event summaries will include the TEAEs only.

The following summaries will be presented:

- A table of number (percentage) of participants with adverse events and number of adverse events with an overview of treatment emergent adverse events (TEAE), related TEAEs, SAE, potential DLT AE leading to discontinuation and AE leading to death will be presented by cohort, treatment, pooled placebo and overall.

- A table of number (percentage) of participants with treatment emergent adverse events and number of treatment emergent adverse events (TEAE), and SAE summarized by SOC and PT, cohort, treatment, pooled placebo and overall. This table will be sorted by decreasing frequency of the overall column.

- A table of number (percentage) of participants with treatment emergent adverse events and number of treatment emergent adverse events (TEAE) summarized by SOC, PT, relationship, cohort, treatment, pooled placebo and overall. If more than one event with the same preferred term occurred for the same participant, then the participant was counted only once for that preferred term under the strongest causality. This table will be sorted by decreasing frequency of the overall column.

- A table of number (percentage) of participants with treatment emergent adverse events and number of TEAE summarized by SOC, PT, maximum severity, cohort, treatment, pooled placebo and overall will be presented. If more than one event occurred with the same preferred term for the same participant, then the participant was counted only once for maximum severity level of that preferred term for the summarization of severity. This table will be sorted by decreasing frequency of the overall column.

- A table of number (percentage) of participants with adverse events leading to discontinuation summarized by cohort, treatment, pooled placebo and overall. This table will be sorted by decreasing frequency of the overall column.

- A table of number (percentage) of participants with adverse events leading to death summarized by cohort, treatment, pooled placebo and overall. This table will be sorted by decreasing frequency of the overall column.

- A table of number (percentage) of participants with adverse events of special interest and number of adverse events of special interest (AESI) summarized by SOC and PT, cohort, treatment, pooled placebo and overall. This table will be sorted by decreasing frequency of the overall column.

- A table of number (percentage) of participants with adverse events and number of treatment emergent adverse events (TEAE) summarized by SOC, PT, relationship with EIA, cohort, treatment, pooled placebo and overall. If more than one event with the same preferred term occurred for the same participant, then the participant was counted only once for that preferred term under the strongest causality. This table will be sorted by decreasing frequency of the overall column.

A listing will be created for all Adverse Events (AEs), which will include treatment received, Verbatim term, preferred term (PT), system organ class (SOC), AE onset date (and time), AE end date (and time), counter measure taken on AE, relationship to study drug, AE outcome, severity, frequency, concomitant medication (if administered), TEAE indicator flag, SAE indicator flag, DLT indicator flag.

4.12.2 Deaths, Serious Adverse Events, and Other Significant Adverse Events

- A by-participant listing of all deaths that occurred during the study
- A by-participant listing of all SAEs
- A by-participant listing of all AES leading to discontinuation from the study
- A by-participant listing of all other significant AEs.

4.12.3 Clinical Laboratory Evaluation

Hematology:

- Red blood cell count (hemoglobin, hematocrit, reticulocytes, platelet count).
- Only at Days -1, 4, 29, 85, and 169
    - mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)
- White blood cell count

- Only at Days -1, 4, 29, 85, and 169
    - lymphocytes, abs and %, monocytes, abs and %, eosinophils, abs and %, neutrophils, abs and %, basophils, abs and %

Clinical Chemistry: alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin (total and direct), lactate dehydrogenase (LDH), total, protein, albumin, creatine kinase (CK), sodium, chloride, potassium, creatinine, blood urea nitrogen (BUN), fasting blood glucose.

Routine Urinalysis: specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick, microscopic examination (if blood or protein is abnormal).

Coagulation: International normalized ratio (INR), PT, PTT

Complement Panel: Bb, C3a, C5a.

Other Screening Test:

- Alcohol breath test
- Rapid SARS-CoV-2 (local laboratory testing)
- Follicle-stimulating hormone (as needed in women of non-childbearing potential only)
- Highly sensitive (urine; serum for confirmation if needed) human chorionic gonadotropin (hCG) pregnancy test (as needed for WOCBP). Any positive urine pregnancy test will be confirmed with a serum pregnancy test.
- Urine drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, and benzodiazepines)
- Viral serology (human immunodeficiency virus antibody, hepatitis B surface antigen, and hepatitis C virus antibody)

Laboratory values (Observed and change from baseline) (hematology, clinical chemistry urinalysis, coagulation and complement panel) will be listed by participant and summarized by time point, cohort, treatment , and pooled placebo. The baseline for the laboratory values will be defined as the last non-missing measurement prior to the date and time of the dose of study intervention. Laboratory values (routine urinalysis, complement panel, coagulation and other screening test) will be listed by participant.

All values outside the clinical reference ranges will be flagged in the data listings. The abnormal values will be flagged with ‘L’ for values below the lower limit of the clinical reference range and ‘H’ for values above the upper limit of the clinical reference range and included in the listings. The Investigator will assess whether the values outside the clinical reference range are clinically significant, and these will be reported as abnormal not clinically significant (NCS) or abnormal clinically significant (CS).

Shift tables will be presented for select laboratory parameters (chemistry and hematology).

4.12.4 Vital Signs

Temperature (by skin refraction), pulse rate, respiratory rate, and blood pressure will be assessed.

Blood pressure and pulse measurements will be assessed in the seated position with a completely automated device. Heart rate will be measured using telemetry during EIAs. Manual techniques will be used only if an automated device is not available. Blood pressure and pulse measurements should

be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).

Temperature will be obtained in degrees Celsius (°C), pulse rate will be counted for a full minute and recorded in beats per minute, and respirations will be counted for a full minute and recorded in breaths per minute.

Vital signs data will be listed by participant including changes from baseline. The baseline for the vital signs measurements will be defined as the last non-missing measurement prior to the date and time of the dose of study intervention. Vital signs will be summarized in tabular format to include descriptive statistics (n, mean, SD, median, minimum, maximum) for absolute values and changes from baseline will be presented by cohort, treatment, and pooled placebo and time-point.

4.12.5 Physical Examination

Physical examinations will include, at a minimum, assessments of the Cardiovascular, Respiratory, Gastrointestinal, Neurological, and Skin systems. Height will also be measured and recorded at Day 1 and weight will be measured and recorded at every visit.

- Investigators should pay special attention to clinical signs related to previous serious illnesses or AEs.
- All physical examinations should include inspection of the injection site.
- Abnormal physical examination findings will be listed.

4.12.6 Alcohol Consumption via Timeline Follow Back

Study participants are to refrain from drinking alcohol throughout the outpatient portion of the study (sentinel participants will refrain from alcohol throughout the in-clinic portion as well). At every visit, the TLFB (Timeline Follow Back) will be used to assess each participant's abstention from alcohol since the previous visit. The TLFB was developed as a procedure to aid recall of past drinking. Currently, self-reports are the only viable method for retrospectively measuring drinking with any precision. Alternative methods exist, but they are either impractical (e.g., continuous direct observation), fraught with problems (e.g., alcohol sweat patches) or they only measure very recent drinking (e.g., biochemical indicators or blood alcohol tests). Consequently, there is no practical alternative technology other than self-reports for retrospectively assessing drinking. TLFB stands as the most exhaustively evaluated method that can be used for this purpose.

Alcohol Consumption via Timeline Follow Back will be summarized and listed by cohort and visit.

4.12.7 Columbia-Suicide Severity Rating Scale

The C-SSRS is a suicidal ideation rating scale created by researchers at Columbia University. It measures an individual's degree of suicidal ideation and behaviour.

The scale identifies behaviors that may be indicative of an individual's intent to commit suicide. The C-SSRS is used extensively across primary care, clinical practice, surveillance, research, and institutional settings and is part of a national and international public health initiative involving the assessment of suicidal ideation and behavior. The C-SSRS requires no mental health training to administer it. The C SSRS will be administered by qualified, trained raters.

The Baseline version will be administered at Screening and at subsequent visits the "C-SSRS Since Last Visit" version will be administered.

C-SSRS will be listed by participant.

4.12.8 Dose Limiting Toxicities

A DLT is defined as:

- an AE of ⩾ Grade 3 intensity (CTCAE Version 5.0) in one participant, unless it is clearly the result of a non-study-related event OR
- any 2 AEs of ⩾ Grade 2 intensity in the same body system in one participant.

DLT will be summarized as follows:

- A table of number (percentage) of participants with treatment emergent adverse events and number of treatment emergent adverse events (TEAE) summarized by cohort, treatment, pooled placebo and overall.

4.12.9 ECG

Standard safety 12-lead ECGs will be performed as shown in the Table 6-3 Schedule of Activities.

The following ECG parameters will be recorded:

- RR-interval (msec)
- QRS-interval (msec)
- PR-interval (msec)
- QT-interval (msec)
- QTc-interval (msec)
- QT-interval corrected using the Fridericia correction formula (QTcF) (msec)
- Heart rate (HR) (beats per minute [bpm])

For QTcF, QT will be corrected as:

$$\text{QTcF} = \frac{QT}{RR^{\frac{1}{3}}}$$

The ECG will be evaluated by the Investigator as 'Normal', 'Abnormal, NCS' or 'Abnormal, CS'.

All ECG parameters will be listed by participant for each dose cohort and time point including changes from baseline.

Baseline is defined as the last non-missing measurement prior to the date and time of the dose of study intervention.

Descriptive statistics (n, mean, SD, median, minimum, maximum) for absolute values and changes from baseline will be presented by cohort, treatment, pooled placebo, and time point.

A summary of the number and percentage of participants with QT/QTc intervals exceeding some predefined upper limits (e.g. >450ms, >480ms, >500ms for measured values as well as. >30ms, >60ms for changes from baseline) of ECG parameters will be displayed in a frequency table.

4.13 Safety Review Committee

A Safety Review Committee (SRC) will conduct periodic reviews and will review all cumulative safety, tolerability, and available PD data on participants across all cohorts, approximately every 2 months during treatment and post dose follow-up. Participant safety will be continuously monitored by the SRC by ongoing review of AEs, laboratory results, and other protocol-specified assessments, to allow safety signal detection and to determine dose advancement from sentinel to expanded and for dose escalation to next cohort throughout the study.

Details about the SRC will be provided in the SRC charter Version 1.0 dated Aug 12, 2021.

SRC Tables and figures will summarized by cohort and overall. SRC Listings will be listed by cohort and participant.

SRC TFLs will be prepared as mentioned in SRC TFLs SHELLs Version 1.0 dated Oct 19, 2021

Summary statistics for laboratory parameters will summarized by cohort and overall for sentinel subjects separately.

4.14 Interim Analysis

No interim analysis is planned for this study.

4.15 Determination of Sample Size

No formal sample size estimations were performed. The following sample sizes were considered sufficient to provide an initial assessment of the safety profile of DCR-AUD in HVs, and adequate for the purpose of describing PK/PD data in HVs.

- 27 HV participants (3 cohorts with 9 participants each, 2 to 1 randomization), plus an additional 9 HV participants (2 to 1 randomization), for a total of 36, if an optional fourth cohort is enrolled.

The sample size is based on clinical rather than statistical considerations.

4.16 Changes in the Conduct of the Study or Planned Analysis

In addition to the planned secondary endpoints, Quantitative assessment of 6 symptom responses during EIAs have been added to this study as a secondary endpoint. More details on this have been provided in respective Pharmacodynamic endpoints and analysis sections.

5 REFERENCES

[1] SAS® Version 9.4 of the SAS System for Personal Computers. Copyright © 2002-2003. SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

[2] Smith, Brian & Vandenhende, Francois & DeSante, Karl & Farid, Nagy & Welch, Pamela & Callaghan, John & Forgue, S.. (2000). Confidence Interval Criteria for Assessment of Dose Proportionality. Pharmaceutical Research. 17. 1278-1283. 10.1023/A:1026451721686.

[3] Hummel J, McKendrick S, Brindley C, French R. Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. Pharm Stat. 2009 Jan-Mar;8(1):38-49. doi: 10.1002/pst.326. PMID: 18386766.

6 APPENDICES

6.1 Schedule of Activities

Table 6-1 Schedule of Activities

	Screening	Visit 1 (In-Clinic)					V1b[a]	Visit 2	Visit 3	Visit 4	Visits 5-6	Visit 7	Visit 8 EOS/ET
Study Day / **Procedure/Assessment**	**-28 to -2**	**-1**	**1**		**2**	**3**	**4**	**8**	**15 ±2 day**	**29 ±2 day**	**57 ±2 day**	**85, 113 ±2 day**	**141 ±2 day**	**169 ±7 day**
			Pre-dose	**Post-dose**										
Informed consent	X													
Clinical site admission/discharge [b]		X					X							
Inclusion/exclusion criteria	X	X	X											
Demographic/baseline characteristics	X													
General medical history	X													
Psychological history	X													
C-SSRS[c]	X	X							X	X		X[d]		X
MINI	X													
Alcohol history/consumption (TLFB)	X	X						X	X	X	X	X	X	X
Diary (AEs, temperance) distribute/collect								X	X	X	X	X	X	X
Drug/alcohol testing (urine, breath) [e]	X	X							X	X	X	X	X	X
Testing for HCV, HIV, and HBV	X													
Randomize to study intervention			X											
Study intervention administration			X											
Medication history/concomitant meds	X	X		X	X	X	X	X	X	X	X	X	X	X

	Screening	Visit 1 (In-Clinic)					V1b[a]	Visit 2	Visit 3	Visit 4	Visits 5-6	Visit 7	Visit 8 EOS/ET
Study Day / **Procedure/Assessment**	**-28 to -2**	**-1**	**1**		**2**	**3**	**4**	**8**	**15 ±2 day**	**29 ±2 day**	**57 ±2 day**	**85, 113 ±2 day**	**141 ±2 day**	**169 ±7 day**
			Pre-dose	**Post-dose**										
12-lead ECG [f]	X	X	X	X	X	X		X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination [g]	X	X	X		X	X	X	X	X	X	X	X	X	X
Pregnancy test (WOCBP only) [h]	X		X							X	X	X	X	X
FSH (if applicable)	X													
Clinical safety laboratory tests [i]	X	X			X		X	X	X	X	X	X	X	X
Complement panel [j]			X	X	X									
Blood sample for ADA		X								X				X
SARS-CoV-2 rapid test (at site)[k]		X								X	X	X		X
Blood sample for genotyping [l]		X												
Plasma collection for PK analysis [m]			X	X	X	X	X		X					
Urine collection for PK analysis [n]				X	X	X	X							
Ethanol interaction assessment [o]		X					X		X	X	X	X		X[p]
Record AEs and SAEs [q]	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: ADA: antidrug antibody; AE: adverse event; CFU: conditional follow-up; C-SSRS: Columbia-Suicide Severity Rating Scale; ECG: electrocardiogram; EIA: ethanol interaction assessment; FSH: follicle stimulating hormone; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; ICF: informed consent form; MINI: Mini-International Neuropsychiatric Interview; PK: pharmacokinetic; SAE: serious adverse event; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TLFB: Timeline Follow Back; WOCBP: women of childbearing potential

[a] Visit 1b is only required for the 3 sentinel group participants in each cohort.

[b] Participants in the expanded cohort must have fasted for at least 6 hours before admission on Day −1 and will receive a standardized meal prior to the EIA. Participants undergoing EIA will be discharged from the clinic on Day 5. Sentinel group participants will be discharged from the clinic on Day 4, following the 72-hour PK blood draw and completion of pooled urine collection.

[c] At Screening, the 'C-SSRS Baseline' form will be administered, at all other visits indicated, the 'C-SSRS Since Last Visit' form will be administered.

[d] C-SSRS to be administered only on Day 85.

[e] Urine for drugs of abuse and breathalyzer for alcohol. Cannabis will not be recorded as a drug of abuse for this study. Participants will be excluded from EIA evaluation that day if they test positive for breath alcohol on day of study visit prior to EIA.

[f] Standard ECGs will be performed 30 minutes predose, and at 60 minutes and 150 minutes postdose. A ±15-minute window around each time point is allowed if multiple assessments are due. If multiple assessments are due, the order of priority should be ECG, vitals, PK, and then other assessments.

[g] Complete physical examinations will be conducted at every visit. Height will be measured and recorded at Day 1 and weight will be measured and recorded at every visit.

[h] If a urine pregnancy test is positive, confirm with serum test for pregnancy (see Section Protocol section 10.4). Local laboratory may be used. Pregnancy testing to be performed every 28 days throughout the study.

[i] Hematology, clinical chemistry, coagulation, and urinalysis samples will be collected at every visit indicated. Only on Days −1, 4, 29, 85, and 169 will additional hematology analytes (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and absolute and % of lymphocytes, monocytes, eosinophils, neutrophils, and basophils) be collected. See Table 6-6 for a complete list of parameters to be assessed.

[j] During in-clinic stay at Day 1 and Day 2, blood samples will be collected for complement predose, and at 2, 8, and 24 hours postdose.

[k] Participants in expanded cohorts will need to undergo SARS-CoV-2 rapid test in compliance with clinic requirements before admittance to the unit for EIA testing. Refer to Study Manual for further details on the timing of this testing.

[l] A separate informed consent form is needed for collection of blood sample for optional genotyping.

[m] At Visit 1, plasma samples for PK analysis will be collected before study intervention administration and at 15 and 30 minutes and 1, 2, 4, 6, 8, 24, 48, and 72 hours after the injection. At Visit 2, only one sample will be collected for PK. If multiple assessments are due, the order of priority should be ECG, vitals, PK, and then other assessments, with the PK sampling being performed preferably at the nominal time point. Table 6-2 details the windows allowed surrounding collection of PK samples.

[n] Pooled urine collection to be done for PK analyses of DCR-AUD concentration. Participants should have an empty bladder prior to administration of study intervention, and 24-hr urine collection to begin with first void after study intervention administration. Urine to be collected at intervals of 0-4, > 4-8, > 8-12, > 12-24, > 24-48, and > 48-72 hours. Urine will be collected using a polypropylene container.

[o] Each cohort will have a sentinel group (n=3) that does not undergo any EIA assessment. For all other participants, all visits on which an EIA is conducted will require an overnight stay in the clinic. Participants will be admitted after an overnight fast and be provided a standardized meal. EIA estimated time is ~2.5 hours and is conducted at all visits except Visit 7 (Day 141). Note that the Day −1 assessment occurs prior to administration of study intervention and is intended to serve as a baseline for biomarker assessments and to confirm eligibility. See Table 6-3 for details of EIA.

[p] Participants who have positive ethanol reaction symptoms at the Day 169 EIA (e.g., nausea, vomiting, or substantial flushing) will return every 28 (±7) days for follow-up EIAs until the positive ethanol reaction symptoms abate. These CFU EIAs will not require overnight admission to the clinic, but all other aspects of the EIA will be conducted. Participants will be observed for no less than 6 hours after ethanol administration and will not be discharged until the Investigator deems it medically safe to do so.

[q] AEs and SAEs will be recorded from time ICF is signed. SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

Table 6-2 Allowed Windows for Collection of Plasma Pharmacokinetic Samples and for Complement Panel

Nominal Postdose Sample Time	Window
15 minutes	± 5 minutes
30 minutes	± 10 minutes
1, 2, 4, 6, and 8 hours	± 15 minutes
≥ 24 hours	± 30 minutes

Table 6-3 Schedule of Activities: Ethanol Interaction Assessments

Time in minutes / Procedures	Pre-EIA	Rest	Test Period							
		− 20	0[a]	15 ±5	30 ±10	45 ±10	60 ±15	90 ±15	120 ±15	150 ±15
Admit/return to clinic (fasted state)[b]	X									
Standardized meal[c]	X									
Venous catheter placement[d]		X								
Alcohol consumption[e]			← X →							
Blood biomarkers[f g]			X	X	X	X	X	X	X	X
EIA 12-lead ECG			X				X			X
SEAS[h]			X		X		X	X	X	X
Facial skin temperature[i]			X	X	X	X	X	X	X	X
EIA vital sign[j]			X	X	X	X	X	X	X	X
Heart rate via telemetry			X	X	X	X	X	X	X	X
EIA AEs[k]			X	X	X	X	X	X	X	X

Abbreviations: AE: adverse event; CFU: conditional follow up; ECG: electrocardiogram; EIA: ethanol interaction assessment; EtOH: ethanol; SEAS: subjective effects of alcohol scale;

a. Time "0" is immediately before the first sip of alcohol. All Time 0 assessments must be performed prior to initiation of alcohol intake.
b. All visits on which an EIA is conducted will require an overnight stay in the clinic except for any CFU visits for participants who have positive ethanol reaction symptoms at Day 169; these do not require an overnight stay.
c. Participants are required to fast for at least 6 hours prior to consuming the standardized meal.
d. Participants will have a venous access catheter placed for sample collection. Participant will be given a≥20-minute rest period following placement of the catheter prior to recording predose ECG, SEAS, vital signs, and facial temperature.
e. After completion of the standardized meal, alcohol will be consumed in 4 aliquots over a 30-minute period. The ethanol will be mixed in a liquid that has no other alcohol, no caffeine, and is low in sugar. Because BAC is higher in women than men when given the same g/kg EtOH dose (~ 15% higher mean BAC), the EtOH dose will be 0.4 g/kg for male participants and 0.34 g/kg for female participants. The total dose of EtOH will not exceed 28 g in men or 24 g in women.
f. Blood samples will be collected via a venous access device for biomarker analysis, which include acetaldehyde, acetate, and ethanol. Blood sample collection and processing will be detailed in the Laboratory Manual.
g. The order of assessments should be blood draw, 12-lead ECG, SEAS, vital signs, and facial temperature.
h. Participants will rate the extent to which they are feeling each of the 14 SEAS effects in the present moment.
i. Facial skin temperature will be measured using a surface scanning thermometer and will be collected prior to blood draws.
j. Vital signs will be recorded prior to blood draws and include blood pressure and pulse/heart rate.
k. Adverse events associated with administration of ethanol will be recorded continuously throughout the test period, beginning with the first sip of alcohol.

6.2 Imputation Rules for Partial Dates

Imputed dates and time will NOT be presented in the listings.

Table **6-4** and Table 6-5 present algorithm for imputing partial dates for TEAE and prior/concomitant medication respectively.

Table 6-4 Algorithm for Treatment-Emergent Adverse Events:

Start/Increase Severity Date	Stop Date	Action
Known	Known	Considered as a treatment-emergent adverse event (TEAE) if start date on or after the date of the first dose of investigational product (IP)
	Partial	Considered as a TEAE if start date on or after the date of the first dose of IP. The last day of the month and the last month (ie, December) will be used if the stop day/month is missing.
	Missing	Considered as a TEAE if start date on or after the date of the first dose of IP
Partial, but known components show that it cannot be on or after first IP taken date	Known	Not a TEAE. The first day of the month and January will be used if the start day/month is missing.
	Partial	Not a TEAE. The first day of the month and January will be used if the start day/month is missing. The last day of the month and the last month (ie, December) will be used if the stop day/month is missing.
	Missing	Not a TEAE. The first day of the month and January will be used if the start day/month is missing.
Partial, could be on or after first IP taken date	Known	Considered as TEAE, if stop date is after first IP taken date. The first IP taken date will be used if start date is in the same month/year with first IP taken date, or the first day of the month and January will be used if the start day/month is after first IP taken date Considered as not TEAE, if stop date is prior to first IP taken date. The first day of the month and January will be used if the start day/month is missing.
	Partial	Considered as TEAE. The first IP taken date will be used if start date is in the same month/year with first IP taken date, or the first day of the month and January will be used if the start day/month is after first IP taken date. The last day of the month and the last month (ie, December) will be used if the stop day/month is missing.
	Missing	Considered as TEAE. The first IP taken date will be used if start date is in the same month/year with first IP taken date, or the first day of the month and January will be used if the start day/month is after first IP taken date.
Missing	Known	Considered as TEAE if stop date is on or after the date of the first dose of IP.
	Partial	The last day of the month and the last month (ie,

Start/Increase Severity Date	Stop Date	Action
		December) will be used if the stop day/month is missing. If the imputed stop date is on or after the first dose of IP considered as a TEAE; if the year is missing, considered as a TEAE
	Missing	Considered as a TEAE

Table 6-5 Algorithm for Prior/Concomitant Medications Categorization:

Start Date	Stop Date	Action
Known	Known	If stop date is prior to the date for the first dose of IP, considered as prior; if stop date is on or after the date for the first dose of IP, considered as concomitant.
	Partial	The last day of the month and the last month (ie, December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date for the first dose of IP, considered as prior; if the imputed stop date is on or after the date for the first dose of IP, considered as concomitant.
	Missing	Considered as concomitant.
Partial	Known	If stop date is prior to the date for the first dose of IP, considered as prior; If stop date is on or after the date for the first dose of IP, considered as concomitant. The first day of the month and January will be used if the start day/month is missing.
	Partial	The last day of the month and the last month (ie, December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date for the first dose of IP, considered as prior; if the imputed stop date is on or after the date for the first dose of IP, considered as concomitant. The first day of the month and January will be used if the start day/month is missing.
	Missing	Considered as concomitant. The first day of the month and January will be used if the start day/month is missing.
Missing	Known	If stop date is prior to the date for the first dose of IP, considered as prior; if stop date is on or after the date for the first dose of IP, considered as concomitant.
	Partial	The last day of the month and the last month (ie, December) will be used if the day/month of stop date is missing. If the impuated stop date is prior to the date for the first dose of IP, considered as prior; if he imputed stop date is on or after the date for the first dose of IP, considered as concomitant.
	Missing	Considered as concomitant.

Table 6-6 Safety Laboratory Assessments

Laboratory Assessments	Parameters	
Hematology	Red blood cell count hemoglobin hematocrit reticulocytes platelet count Only at Days -1, 4, 29, 85, and 169 mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC)	White blood cell count Only at Days -1, 4, 29, 85, and 169 lymphocytes, abs and % monocytes, abs and % eosinophils, abs and % neutrophils, abs and % basophils, abs and %
Clinical Chemistry	alanine transaminase (ALT) aspartate transaminase (AST) gamma-glutamyl transferase (GGT) alkaline phosphatase (ALP) bilirubin (total and direct) lactate dehydrogenase (LDH) total protein albumin	creatine kinase (CK) sodium chloride potassium creatinine blood urea nitrogen (BUN) fasting blood glucose
Routine Urinalysis	specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick microscopic examination (if blood or protein is abnormal)	
Coagulation Parameters	International normalized ratio (INR), PT, PTT	
Complement panel	Bb, C3a, C5a	
Other Screening Tests	• Alcohol breath test • Rapid SARS-CoV-2 (local laboratory testing) • Follicle-stimulating hormone (as needed in women of non-childbearing potential only) • Highly sensitive (urine; serum for confirmation if needed) human chorionic gonadotropin (hCG) pregnancy test (as needed for WOCBP). Any positive urine pregnancy test will be confirmed with a serum pregnancy test. • Urine drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, and benzodiazepines) • Viral serology (human immunodeficiency virus antibody, hepatitis B surface antigen, and hepatitis C virus antibody)	

[redacted]

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