Statistical Analysis Plan

Protocol Title	A Phase 2 Study of ANC-501 in the Treatment of Adults with Major Depressive Disorder
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This study will be conducted in compliance with the protocol, Good Clinical Practice and all other applicable regulatory requirements, including the archiving of essential documents.

Signatures/Approvals

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Philip Perera (Sep 30, 2023 07:34 PDT)

Signing Reason: I approve this document.

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SAP Version	Changes from Previous Version
1.0	Not applicable.
1.1	Clarify reason for change in definition of efficacy population and remove language pertaining to a treatment policy estimand.

List of Abbreviations

ADaM	Analysis Data Model
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
C-SSRS	Columbia-Suicide Severity Rating Scale
CGI-I	Clinical Global Impression – Improvement
CGI-S	Clinical Global Impression – Severity
CRF	Case Report Form
CRO	Contract Research Organization
CS	Clinically Significant
CTQ	Childhood Trauma Questionnaire (CTQ)
ECG	Electrocardiogram
EDC	Electronic Data Capture
EFF	Efficacy Population
EOT	End of Treatment
ET	Early Termination
GAD-7	General Anxiety Disorder (7-item)
HAM-A	Hamilton Anxiety Scale
HAMD-6	Hamilton Depression Rating Scale (6-item)
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ITT	Intent to Treat
LOCS III	Lens Opacities Classification System III
MADRS	Montgomery-Åsberg Depression Rating Scale
MDD	Major Depressive Disorder
MedDRA	Medical Dictionary of Regulatory Affairs
NCS	Not Clinically Significant
PGI-I	Patient Global Impression of Improvement (PGI-I)

PK	Pharmacokinetics
SAE	Serious Adverse Event
SAF	Safety Population
SAP	Statistical Analysis Plan
SCID-5-CT	Structured Clinical Interview for DSM-5 – Clinical Trials Version
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SF-36	Short Form-Health Survey (36-item)
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TLF	Tables, Listings, and Figures
WHO	World Health Organization

1. Introduction

EmbarkNeuro is conducting a study to investigate a new treatment for Major Depressive Disorder (MDD). The study background, design and subject assessments for the study are described in the study specific protocol.

The statistical methods to be implemented during the analyses of data collected within the scope of this study will be outlined in this document. The purpose of this plan is to provide specific guidelines from which the statistical analysis will proceed. Any deviations from this plan will be documented in the clinical study report.

2. Study Objectives

2.1. Primary Objectives

The primary objectives of this study are:

- To evaluate the effect of treatment with ANC-501 capsules on depressive symptoms in subjects with MDD
- To evaluate the safety and tolerability of ANC-501 capsules in subjects with MDD

2.2. Secondary Objectives

The secondary objectives of this study are:

- To evaluate effect of ANC-501 at all time points and percent reduction in total symptom scores compared with baseline
- To assess the pharmacokinetic (PK) profile of ANC-501 capsules in plasma using a sparse sampling model

2.3. Exploratory Objectives

The exploratory objectives of this study are:

- To evaluate changes in 12-hour urine, plasma, and saliva cortisol
- To evaluate changes in biometrics
- To evaluate biological samples as potential biomarkers and metabolites
- Patient reported changes in depression and anxiety symptoms

3. Study Design

This is a single-arm, open-label Phase 2 study to assess the safety, tolerability, PK, and efficacy of ANC-501 oral capsules as adjunctive treatment in subjects diagnosed with MDD.

With the exception of subjects permitted to use current stable antidepressant treatment, initiation of psychotropic medications and other medications that may potentially have an impact on efficacy or safety endpoints will not be allowed between screening and completion of the Day 56 assessments. Psychotropic medications, which must have been initiated at least 8 weeks prior to screening, must remain at a stable dose until completion of the Day 56 assessments. During the study, efficacy and safety assessments will be performed. Blood samples will also be collected for PK analysis of ANC-501 and future exploratory biomarker and genetic analysis. A wearable watch-like device will collect data on biometrics, which may be used for exploratory analyses.

Subjects will receive 50 mg ANC-501 Capsules (5 ξ 10 mg capsules) orally once daily at least 1 hour prior to the morning meal whenever possible for 8 weeks beginning with the first dose administered in the clinic on Day 1 and will return to the clinic for scheduled visits on Days 8, 15, 29, 43, and 56. Follow-up visits will occur on Day 70 and 112. All scheduled visits after Day 1 allow a visit window of ± 3 days.

Total duration of participation is 20 weeks (up to 30 days screening, 8 weeks dosing, 8 weeks follow-up).

A summary of the schedule of events is provided in Table 1 below.

Table 1: Schedule of Events

Study Procedure	**Screening Period**	**Open-Label Treatment Period [a]**						**Follow-up Period [b]**	
Visit Days **Window**	**-30 to -1**	**Day 1 [c]**	**Day 8** **(±3 days)**	**Day 15** **(±3 days)**	**Day 29** **(±3 days)**	**Day 43** **(±3 days)**	**Day 56/ET** **(±3 days)**	**Day 70** **(±3 days)**	**Day 112** **(±3 days)**
Informed Consent	X								
Demographics	X								
Medical history	X								
Full physical examination	X						X		
Body weight/height/BMI [d]	X	X							
Vitals [e]	X	X	X				X		
12-lead ECG		X	X				X		
Safety laboratory tests [f]	X	X	X				X	X	
Urine drug and alcohol breath test screen [g]	X	X							
Urine pregnancy test [h]	X	X							
Hepatitis and HIV serology	X								
Sparse PK blood sampling [i]		X	X	X	X	X	X		

Exploratory biomarker sampling [j]		X	X				X		
Genetic sampling [k]		X							
Ophthalmic exam and LOCS III [l]	X						X	X	X
SCID-5-CT diagnostic interview	X								
MADRS	X	X	X	X	X	X	X	X	
HAM-A	X	X	X	X	X	X	X	X	
CGI-S	X	X	X	X	X	X	X	X	
C-SSRS [m]	X	X	X	X	X	X	X	X	
HAMD-6 [n]		X							
GAD-7 [n]		X							
SF-36 [n]		X							
EMAW Orienting Questions [n]		X							
CTQ	X								
CGI-I			X	X	X	X	X	X	
PGI-I			X	X	X	X	X	X	
SAFER Interview (central rater) [o]	X								

Inclusion/Exclusion Criteria Review	X	X							
12-hour urine collection for cortisol instructions [p]	X						X		
Plasma and saliva cortisol sample collection	X						X		
Wearable monitoring instructions and Mobile application training		X							
Continuous Wearable monitoring [q]		X							
Site download of Wearable data			X	X	X	X	X		
ANC-501 dosing [r]		X							
ANC-501 Dosing Diary dispensed and reviewed by site		X							
Dispense study medication		X	X	X	X	X			
Adverse events [s]	X	X							
Prior/Concomitant Medications [t]	X	X							

Abbreviations: AE = Adverse Event; BMI = body mass index; CGI-I = Clinical Global Impression-Improvement; CGI-S = Clinical Global Impression-Severity; C-SSRS = Columbia-Suicide Severity Rating Scale; CTQ = Childhood Trauma Questionnaire, 28-item; ECG = electrocardiogram; ET = early termination; HAM-A = Hamilton Anxiety Rating Scale; HIV = human immunodeficiency virus; LOCS III= Lens Opacities Classification System III; MADRS = Montgomery-Åsberg Depression Rating Scale; MDD = major depressive disorder; MGH ATRQ = Massachusetts General Hospital Antidepressant Treatment Response Questionnaire; PGI-I = Patient Global Impression of Improvement; PK = pharmacokinetic; SAE = serious adverse event; SAFER = State vs trait, Assessability, Face

and Ecological validity, Rule of 3Ps (pervasive, persistent, and pathological); SCID-5-CT = Structured Clinical Interview for DSM-5 – Clinical Trials Version; HAMD-6= Hamilton Depression Rating Scale (6-item); GAD-7= General Anxiety Disorder (7-item); SF-36 = Short Form-Health Survey (36-item).

a. Subjects who discontinue study treatment before completing the study and those who prematurely withdraw from the study for any reason should undergo Day 56 visit procedures in-clinic as soon as possible and schedule the Day 70 safety assessments (at +2 weeks post ET visit) and Day 112 follow-up ophthalmic visit (at +8 weeks post ET visit).
b. Subjects will return to the site on Day 70 and 112 for a follow-up visit.
c. Day 1 procedures are to be completed prior to dosing, except for sparse PK sampling which will be performed prior to dosing and between 0.75 and 2 hours post-dose.
d. Body weight and height will be measured at screening and BMI will be calculated. Only body weight and BMI calculation will be performed at subsequent visits.
e. Vital signs include oral temperature (°C), respiratory rate, heart rate, and blood pressure (supine and standing).
f. Safety laboratory tests will include hematology, serum chemistry, coagulation, and urinalysis.
g. Urine toxicology for selected drugs of abuse (barbiturates, amphetamines, cocaine, opioids, cannabis) and breath test for alcohol.
h. Urine pregnancy test will be conducted only in female subjects of childbearing potential. Female subjects who prematurely discontinue will have a pregnancy test performed at the ET visit.
i. A blood sample for sparse PK sampling will be collected pre-dose and between 0.75 and 2.0 hours post-dose at the Day 1 visit, and between 0.75 and 2.0 hours post-dose at all other indicated visits whenever possible (with date/time of previous dose provided by the subject).
j. A blood sample for exploratory biomarker analysis will be collected in conjunction with PK sampling on Days 1 (pre- post-dose), 8, and 56.
k. A genetic sample (blood) for exploratory biomarker testing will be collected before the first dose of drug.
l. Ophthalmic exam, including slit-lamp imaging, may be performed by an appropriately trained and qualified individual as delegated by the PI and documented in the Study Delegation Log. However, a licensed ophthalmologist or optometrist must oversee this work and will make the determination on subject eligibility as per inclusion criteria #4. Slit lamp imaging of the lens of both eyes will be taken and sent to a central reader for assessment using the LOCS III. Ideally the exam would be performed after cortisol results are known and the subject is otherwise deemed eligible to continue with screening assessments. Additional slit lamp imaging of the lens of both eyes may be performed during the treatment period and sent to a central reader for assessment using the LOCS III, per Investigator's discretion.
m. The "Baseline/Screening" C-SSRS form will be completed during screening. The "Since Last Visit" C-SSRS form will be completed at any time of day at all subsequent time points.
n. The Short Form-Health Survey (36-item) (SF-36) weekly. The Hamilton Depression Rating Scale (6-item) (HAMD-6) and the General Anxiety Disorder (7-item) (GAD-7) will be completed up to twice a day for a minimum of three days per week (to achieve 6 collections per week). Subjects will answer EMA orienting questions up to three times per day in conjunction with the other assessments being collected via the EMA application on their cell phones or the provided device.
o. Conducted by central reader after Screening visit, but prior to Day 1 visit. The MADRS, MGH ATRQ and confirmation of MDD will be conducted by central rater for determination of eligibility.
p. Study site personnel will dispense equipment and train subjects on 12-hour urine collection for measuring cortisol level. At the Day 43 visit, study site personnel will dispense equipment and retrain subjects on the 12hour urine collection to measure cortisol levels (the collection will be returned to the site at the Day 56 visit). On Day 54, the site will contact the subject to remind them to begin collection on Day 55.
q. Subjects will wear their wearable watch-like device throughout the day and night except when charging.
r. At the Day 1 visit, subjects confirmed as eligible will receive a 50 mg dose of study drug at the study site prior to eating. All other doses will be taken by the subjects at home

s. All AEs will be recorded from study drug administration on Day 1 until Day 112visit. Serious adverse events that occur subsequent to the signing of the informed consent (i.e., during the Screening Period) as a result of study participation will be recorded, as well as all SAEs occurring from study drug administration on Day 1 until Day 112 visit.

t. Includes medications taken within 30 days prior to informed consent and throughout the study

4. Analysis Populations

Safety Population (SAF): The Safety population will consist of all subjects who received at least one dose of ANC-501. This population will be used for all Safety analyses.

> Enrolled Subjects: The date the first dose of ANC-501 is administered is the date a subject is considered enrolled into the study. Enrolled subjects and the safety population are equivalent.

Efficacy Population (EFF): The Efficacy population will consist of all subjects who received at least one dose of ANC-501 and have completed treatment. This population will be used for all Efficacy analyses.

> EFF differs from the protocol due to two subjects who were discontinued near the beginning of the study due to a failed Day 1 drug screen.

5. Statistical Methods

The statistical analyses will be reported using tables, listings, and figures (TLFs). Numbering for TLFs will be based on the recommended numbering convention provided by the International Conference on Harmonization (ICH). Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums unless mentioned otherwise in this statistical analysis plan (SAP). Categorical variables will be summarized by counts and percentage of subjects in corresponding categories. Footnotes will be used to specify the percent calculation when applicable.

Tables will be summarized for scheduled visits, and individual subject data obtained via the electronic data capture (EDC) system and from external vendors may be presented in listings, as indicated in this SAP. If there are multiple readings taken per visit, the most recent reading from that visit will be using in summaries, unless there are pre-dose and post-dose readings taken at the same visit, as mentioned elsewhere in this SAP.

The analyses described in this plan are considered *a priori*, in that they have been defined prior to database lock. Any analysis added after the database lock will be considered post hoc and exploratory. Post hoc analyses will be labeled as such on the output and identified in the CSR. All analyses and tabulations will be performed using SAS version 9.4 or higher. TLFs will be presented in .pdf format. SDTM datasets will be produced using Version 3.3 of the Implementation Guide. ADaM datasets will be produced using Version 1.0 of the Implementation Guide. Upon completion, all SAS programs used to

produce datasets (SDTM, ADaM) and tables will be validated by an independent programmer. The validation process will be used to confirm that statistically valid methods have been implemented and that all data manipulations and calculations are accurate. Checks will be made to ensure accuracy, consistency with this SAP, consistency within tables, and consistency between tables and corresponding listings.

5.1. Handling Missing Data

For incomplete dates related to adverse events and concomitant medications, the dates will be imputed as follows:

- Incomplete start dates missing day with known month will be imputed as the later of (a) day of first dose started in that month or (b) first of the month.
- Incomplete start dates missing month and day will be imputed as the later of (a) month and day of first dose started in that year or (b) January 1.
- Dates missing year regardless of month or day will be considered as missing.
- An unknown start time with known or imputed date will be imputed with a time of 23:59 for events or medications on Day 1 or as 00:00 on other days.
- An unknown end time with known or imputed date will be imputed the same as a missing start time unless the event or medication is ongoing.
- Events with completely unknown start dates will be considered as treatment-emergent adverse events unless otherwise indicated by a known end date.
- Medications with completely unknown start dates will be considered as concomitant medications unless otherwise indicated by a known end date.
- Any imputed start date or time must come before any known end date or time.
- Missing dates, date parts, and time will be shown as missing in the listings.

For missing values not described in this section of the SAP, imputation will not be performed.

5.2. Definitions

- The baseline measurement for a variable is defined as the most recent non-missing value prior to study treatment.

- The change from baseline measurement for a variable at a post-baseline visit will be derived as the measurement taken at the specified visit minus the baseline measurement.

- Treatment Duration will be derived as the total number of days on treatment as follows: Date of Last Dose – Date of First Dose + 1.

- Study Duration will be defined as the total number of days in the study as follows: Date of Last Visit – Date of Informed Consent + 1.

- Treatment Period is defined to be the period that the subject is active on treatment: Date of First Dose through Date of Last Dose.

- Treatment Compliance over the Treatment Period will be calculated as follows based on drug accountability: Treatment Compliance (%) = Number of capsules taken / Number of capsules expected to be taken ξ 100.

- Morning Meal Compliance over the Treatment Period will be calculated as follows based on Day 1 administration and the dosing diary: Morning Meal Compliance is Yes if all responses to the following questions are yes; otherwise, Morning Meal Compliance is No:

    - Was subject dosed at least 1 hour prior to morning meal?
    - Since last visit, has subject been compliant with dosing at least 1 hour prior to the morning meal?

- MADRS responders are defined as subjects who experienced a >= 50% reduction in MADRS total score compared to baseline.

- MADRS remitters are defined as subjects with a MADRS total score <= 10.

- CGI-I improvers are defined as subjects who reported “Very much improved” or “Much improved” on the CGI-I scale.

- An adverse event is defined as Related if the event is reported as Definitely Related, Probably Related, or Possibly Related.

- Adverse Events of Special Interest are defined as a change from baseline of ζ 0.5 in Nuclear Opalescence, Cortical Lens Opacity, or Posterior Subcapsular Lens Opacity on the LOCS III scale, or any other ophthalmic results deemed clinically significant by the investigator.

- Age at First MDD Diagnosis will be calculated as follows: Year of the First MDD Diagnosis – Year of Birth, where the Year of the First MDD Diagnosis is defined as the year from the earliest start date of the major depressive episodes determined in Section 7.4 of this SAP.

- Number of Prior MDD Episodes will be calculated as follows: The number of MDD Episodes with a Start Year that is prior to the Year of the Day 1 Visit,

where MDD Episodes are defined as the major depressive episodes determined in Section 7.4 of this SAP.

- Duration of Current MDD Episode will be calculated as follows: Year of the Day 1 Visit – Start Year of the Current MDD Episode, where the Start Year of the Current MDD Episode is defined as the year from the most recent start date prior to the Day 1 Visit of the major depressive episodes determined in Section 7.4 of this SAP.

- Duration from First MDD Diagnosis will be calculated as follows: Year of Day 1 Visit – Year of the First MDD Diagnosis, where the Year of the First MDD Diagnosis is defined as the year from the earliest start date of the major depressive episodes determined in Section 7.4 of this SAP.

6. Analysis Considerations

The objectives of this study are to evaluate the safety and tolerability, and to evaluate the efficacy, of ANC-501 capsules in subjects with MDD.

6.1. Determination of Sample Size

Up to 20 subjects will be enrolled. The sample size was selected based on clinical and not statistical considerations.

6.2. Adjustments for Covariates

No adjustment for covariates is planned.

6.3. Interim Analysis and Data Monitoring

No interim analyses are planned.

6.4. Examination of Subgroups

No subgroup analyses are planned.

6.5. Multicenter Studies

All sites will be pooled for this analysis.

6.6. Multiplicity Comparison/Multiplicity

Not Applicable.

7. Population Analysis

The subjects will be summarized using descriptive statistics. All population summaries will be based on the set of subjects mentioned in the relevant section of this SAP.

Results for population parameters will also be listed by subject according to the relevant section in this SAP.

7.1. Subject Disposition

Information regarding subject disposition will be summarized using all subjects. Summaries will include the number of screen failures, the number of enrolled subjects, the number of subjects in the safety and efficacy populations, number of subjects completing the study treatment, number of subjects who discontinue the study treatment early, number of subjects completing the study, and number of subjects who discontinue the study early. For those who discontinue the study treatment early or discontinue the study early, the primary reason for discontinuation will be summarized.

A listing of subjects showing screen failures, analysis populations, study treatment status, study status, reason for discontinuing study treatment early, if applicable, and reason for discontinuing the study early, if applicable, will also be provided. Additionally, a listing of inclusion/exclusion criteria not met will be provided.

7.2. Protocol Deviations

Protocol deviations will be collected throughout the duration of the study. Protocol deviations will be assigned a sponsor-defined category and subcategory and classified as major or minor. A categorical summary of all major subject level deviations will be provided by deviation category and subcategory.

A listing of all subject level deviations will also be provided.

7.3. Demographic and Baseline Characteristics

Demographics variables will include age, sex and childbearing potential if females, race, ethnicity, height at baseline, weight at baseline, and BMI at baseline. Baseline characteristics will include the Montgomery-Åsberg Depression Rating Scale (MADRS) total score obtained at the SAFER screening interview and the Childhood Trauma Questionnaire (CTQ) subscale scores (Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, Physical Neglect) and CTQ Minimization/Denial scale score.

Demographics and baseline characteristics will be summarized for the safety and efficacy analysis populations, and a listing of demographics will also be provided.

7.4. Baseline Major Depressive Disorder Characteristics

Major depressive episodes will be determined by using the Medical Dictionary for Regulatory Activities (MedDRA) coded terms of "Depression" and "Major depression" from medical history. The Age at First MDD Diagnosis (years), Number of Prior MDD Episodes, Duration of Current MDD Episode (years), and Duration from First MDD Diagnosis (years) will be calculated as defined in Section 5.2 of this SAP.

A summary will be provided for the Age at First MDD Diagnosis, Number of Prior MDD Episodes, Duration of Current MDD Episode, Duration from First MDD Diagnosis, baseline MADRS total score, and baseline HAM-A total score using the safety population.

A listing of major depressive episodes from medical history with their start and end dates, identified by the aforementioned MedDRA coded terms, and the Age at First MDD Diagnosis, Number of Prior MDD Episodes, Duration of Current MDD Episode, and Duration from First MDD Diagnosis will also be provided.

7.5. Screening Mental Health Assessments

7.5.1. Structured Clinical Interview for DSM-5 – Clinical Trials Version

Subjects will be screened to determine whether they fulfill the criteria for diagnosis of MDD according to the Structured Clinical Interview for DSM-5 – Clinical Trials Version (SCID-5-CT). No listing or summary table will be provided.

7.5.2. SAFER Interview

The SAFER interview is conducted for confirmation of MDD. The interview uses the MADRS and also makes use of the Massachusetts General Hospital Antidepressant Treatment History Questionnaire (MGH ATRQ) for assessment of current antidepressant therapy (ADT), adequacy of duration, and dose of prior and current ADT, as well as degree of improvement. No listing or summary table will be provided.

7.5.3. Childhood Trauma Questionnaire

CTQ is assessed at screening only. It is a retrospective measure of childhood trauma and contains five subscales (Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, Physical Neglect) and a Minimalization/Denial scale. The results will be

presented in a listing. No summary table will be provided. The CTQ scores are addressed in Section 7.3 of this SAP.

7.6. Medical History

Medical history verbatim terms captured via the EDC system will be mapped to System Organ Class (SOC) and Preferred Term (PT) using MedDRA version 25.0. Any new, untoward, or worsening medical or psychiatric condition reported after first administration of the investigational product is to be captured as an adverse event.

Medical history events will be summarized by SOC and PT using the safety population. The summary will present the number and percent of subjects having each event. Subjects may have more than one event per SOC and PT. However, a subject is counted once if one or more events at the same level of SOC and PT is reported for the subject. The summary will be displayed in descending order of incidence of SOC and then descending order of PT within SOC.

A listing of medical history events will also be provided.

7.7. Prior and Concomitant Medications

Prior and concomitant medication verbatim terms captured via the EDC system will be mapped to Anatomical Therapeutic Chemical (ATC) classification and preferred name using the World Health Organization Drug (WHODrug) Dictionary Global March 2022 release.

Prior and concomitant medications (defined below) will be summarized separately by WHO ATC classification (using ATC4 and then using the closest available parental ATC classification if ATC4 is not available) and preferred name using the safety population.

- Prior Medication: Medications with a start date prior to first dose date will be considered as prior medications regardless of when the medications ended or whether the medications are ongoing.
- Concomitant Medication: Prior medications that continue to be taken on or after the first dose date or new medications with a start date on or after first dose date will be considered concomitant medications.

  Note that these definitions are not mutually exclusive in that it is possible for a medication to be both prior and concomitant if that medication started prior to first dose and continued after first dose, such as with for example a high blood pressure medication.

These summaries will present the number and percent of subjects using each medication. Subjects may have more than one medication per ATC classification and preferred name. However, a subject is counted once if one or more medications at the same level of ATC classification and preferred name is reported for the subject. Each summary will be displayed in descending order of incidence of ATC classification and then descending order of preferred name within ATC classifications.

A listing of prior and concomitant medications will also be provided. In the listing, each medication will be marked as prior, concomitant, or prior and concomitant.

7.8. Duration and Compliance

Treatment Duration, Study Duration, Treatment Compliance, and Morning Meal Compliance will be derived as stated in Section 5.2 of this SAP. A summary will be provided for Treatment Duration, Study Duration, and Compliance as continuous, and for Morning Meal Compliance as categorical, using the safety population.

A listing of Treatment Duration, Study Duration, Treatment Compliance, and Morning Meal Compliance, and a listing of dosing diary information, will also be provided. Additionally, a listing of study drug administration (in clinic) and a listing of drug accountability will be provided.

8. Efficacy Analysis

The efficacy objectives of this study are to evaluate the efficacy of ANC-501 capsules in subjects with MDD. There will be no imputations for missing data except as outlined in Section 5.1 of this SAP.

The efficacy endpoints will be summarized using descriptive statistics. All efficacy analyses will be based on the efficacy population.

Results for efficacy parameters will also be listed by subject according to the relevant section in this SAP.

8.1. Efficacy Parameters

The primary efficacy parameter is the change from baseline to Day 56 in the Montgomery-Åsberg Depression Rating Scale (MADRS) total score.

Secondary parameters for efficacy to be considered include:

- Change from baseline in MADRS total score at all scheduled post-baseline visits

- Percentage of MADRS responders (>= 50% reduction in total score) at all scheduled post-baseline visits
- Percentage of MADRS remitters (total score <= 10) at all scheduled post-baseline visits
- Change from baseline in Hamilton Anxiety Scale (HAM-A) total score at all scheduled post-baseline visits
- Change from baseline in Clinical Global Impression-Severity (CGI-S) score at all scheduled post-baseline visits
- Short Form-Health Survey (36-item) (SF-36) total score and subscale scores from Baseline to Day 56 and at all time points
- Percentage of Clinical Global Impression-Improvement (CGI-I) improvers ("Very much improved" or "Much improved") at all scheduled post-baseline visits
- Patient Global Impression of Improvement (PGI-I) score at Day 56

Exploratory parameters for efficacy to be considered include:

- Change in Hamilton Depression Rating Scale (6-item) (HAMD-6) scores from baseline across all time points
- Change in General Anxiety Disorder (7-item) (GAD-7) from baseline across all time points

8.2. Primary Efficacy Analysis

The primary efficacy objective of this study is to evaluate the effect of treatment with ANC-501 capsules on depressive symptoms in subjects with MDD.

8.2.1. MADRS Total Score – Day 56 Only

The primary efficacy endpoint is the change from baseline to Day 56 in Montgomery-Åsberg Depression Rating Scale (MADRS) total score.

The MADRS contains 10 questions, each rated on a 7-point scale. The total score is calculated by summing the 10 item scores and will range from 0 to 60. A higher score indicates more severe depression.

Summary statistics on the observed and change from baseline MADRS total score will be displayed at baseline and Day 56. In addition, a one-sample two-sided t-test will be performed and the 95% confidence interval and p-value for the mean change from baseline (i.e., mean difference) will be included.

MADRS total score will be shown on the listing described in Section 8.3.1 of this SAP.

One figure will be provided showing a line plot of baseline MADRS total score and Day 56 MADRS total score for all subjects, one line per subject.

8.3. Secondary Efficacy Analysis

The secondary efficacy objective of this study is to evaluate the effect of ANC-501 at scheduled visits and percent reduction in total symptom scores compared with baseline.

8.3.1. MADRS Total Score – Scheduled Visits

MADRS scores will be collected at Screening, Day 1, Day 8, Day 15, Day 29, Day 43, Day 56, and Day 70. The total score will be calculated as described in Section 8.2.1.

Summary statistics on the observed and change from baseline MADRS total score will be displayed at baseline and all scheduled visits. Additionally, summary statistics on the observed and change from baseline MADRS item scores will be displayed at baseline and all scheduled visits.

A listing of MADRS total score, MADRS responder (Yes, No) as described in Section 8.3.2 of this SAP, MADRS remitter (Yes, No) as described in Section 8.3.3 of this SAP, and the ten MADRS item scores will also be provided. MADRS responder and MADRS remitter will appear in the listing starting on Day 8.

Two figures will be provided, one showing the mean MADRS total score at baseline and at each post-baseline scheduled visit, and the other showing the mean change from baseline MADRS total score at baseline (i.e., zero) and each post-baseline scheduled visit. Both figures will including standard error bars at baseline and each post-baseline visit, where standard error = standard deviation / $\sqrt{n}$, except for mean change from baseline in MADRS total score at baseline.

8.3.2. MADRS Responders

MADRS responders are defined as subjects who experienced a >= 50% reduction in total score from baseline. The total score will be calculated as described in Section 8.2.1.

For all subjects with a MADRS measurement at the corresponding visit, the frequency and percentage of MADRS responders (Yes, No) will be displayed at all scheduled post-baseline visits. MADRS responders will be shown on the listing described in Section 8.3.1 of this SAP.

8.3.3. MADRS Remitters

MADRS remitters are defined as subjects with a total score <= 10. The total score will be calculated as described in Section 8.2.1.

For all subjects with a MADRS measurement at the corresponding visit, the frequency and percentages of MADRS remitters (Yes, No) will be displayed at all scheduled post-baseline visits. MADRS remitters will be shown on the listing described in Section 8.3.1 of this SAP.

8.3.4. Hamilton Anxiety Scale (HAM-A)

Hamilton Anxiety Scale (HAM-A) scores will be collected at Screening, Day 1, Day 8, Day 15, Day 29, Day 43, Day 56, and Day 70. HAM-A consists of 14 questions, each rated on a 5-point scale. The total score is calculated by summing the responses to all 14 questions and will range from 0 to 56. A higher score indicates more severe anxiety.

Summary statistics on the observed and change from baseline in HAM-A total score will be displayed at baseline and all scheduled visits.

A listing of the fourteen HAM-A items as well as the HAM-A total score will also be provided.

One figure will be provided showing the mean HAM-A total score at baseline and at each post-baseline scheduled visit. The figure will including standard error bars at baseline and each post-baseline visit, where standard error = standard deviation / $\sqrt{n}$.

8.3.5. Clinical Global Impression-Severity (CGI-S)

The Clinical Global Impression-Severity (CGI-S) score will be collected at Screening, Day 1, Day 8, Day 15, Day 29, Day 43, Day 56, and Day 70. CGI-S consists of one question, where the clinician is asked, “Considering your total clinical experience with this particular population, how mentally ill is the patient at this time?” This question is rated on a scale from 0 to 7, where a higher score indicates greater severity of mental illness, as follows:

0 = Not assessed
1 = Normal, not at all ill
2 = Borderline mentally ill
3 = Mildly ill
4 = Moderately ill
5 = Markedly ill

6 = Severely ill
7 = Among the most extremely ill patients

Summary statistics on the observed and change from baseline in the CGI-S score will be displayed at baseline and all scheduled visits. A value of zero will be treated as missing and not included in the summary statistics.

CGI-S score will be shown on the listing described in Section 8.3.7 of this SAP.

One figure will be provided showing the mean CGI-S score at baseline and at each post-baseline scheduled visit. The figure will including standard error bars at baseline and each post-baseline visit, where standard error = standard deviation / $\sqrt{n}$.

8.3.6. Short Form-Health Survey (36-item) (SF-36)

Short Form-Health Survey (36-item) (SF-36) consists of 36 questions. Each item's score will be recoded to a value on a scale from 0 to 100. For items 1, 2, 20, 22, 34, and 36, the scores are recoded as 1=100, 2=75, 3=50, 4=25, 5=0. For items 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, scores are recoded as 1=0, 2=50, 3=100. For items 13, 14, 15, 16, 17, 18, and 19, scores are recoded as 1=0 and 2=100. For items 21, 23, 26, 27, and 30, scores are recoded as 1=100, 2=80, 3=60, 4=40, 5=20, 6=0. For items 24, 25, 28, 29, and 31, scores are recoded as 1=0, 2=20, 3=40, 4=60, 5=80, 6=100. For items 32, 33, and 35, scores are recoded as 1=0, 2=25, 3=50, 4=75, 5=100.

For each of the 8 scales, the corresponding items are averaged for a scale score. Items that are left blank (missing data) are not considered when calculating the scale scores.

The scales and corresponding items are as follows: physical functioning (PF) (items 3-12), role limitations due to physical health (RP) (items 13-16), role limitations due to emotional problems (RE) (items 17-19), energy/fatigue (ENGY) (items 23, 27, 29, 31), emotional well-being (EMWB) (items 24-26, 28, 30), social functioning (SF) (items 20, 32), Pain (PAIN) (items 21-22), and general health (GH) (items 1, 33-36).

The physical health summary measure includes four scales: PF (10 items), RP (4 items), PAIN (2 items), and GH (5 items). The mental health summary measure includes four scales: RE (3 items), ENGY (4 items), EMWB (5 items), and SF (2 items). The total score is an average of all 36 items. A higher score indicates a more favorable physical and mental health state.

All SF-36 summaries, listings, figures, and analyses will be described in a separate analysis plan and performed separately by an external vendor, including any analysis of orienting questions.

8.3.7. Clinical Global Impression-Improvement (CGI-I)

The Clinical Global Impression-Improvement (CGI-I) score will be collected on Day 8, Day 15, Day 29, Day 43, Day 56, and Day 70. CGI-I consists of one question, where the clinician is asked, "Compared to his condition at admission to the project, how much has he changed?" This question is rated on a scale from 0 to 7, where a higher score indicates greater lack of improvement, as follows:

0 = Not assessed
1 = Very much improved
2 = Much improved
3 = Minimally improved
4 = No change
5 = Minimally worse
6 = Much worse
7 = Very much worse

A CGI-I improver is defined as a subject with a CGI-I score of "Very much improved" or "Much improved".

A categorical summary of CGI-I improvers (Yes, No) will be displayed at all scheduled post-baseline visits. A value of "Not assessed" will be treated as missing and not included in the categorical summary.

A listing of the CGI-S and CGI-I scores will also be provided.

8.3.8. Patient Global Impression-Improvement (PGI-I)

The Patient Global Impression of Improvement (PGI-I) score will be collected on Day 8, Day 15, Day 29, Day 43, Day 56, and Day 70. PCGI-I consists of one question where the subject is asked to describe his or her current condition with how it was before treatment. This question is rated on a scale from 0 to 7, where a higher score indicates greater dissatisfaction with treatment, as follows:

0 = Not assessed
1 = Very much better
2 = Much better
3 = A little better
4 = No change
5 = A little worse
6 = Much worse
7 = Very much worse

Summary statistics on the observed and change from baseline in the PGI-I score will be displayed at baseline and Day 56. A value of zero will be treated as missing and not included in the summary statistics.

A listing of the PGI-I score will also be provided.

8.4. Exploratory Efficacy Analysis

The exploratory efficacy objective of this study is to evaluate subject-reported changes in depression and anxiety symptoms.

8.4.1. Hamilton Depression Rating Scale (6-item) (HAMD-6)

The Hamilton Depression Rating Scale (6-item) (HAMD-6) measures six core symptoms of depression on a 0-4-point scale for items 1 to 5 and 0-2 points for item 6. The total score is calculated by summing the 6 item scores and will range from 0 to 22. A higher score indicates more severe depression.

All HAMD-6 summaries, listings, figures, and analyses will be described in a separate analysis plan and performed separately by an external vendor.

8.4.2. General Anxiety Disorder (7-item) (GAD-7)

The General Anxiety Disorder (7-item) (GAD-7) Scale consists of 7 items scored on a 4-point scale. The total score is calculated as the sum of all item scores and ranges from 0 to 21. A higher score indicates more severe anxiety.

All GAD-7 summaries, listings, figures, and analyses will be described in a separate analysis plan and performed separately by an external vendor.

9. Safety Analysis

The primary safety objectives of this study are to evaluate the overall safety of ANC-501 capsules in subjects with MDD. There will be no imputations for missing data except as outlined in Section 5.1 of this SAP.

The safety endpoints will be summarized using descriptive statistics. All safety analyses will be based on the safety population.

Results for safety parameters will also be listed by subject according to the relevant section in this SAP.

9.1. Safety Parameters

Primary parameters for safety to be considered include:

- Frequency and severity of adverse events (AEs)
- Vital signs
- Body weight
- Electrocardiograms (ECGs)
- Clinical laboratory tests
- Physical examination
- Lens Opacities Classification System III (LOCS III)
- Emergent behavior using Columbia-Suicide Severity Rating Scale (C-SSRS)

The secondary safety parameter is the plasma concentrations of ANC-501.

Exploratory parameters for safety to be considered include:

- Changes in 12-hour urine, plasma, and saliva cortisol from baseline to Day 56
- Changes in biometrics using the wearable watch-like device
- Baseline levels and/or changes in exploratory biomarkers through Day 56

9.2. Primary Safety Analysis

The primary safety objective of this study is to evaluate the safety and tolerability of ANC-501 capsules in subjects with MDD.

9.2.1. Adverse Events

Adverse events (AEs) summaries will only consider treatment-emergent adverse events (TEAEs), using the safety population. TEAEs are defined as those AEs that occurred after dosing and those existing AEs that worsened during the study. If it cannot be determined whether the AE is treatment-emergent due to an incomplete or missing onset date or time, the AE will be considered a treatment-emergent AE. AEs with an onset day after the last day study medication was applied will not be considered TEAEs.

An overall categorical summary of TEAEs will be provided where the total number of TEAEs, the number and percent of subjects with at least one mild, moderate, or severe TEAE, and the number and percent of each of the following items will be displayed:

- Subjects with at Least One TEAE
- Subjects with at Least One TEAE Related to Study Treatment
- Subjects with at Least One TEAE Leading to Discontinuation of Study Treatment

- Subjects with at Least One TEAE of Special Interest
- Subjects with at Least One Serious TEAE
- Subjects with at Least One TEAE Leading to Death

AEs of Special Interest and Related AEs are defined as stated in Section 5.2 of this SAP. If a subject reports one or more events within the same SOC and PT, the subject is classified (Related/Not Related) according to the most related relationship within the same SOC and PT.

In addition, for each bulleted item in the above list, categorical summaries for TEAEs by system organ class (SOC) and preferred term (PT) via MedDRA version 25.0 will be provided, and subjects having more than one TEAE may appear in more than one SOC or PT but will be counted at most once per each SOC and PT. Furthermore, a summary of TEAEs by SOC, PT, and maximum relatedness (Related, Not Related) will be provided where the maximum relatedness will be used for subjects with multiple TEAEs within the same SOC and PT, and a summary of TEAEs by SOC, PT, and maximum severity (Mild, Moderate, Severe) will be provided where the maximum severity will be used for subjects with multiple TEAEs within the same SOC and PT. Summaries that are displayed by SOC and PT will be ordered by descending incidence of SOC and then PT within each SOC, and if applicable ascending severity within SOC and PT.

A listing of adverse events and a listing of serious adverse events will also be provided.

9.2.2. Vital Signs, Height, and Weight

Blood pressure (standing and supine), heart rate, respiration rate, temperature, weight, and BMI will be collected at screening, Day 1, Day 8, and Day 56. Height will be collected at screening only. A summary of change from baseline in the vital signs at baseline and all scheduled post-baseline visits will be provided.

A listing of vital signs will also be provided.

9.2.3. Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be performed on Day 1 prior to dosing, Day 8, and Day 56. A summary for change from baseline in the ECG parameters (Ventricular Rate, PR Interval, RR Interval, QRS Interval, QT Interval, QTcB Interval, QTcF Interval) at baseline and all scheduled post-baseline visits will be provided.

The ECG results are to be interpreted as follows: Normal; Abnormal, NCS (not clinically significant); Abnormal, CS (clinically significant). A shift table of the interpretation will

be presented for all scheduled post-baseline visits. Categories for the shift table will be as follows: Missing; Normal; Abnormal, NCS; Abnormal, CS; Total.

Additionally, a categorical summary of maximum post-baseline QTcB Interval (> 0 to <= 500 msec, > 500 msec), maximum post-baseline QTcF Interval (> 0 to <= 500 msec, > 500 msec), and maximum change from baseline (> 0 to < 30 msec, >= 30 to <= 60 msec, > 60 msec) for both QTcB and QTcF will be provided.

A listing of ECG data will also be provided.

9.2.4. Clinical Laboratory Tests

Laboratory evaluations (hematology, serum chemistry, coagulation, urinalysis) will be conducted at Screening, Day 1, Day 8, Day 56, and Day 70. Any clinically significant laboratory result after baseline is to be captured as an adverse event and thus included in the summary of adverse events.

Summary statistics for the observed and change from baseline continuous laboratory results will be displayed at baseline and all scheduled post-baseline visits via the following tables:

- Hematology
- Serum Chemistry
- Coagulation
- Urinalysis

For categorical urinalysis results, frequency counts and percentages will be presented.

For laboratory parameters (hematology, serum chemistry, coagulation) where normal ranges are available, shift tables will be presented for all scheduled post-baseline visits. Categories for the shift tables will be Missing, Low, Normal, High, and Total.

A listing for each category of laboratory results shown in the above bulleted list as well as a listing for serology will also be provided, and values will be flagged as Low, Normal, or High relative to normal ranges, if available, and included in the listings. In addition, a listing will be provided for urine drug screen results and a listing will be provided for alcohol breath test screen results.

9.2.5. Physical Examination

A physical examination (PE) will be conducted during the screening period and at end of treatment. Any abnormalities noted prior to first administration of the investigational

product are to be captured as medical history. Findings after first administration of the investigational product that meet the criteria of an AE are to be captured as an AE.

A listing of PE results will be provided. No summary tables will be provided.

9.2.6. Lens Opacities Classification System III (LOCS III)

An ophthalmic exam including slit-lamp imaging may be performed and would be assessed using LOCS III. Any result that is an AE of Special Interest as defined in Section 5.2 of this SAP is to be recorded.

LOCS III data are collected at Screening, Day 56, Day 70, and Day 112. A summary for change from baseline in four LOCS III parameters (Cortical Lens Opacity, Nuclear Color, Nuclear Opalescence, Posterior Subcapsular Lens Opacity) by eye (OD, OS) at all scheduled post-baseline visits will be provided.

In addition, a shift table for each of the LOCS III parameters by eye will be provided. The possible values for Cortical Lens Opacity (C) range from 0.1 to 5.9, for Nuclear Color (NC) range from 0.1 to 6.9, for Nuclear Opalescence (NO) range from 0.1 to 6.9, and for Posterior Subcapsular Lens Opacity (P) range from 0.1 to 5.9. The exclusion criteria based on LOCS III are as follows:

- C with a LOCS III classification > 2.0 in either eye
- NC with a LOCS III classification > 3.5 in either eye
- NO with a LOCS III classification > 3.0 in either eye
- P with a LOCS III classification > 0.3 in either eye

Given the above exclusion criteria and the definition of AEs of Special Interest as provided in Section 5.2 of this SAP, the categories for the shift tables will be defined as follows, where Nuclear Color will follow the same shift table categorization strategy as Nuclear Opalescence, Cortical Lens Opacity, and Posterior Subcapsular Lens Opacity:

Baseline categorization:

- C: Missing, 0.1 to <= 1.0, > 1.0 to <= 2.0, > 2.0, Total
- NC: Missing, 0.1 to <= 1.5, > 1.5 to <= 2.5, > 2.5 to <= 3.5, > 3.5, Total
- NO: Missing, 0.1 to <= 1.0, > 1.0 to <= 2.0, > 2.0 to <= 3.0, > 3.0, Total
- P: Missing, 0.1 to <= 0.2, > 0.2 to <= 0.3, > 0.3, Total

Post-baseline categorization:

- Change: Missing, <= -0.5, > -0.5 to < 0, No Change, > 0 to < 0.5, >= 0.5, Total

Primary ophthalmic data (assessment of first reading) will be used for summaries, and a listing of primary ophthalmic data will also be provided. Secondary ophthalmic data (secondary readings) will not be summarized or listed.

9.2.7. Columbia-Suicide Severity Rating Scale (C-SSRS)

The Columbia-Suicide Severity Rating Scale (C-SSRS) will be used to assess suicidal ideation and behavior. The "Baseline/Screening" C-SSRS form will be completed at screening. The "Since Last Visit" C-SSRS form will be completed at all other visits (Day 1, Day 8, Day 15, Day 29, Day 43, Day 56, Day 70).

The "Baseline/Screening" C-SSRS form contains the following possible types of responses per each item: response based on Past Month, response based on Life, and/or Describe event. The "Since Last Visit" C-SSRS form contains the following possible types of responses per each item: response based on Since Last Visit and/or Describe event. Not all types of responses are present for all items.

C-SSRS Suicidal Ideation contains the following five ordered Yes/No response items:

1. Wish to be Dead
2. Non-Specific Active Suicidal Thoughts
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan
5. Active Suicidal Ideation with Specific Plan and Intent

C-SSRS Intensity of Ideation contains the following six numeric response items:

- Most Sever Ideation (1-5)
- Frequency – How many times have you had these thoughts (1-5)?
- Duration – When you have the thoughts how long do they last (1-5)?
- Controllability – Could/can you stop thinking about killing yourself or wanting to die if you want to (1-6)?
- Deterrents – Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide (1-6)?
- Reasons for Ideation – What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both (1-6)?

The Suicidal Ideation Subscale Score is determined as follows. For the five C-SSRS Suicidal Ideation items, Yes/No responses are recorded. The maximum item number where Yes is the response is the Suicidal Ideation Subscale Score, i.e., the most severe ideation endorsed is the score. Zero is to be assigned (a) if all responses to Categories 1-5 are No or (b) if responses to Categories 1-2 are both No and responses to Categories 3-5 are missing. If a score is not able to be determined, them it will be left as missing.

The Intensity of Ideation Subscale Score is determined as follows. The sum of responses on five C-SSRS Intensity of Ideation items (Frequency, Duration, Controllability, Deterrents, Reasons for Ideation) is the Intensity of Ideation Subscale Score. If the Suicidal Ideation Subscale Score is zero or missing, the Intensity of Ideation Subscale Score is also zero or missing, respectively. Higher values for the Intensity of Ideation Subscale Score indicate more intense ideation.

Summary statistics for the Suicidal Ideation Subscale Score and Intensity of Ideation Subscale Score from the "Baseline/Screening" C-SSRS form and "Since Last Visit" C-SSRS form will be presented by scheduled visit, where the screening visit will contain Past Month and Life summaries and other scheduled visits will contain the Since Last Visit summary.

A shift table of Suicidal Ideation Items 1 and 2 (Wish to be Dead, Non-Specific Active Suicidal Thoughts) will be presented using "Since Last Visit" for all scheduled post-baseline visits. Categories for the shift table will be Missing, No, Yes, and Total.

A listing of C-SSRS Suicidal Ideation and C-SSRS Intensity of Ideation will be provided. The Suicidal Ideation Subscale Score and Intensity of Ideation Subscale Score will be included in the listing.

9.3. Secondary Safety Analysis

The secondary safety objective of this study is to assess the pharmacokinetic profile of ANC-501 capsules in plasma using a sparse sampling model.

9.3.1. Pharmacokinetics

The secondary endpoint is the plasma concentrations of ANC-501.

Sparse pharmacokinetic (PK) blood sampling will be collected pre-dose and between 0.75 and 2 hours post-dose on Day 1 and between 0.75 and 2 hours post-dose, whenever possible, on Day 8, Day 15, Day 29, Day 43, and Day 56.

Summary statistics (number, arithmetic mean, standard deviation, median, minimum, maximum, geometric mean, geometric coefficient of variation) for the observed PK concentration data will be displayed by scheduled visit for the ANC-501 analyte using the safety population. Reported values marked as Below Limit of Quantitation (BLQ) will be set to 0.5 ξ Lower Limit of Quantitation (LLOQ), and reported values marked as Above Limit of Quantitation (ALQ) will be set to 1.0 ξ Upper Limit of Quantitation (ULOQ), for summarization purposes. LLOQ is 2.50 ng/mL, and ULOQ is 2,500 ng/mL.

A listing of PK concentration results will also be provided.

Any additional PK summaries, listings, figures, and analyses will be described in a separate analysis plan and performed separately by an external vendor.

9.4. Exploratory Safety Analysis

The exploratory safety objectives of this study are to evaluate changes in 12-hour urine, plasma, and saliva cortisol, to evaluate changes in biometrics, and to evaluate biological samples as potential biomarkers and metabolites.

9.4.1. Cortisol

Cortisol sampling (12-hour urine cortisol, plasma and/or saliva cortisol) will be performed at screening and Day 56.

Summary statistics for the observed and change from baseline cortisol level will be displayed at baseline and all scheduled post-baseline visits.

A listing for cortisol level will also be provided. The type of sampling will be included in the listing.

9.4.2. Wearable Device Biometrics

Subjects are to wear their watch-like device throughout the day and night between Day 1 to Day 56 except when charging. The wearable device collects actigraphy data (such as total daily steps, hour with the most steps, waking hour with the least steps, total minutes of sleep divided into daytime sleep and nighttime sleep, resting heart rate, average daily heart rate, and/or heart rate variation) on a daily basis. As the first round of daily data comes after Day 1 dosing, there is no baseline measure.

All wearable device biometric summaries, listings, figures, and analyses will be described in a separate analysis plan and performed separately by an external vendor.

9.4.3. Biomarkers

Biomarker samples will be collected on Day 1 (pre-dose, post-dose), Day 8, and Day 56.

All biomarker summaries, listings, figures, and analyses will be described in a separate analysis plan and performed separately.

10. Tables, Listings, and Figures

Tables will generally be presented with parameter and visits as applicable in the first column followed by the summary column(s). Descriptive statistics such as mean, median, minimum, and maximum will typically be presented to one decimal place and standard deviation will typically be presented to two decimal places. Numeric laboratory tables may vary for each test depending on the precision of the test.

Listings will be sorted by subject ID and visit within subject ID as appropriate. Data reported in EDC, external vendor data, and/or derived values may be included in the listings, as indicated in this SAP.

Tables, listings, and figures will be maintained outside of this document and may be amended as needed. The spacing, overall presentation, or layout of the displays may need to be altered once data are available.

11. Protocol and SAP Differences

If there are any differences between the study protocol and this SAP, this SAP takes precedent.

12. References

EmbarkNeuro, Inc. ANC501D0005: A Phase 2 Study of ANC-501 in the Treatment of Adults with Major Depressive Disorder, Protocol Version 6.0, Amendment 05. 03 March 2023.