# CLINICAL STUDY PROTOCOL

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| **Protocol Title:** | A Phase 2 Study of ANC-501 in the Treatment of Adults with Major Depressive Disorder |
| **Protocol Number:** | ANC501D0005 |
| **Study Drug:** | ANC-501 |
| **Brief Title:** | ANC-501 in Adults with Major Depressive Disorder |
| **Phase of Development:** | 2 |
| **Sponsor Name and Address:** | Ancora Bio d/b/a EmbarkNeuro, Inc.<br>1111 Broadway<br>Suite 1300<br>Oakland, CA 95607 |
| **Sponsor Contact:** | Philip Perera, MD, MBA<br>Chief Medical Officer<br>philip.perera@embarkneuro.com |
| **Regulatory Agency Identifier Number(s):** | IND 119048 |

| Version Number: | Date |
|---|---|
| Protocol Version 6.0, Amendment 05 | 03 March 2023 |
| Protocol Version 5.0, Amendment 04 | 17 January 2023 |
| Protocol Version 4.0, Amendment 03 | 21 November 2022 |
| Protocol Version 3.0, Amendment 02 | 22 August 2022 |
| Protocol Version 2.0, Amendment 01 | 04 May 2022 |
| Original Protocol 1.0 | 09 March 2022 |

This clinical study will be conducted in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements.

# CONFIDENTIALITY STATEMENT

The confidential information in this document is provided to you as an Investigator or consultant for review by you, your staff, and the applicable Institutional Review Board/Independent Ethics Committee. Your acceptance of this document constitutes agreement that you will not disclose the information contained herein to others without written authorization from the Sponsor.

# PROTOCOL SIGNATURE PAGE

**Protocol:** ANC501D0005 Version 6.0, Amendment 05

I, the undersigned, have read this protocol and agree that it contains all necessary information required to conduct the study.

DocuSigned by:
Stephen J Kanes MD PhD
Signer Name: Stephen J Kanes MD PhD
Signing Reason: I approve this document
Signing Time: 07-Mar-2023 | 2:47:47 AM PST
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07-Mar-2023

Stephen J. Kanes, MD, PhD
Chief Executive Officer
EmbarkNeuro

Date

# INVESTIGATOR SIGNATURE

By signing below, I agree that:

I have read this protocol. I agree that it contains all necessary details for carrying out the study as described. I will conduct this study in accordance with the design and specific provisions of this protocol. I will not initiate the study until it has received approval from the Institutional Review Board/Independent Ethics Committee. I will provide copies of this protocol and access to all information furnished by EmbarkNeuro to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the study drug and study procedures. I will inform them that this information is confidential and proprietary to EmbarkNeuro and that may not be further disclosed to third parties. I understand that the study may be terminated or enrollment suspended at any time by EmbarkNeuro with or without cause, or by me if it becomes necessary to protect the best interests of the study patients.

I agree to conduct this study in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the International Council for Harmonisation guidelines for Good Clinical Practice, and applicable regional regulations.

**INVESTIGATOR SIGNATURE**

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| Investigator Signature | Date |
| Investigator Printed Name | |

# SUMMARY OF MAJOR PROTOCOL CHANGES

| Protocol Version | Summary of change | Rationale for change |
|---|---|---|
| 6.0, A05 | Change inclusion # 8 | Allows less restrictive MADRS cutoff score from minimum of 28 to minimum of 26 |
| 6.0, A05 | Change inclusion # 9 | Allows screening of subjects with fewer weeks on continuous antidepressant therapy<br><br>Broadens the types of concomitant antidepressants allowed |
| 6.0, A05 | Change inclusion # 11 | Allows less restrictive MADRS cutoff score from minimum of 28 to minimum of 26 |
| 6.0, A05 | Added clarification language for the screening period | Clarifies the restriction around medication changes during the screening period<br><br>Clarifies the screening period does not need to be a full 30 days |
| 6.0, A05 | Updated exclusion #22 to be consistent with change in Inclusion #9 | Clarifies the length of treatment on a stable dose prior to day1 |
| 5.0, A04 | Removed exclusion #5 | Conforms with FDA IND advice |
| 5.0, A04 | Updated exclusion #18 (previously #19) to indicate positive alcohol breath test | New exclusion #19 covers the urine drug screen |
| 5.0, A04 | Added new exclusion #19 to clarify the positive urine drug test criteria | Offers specific guidance to investigators and no longer eliminates subjects with recreational cannabis use |
| 5.0, A04 | Updated abbreviation for CTQ | Reflects the appropriate 28-Item CTQ used in this study |

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| 5.0, A04 | Removed the word “cannabis” from footnote ‘g’ in the Schedule of Events | Consistent with exclusion #19 |
| 5.0, A04 | Clarified footnote ‘i' in the Schedule of Events | Offers greater latitude for blood draw times |
| 4.0, A03 | Updated Sponsor name throughout document from Ancora Bio to EmbarkNeuro | Ancora Bio is now doing business as EmbarkNeuro |
| 4.0, A03 | Clarified the conversion from nmol/L to mcg/L for 12-hour urine cortisol (>22.7 nmol/L = greater than or equal to 8.3 mcg/L). | Clarification |
| 4.0, A03 | Clarification to that exclusion #16 history of seizures does not include isolated febrile seizures of childhood | Clarification |
| 4.0, A03 | Clarification that the eye exam will be referred to as an ophthalmic exam instead of an ophthalmological exam since optometrists are now permitted into the study | Clarification which conforms to the current standard of practice |
| 4.0, A03 | The addition of an optometrist has been included along with an ophthalmologist | Conforms to the current standard of practice |
| 3.0, A02 | LOCS III scale changes from baseline in the LOCS III grade of ≥0.5 (Nuclear Opalescence), ≥0.5 (Cortical). ≥0.5 (Posterior Subcapsular) in either eye or any changes considered to be clinically significant (e.g., incidence of cataract) will be reported as an adverse event and as an event of special interest. | Response to FDA comments |
| 3.0, A02 | Clarified C-SSRS exclusion criterion #2 | Clarification to the critical questions and the timeframe. |
| 3.0 A02 | Clarification of trained personnel qualified to administer Ophthalmic exam and slit-lamp imaging | Conforms to the current standard of care |
| 3.0 A02 | Addition of Dosing Diary | Improves ability to monitor study drug accountability and compliance |
| 2.0, A01 | Updated list of abbreviations | To reflect the updates deleted and added, as applicable |
| 2.0, A01 | Updated follow-up visits to include Day 112 for all subjects | Response to FDA comments |

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| 2.0, A01 | Updated method of alcohol test at screening | Clarification |
| 2.0, A01 | Updated list of scales patients will be completing | Clarification |
| 2.0, A01 | Change from AREDS to LOCS III grading scale to detect changes in lens opacities | Response to FDA comments |
| 2.0, A01 | Added word “emergent” to the C-SSRS endpoint | Clarification |
| 2.0, A01 | Updated inclusion #4 to include BCVA and clarify what is meant by significant findings | Clarification |
| 2.0, A01 | Updated Exclusion #1 to include current MDD episode | Clarification |
| 2.0, A01 | Added new Exclusion (now #5) for History of implantation of an artificial lens or missing lens in one or both eyes | Improves ability to identify risk of lens opacity |
| 2.0, A01 | Added new Exclusion (now #6) to read as follows<br>Based on an ophthalmic examination performed at Screening, patient must not have any of the following:<br>• Nuclear opalescence (NO) with a LOCSIII classification > 3.0 in either eye<br>• Nuclear color (NC) with a LOCSIII classification > 3.5 in either eye<br>• Cortical lens opacities (C) with a LOCSIII classification > 2.0 in either eye<br>• Posterior subcapsular lens opacities (P) with a LOCSIII classification > 0.3 in either | Response to FDA comments |
| 2.0, A01 | Updated Exclusion #18 to remove benzodiazepines from exclusion | Clarification |
| 2.0, A01 | Updated Exclusion #19 to clarify method and type drug tests at screening | Clarified breath test or urine drug screen |
| 2.0, A01 | Updated Schedule of Events to reflect other updates in this Summary of Changes | Clarification |
| 2.0, A01 | Include HAMD-6 and GAD-7 as exploratory endpoints | Would like to assess patient reported changes in depression and anxiety symptoms |

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| 2.0, A01 | Included Section 8.4.9 to include Events of Special Interest – Lens Opacity | Response to FDA comments |
| 2.0, A01 | Removed mention of Local laboratories | Clarification |
| 2.0, A01 | Removed use of AiCure platform throughout the document | AiCure no longer being used for drug adherence |
| 2.0, A01 | Administration changes/corrections throughout | Clarifications/corrections |

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## LIST OF TABLES

# LIST OF ABBREVIATIONS

| Abbreviation | Term |
|---|---|
| ACTH | adrenocorticotropic hormone |
| ADT | antidepressant therapy |
| AE | adverse event |
| ALT | alanine aminotransferase |
| aPTT | activated partial thromboplastin time |
| AST | aspartate aminotransferase |
| AUC | area under the concentration-time curve |
| AVP | arginine vasopressin |
| BCVA | best corrected visual acuity |
| BMI | body mass index |
| BUN | blood urea nitrogen |
| CGI-I | Clinical Global Impression-Improvement |
| CGI-S | Clinical Global Impression-Severity |
| $CL_r$ | renal clearance |
| $C_{max}$ | maximum plasma concentration |
| CONSORT | Consolidated Standards of Reporting Trials |
| COVID-19 | coronavirus disease 2019 |
| CRF | corticotropin-releasing factor |
| CRH | corticotropin-releasing hormone |
| CRO | clinical research organization |
| C-SSRS | Columbia-Suicide Severity Rating Scale |
| CTQ | Childhood Trauma Questionnaire |
| CYP | cytochrome P450 |
| DSM-IV | Diagnostic and Statistical Manual of Mental Disorders, fourth edition |
| ECG | electrocardiogram |
| eCRF | electronic case report form |
| ECT | electroconvulsive therapy |
| EMA | Ecological Momentary Assessment |
| ENT | ears, nose, and throat |
| ET | early termination |
| FDA | Food and Drug Administration |

| Abbreviation | Term |
|---|---|
| GAD-7 | General Anxiety Disorder (7-item) |
| GCP | Good Clinical Practice |
| HAM-A | Hamilton Anxiety Scale |
| HAMD-6 | Hamilton Depression Rating Scale (6-item) |
| HBsAg | hepatitis B surface antigen |
| HCV Ab | hepatitis C virus antibody |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | human immunodeficiency virus |
| HPA | hypothalamus-pituitary-adrenal |
| IB | Investigator's Brochure |
| ICF | informed consent form |
| ICH | International Council for Harmonisation |
| IND | Investigational New Drug |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| IRT | Interactive Response Technology |
| LOCS III | Lens Opacities Classification System III |
| MAD | multiple ascending dose |
| MADRS | Montgomery-Åsberg Depression Rating Scale |
| MDD | major depressive disorder |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MGH | Massachusetts General Hospital |
| MGH ATRQ | Massachusetts General Hospital Antidepressant Treatment Response Questionnaire |
| mRNA | messenger RNA |
| NO | Nuclear Opalescence |
| PET | positron emission tomography |
| PGI-I | Patient Global Impression of Improvement |
| PK | pharmacokinetic(s) |
| PT | Preferred Term; prothrombin time |
| RBC | Red blood cell(s) |

| Abbreviation | Term |
|---|---|
| rTMS | repetitive transcranial magnetic stimulation |
| SAD | single ascending dose |
| SAE | serious adverse event |
| SAFER | State vs trait, Assessability, Face and Ecological validity, Rule of 3Ps [pervasive, persistent, and pathological] |
| SAP | Statistical Analysis Plan |
| SCID-5-CT | Structured Clinical Interview for DSM-5 – Clinical Trials Version |
| SF-36 | Short Form-Health Survey (36-item) |
| SNRI | serotonin norepinephrine reuptake inhibitor |
| SOC | System Organ Class |
| SSRI | selective serotonin reuptake inhibitor |
| STAR*D | Sequenced Treatment Alternatives to Relieve Depression |
| $t_{½}$ | apparent half-life |
| TRD | treatment-resistant depression |
| US | United States |
| $V_{1b}$ | vasopressin receptor 1b |
| WBC | white blood cell(s) |

# 1. PROTOCOL SUMMARY

## 1.1. Synopsis

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| **Sponsor:** | **EmbarkNeuro** | | |
| **Study Title:** | A Phase 2 Study of ANC-501 in the Treatment of Adults with Major Depressive Disorder | | |
| **Protocol Number:** | ANC501D0005 | **Phase of Development:** | 2 |
| **Investigational Product:** | ANC-501 Capsules | | |
| **Study Design and Duration:** | This is a single-arm, open-label Phase 2 study to assess the safety, tolerability, pharmacokinetics (PK), and efficacy of ANC-501 oral capsules as adjunctive treatment in subjects diagnosed with major depressive disorder (MDD).<br>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 and blood samples collected for PK analysis of ANC-501and future exploratory biomarker and genetic analysis. A wearable watch-like device will collect data on biometrics, which may be used for exploratory analyses.<br>Subjects will receive 50 mg ANC-501 Capsules (5 × 10 mg capsules) orally once daily at least 1 hour prior to the morning meal 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 visits after Day 1 allow a visit window of ±3 days.<br>Total duration of participation is 20 weeks (up to 30 days screening, 8 weeks dosing, 8 weeks follow-up).<br>The following is an overview of study procedures/assessments (refer to the Schedule of Events [Section 1.3] for specific procedures/assessments and their timing, with further details located in Section 8):<br>**Screening Period (Day -30 to Day -1)**<br>After providing informed consent, eligibility assessments will include review of demographics, medical history, prior/concomitant medications, full physical examination, vital sign assessments, body weight/height/body mass index (BMI) measurements, urine pregnancy test (for female subjects of childbearing potential), urine drug and alcohol breath test screen, safety laboratory tests (i.e., hematology, serum chemistry, coagulation, and urinalysis), hepatitis and human immunodeficiency virus (HIV) screen, and plasma, urine and saliva cortisol collection.<br>The Structured Clinical Interview for DSM-5 – Clinical Trials Version (SCID-5-CT) will be employed for the determination of MDD and will be performed by a qualified, trained, validated healthcare professional.<br>Qualified staff will also complete the Montgomery-Åsberg Depression Rating Scale (MADRS), Hamilton Anxiety Rating Scale (HAM-A), Clinical Global Impression-Severity (CGI-S), Columbia-Suicide Severity Rating Scale (C-SSRS), and Childhood Trauma Questionnaire (CTQ).<br>Ophthalmic exams, 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 classification.

Study site personnel will dispense equipment for 12-hour urine collection to measure cortisol levels.

A call between the subject and the qualified central rater will be scheduled to allow the rater to administer the Massachusetts General Hospital Antidepressant Treatment Response 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, to ensure only patients with inadequate response (<50% improvement) are included. The central rater will also complete the MADRS assessment and SAFER inventory (State vs trait, Assessability, Face and Ecological validity, Rule of 3Ps [pervasive, persistent, and pathological]) for confirmation of eligibility (diagnosis, severity, and treatment history). Results will be communicated to the site prior to Day 1.

No changes to medications are allowed during the screening period, and screening can be less than 30 days if all other criteria are met.

**Treatment Period (Days 1, 8, 15, 29, 43, 56)**

At the Day 1 visit only, subjects confirmed as eligible will receive a 50 mg dose of study drug prior to eating. Subjects who cannot tolerate 50 mg will receive 40 mg for the remainder of the study. Subjects who cannot tolerate 40 mg will be terminated from the study. Before releasing subjects, study site personnel will dispense and train subjects on a dosing diary, the wearable watch-like device and mobile application. ANC-501 will be dispensed to subjects with instructions to take the medication every morning at least 1 hour prior to eating breakfast.

During visits, safety procedures/assessments and administration of assessments/scales (including from Day 8 onward, the Clinical Global Impression-Improvement [CGI-I] and the Patient Global Impression of Improvement [PGI-I]) will be conducted. During specific visits, PK blood samples will be collected, as well as blood samples for future exploratory biomarker and genetic analysis. Concomitant medications and dosing diary will be reviewed, the dosing diary will be returned to the subject, and adverse events (AEs) recorded. At all visits after Day 1, recording/accounting for any returned/unused ANC-501 will be performed and ANC-501 will be dispensed to subjects with a reminder to take the medication every morning at least 1 hour prior to eating breakfast.

**At home activities**

Subjects will take study medication every morning (at least 1 hour prior to eating breakfast), make entries into the dosing diary, and wear their watch-like device throughout the day and night except when charging. Subjects will also complete EMA orienting questions, the Hamilton Depression Rating Scale (6-item) (HAMD-6) and the General Anxiety Disorder (7-item) (GAD-7) twice a day for three days per week, and the Short Form-Health Survey (36-item) (SF-36) weekly using the EMA Wellness smartphone/tablet application.

At the Day 43 visit, study site personnel will dispense equipment and retrain subjects on the 12-hour 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.

At the Day 56 visit, subjects will take their last study medication and visit the site to return their 12-hour urine collection, all remaining medication, the dosing diary, and wearable watch-like devices. Study procedures/assessments will be conducted, including an ophthalmic exam and slit lamp imaging of the lenses of both eyes. Images will be sent to a

central reader for assessment using the LOCS III. Collection of plasma and saliva cortisol samples will also be conducted.

The same rater should perform the CGI-S, CGI-I, and MADRS assessments throughout the study on a given subject when possible.

**Follow-up Period (Day 70 and Day 112)**

Subjects will return to the site for a follow-up visit on Day 70 and Day 112. Study procedures/assessments will be conducted, including an ophthalmic exam and slit lamp imaging of the lenses of both eyes. Images will be sent to a central reader for assessment using the LOCS III. Concomitant medications will be reviewed, and AEs recorded.

**Objectives and Endpoints:**

| Objectives | Endpoints |
|---|---|
| **Primary** | |
| • To evaluate the effect of treatment with ANC-501 capsules on depressive symptoms in subjects with major depressive disorder (MDD) | • Change from baseline (Day 1) to Day 56 in Montgomery-Åsberg Depression Rating Scale (MADRS) total score |
| • To evaluate the safety and tolerability of ANC-501 capsules in subjects with MDD | • Safety and tolerability as assessed by:<br>o Frequency and severity of adverse events (AEs)<br>o Vital signs<br>o Body weight<br>o Electrocardiograms (ECGs)<br>o Clinical laboratory tests<br>o Physical examination<br>o Lens Opacities Classification System III (LOCS III)<br>o Emergent behavior using Columbia-Suicide Severity Rating Scale (C-SSRS) |
| **Secondary** | |
| • To evaluate effect of ANC-501 at all time points and percent reduction in total symptom scores compared with baseline. | • Change from baseline (Day 1) in the MADRS total score at all time points<br>• Percentage of MADRS responders (≥50% reduction in total score)<br>• Percentage of MADRS remitters (total score ≤10)<br>• Change in Hamilton Anxiety Scale (HAM-A) total score from Baseline to Day 56<br>• Change in Clinical Global Impression-Severity (CGI-S) score from Baseline to Day 56<br>• Short Form-Health Survey (36-item) (SF-36) total score and subscale scores from Baseline to Day 56 and at all time points<br>• Percentage of Clinical Global Impression-Improvement (CGI-I) improvers (“Very much improved” or “Much improved”) |

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| | • To assess the PK profile of ANC-501 Capsules in plasma using a sparse sampling model<br>• Patient Global Impression of Improvement (PGI-I) score at Day 56<br>• Plasma concentrations of ANC-501 |

| Objectives | Endpoints |
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| **Exploratory** | |
| • To evaluate changes in 12-hour urine, plasma, and saliva cortisol<br>• To evaluate changes in biometrics<br>• To evaluate biological samples as potential biomarkers and metabolites<br>• Patient reported changes in Depression and Anxiety symptoms | • Changes in 12-hour urine, plasma, and saliva cortisol from baseline to Day 56<br>• Changes in biometrics using the wearable watch-like device<br>• Baseline levels and/or changes in exploratory biomarkers through Day 56<br>• Changes in Hamilton Depression Rating Scale (6-item) (HAMD-6) scores from baseline across all time points using ePRO.<br>• Changes in General Anxiety Disorder (7-item) (GAD-7) from baseline across all time points using ePRO. |

**Population:**

**Inclusion Criteria**

Patients who meet ALL the following inclusion criteria will be eligible to participate in the study:

1. Demonstrate understanding of the study procedures, restrictions, and willingness to participate as evidenced by voluntarily signing and dating the written informed consent form.
2. Adult male or female between 18 and 65 years of age, inclusive.
3. Good physical health and has no clinically significant findings, as determined by the Investigator, on physical examination, 12-lead electrocardiogram (ECG), vitals, or clinical laboratory tests.
4. Has no clinically significant findings on the ophthalmic examination including, Best Corrected Visual Acuity (BCVA) worse than 20/30 or, in the opinion of the ophthalmologist or optometrist, any cataract that may become clinically significant and/or need surgical intervention during the course of the trial.
5. Able and willing to complete all study requirements.
6. Diagnosis of current episode of major depressive disorder (MDD) at least 8 weeks prior to screening, confirmed by Structured Clinical Interview for DSM-5 – Clinical Trials Version (SCID-5-CT).
7. Have not responded to their current antidepressant therapy or to dose adjustment/treatment changes following a loss of response to their current antidepressant therapy.

8. Complete the SAFER (State vs trait, Assessability, Face and Ecological validity, Rule of 3Ps [pervasive, persistent, and pathological]) interview, administered via teleconference by a Massachusetts General Hospital (MGH) psychiatrist or psychologist, and receive a passing score which confirms a diagnosis of MDD, and a minimum Montgomery-Åsberg Depression Rating Scale (MADRS) total score of 26. The SAFER interview will also confirm the subject's treatment history and response to antidepressant therapies (ADTs) through the administration of the MGH Antidepressant Treatment Response Questionnaire (ATRQ).
9. Receiving a stable dose of the same antidepressant (selective serotonin reuptake inhibitor [SSRI], serotonin and norepinephrine reuptake inhibitor [SNRI], bupropion or trazodone monotherapy) for the current episode for at least 6 weeks of continuous treatment, which can include some or all of the screening period, with 4 weeks on a stable dose prior to day 1 and has an inadequate response (<50% improvement) using the MGH ATRQ.
10. Willing to maintain stable dose and delay start of other antidepressant or antianxiety medications and any new pharmacotherapy regimens, including benzodiazepine anxiolytics, during the screening and treatment periods.
11. Has a MADRS total score of ≥26 at screening and Day 1 (prior to dosing).
12. Has a 12-hour urine cortisol level >22.7 nmol/L (greater than or equal to 8.3 mcg/L).
13. Body mass index (BMI) ≥18 and ≤38 kg/m$^2$ at screening using the formula: $weight\ (kg)/height\ (m)^2$.
14. Agrees to practice an acceptable method of highly effective birth control at screening, throughout study participation, and for 90 days after the last dose of study drug. Highly effective methods of birth control include sexual abstinence (for males and females); vasectomy; or a condom with spermicide in combination with a highly effective female partner's method (for males); and hormonal methods of contraception (i.e., established use of oral, implantable, injectable, or transdermal hormones); placement of an intrauterine device; placement of an intrauterine system; and mechanical/barrier method of contraception (i.e., condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [foam, gel, film, cream, or suppository]) (for females).

**Exclusion Criteria**

Patients who meet any of the following exclusion criteria will be excluded from participation in the study:

1. History of suicide attempt in the past 2 years or within current MDD episode
2. Active suicidal ideation with plan or a "yes" response to items 4 or 5 on the C-SSRS in the past 24 months is exclusionary as assessed at Screening and Baseline (Day 1).
3. Inadequate response to >2 prior ADTs (not including current antidepressant) of at least 6 weeks duration each for the episode current at screening.
4. Recent history or active clinically significant manifestations of metabolic, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, musculoskeletal, dermatological, urogenital, neurological, ophthalmic (especially cataracts), or ears, nose, and throat (ENT) disorders, or any other acute or chronic condition that, in the Investigator's opinion, would limit the subject's ability to complete or participate in this clinical study.
5. Based on an ophthalmic examination performed at Screening, patient must not have any of the following:
    - Nuclear opalescence (NO) with a LOCSIII classification > 3.0 in either eye
    - Nuclear color (NC) with a LOCSIII classification > 3.5 in either eye
    - Cortical lens opacities (C) with a LOCSIII classification > 2.0 in either eye

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| | • Posterior subcapsular lens opacities (P) with a LOCSIII classification > 0.3 in either<br>6. History or presence of intellectual disability, pervasive developmental disorder, cognitive disorder, neurodegenerative disorder (e.g., Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease), or brain injury.<br>7. Receipt of electroconvulsive therapy (ECT) within 12 months of screening, lifetime receipt of more than one course of ECT, or plans to receive ECT during the study.<br>8. Receipt of repetitive transcranial magnetic stimulation (rTMS) within 12 months of screening or planned to receive rTMS during the study.<br>9. Plans to initiate or terminate cognitive or behavioral psychotherapy or alter the frequency of ongoing therapy during this study.<br>10. Works night shifts or needs to work night shifts during the study.<br>11. Females who are pregnant, intend to become pregnant (within 90 days of the last dose of ANC-501), are breastfeeding, or have a positive pregnancy test at screening or on Day 1 prior to study drug administration.<br>12. Known allergy to ANC-501, or related compounds.<br>13. Detectable hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab), or human immunodeficiency virus (HIV) antibody at screening.<br>14. Active psychosis per Investigator assessment.<br>15. Medical history of seizures (does not include isolated febrile seizures of childhood).<br>16. Medical history of bipolar disorder, schizophrenia, and/or schizoaffective disorder.<br>17. History of alcohol or substance use disorder in the 12 months prior to screening.<br>18. Positive alcohol breath test.<br>19. Positive urine drug screen for hallucinogens, psychoactive inhalants, stimulants, opioids, barbiturates, non-prescribed anxiolytics or other non-prescribed sedatives. Does not include positive test for cannabinoids, tobacco or caffeine, unless the subject meets criteria for substance use disorder for these substances.<br>20. Exposure to another investigational medication or device within 30 days or 5 half-lives (whichever is longer) prior to screening.<br>21. Has been previously treated in this study or randomized or treated in any other study employing ANC-501 (i.e., subject may not have received study drug and then re-enrolled).<br>22. Administration of drugs to treat psychiatric or neurologic conditions that have not been taken at a stable dose for at least 4 weeks prior to day 1.<br>23. Need/plan to take moderate to strong inhibitors and/or inducers of cytochrome P450 (CYP)3A4 (food, beverages, medications, and supplements).<br>24. Any condition not identified in the protocol that in the opinion of the Investigator would confound the evaluation and interpretation of the study data or may put the subject at risk. |
| **Dosage Forms and Route of Administration:** | 50 mg ANC-501 Capsules (5 × 10 mg capsules), administered orally. |
| **Dose Adjustment for Safety or Tolerability Reasons:** | During the Open-Label Treatment Period, subjects may receive study drug as long as there are no dose-limiting safety/tolerability concerns as determined by the Investigator. Subjects who experience moderate or severe AEs while receiving the 50 mg dose of study drug which, according to the clinical judgement of the Investigator, are related to study drug, |

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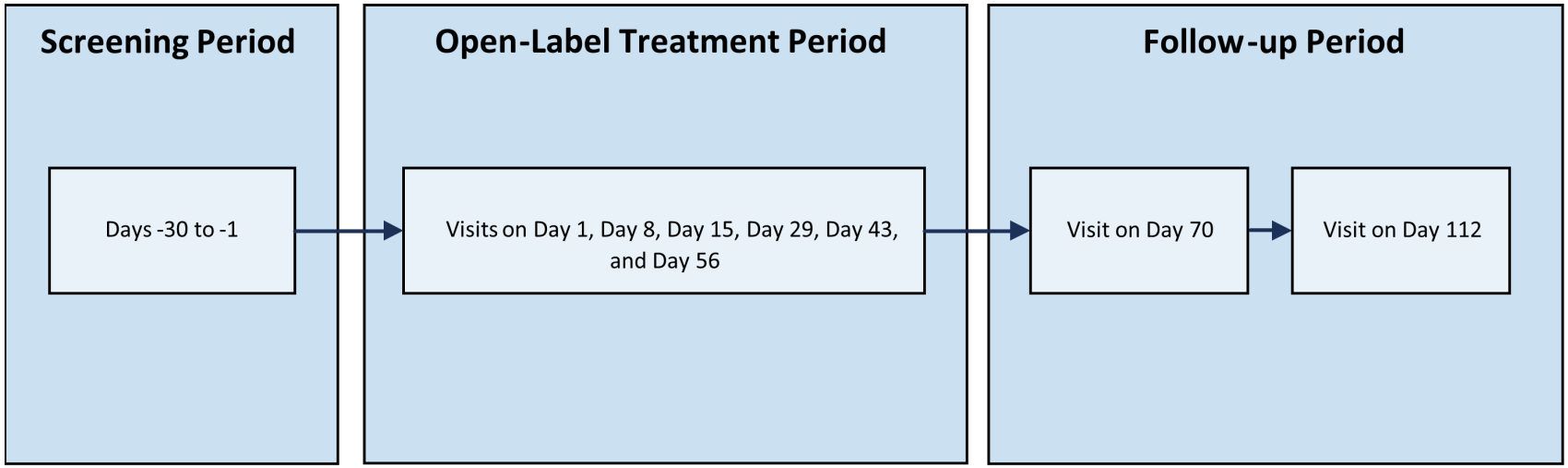
|---|---|
| | may receive 40 mg for the remainder of the Open-Label Treatment Period if the AEs are intolerable. Subjects who experience moderate or severe related AEs while receiving the 40 mg dose of study drug might not be able to continue receiving study drug, based on the evaluation and clinical judgment of the Investigator, and may be discontinued from further treatment. |
| Statistical Methods: | **General**<br>Continuous endpoints will be summarized with n, mean, standard deviation, median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively. For categorical endpoints, descriptive summaries will include counts and percentages.<br><br>**Analysis Populations and Methods**<br>The Safety Population (defined as all subjects administered study drug) will be used to provide descriptive summaries of safety data. Treatment-emergent adverse events (TEAEs) will be classified by type, incidence, severity, and causality. The overall incidence of AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT). Data for vital signs, clinical laboratory tests, ECG, physical examinations, and concomitant medication usage will also be summarized. Out-of-range safety endpoints may be categorized as low or high, where applicable.<br><br>Suicidality data collected using the C-SSRS at baseline and at each visit during the active Open-Label Treatment Period will be listed for all subjects. The C-SSRS listings will include behavior type and/or category for suicidal ideation and suicidal behavior of the C-SSRS.<br><br>Slit lamp imaging performed during the study showing a change of greater than or equal to 0.5 on the LOCS III scale in either eye or any new incidence of cataract will be considered adverse events and events of special interest.<br><br>The Efficacy Population (defined as all subjects in the Safety Population who complete at least one day of dosing of study drug and have at least one post-baseline efficacy evaluation) will be used to analyze efficacy data. Efficacy data will be analyzed using appropriate descriptive statistics and pre-specified statistical methods, as well as other data presentation methods where applicable; subject listings will be provided for all efficacy data. Efficacy data will be summarized descriptively. |
| **Sample Size Determination:** | Up to 20 subjects will be enrolled. The sample size was selected based on clinical and not statistical considerations. |

## 1.2. Study Schema



## 1.3. 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] | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |
| GAD-7 [n] | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |

| 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)** |
| SF-36 [n] | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |
| EMAW Orienting Questions [n] | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |
| 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] | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |
| Site download of Wearable data | | | X | X | X | X | X | | |
| ANC-501 dosing [r] | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |
| ANC-501 Dosing Diary dispensed and reviewed by site | | ◄——— | ——— | ——— | ——— | ——— | ———► | | |
| Dispense study medication | | X | X | X | X | X | | | |
| Adverse Events [s] | X | ◄——— | ——— | ——— | ——— | ——— | ——— | ——— | ———► |
| Prior/ConcomitantMedications [t] | 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 immunodeficiencyvirus; 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

| 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) |

Anxiety Disorder (7-item); SF-36 = Short Form-Health Survey (36-item).

**Schedule of Events (continued)**

Refer to Section 8 for additional details regarding assessments/procedures.

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. Refer to Table 4 for details.
g. Urine toxicology for selected drugs of abuse (barbiturates, amphetamines, cocaine, opioids,) 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 sparce PK sampling will be collected pre-dose and between 0.75 and 2 hours post-dose at the Day 1 visit and between 0.75 and 2 hours post-dose, whenever possible, at other indicated visits (with the date and time of the 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 12-hour 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 112 visit. 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.

# 2. INTRODUCTION

## 2.1. Major Depressive Disorder

Depression is ranked as the leading cause of disability worldwide by the World Health Organization.[1] Major depressive disorder (MDD) is a psychiatric disorder that is characterized by the occurrence of one or more major depressive episodes, along with an absence of any history of manic, mixed, or hypomanic episodes. It is a serious, often recurrent medical condition, which is associated with a 15.9% lifetime risk of suicide attempt.[2] Results of the World Mental Health Survey Initiative (2011) found that the average lifetime incidence of Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) major depressive episodes was 14.6%, with a 12-month prevalence of 5.5% in higher income counties.[3] Similarly, the National Comorbidity Survey-Replication[4] found the incidence of depression to be 6.6% over 12-months, with a 16.2% lifetime incidence. In addition to the high mortality rate due to suicide, depressed patients are more likely to develop coronary artery disease and type 2 diabetes.[5]

Current treatments available for depression include psychotherapy (for mild depression), antidepressant medications, a combination of medication and psychotherapy (for mild-to-moderate depression), and electroconvulsive therapy (moderate-to-severe depression). Despite the availability of numerous antidepressant drugs (e.g., monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors [SSRIs], serotonin norepinephrine reuptake inhibitors [SNRIs]), there remains an unmet need for the pharmacological treatment of MDD. With currently available medications, alleviation of depressive symptoms is often not observed until several weeks of treatment have been received. Even after an adequate duration of treatment, the most commonly used treatments today (e.g., SSRIs and SNRIs) only result in approximately 35% to 45% of patients achieving clinical remission.[6] Approximately 30% of patients are resistant to a series of treatments according to STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study.[7] Moreover, side effects are still a concern, even with newer medications. Thus, there is a clear need for efficacious and well-tolerated agents that can effectively treat depression and prevent recurrent episodes of depression.

Stress has also been hypothesized to be a pivotal factor in the pathophysiology of depression, specifically by way of the hypothalamus-pituitary-adrenal (HPA) axis, which appears to be a prominent mechanism by which the brain reacts to both acute and chronic stress through feedback loops involving several neuroendocrine hormones, including adrenocorticotropic hormone (ACTH) and cortisol. In addition, both corticotropin-releasing factor (CRF) and arginine vasopressin (AVP), both of which are produced in the paraventricular nucleus of the hypothalamus[8,9] are considered primary factors in the regulation of HPA axis activity. Receptor subtypes for these neuropeptides, which may be involved in the regulation of HPA axis activity, have attracted much attention as potential targets for the treatment of depression and anxiety.

ANC-501 is an investigational new drug with antagonistic activity of the vasopressin receptor 1b ($V_{1b}$ receptor), which plays a role in the modulation of stress and mood. Based on nonclinical studies conducted, ANC-501 appears to be a promising candidate for clinical development with a novel mode of action that may benefit MDD patients.

## 2.2. Mechanism of Action of Vasopressin Receptor 1b (V1b Receptor)

Arginine vasopressin, a cyclic nonapeptide, together with CRF, are principal factors in the regulation of ACTH release from the anterior pituitary,[8] and have been reported to play an important role in mood regulation. Arginine vasopressin is synthesized in the parvocellular division of the paraventricular nucleus of the hypothalamus, which is linked to activation of the HPA axis, and upon stress exposure, AVP is released from the median eminence into the pituitary portal circulation, where it strongly potentiates the effects of CRF on the release of ACTH. Clinically, the plasma AVP levels are elevated in patients with major depression, compared with healthy controls.[10,11] In previous studies, subjects with melancholic-type or anxious-retarded depression exhibited elevated plasma AVP levels. Plasma AVP levels are also reportedly positively correlated with cortisol levels during depression, particularly in suicide victims.[12] Moreover, the quantities of AVP-immunoreactive neurons and AVP messenger RNA (mRNA) in the paraventricular nucleus are increased during depression,[13,14] particularly in subjects in the melancholic subgroup,[13] and pituitary AVP responsibility in the regulation of the HPA axis is increased.[15] The involvement of the AVP system in anxiety disorders has also been suggested.

AVP exerts its effects via three receptor subtypes: $V_{1a}$, $V_{1b,}$ and $V_2$. These subtypes are G-protein coupled receptors.[16] Among them, the $V_{1b}$ receptor is mainly expressed in the anterior pituitary and is thought to be involved in the regulation of HPA axis activity and emotional processes.[17] Indeed, SSR149415, a non-peptide $V_{1b}$ receptor antagonist with high affinity and potent antagonistic activity for the $V_{1b}$ receptor,[18] has been reported to attenuate increases in plasma ACTH induced by corticotropin-releasing hormone (CRH)+AVP,[19] and to exhibit antidepressant- and anxiolytic-like activities in a variety of rodent models of depression and anxiety, with more pronounced effects in models involving stressful situations.[18,19,20] Therefore, blockade of $V_{1b}$ receptor may provide a novel approach to treating stress-related disorders such as depression and anxiety disorders.

ANC-501 is a potent and selective antagonist of $V_{1b}$ receptor in both in vitro and in vivo studies and has been shown to possess antidepressant activity in several animal models of depression, including one which mimics severely impaired HPA axis function.

## 2.3. Rationale for Targeting V1b Receptor in Treatment of Depression

A series of in vitro studies was conducted to define the affinity of ANC-501 to the $V_{1b}$ receptor, as well as a battery of in vivo studies to characterize the efficacy profile in MDD and its anxiolytic-like potential in animal models.

During in vivo rat studies, the immobility time in the forced swimming test and the hyperemotionality in a rat olfactory bulbectomy model, both of which are indicative of depressive states, were each significantly reduced by treatment with ANC-501. In the olfactory bulbectomy model, ANC-501 exhibited efficacy following chronic treatment for 14 days, as did fluvoxamine. In addition, ANC-501 significantly improved depressive-like behavior induced by repeated corticosterone injection, a depression model which has been reported to be resistant to fluvoxamine and imipramine,[21] indicating that ANC-501 may be effective for patients with severely impaired HPA axis function. In an anxiety model (social interaction behavior in rats), ANC-501 demonstrated anxiolytic activity as well. This preclinical evidence further suggests

that a $V_{1b}$ receptor antagonist may be useful in the treatment of MDD with co-morbid anxiety disorders.

### 2.3.1. Evaluation in Humans

Three Phase 1 studies (a single ascending dose [SAD] study [TS121-US101], a multiple ascending dose [MAD] study [TS121-US102], and a PET receptor occupancy study [TS121-US103]) and one Phase 2 study (TS121-US201) have been conducted in the United States.

Pharmacokinetic (PK) studies indicate that following single and multiple oral administration of ANC-501, increases in plasma ANC-501 peak and total exposures (maximum plasma concentration [$C_{max}$] and area under the concentration-time curves [AUCs]) were observed with increases in dose. ANC-501 reached $C_{max}$ within 4 hours after dosing and declined with apparent half-life ($t_{½}$) of approximately 20 hours. No food effect was observed on AUCs of ANC-501, while $C_{max}$ was reduced under fed conditions. In the MAD study, the plasma trough concentrations of ANC-501 appeared to reach steady state by 5 days after dosing and the exposures accumulated to approximately 2 times. Low renal clearance ($CL_r$) suggested minimal contribution of the urinary excretion of ANC-501. Though two metabolites (M5 and M9) were found in plasma, the unchanged form was proposed as a predominant component. In addition, PET study revealed the relationship between plasma exposure of ANC-501 and $V_{1b}$ receptor occupancy in the pituitary.

Overall, 78 healthy and 32 adult subjects with MDD were administered ANC-501 to date. In the Phase 2 study, there was no statistically significant difference observed in the primary endpoint (Montgomery-Åsberg Depression Rating Scale [MADRS] Change from Baseline to Week 6positive trends were observed for the scores of depression scales in ANC-501 treated groups (10 mg and 50 mg) versus placebo. The lack of statistical significance may be attributable to the small number of subjects enrolled.

**Safety Findings in Clinical Studies**

A top dose of 50 mg for 6 weeks was deemed safe and well tolerated. There were no deaths or serious adverse events (SAEs) reported in any of the studies. No safety trends were observed in clinical laboratory, electrocardiogram (ECG), vital signs, physical examinations, or cardiac telemetry. In consideration of the nonclinical findings at the time of the MAD study, comprehensive eye examinations were performed. In Phase 2a study, eye examinations with a standardized lens opacity grading scale (Lens Opacities Classification System III [LOCS III]), were conducted to evaluate this risk in the study. Increase from baseline in the LOCS III grade of ≥0.5 (Nuclear Opalescence [NO]), ≥0.8 (Cortical) or ≥0.5 (Posterior Subcapsular), or changes considered to be clinically significant as per the ophthalmologist or optometrist, were defined in the protocol as events of clinical significance. Two events of lenticular opacities were reported in Phase 2a study TS121-US201 in patients dosed with ANC-501 in the 50 mg dose group. One of 2 patients had an increase of NO score from baseline at Visit 6 in the left and right eye of 0.9 and 0.8 points, respectively, which was reportable as an event of clinical significance per protocol. Per the Investigator, the difference from the initial LOCS III assessment did not reflect actual change in lens opacity but rather intra-observer variability. The Investigator rated the event as mild in intensity and considered it to be not related to the study drug. The second patient had an increase of NO score from baseline at visit 6 in the unilateral (right) eye of 0.7 points, which was

reportable as an event of clinical significance per protocol. The Investigator rated this event as mild in intensity and considered it to be possibly related to the study drug. The nonclinical studies demonstrated changes in the lens in a select number of study animals that were noted either in the posterior pole of the lens or affecting cortical lens fibers. In the clinical program, neither of the 2 patients with reports of progression of NO that met the level of clinical significance according to the study protocol were noted to have posterior polar or cortical changes. Further, the LOCS III scoring has a standardized range for NO from 1–6, with 1 being the lowest standard image available for comparison. The scores reported for one patient were 0.3 at baseline and increasing to 1.0 at the time of the event. The initial score is below the level of the first comparative image on the LOCS scale template, and thus inter-visit variability could account for the noted difference. The second patient had changes noted in both eyes, with 0.7 and 0.6 noted at baseline in the right and left eye, respectively and 1.5 noted at the time of the reported event in both eyes. Given the relative subjective nature of lens grading, and the fact that at initial examination the scoring was below the lowest standard image standard, intra-grader variability likely accounted for these changes, as noted by the Investigator for the second patient.

In the Phase 2 study, there was no statistically significant difference observed in the primary endpoint (Montgomery-Åsberg Depression Rating Scale [MADRS] Change from Baseline to Week 6), although positive trends were observed for the scores of depression scales in ANC-501 treated groups (10 mg and 50 mg) versus placebo. The lack of statistical significance may be attributable to the small number of subjects enrolled.

In conclusion, these results suggest that ANC-501 (ANC-501) may be useful for the treatment of patients with MDD.

## 2.4. Risk/Benefit

Three Phase 1 studies, a SAD study (TS121-US101), a MAD study (TS121-US102) and a PET receptor occupancy study (TS121-US103), and one Phase 2 study in MDD (TS121-US201) have been conducted in the US. Overall, 78 healthy male and female adult subjects and 32 adult subjects with MDD were administered ANC-501 to date. A top dose of 50 mg for 6 weeks was deemed safe and well-tolerated. There were no deaths or SAEs reported in any of the studies. All events across trials were either mild or moderate in intensity. Moreover, no safety trends were observed in clinical laboratory, ECG, vital signs, physical examinations, or cardiac telemetry. In consideration of the nonclinical findings (rat) at the time of the MAD study, comprehensive prospective slit lamp examinations were performed in the Phase 2 trial. Two events of lenticular opacity were reported as clinically significant by prespecified criteria on the LOCS III rating scale in 2 subjects (1 event each) in the 50 mg group. These events were rated as mild in intensity, were not accompanied by any changes in vision, and did not require treatment. One was judged as “possibly related” and the other was judged as “not related” to ANC 501. While Study TS121-US201 was not enrolled to completion, patients treated with ANC-501 in combination with their underlying antidepressant showed numerical improvement compared to placebo. Consistent with the hypothesized mechanism of action of ANC-501, those patients with elevated baseline 12-hour urine cortisol concentration showed substantially larger separation from placebo at the end of 6 weeks in both dose groups tested. In the case of the high cortisol 50 mg sub-group, these differences continued to increase throughout the follow-up period.

Approximately 30% of patients are resistant to a series of treatments according to the STAR*D study. There is little data-driven guidance on next steps to treat MDD patients in the event of a first-line failure. Importantly, side effects are a major concern for patients particularly the use of antipsychotic agents as adjunctive therapies and those used to treat treatment-resistant depression (TRD). Thus, there is a clear need for new, efficacious, well-tolerated agents to both treat depression episodes and prevent recurrent episodes of depression.

The safety profile of ANC-501, based on the previously conducted trials and preliminary clinical data, suggest that ANC-501 may also be well tolerated in patients and efficacious as an adjunctive treatment in select MDD patients. The current significant unmet need in the treatment of depression, remaining as a number one cause of disability worldwide, justifies a favorable risk-benefit ratio, and investigation of ANC-501 in MDD.

# 3. OBJECTIVES AND ENDPOINTS

| Objectives | Endpoints |
|---|---|
| **Primary** | |
| • To evaluate the effect of treatment with ANC-501 capsules on depressive symptoms in subjects with major depressive disorder (MDD) | • Change from baseline (Day 1) to Day 56 in Montgomery-Åsberg Depression Rating Scale (MADRS) total score |
| • To evaluate the safety and tolerability of ANC-501 capsules in subjects with MDD | • Safety and tolerability as assessed by:<br>o Frequency and severity of adverse events (AEs)<br>o Vital signs<br>o Body weight<br>o Electrocardiograms (ECGs)<br>o Clinical laboratory tests<br>o Physical examination<br>o Lens Opacities Classification System III (LOCS III)<br>o Emergent Suicidal behavior using Columbia-Suicide Severity Rating Scale (C-SSRS) |
| **Secondary** | |
| • To evaluate effect of ANC-501 at all time points and percent reduction in total symptom scores compared with baseline. | • Change from baseline (Day 1) in the MADRS total score at all time points<br>• Percentage of MADRS responders (≥50% reduction in total score)<br>• Percentage of MADRS remitters (total score ≤10)<br>• Change in Hamilton Anxiety Scale (HAM-A) total score from Baseline to Day 56<br>• Change in Clinical Global Impression-Severity (CGI-S) score from Baseline to Day 56<br>• Short Form-Health Survey (36-item) (SF-36) total score and subscale scores from Baseline to Day 56 and at all time points<br>• Percentage of Clinical Global Impression-Improvement (CGI-I) improvers (“Very much improved” or “Much improved”)<br>• Patient Global Impression of Improvement (PGI-I) score at Day 56 |
| • To assess the pharmacokinetic (PK) profile of ANC-501 Capsules in plasma using a sparse sampling model | • Plasma concentrations of ANC-501 |
| **Exploratory** | |
| • To evaluate changes in 12-hour urine, plasma, and saliva cortisol | • Changes in 12-hour urine, plasma, and saliva cortisol from baseline to Day 56 |
| • To evaluate changes in biometrics | • Changes in biometrics using the wearable watch-like device |
| • To evaluate biological samples as potential biomarkers and metabolites | • Baseline levels and/or changes in exploratory biomarkers through Day 56 |
| • Patient reported changes in Depression and Anxiety symptoms | • Changes in Hamilton Depression Rating Scale (6-item) (HAMD-6) scores from baseline across all time points using ePRO<br>• Changes in General Anxiety Disorder (7-item) (GAD-7) from baseline across all time points using ePRO |

# 4. STUDY DESIGN

## 4.1. Overall Design

This is a single-arm, open-label Phase 2 study to assess the safety, tolerability, pharmacokinetics (PK), and efficacy of ANC-501 oral capsules as adjunctive treatment in subjects diagnosed with major depressive disorder (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 and blood samples collected for PK analysis of ANC-501and 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 × 10 mg capsules) orally once daily at least 1 hour prior to the morning meal 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 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).

The following is an overview of study procedures/assessments (refer to the Schedule of Events [Section 1.3] for specific procedures/assessments and their timing, with further details located in Section 8):

**Screening Period (Day -30 to Day -1)**

After providing informed consent, eligibility assessments will include review of demographics, medical history, prior/concomitant medications, full physical examination, vital sign assessments, body weight/height/body mass index (BMI) measurements, urine pregnancy test (for female subjects of childbearing potential), urine drug and alcohol breath test screen, safety laboratory tests (i.e., hematology, serum chemistry, coagulation, and urinalysis), hepatitis and human immunodeficiency virus (HIV) screen, and plasma and saliva cortisol sample collection.

The Structured Clinical Interview for DSM-5 – Clinical Trials Version (SCID-5-CT) will be employed for the determination of MDD and will be performed by a qualified, trained, validated healthcare professional.

Qualified staff will also complete the Montgomery-Åsberg Depression Rating Scale (MADRS), Hamilton Anxiety Rating Scale (HAM-A), Clinical Global Impression-Severity (CGI-S), Columbia-Suicide Severity Rating Scale (C-SSRS), and Childhood Trauma Questionnaire (CTQ).

Ophthalmic exams, 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 classification.

Study site personnel will dispense equipment for 12-hour urine collection to measure cortisol levels.

A call between the subject and the qualified central rater will be scheduled to allow the rater to administer the Massachusetts General Hospital Antidepressant Treatment Response 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, to ensure only patients with inadequate response (<50% improvement) are included. The central rater will also complete the MADRS assessment and SAFER inventory (State vs trait, Assessability, Face and Ecological validity, Rule of 3Ps [pervasive, persistent, and pathological]) for confirmation of eligibility (diagnosis, severity, and treatment history). Results will be communicated to the site prior to Day 1.

No changes to medications are allowed during the screening period, and screening can be less than 30 days if all other criteria are met.

**Open-Label Treatment Period (Days 1, 8, 15, 29, 43, 56)**

At the Day 1 visit only, subjects confirmed as eligible will receive a 50 mg dose of study drug prior to eating. Subjects who cannot tolerate 50 mg will receive 40 mg for the remainder of the study. Subjects who cannot tolerate 40 mg will be terminated from the study. Before releasing subjects, study site personnel will dispense and train subjects on a dosing diary, the wearable watch-like device and mobile application. ANC-501 will be dispensed to subjects with instructions to take the medication every morning at least 1 hour prior to eating breakfast.

During visits, safety procedures/assessments and administration of assessments/scales (including from Day 8 onward, the Clinical Global Impression-Improvement [CGI-I] and the Patient Global Impression of Improvement [PGI-I]) will be conducted. During specific visits, PK blood samples will be collected, as well as blood samples for future exploratory biomarker and genetic analysis. Concomitant medications and dosing diary will be reviewed, the dosing diary will be returned to the subject, and adverse events (AEs) recorded. At all visits after Day 1, recording/accounting for any returned/unused ANC-501 will be performed and ANC-501 will be dispensed to subjects with a reminder to take the medication every morning at least 1 hour prior to eating breakfast.

**At home activities**

Subjects will take study medication every morning (at least 1 hour prior to eating breakfast), make entries into the dosing diary, and wear their wearable watch-like device throughout the day and night except when charging. Subjects will also complete EMA orienting questions, the Hamilton Depression Rating Scale (6-item) (HAMD-6) and the General Anxiety Disorder (7-item) (GAD-7) twice a day for three days per week, and the Short Form-Health Survey (36-item) (SF-36) weekly in the EMA Wellness smartphone/tablet application.

At the Day 43 visit, study site personnel will dispense equipment and retrain subjects on the 12-hour 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.

At the Day 56 visit, subjects will take their last study medication and visit the site to return their 12-hour urine collection, all remaining medication, the dosing diary, and wearable watches. Study procedures/assessments will be conducted, including an ophthalmic exam and slit lamp imaging of the lenses of both eyes. Images will be sent to a central reader for assessment using the LOCS III. Collection of plasma and saliva cortisol samples will also be conducted.

The same rater should perform the CGI-S, CGI-I, and MADRS assessments throughout the study on a given subject when possible.

**Follow-up Period (Day 70 and Day 112)**

Subjects will return to the site for a follow-up visit on Day 70 and Day 112. Study procedures/assessments will be conducted, including an ophthalmic exam and slit lamp imaging of the lenses of both eyes. Images will be sent to a central reader for assessment using the LOCS III. Concomitant medications will be reviewed, and Aes recorded.

## 4.2. Justification for Dose

ANC-501 dose selection was based on accumulated experience from preclinical studies and previous clinical trials. The SAD study (TS121-US101) and MAD study (TS121-US102) provide safety and tolerability support for the current doses. A PET receptor occupancy study (TS121-US103) provides rationale for doses of 10 mg and 50 mg orally once daily. From results of the PET receptor occupancy study, 10 mg and 50 mg doses were modeled to provide approximately 55% and 75% receptor occupancy, respectively. In the Phase 2 study (TS121-US201), the 50 mg dose suggested efficacy in the sub-population of subjects with higher cortisol levels.

## 4.3. End of Study Definition

End of study is defined as the date when the last subject has completed the final scheduled procedure shown in the Schedule of Events (Section 1.3).

# 5. STUDY POPULATION

Subject eligibility must be verified prior to enrollment. Prospective approval of protocol deviations to recruitment and enrollment criteria (also known as protocol waivers or exemptions) is not permitted.

## 5.1. Inclusion Criteria

Patients who meet ALL the following inclusion criteria will be eligible to participate in the study:

1. Demonstrate understanding of the study procedures, restrictions, and willingness to participate as evidenced by voluntarily signing and dating the written informed consent form.
2. Adult male or female between 18 and 65 years of age, inclusive.
3. Good physical health and has no clinically significant findings, as determined by the Investigator, on physical examination, 12-lead electrocardiogram (ECG), vitals, or clinical laboratory tests.
4. Has no clinically significant findings on the ophthalmic examination, including Best Corrected Visual Acuity (BCVA) worse than 20/30 or, in the opinion of the ophthalmologist or optometrist, any cataract that may become clinically significant and/or need surgical intervention during the course of the trial.
5. Able and willing to complete all study requirements.
6. Diagnosis of current episode of major depressive disorder (MDD) at least 8 weeks prior to screening confirmed by Structured Clinical Interview for DSM-5 – Clinical Trials Version (SCID-5-CT).
7. Have not responded to their current antidepressant therapy or to dose adjustment/treatment changes following a loss of response to their current antidepressant therapy.
8. Complete the SAFER interview, administered via teleconference by a Massachusetts General Hospital (MGH) psychiatrist or psychologist, and receive a passing score which confirms a diagnosis of MDD, and a minimum Montgomery-Åsberg Depression Rating Scale (MADRS) total score of 26. The SAFER interview will also confirm the subject's treatment history and response to antidepressant therapies (ADTs) through the administration of the MGH Antidepressant Treatment Response Questionnaire (ATRQ).
9. Receiving a stable dose of the same antidepressant (selective serotonin reuptake inhibitor [SSRI], serotonin and norepinephrine reuptake inhibitor [SNRI], bupropion or trazodone monotherapy) for the current episode for at least 6 weeks of continuous treatment, which can include some or all of the screening period, with 4 weeks on a stable dose prior to day 1 and has an inadequate response (<50% improvement) using the MGH ATRQ.
10. Willing to maintain stable dose and delay start of other antidepressant or antianxiety medications and any new pharmacotherapy regimens, including benzodiazepine anxiolytics, during the screening and treatment periods.

11. Has a MADRS total score of ≥26 at screening and Day 1 (prior to dosing).
12. Has a 12-hour urine cortisol level >22.7 nmol/L (greater than or equal to 8.3 mcg/L).
13. Body mass index (BMI) ≥18 and ≤38 kg/m$^2$ at screening using the formula: $weight\ (kg)/height\ (m)^2$.
14. Agrees to practice an acceptable method of highly effective birth control at screening, throughout study participation, and for 90 days after the last dose of study drug. Highly effective methods of birth control include sexual abstinence (for males and females); vasectomy; or a condom with spermicide in combination with a highly effective female partner's method (for males); and hormonal methods of contraception (i.e., established use of oral, implantable, injectable, or transdermal hormones); placement of an intrauterine device; placement of an intrauterine system; and mechanical/barrier method of contraception (i.e., condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [foam, gel, film, cream, or suppository]) (for females).

## 5.2. Exclusion Criteria

Patients who meet any of the following exclusion criteria will be excluded from participation in the study:

1. History of suicide attempt in the past 2 years or within current MDD episode.
2. Active suicidal ideation with plan or a "yes" response to items 4 or 5 on the C-SSRS in the past 24 months is exclusionary as assessed at Screening and Baseline (Day 1).
3. Inadequate response to >2 prior ADTs (not including current antidepressant) of at least 6 weeks duration each for the episode current at screening.
4. Recent history or active clinically significant manifestations of metabolic, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, musculoskeletal, dermatological, urogenital, neurological, ophthalmic (especially cataracts), or ears, nose, and throat (ENT) disorders, or any other acute or chronic condition that, in the Investigator's opinion, would limit the subject's ability to complete or participate in this clinical study.
5. Based on an ophthalmic examination performed at Screening, patient must not have any of the following:
    - Nuclear opalescence (NO) with a LOCSIII classification > 3.0 in either eye
    - Nuclear color (NC) with a LOCSIII classification > 3.5 in either eye
    - Cortical lens opacities (C) with a LOCSIII classification > 2.0 in either eye
    - Posterior subcapsular lens opacities (P) with a LOCSIII classification > 0.3 in either
6. History or presence of intellectual disability, pervasive developmental disorder, cognitive disorder, neurodegenerative disorder (e.g., Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease), or brain injury.

7. Receipt of electroconvulsive therapy (ECT) within 12 months of screening, lifetime receipt of more than one course of ECT, or plans to receive ECT during the study.
8. Receipt of repetitive transcranial magnetic stimulation (rTMS) within 12 months of screening or planned to receive rTMS during the study.
9. Plans to initiate or terminate cognitive or behavioral psychotherapy or alter the frequency of ongoing therapy during this study.
10. Works night shifts or needs to work night shifts during the study.
11. Females who are pregnant, intend to become pregnant (within 90 days of the last dose of ANC-501), are breastfeeding, or have a positive pregnancy test at screening or on Day 1 prior to study drug administration.
12. Known allergy to ANC-501, or related compounds.
13. Detectable hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab), or human immunodeficiency virus (HIV) antibody at screening.
14. Active psychosis per Investigator assessment.
15. Medical history of seizures (does not include isolated febrile seizures of childhood).
16. Medical history of bipolar disorder, schizophrenia, and/or schizoaffective disorder.
17. History of alcohol or substance use disorder in the 12 months prior to screening.
18. Positive alcohol breath test.
19. Positive urine drug screen for hallucinogens, psychoactive inhalants, stimulants, opioids, barbiturates, non-prescribed anxiolytics or other non-prescribed sedatives. Does not include positive test for cannabinoids, tobacco or caffeine, unless the subject meets criteria for substance use disorder for these substances.
20. Exposure to another investigational medication or device within 30 days or 5 half-lives (whichever is longer) prior to screening.
21. Has been previously treated in this study or randomized or treated in any other study employing ANC501 (i.e., subject may not have received study drug and then reenrolled).
22. Administration of drugs to treat psychiatric or neurologic conditions that have not been taken at a stable dose for at least 4 weeks prior to day 1.
23. Need/plan to take moderate to strong inhibitors and/or inducers of cytochrome P450 (CYP)3A4 (food, beverages, medications, and supplements).
24. Any condition not identified in the protocol that in the opinion of the Investigator would confound the evaluation and interpretation of the study data or may put the subject at risk.

## 5.3. Subject Enrollment

Subject eligibility must be verified prior to enrollment. Upon eligibility verification, subject information will be entered into an Interactive Response Technology (IRT) system. The first dose of study drug should be administered within 72 hours (all efforts should be made to initiate

treatment as soon as possible after registration) following entry into the IRT. The date the first dose of study drug is administered is the date the subject is considered enrolled into the study.

## 5.4. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but not subsequently enrolled in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE) that occurs after completion of the informed consent form (ICF).

Subjects who do not meet the criteria for participation in this study (i.e., screen failures) may be rescreened after discussion with the Medical Monitor. Previously completed study assessments remaining within the protocol-defined window will be accepted for rescreening.

# 6. STUDY TREATMENT

## 6.1. Study Treatment Administered

Subjects will receive 50 mg ANC-501 Capsules (5 × 10 mg capsules) orally once daily at least 1 hour prior to the morning meal for 8 weeks beginning with the first dose administered in the clinic on Day 1.

## 6.2. Handling, Storage, and Accountability

ANC-501 Capsules are stored in child-resistant 35-count bottles at controlled room temperature (15–25°C/59–77°F).

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received, and any discrepancies are reported and resolved before use of the study drug.

Only subjects enrolled in the study may receive study drug, which may only be supplied or administered by authorized site staff. All study drug must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the Investigator and authorized site staff.

The Investigator is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). The Investigator or designee will maintain a record of all study drug received and dispensed.

Further guidance and information for the final disposition of unused study drug are provided in the Pharmacy Manual.

## 6.3. Measures to Minimize Bias: Randomization and Blinding

Not applicable; this is an open-label study.

## 6.4. Treatment Compliance

Drug accountability will be performed by the study site using appropriate source documentation and recorded in the eCRF.

## 6.5. Dose Modifications

During the Open-Label Treatment Period, subjects will be able to receive study drug as long as there are no dose-limiting safety/tolerability concerns as determined by the Investigator. Subjects who experience moderate or severe AEs while receiving the 50 mg dose of study drug which, according to the clinical judgement of the Investigator, are related to study drug, may receive 40 mg for the remainder of the Open-Label Treatment Period if the AEs are intolerable. Subjects who experience moderate or severe related AEs while receiving the 40 mg dose of study drug might not be able to continue receiving study drug based on the evaluation and clinical judgment of the Investigator and may be discontinued from further treatment.

## 6.6. Continued Access to Study Drug after the End of Study

There are currently no plans to provide ANC-501 to subjects after the study ends.

## 6.7. Treatment of Overdose

No information regarding overdose of ANC-501 in humans is available. The subject should be closely monitored for signs of toxicity, and the Medical Monitor notified immediately. There is no specific antidote for overdose of ANC-501, and general supportive care should be provided.

## 6.8. Concomitant Medications and Therapies

Any medication or vaccine(s) (including over-the-counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements) that the subject received within 30 days prior to informed consent or receives during the study must be recorded along with the reason for use, start and end dates, dose, and frequency. The Medical Monitor should be contacted if there are any questions regarding prior or concomitant therapy.

Subjects may not have received another investigational medication or device within 30 days prior to screening. 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.

Strong inhibitors and/or inducers of CYP3A4 (food, beverages, medications, and supplements) are not permitted.

# 7. DISCONTINUATION OF STUDY TREATMENT AND SUBJECT WITHDRAWAL

## 7.1. Discontinuation of Study Treatment

A subject must be discontinued from study treatment for any of the following:

1. Unacceptable toxicity, despite maximal supportive care and/or dose reduction.
2. Subjects exhibiting a change in the LOCS III greater than or equal to 0.5 in Nuclear Opalescence, Cortical Opalescence or Posterior Subcapsular Opalescence in either eye or clinically significant eye changes (e.g., incident of cataract).
3. Investigator decision (may include the need for antidepressant therapy or other therapy not otherwise permitted in the study, including ECT or changes to cognitive/behavioral psychotherapy) or noncompliance with study requirements.
4. Subject becomes or intends to become pregnant.
5. Subject withdrawal of consent for further study treatment.
6. Study termination by the Sponsor.

Subjects discontinuing treatment should complete the Early Termination visit procedures and enter the Follow-up Period.

## 7.2. Subject Withdrawal from Study

A subject will be withdrawn from all study participation (i.e., discontinued without further follow-up):

1. Withdrawal of consent to further follow-up assessments, irrespective of the reason
2. Lost to follow-up
    a. A subject will be considered lost to follow-up after repeated failure to attend scheduled study visits, and who is unable to be contacted by the study site. Only after 3 phone calls (if possible) are attempted and no response received from a certified letter (or equivalent local method) to the subject's last known mailing address should a subject be considered lost to follow-up. All contact attempts should be documented in the subject's source records.
3. Death

# 8. STUDY ASSESSMENTS AND PROCEDURES

Written informed consent must be provided before any study-related procedures/assessments are performed. Refer to the Schedule of Events (Section 1.3) for procedures/assessments and timing.

## 8.1. General Study Assessments

General study assessments are outlined in Table 1.

**Table 1: General Study Assessments**

| Assessment | Details |
|---|---|
| Review of inclusion/exclusion criteria | Eligibility is to be confirmed prior to the first dose of study treatment (see Section 5.1 and Section 5.2 for eligibility criteria). |
| Medical history, demographics | Demographics (age, gender, race, ethnicity), relevant medical history (past and concurrent). |
| Height and body weight | Height is measured at screening only (for body mass index [BMI] calculation). |
| Prior and concomitant medications/concomitant therapy | Record all medications that are taken within 30 days prior to informed consent and throughout the study. |
| Structured Clinical Interview for DSM-5 – Clinical Trials Version (SCID-5-CT) Diagnostic Interview | Subjects will be screened to determine whether they fulfill the criteria for diagnosis of MDD according to the SCID-5-CT, which will be performed by a qualified, trained, validated healthcare professional. |
| SAFER Interview | The interview will be conducted by a central rater for determination of eligibility. The SAFER inventory for confirmation of eligibility (diagnosis, severity, and treatment history) includes confirmation of MDD using the MADRS, and 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, so that only patients with inadequate response (<50% improvement) are included.<br>Site staff will schedule the interview for the central rater, who will contact the patient as scheduled and conduct the interview. Results will be communicated to the site prior to Day 1. |
| Childhood Trauma Questionnaire (CTQ) | Retrospective measure of childhood trauma containing 5 subscales (emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect). Assessed at screening only. |

## 8.2. Efficacy Assessments

Efficacy assessments are outlined in Table 2.

**Table 2: Efficacy Assessments**

| Assessment | Details |
|---|---|
| Montgomery-Åsberg Depression Rating Scale (MADRS) | 10 questions, each rated on a 7-point scale, to stratify severity of depressive episodes in adults.<br>Includes change from baseline in the MADRS total score at all time points, percentage of MADRS responders (≥50% reduction in total score), percentage of MADRS remitters (total score ≤10).<br>The same rater should perform MADRS assessments throughout the study on a given patient when possible. |
| Hamilton Anxiety Scale (HAM-A) | 14 questions, each rated on a 5-point scale, to rate the level of anxiety.<br>Includes change in HAM-A total score from baseline to Day 56, and change in total score at all time points. |
| Clinical Global Impression-Severity (CGI-S) | 7-point scale for clinician to rate the severity of the patient's illness at the time of assessment, relative to past experience with patients having the same diagnosis.<br>Includes change in CGI-S total score from baseline to Day 56, and change in total score at all time points.<br>The same rater should perform CGI assessments throughout the study on a given patient when possible. |
| Clinical Global Impression-Improvement (CGI-I) | 7-point scale for clinician to assess how much the patient's illness has improved or worsened relative to a baseline state.<br>Includes percentage of CGI-I improvers ("Very much improved" or "Much improved") at all time points.<br>The same rater should perform CGI assessments throughout the study on a given patient when possible. |
| Patient Global Impression of Improvement (PGI-I) | 7-point scale used to evaluate patient satisfaction with treatment.<br>Includes PGI-I score at Day 56, and score at all time points. |
| Hamilton Depression Rating Scale (6-item) (HAMD-6) | 6-point scale derived from the 17-item Hamilton Rating Scale for Depression to evaluate severity of depression. Subjects will complete the HADM-6 twice a day for three days per week. |
| General Anxiety Disorder (7-item) (GAD-7) | 7-point scale to measure the level of anxiety. Subjects will complete the GAD-7 twice a day for three days per week. |
| Short Form-Health Survey (36-item) (SF-36) | 36-question survey self-reported measure of physical and mental health.<br>Includes total score from baseline to Day 56, and change from baseline in total score at all time points. |

## 8.3. Safety Assessments

Safety assessments are outlined in Table 3.

**Table 3: Safety Assessments**

| Assessment | Details |
|---|---|
| AE monitoring | Serious adverse events (SAEs) 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. All adverse events (AEs) and SAEs occurring subsequent to the first dose of study drug on Day 1 will be recorded. |

| Assessment | Details |
|---|---|
| Physical examination | Physical examination and review of relevant systems at screening, including body weight and height. |
| Vital signs | Oral temperature (°C), respiratory rate, heart rate, and blood pressure (supine and standing). |
| 12-lead electrocardiograms | A standard 12-lead ECG (ventricular rate, RR, PR, QRS, QT, QTcB, and QTcF) will be performed by the Investigator or designee after the subject has been in the supine position for at least 5 minutes at specified visits. The subject must be in a supine position while the ECG is obtained. Electrocardiogram tracings will be reviewed and interpreted by a qualified clinician. Electrocardiogram tracings and results with Investigator's interpretation are to be included and summarized in the subject's study records.<br>The date, time, subject demographics (e.g., age, and sex) and subject number should be recorded on the ECG. |
| Ophthalmic examination | 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. Additional slit lamp imaging of the lenses of both eyes may be performed during the treatment period and sent to a central reader for assessment using the LOCS III as per investigator's discretion.<br>Slit lamp imaging performed during the study showing a change of greater than or equal to 0.5 on the LOCS III scale and any changes determined by the investigator to be clinically significant will be recorded and considered an adverse event of special interest.<br>Clinical significance is defined as a class I lens event (minimal change) [increase from baseline in the LOCS III grade of ≥0.5 (Nuclear Opalescence), ≥0.5 (Cortical) or ≥0.5 (Posterior Subcapsular)] and any changes considered to be clinically significant (e.g., incidence of cataract). |
| Columbia-Suicide Severity Rating Scale (C-SSRS) | Rating scale used to assess suicidal ideation and behavior.<br>The "Baseline/Screening" C-SSRS form will be completed at screening. The "Since Last Visit" C-SSRS form will be completed at any time of day at all subsequent time points. |
| Safety laboratory tests | See Table 4. |

Abbreviations: AE = adverse event; ECG = electrocardiogram; ET = Early Termination; PK = pharmacokinetic; QTcF = QT interval corrected for heart rate using Fridericia's formula.

### 8.3.1. Laboratory Assessments

See Table 4 for the list of clinical laboratory tests to be performed and the Schedule of Events (Section 1.3) for timing and frequency.

**Table 4: Laboratory Assessments**

| Laboratory Test | Parameters |
|---|---|
| Hematology | Hemoglobin, hematocrit, RBC (including mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration), WBC with differential (neutrophils [absolute count] and lymphocytes, monocytes, eosinophils, basophils), and platelet count. |

| Laboratory Test | Parameters |
|---|---|
| Coagulation | PT, INR, aPTT. |
| Serum chemistry | Non-fasting: albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, amylase/lipase, bicarbonate, bilirubin (total and direct), BUN (urea where BUN not tested), calcium, chloride, creatinine, glucose, magnesium, phosphorus, potassium, sodium, and total protein. Subjects found to have treatment-emergent laboratory toxicity of Grades 3 or 4 will be monitored at least weekly until resolution. |
| Urinalysis | pH, protein, ketones, bilirubin, nitrite, blood, leukocytes, specific gravity, urobilinogen. Reflex to microscopic exam when positive for protein, blood, nitrite, or leukocytes. |
| Pregnancy test | Urine pregnancy test will be conducted in only female subjects of childbearing potential. Female subjects who prematurely discontinue will have a pregnancy test performed at the ET visit. |
| Serology | HBsAg, HCV Ab, HIV antibody |
| Urine drug and alcohol screen | Urine toxicology for selected drugs of abuse (barbiturates, amphetamines, cocaine, opioids) and breath test for alcohol. |
| Plasma, saliva, and 12-hour urine collection for measurement of cortisol level, | Study site personnel will dispense the equipment and train subjects on how to collect a 12-hour urine collection for measuring cortisol level.<br>Saliva collection at screening should be a morning sample. |

Abbreviations: ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; ET = early termination; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HIV = human immunodeficiency virus; INR = International Normalized Ratio; PT = prothrombin time; RBC = red blood cell; WBC = white blood cell.

#### 8.3.1.1. Reporting and Evaluation of Laboratory Test Results

Adverse events will be collected from the first dose of ANC-501, therefore the screening laboratory results are considered baseline. All laboratory test results (except for those at screening, which are considered baseline) must be reviewed for clinical significance by the Investigator. Laboratory test results outside the normal range (hematology, coagulation, chemistry, urinalysis) that worsen in severity from baseline and are deemed clinically significant by the Investigator should be reported as AEs. A new laboratory abnormality resulting in study drug interruption, dose reduction, treatment discontinuation, intervention, or otherwise having an impact on the subject should be reported as an AE, unless it is part of a clinical diagnosis already reported as an AE.

All laboratory values outside the normal range are to be evaluated.

#### 8.3.1.2. Repeat Testing

Repeat testing of any clinically significant laboratory test will be performed until the value returns to within the normal range, returns to the baseline level, or clinically stabilizes.

### 8.3.2. COVID-19 Pandemic Accommodations

The US Food and Drug Administration (FDA) has issued guidance to provide general considerations to assist sponsors in assuring the safety of study participants, maintaining compliance with Good Clinical Practice (GCP), and minimizing risks to trial integrity during the coronavirus disease 2019 (COVID-19) public health emergency. It is recognized that the COVID-19 public health emergency may impact the conduct of clinical trials. Challenges may arise, for example, from quarantines, site closures, travel limitations, interruptions to the supply chain for the investigational product, or other considerations if site personnel or study subjects become infected with COVID-19. These challenges may lead to difficulties in meeting protocol-specified procedures, including administering or using the investigational product or adhering to protocol-mandated visits and laboratory/diagnostic testing. Protocol modifications may be required, and there may be unavoidable protocol deviations due to COVID-19 illness and/or COVID-19 public health control measures. EmbarkNeuro, or its affiliated clinical research organizations (CROs), representatives, and clinical sites may need to make decisions regarding continuing study recruitment, continuing use of the investigational product for subjects already participating in the study, and the need to change subject monitoring during the study. EmbarkNeuro acknowledges that it is critical that study subjects are kept informed of changes to the study and monitoring plans that could impact them. EmbarkNeuro, following consultation with clinical investigators and Institutional Review Boards (IRBs), may determine that the protection of a subject's safety, welfare, and rights is best served by continuing a subject in the study as per the protocol or by discontinuing the administration or use of the investigational product or even participation in the study. In addition, home health services may be utilized in this study per FDA guidance to enable subjects to remain on study and have testing performed per the protocol. It is generally the practice of EmbarkNeuro to implement protocol changes only after review and approval by the IRB, and in some cases, by the FDA. EmbarkNeuro or its affiliated CROs and clinical investigators will engage with IRBs as early as possible when urgent or emergent changes to the protocol or informed consent are anticipated as a result of COVID-19. Such changes to the protocol or investigational plan to minimize or eliminate immediate hazards or to protect the life and well-being of research subjects (e.g., to limit exposure to COVID-19) may be implemented without IRB approval or before filing an amendment to the Investigational New Drug (IND) application, but will be reported afterwards. These considerations are intended to remain in effect only for the duration of the public health emergency related to COVID-19.

## 8.4. Adverse Events, Serious Adverse Events, and Other Safety Reporting

### 8.4.1. Definition of Adverse Event

| **AE Definition** |
| --- |
| An AE is defined as any untoward medical occurrence in a clinical study subject administered a pharmaceutical product and does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a treatment, whether or not related to the treatment. |

#### 8.4.1.1. Events Meeting the Adverse Event Definition

| Events Meeting the AE Definition |
|---|
| • Laboratory test results outside the normal range (hematology, coagulation, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, vital signs measurements, questionnaires), including any that worsen in severity from baseline, which are considered clinically significant in the medical and scientific judgement of the Investigator.<br>• Exacerbation of a chronic or intermittent pre-existing condition resulting in an increase in severity.<br>• Condition detected or diagnosed that is new in onset or increased in severity from the baseline condition.<br>• Signs, symptoms, or the clinical sequelae of a suspected intervention-intervention interaction.<br>• Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. An overdose or incorrect administration of study treatment is not itself an AE, but it may result in an AE. |

#### 8.4.1.2. Events NOT Meeting the Adverse Event Definition

| Events NOT Meeting the AE Definition |
|---|
| • Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition.<br>• The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the patient's condition.<br>• Medical or surgical procedure (e.g., endoscopy, appendectomy). The condition that leads to the procedure is the AE.<br>• Pre-planned medical or surgical procedures, or pre-planned treatments or hospital admissions used to facilitate study visit arrival or procedures.<br>• Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).<br>• Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen in severity. |

### 8.4.2. Definition of Serious Adverse Event (SAE)

**An SAE is defined as any untoward medical occurrence that at any dose:**

**1. Results in death**

**2. Is life-threatening**

- The term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

**3. Requires inpatient hospitalization or prolongation of existing hospitalization**

- In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for preplanned procedure and/or study-related treatment or procedure that did not worsen from baseline is not considered an AE.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

**4. Results in persistent disability/incapacity**

- Disability is defined as a substantial disruption of a person's ability to conduct normal life functions, either reported or per clinical judgment.

**5. Is a congenital anomaly/birth defect (if exposure to product just before conception or during pregnancy resulted in an adverse outcome in the child)**

**6. Other situations (Important medical events):**

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious (e.g., invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, development of drug dependency or drug abuse).
- Is any other important medical event that based upon appropriate medical judgment may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the definitions above (e.g., may not result in death, be life threatening, or require hospitalization).

### 8.4.3. Time Period for Collecting AE and SAE Information

All AEs will be recorded from study drug administration on Day 1 until Day 112 (see Section 8.3.1.1 regarding laboratory-associated AE assessments).

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. If the Investigator becomes aware of an SAE that is possibly related to the investigational drug treatment at any time after the final follow-up visit, the Sponsor should be notified immediately (i.e., within 24 hours). Report the SAE using the method described below.

### 8.4.4. Recording of Adverse Events and/or Serious Adverse Events

#### 8.4.4.1. Terms of Reported Adverse Events

All AEs will be recorded in the electronic case report form (eCRF), including start and stop dates, severity/grade, study drug relationship, and event outcome.

The Investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) should be documented as the AE.

#### 8.4.4.2. Detection of Adverse Events and Serious Adverse Events

The Investigator will record all observed and reported AEs. In addition, each subject will be questioned with regard to AEs, but not occurrence of specific AEs.

#### 8.4.4.3. Severity of Adverse Events

Severity of AEs will be graded as:

- **Mild:** event may be noticeable to the subject; does not influence daily activities; usually does not require intervention
- **Moderate:** event may be of sufficient severity to make the subject uncomfortable; performance of daily activities may be influenced; intervention may be needed
- **Severe:** event may cause severe discomfort; usually interferes with daily activities; subject may not be able to continue in the study; treatment or other intervention usually needed

If the severity of an AE changes more than once a day, the maximum severity for the event should be listed. If the severity changes over a number of days, these mini-events or changes should be recorded separately (i.e., having distinct onset dates).

#### 8.4.4.4. Causal Relationship with Study Treatment

| **Assessment of Causality** |
|---|
| • The Investigator is obligated to assess the relationship between study drug and each occurrence of each AE/SAE. A “reasonable possibility” of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship. Investigators |

**Assessment of Causality**

should use their knowledge of the subject's medical/disease history, the specific circumstances of the event, and potential alternative causes to determine if an AE is considered related to the study drug:

- Definitely: The event has a plausible time relationship to drug intake, cannot be explained by the disease under study or other drugs, or has a definitive pharmacologic relationship to the study drug.
- Probably: The event has a reasonable time relationship to drug intake, and is unlikely to be attributed to the disease under study or other drugs.
- Possibly: The event has a reasonable time relationship to drug intake, and could also be attributed to the disease under study or other drugs.
- Not related: The event has a time relationship to drug intake that makes a relationship impossible, or is clearly attributable to the disease under study, to other drugs, or to other factors or events.

- The Investigator will consult the Investigator's Brochure (IB) in the assessment and will use clinical judgment in the determination.
- For each AE/SAE, the Investigator **must** document in the medical notes that the event has been reviewed and an assessment of causality provided.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor or designee. However, it is very important that the Investigator always assessment causality for every event before the initial transmission of the SAE data. The Investigator may change causality determination in light of follow-up information, and send an SAE follow-up report with the updated causality assessment.

#### 8.4.4.5. Follow-up of Adverse Events

Adverse events should be followed until the AE has resolved, returned to baseline, or stabilized; withdrawal of consent; or death.

### 8.4.5. Reporting of Serious Adverse Events (SAE)

The Sponsor or designee must be notified using an SAE Report form within 24 hours of the Investigator becoming aware of an SAE. This timeframe is also applicable to follow-up information for previous reported SAEs.

The Investigator is obligated to obtain supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor or designee to elucidate the nature and/or causality of the event as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, radiographic imaging, or consultation with other health care professionals.

The SAE Report form and redacted medical records should be submitted to safety@safeharborpv.com or via fax to (919) 591-0004.

Follow-up information will be reported in the same method as above.

### 8.4.6. Reporting of Deaths

Death must be reported if it occurs during the SAE reporting period as noted in Section 8.4.3.

### 8.4.7. Pregnancy

Details of all pregnancies in female subjects or in female partners of male subjects (after appropriate consent for use of medical information has been obtained) will be reported to the Sponsor or designee at the email noted in Section 8.4.5 within 24 hours of the Investigator's awareness, using a Pregnancy Report form.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.

The subject/pregnant female partner will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject/pregnant female partner and the neonate, and forward to the Sponsor or designee.

Any female subject who becomes pregnant while participating in the study will discontinue study drug.

### 8.4.8. Overdose

Refer to Section 6.7 for information regarding ANC-501 overdose.

### 8.4.9. Events of Special Interest- Lens Opacity

Lens opacity is an event of special interest in this study. Slit lamp imaging of the lenses of both eyes will be taken at Screening, Day 56, follow-up Day 70 and follow-up Day 112 and sent to a central reader for assessment using the LOCS III. Additional slit lamp imaging may be performed during the treatment period and sent to a central reader for assessment using the LOCS III as per the investigator's discretion.

LOCS III scale changes from baseline of ≥0.5 (Nuclear Opalescence), ≥0.5 (Cortical). ≥0.5 (Posterior Subcapsular) and any changes considered to be clinically significant (e.g., incidence of cataract) are considered adverse events and events of special interest. Any changes considered to be clinically significant will be immediately reported to the Sponsor and/or designee within 24 hours of becoming aware of the event whether or not the event is deemed to be related to investigational product.

## 8.5. Pharmacokinetics

The serum concentrations and calculated PK parameters of ANC-501 will be assessed based on blood samples collected from subjects during the study. Collection time points for the assessment of PK are detailed in the Schedule of Events (Section 1.3). Additional details regarding sample collection timing will be included in a separate Laboratory Manual.

## 8.6. Biomarker Evaluations

Blood samples will be collected for future exploratory biomarker and genetic analysis. Collection time points are detailed in the Schedule of Events (Section 1.3).

# 9. STATISTICAL CONSIDERATIONS

## 9.1. Statistical Hypotheses

No formal statistical hypotheses testing will be performed.

## 9.2. Sample Size Determination

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

## 9.3. Analysis Populations

For the purposes of analysis, the following populations are defined:

**Table 5: Definitions of Analysis Populations**

| Analysis Population | Definition |
|---|---|
| Safety | Includes all subjects who received at least one dose of ANC-501. This is the primary population for exposure and safety analyses. |
| Efficacy | Includes all subjects who received at least one dose of ANC-501 and have at least one post-baseline efficacy evaluation. This is the primary population for efficacy endpoints. |

## 9.4. Statistical Analyses

### 9.4.1. General Considerations

A Statistical Analysis Plan (SAP) will be prepared as a separate document. The SAP will include a more technical and detailed description of the planned statistical summaries and will be finalized prior to closing of database.

Continuous endpoints will be summarized with n, mean, standard deviation, median, minimum, and maximum. In addition, change from baseline values will be calculated at each time point and summarized descriptively. For categorical endpoints, descriptive summaries will include counts and percentages.

Methodology and analyses for missing data will be defined in the SAP.

### 9.4.2. Analysis of Safety

The Safety Population will be used to provide descriptive summaries of safety data. Treatment-emergent adverse events will be classified by type, incidence, severity, and causality. The overall incidence of adverse events will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT). Data for vital signs, clinical laboratory tests, ECG, physical examinations, and concomitant medication usage will also be summarized.

Changes in LOCS III will be assessed from Screening to Day 56, 70 and 112 visits. An increase from baseline in the LOCS III grade of ≥0.5 (Nuclear Opalescence), ≥0.5 (Cortical) or ≥0.5 (Posterior Subcapsular)] in either eye and any changes considered to be clinically significant

(e.g., incidence of cataract) as per the ophthalmologist or optometrist will be reported as an adverse event and an event of special interest.

Suicidality data collected using the C-SSRS at baseline and at each visit during the active Open-Label Treatment Period will be listed for all subjects. The C-SSRS listings will include behavior type and/or category for suicidal ideation and suicidal behavior of the C-SSRS.

### 9.4.3. Analysis of Efficacy

The Efficacy Population will be used to analyze efficacy data. Efficacy data will be analyzed using appropriate descriptive statistics and pre-specified statistical methods, as well as other data presentation methods where applicable; subject listings will be provided for all efficacy data. Efficacy data will be summarized descriptively.

### 9.4.4. Analysis of Pharmacokinetics

Plasma concentrations of ANC-501 will be listed by individual and summarized by treatment in tabular format using descriptive statistics (number, arithmetic mean, standard deviation, median, minimum, maximum, geometric mean, and geometric coefficient of variation). ANC-501 plasma concentrations will be plotted against time points by dose level/cohort (mean and individual).

ANC-501 concentration data may be combined with concentration data from other studies to build a PK model.

## 9.5. Interim Analysis

No formal interim analysis is planned.

# 10. ADMINISTRATIVE CONSIDERATIONS

## 10.1. Protocol Compliance/Deviations

The Investigator agrees to conduct the study in compliance with the protocol and all amendments. Any protocol deviation(s) should be recorded in the source documents along with a clear description of the deviation(s) and cause.

## 10.2. Study Termination

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the Institutional Review Board (IRB), issues with study drug, or at the discretion of the Sponsor. In addition, the Sponsor retains the right to suspend or discontinue ANC-501 development at any time.

The Sponsor will notify the Investigator if the study is prematurely terminated. The Investigator must promptly contact all subjects to arrange final study visit(s) and procedures, as directed by the Sponsor.

## 10.3. Case Report Forms

An eCRF is required for each enrolled subject. For screen failure subjects, a minimal set of data will be collected as noted in Section 5.4. Data entered into the eCRF must be consistent with the source documents.

The Investigator has ultimate responsibility for the collection and reporting of all data entered in the eCRF and any other data collection forms, and will be electronically signed by the Investigator to attest that the data contained in the eCRF are accurate.

## 10.4. Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents (e.g., hospital records, patient hard-copy/electronic files, clinical/office charts, pharmacy dispensing records, X-rays) are filed at the Investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, International Council for Harmonisation Good Clinical Practice guideline E6 (ICH GCP), and all applicable regulatory requirements.

## 10.5. Data Protection

Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; names or any information which would make the subject identifiable will not be transferred.

The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject, who will be required to give consent for the data to be used as described in the informed consent form (ICF).

The subject must also be informed that medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

## 10.6. Recordkeeping

The Investigator agrees to keep and maintain records that would permit the Sponsor and regulatory authorities to evaluate/audit the study. These include the identity of subjects, IRB correspondence, original signed ICFs, source documents, the eCRFs, SAE/Pregnancy Report forms, drug storage/accountability/disposition records, and correspondence (e.g., letters, email, meeting minutes, telephone call records) with the Sponsor or designee. Records should be retained by the Investigator for a period of 2 years after the last marketing application approval (or if not approved, for 2 years following the discontinuation of the investigational use), according to the ICH guidelines, local regulations, or as specified in the clinical study agreement, whichever is longer.

If the Investigator becomes unable to retain study records for any reason (e.g., retirement, relocation), they must be transferred to the Sponsor or to a designee acceptable to the Sponsor (e.g., another investigator, another institution, or independent third party).

## 10.7. Quality Control and Quality Assurance

The Sponsor or designee will conduct periodic visits or audits during or after the study to ensure that the protocol and GCP are being followed. The monitors may review the drug storage area, study drug stocks, drug storage/accountability/disposition records, source documents, and regulatory documentation. The Investigator and institution will permit direct access to the physical spaces, records, and source documents to perform this verification to the Sponsor or designee monitors/auditors, IRB members, and appropriate regulatory authority inspectors.

The Investigator must immediately notify the Sponsor or its designee of any regulatory inspection notification, will cooperate with the Sponsor or designee to prepare the study site for the inspection, will be present during the inspection along with relevant study staff, will allow the Sponsor or designee to be present during the inspection, and will provide copies of any inspection findings.

## 10.8. Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial

certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

# 11. ETHICS

## 11.1. Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), ICH GCP Guidelines, and the Declaration of Helsinki (World Medical Association 1996 and 2008).

In addition, the study will be conducted in accordance with the protocol, ICH GCP, and applicable local regulatory requirements and laws.

## 11.2. Written Informed Consent

An informed consent form (ICF) meeting the requirements of 21 CFR §50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements (where applicable), must be reviewed and approved by the Sponsor, approved by the IRB before use, and available for inspection.

The Investigator or authorized representative will explain the nature of the study to the subject and answer all questions regarding the study. Subjects must be informed that their participation is voluntary, and if they choose to participate, will be required to sign the approved ICF. The medical record must include a statement that written informed consent was obtained before any study procedures were performed, along with the date written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF. The original signed ICF will be retained in the Investigator's records, with a copy provided to the subject.

Subjects must be re-consented to the most current version of the ICF during their participation in the study.

## 11.3. Institutional Review Board/Independent Ethics Committee

The protocol, protocol amendments, ICF, Investigator's Brochure (IB), and other relevant documents (e.g., advertisements) must be submitted to an IRB by the Investigator, and reviewed and approved by the IRB before the study is initiated.

Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study subjects. Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The Investigator will provide written summaries of the status of the study and any other significant safety findings to the IRB annually or more frequently in accordance with its requirements, policies, and procedures. At the end of the study, the Investigator will notify the IRB of the conclusion of the study and its outcome.

# 12. PUBLICATION POLICY

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

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