A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, and Pharmacokinetics of Multiple Ascending Doses of AZD4041 in Healthy Adult Subjects

Protocol Number:	D7460C00002
Altasciences Project Number:	AZN-P3-000
Investigational Product:	AZD4041
Phase of Development:	Phase 1
Sponsor:	AstraZeneca AB 151 85 Södertälje Sweden

COMPLIANCE

The study will be conducted in accordance with standards of Good Clinical Practice, as defined by the International Council for Harmonisation and all applicable federal and local regulations.

Protocol Version	Date
2.0 (Amendment 1)	**03 February 2022**

SUMMARY OF CHANGES

PROTOCOL AMENDMENT 01 – SUMMARY OF CHANGES

Description of Change Made	Section/Location	Rationale
Safety laboratory assessment for sentinel dosing group changed from Day 3 (in text) to Day 2 to align with Schedule of Activities (SOA) Table	Synopsis (Study Design) and Section 3.1	Corrected for accuracy
Clarification of the evaluation of the endpoint: CCI [redacted]	CCI [redacted]	Added to align with study objectives and design
Updated cross reference for inclusion criteria regarding men who are biologically capable of fathering children From: *Table 16-1* To: ***Appendix 7***	Section 4.1	To align with the changes in Section 4.7
Contraception Guidance heading added and text changed.	Section 4.7	To correct formatting errors
Changed: *"Men who are biologically capable of fathering children must agree and commit to use of adequate forms of double-barrier contraception and refrain from sperm donation for the duration of the treatment period and for no less than 120 days (4 months) after the last administration of study drug. A male subject is considered capable of fathering children even if his sexual partner is sterile or using contraceptives."* To:	Section 4.7	Updated to improve understanding and clarity

"Contraception guidance for male and female subjects can be found in Appendix 7"		
The text in Section 5.2.3 Method of Assigning Subjects to Treatment groups was updated: Deleted: "*Subjects who sign the ICF and are randomized but do not receive the study treatment may be replaced. Subjects who sign the ICF, are randomized, receive the study treatment, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced."* *Added:* ***"Subjects who sign the ICF, are randomized, receive the study treatment, and subsequently withdraw, or are withdrawn or discontinued from the study, should not generally be replaced. However, consideration may be given to the replacement of such subjects in order to enable the completion of at least 10 subjects for the purposes of Safety Review Committee and dose escalation decisions. Replacement of subjects under these circumstances will be at the discretion of the Principal Investigator in consultation and agreement with the Medical Monitor and Sponsor Physician. Any decision to replace subjects should not conflict with the pre-defined individual subject or cohort stopping rules (Section 4.4.2.1) or otherwise jeopardise the safe conduct of the study."***	Section 5.2.3	To ensure that a sufficient number of evaluable subjects complete each cohort.

Administration of Study Drug: adding "***with approximately 240 mL of non-carbonated room temperature***"	Section 5.3	To align with the process followed in the preceding SAD study
Changed: *On Days 1 and 14, water will be permitted as needed except from 1 hour pre-dose until 1 hour after dosing.* To: *On Days 1 and 14, water will be permitted as needed except from 1 hour pre-dose until 1 hour after dosing* ***(with the exception of the water to be administered during drug administration)****.*	Section 5.5	To align with the revised text for section 5.3 (above)
Addition of Footnote to the SOA table for activities that need to be performed on Days -2 and -1. ***"Activities need to be completed only once and should be performed based on the order of assessments and timing defined for specific assessments."***	Table 6.1 Schedule of Activities	This was to provide clarity on the timings and order of assessments for each specific assessment.
Study assessments were adjusted for Endocrinology, pregnancy test, blood sampling for investigative metabolite, and PD biomarker sampling to reflect accurate time points for each assessment. Change: Endocrinology assessments ***added to day 1*** and *removed from days 2, 3-13, 15, 16 and 17*. Change: Footnote for endocrinology assessment added: ***"Male hormones (FSH, testosterone, LH, Inhibin B) are to be collected: Day-1 (1 sample preferably in the morning); Day 1 (2 samples: pre-dose and 1.5 hours***	Table 6.1 Schedule of Activities	To provide clarity on sampling timepoints

post-dose); Day 14 (2 samples: pre-dose and 1.5 hours post-dose).” Added: To 12-lead dECG footnote ***“12-lead digital Holter is used for dECG extraction on Day 17”*** Change: Serum Pregnancy test *added to Day -2 to -1* Change: Blood sample for metabolite investigation *was* ***added to Day 1*** Change: PD blood sample *removed from day 14* and **added to Day 15** since the timepoint falls on day 15.		
Physical Examination worksheets for physical and neurological examination Appendices were updated from Appendix 11 and 12 to Appendix 12 and 13 for accuracy.	Section 6.2.2	Corrected for accuracy
ECG Recording Table 6-4 Updated: ***Added*** *Day -1:* ***in the morning at the start of*** *24-hour continuous wired ECG monitoring* ***Day7*** timepoint.	Table 6-4	Updated to add clarity and improve safety data evaluation
The dECG acceptable window of assessments Appendix Cross-links were updated from Appendix 10 to Appendix 11 for accuracy.	Section 6.2.5	Corrected for accuracy
Addition of text to Section 6.2.5 CCI [redacted]	Section 6.2.5	CCI [redacted]

CCI [redacted]		
Added: Telemetry Updated Added: Footnote to Table 6-7 ***"It is recommended that ECG telemetry on Day 1 and Day 14 should be continued for up to 48 hours post dose when subjects are at rest."***	Table 6-7	To ensure accurate data capture and guidance to the investigators
Table 6-14 The number of endocrinology samples was reduced (from 8 to 5). However, of the 5 samples, only 3 sample timepoints will require additional blood from the subject (other samples are included in the standard biochemistry/hematology assessments). As such the volume of blood required for the assessments has been reduced from 32 mL to 10.5 mL.	Table 6-14	To provide clarity on sampling volumes
Added: CCI [redacted] Modified The following text: CCI [redacted]	Section 8.6.3	For clarification purposes

CCI [redacted]		
Appendix 6 Endocrinology Assessment for TSH footnote was updated to remove footnote b. This was done to ensure that all subjects (not just women) have their TSH evaluated during screening. Footnote d was also updated to: *Males only at Day -1, Day 1 and Day 14*	Appendix 6	Corrected for accuracy
Appendix 7: Male contraception guidance was updated from 90 days to 120 days to align with Study Inclusion/Exclusion Criteria (Section 4)	Appendix 7	Corrected for accuracy
Append 11 Digital ECG Updated to include Day 7 procedure to align with protocol	Appendix 11	Update for consistency

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STUDY SYNOPSIS

Name of Sponsor/Company:	AstraZeneca AB
Name of Product:	AZD4041
Title of Study:	A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, and Pharmacokinetics of Multiple Ascending Doses of AZD4041 in Healthy Adult Subjects
Study Development Phase:	Phase 1
Objectives:	**Primary objective:** • To evaluate the safety and tolerability of orally administered AZD4041 in healthy subjects following daily doses for 14 days (or to steady state). **Secondary objectives:** • To characterize the multiple dose pharmacokinetics (PK) of AZD4041 and assess the time required to reach steady state, the degree of accumulation, and the time dependency of its PK. • CCI [redacted] • To assess the central nervous system (CNS) penetration potential of AZD4041 by quantification of AZD4041 concentration in the cerebrospinal fluid (CSF) at steady state. **Exploratory objective:** CCI [redacted]

Endpoints:	**Primary endpoints:** • Adverse events (AEs) • Vital signs • Clinical laboratory tests • 12-lead electrocardiogram (ECG) (digital ECG [dECG] and safety ECG) and telemetry • Columbia Suicide Severity Rating Scale (C-SSRS) questionnaire • Physical and neurological examination • Measurement of male hormone levels: testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and inhibin B. **Secondary endpoints:** The following AZD4041 PK endpoints will be estimated: • Plasma: C_{max}, T_{max}, AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, AUC_{τ}, C_{trough}, C_{τ}, $t_{1/2,z}$, $t_{1/2Eff}$, CL/F, V_z/F, $R_{AC}(C_{max})$, and $R_{AC}(AUC)$, λ_z • Urine: Ae_{τ}, fe/F, and CL_R • CSF (Cohorts 2 and 3 only): CSF concentration (ng/mL) reported as a percentage of total and free plasma concentration CCI • **Exploratory endpoints:** CCI
Investigational Product, Dose, and Mode of Administration (proposed):	CCI Manufacturer of Drug Substance: AstraZeneca Mode of administration: Oral
Placebo, Dose, and Mode of Administration:	CCI Manufacturer: MEDISCA

	Mode of administration: Oral
Study Design:	This is a Phase 1, randomized, double-blind, placebo-controlled, multiple ascending dose (MAD) study in healthy male and female adult subjects. The study will include up to 48 subjects (12 subjects per cohort) who will be randomized 9:3 to active drug or placebo. Each cohort will receive AZD4041 or placebo in a MAD order or until a maximum tolerated dose (MTD) is defined. If the MTD has not been defined and the maximum allowed exposure has not been reached after 3 cohorts, 1 additional cohort may be added. Enrolment of an additional cohort will be subject to a review of safety, tolerability and PK data from completed preceding cohorts, and will be based on the scientific rationale to explore additional dose levels with respect to predicted therapeutic dose levels and agreed exposure limits. Screening will occur within 28 days prior to the first study drug administration. Screening data will be reviewed to determine subject eligibility prior to the first study drug administration. Subjects who meet all inclusion criteria and none of the exclusion criteria and who consent to participation will be admitted to the study site the evening of Day -2 for baseline evaluations before dosing on the morning of Day 1. All baseline safety evaluation results should be available prior to first study drug administration and continued eligibility should be confirmed. The protocol makes provision for the evaluation of AZD4041 across 3 cohorts of subjects (N=12 subjects per cohort). For each cohort, 9 subjects will be randomly assigned to receive AZD4041 and 3 subjects will be assigned to receive placebo (see Synopsis Table 1-1). Within each cohort, 2 subjects will be randomized initially to AZD4041 or placebo (1:1 ratio) to allow a sentinel dosing approach. Providing no clinically significant issues have been noted after the first 3 doses of the initial 2 (sentinel) subjects in a cohort and provided the Day 2 safety laboratory tests for the 2 subjects have been reviewed, the remaining 10 subjects will be randomised to AZD4041 or placebo in an 8:2 ratio and dosed in 2 separate groups with at least 24 hours between groups to monitor for safety and tolerability. All subjects will receive either AZD4041 or placebo administered once daily for 14 days. **Synopsis Table 1-1. Proposed Dose Levels** **Cohort[a] / N[b] / Dose[c] / Regimen** 1 / 9:3 / Adaptive / Oral solution of AZD4041 or placebo once daily for 14 consecutive days (Day 1 to Day 14) 2 / 9:3 / Adaptive / Oral solution of AZD4041 or placebo once daily for 14 consecutive days (Day 1 to Day 14) 3 / 9:3 / Adaptive / Oral solution of AZD4041 or placebo once daily for 14 consecutive days (Day 1 to Day 14) a. An additional cohort may be added if the maximum tolerated dose has not been defined and the maximum allowed exposure has not been reached after 3 cohorts

	b. Active:Placebo c. Or until maximum tolerated dose is defined Safety will be assessed during the study through physical and neurological examinations, vital signs, ECGs (safety and dECG), telemetry, C-SSRS questionnaires, clinical laboratory assessments, and AE questioning. CCI [redacted] CCI [redacted] In addition, subjects from Cohorts 2 and 3 will have lumbar punctures performed on one occasion to evaluate CSF levels of AZD4041 CCI [redacted]. Subjects are confined to the clinical site from the evening of Day -2 to Day 17. A follow-up visit will occur 10 to 14 days after discharge (Days 27 to 31). Subjects who terminate the study early will perform Day 17 scheduled procedures at the time of Early Termination. The Safety Review Committee (SRC) will review the safety and PK data of the previous cohort prior to initiating dosing of the subsequent cohort. Complete safety, tolerability and PK datasets from at least 10 subjects in the previous cohort must be reviewed during any safety meeting. Scope for dose escalation will be determined by the SRC based on an evaluation of the observed AZD4041 safety, tolerability and PK data from the completed cohort. Where dose escalation is not possible following completion of a given dose-level cohort, the protocol makes provision for an evaluation of the same dose or a lower dose in the next scheduled cohort at the discretion of the SRC.
Duration of Treatment and Subject Confinement:	Duration of clinical trial (per subject): Screening: Day -28 to Day -1 (up to 28 days) Treatment period: Subjects will be confined to the clinical site from the evening of Day -2 until 72 hours following the last study drug administration (Day 17). Follow-up visit: Subjects will return to the clinical site approximately 10 to 14 days after discharge for a follow-up visit (Days 27 to 31). Total study duration: Up to 59 days (including Screening)
Safety Meetings for Dose Escalation and Adjustments:	Following completion of each cohort, a SRC will review all safety, tolerability, and PK data to evaluate scope for progression to the next dose cohort. Data from at least 10 subjects in the previous cohort must be reviewed before dose escalation can take place. The SRC will be comprised, at a minimum, of the clinical research unit's (CRU) Principal Investigator, Study Manager, Medical Monitor and the Sponsor's Study Physician. During the study, each prescheduled cohort may be conducted at the dose outlined in the protocol or may be adjusted to allow for an evaluation of a lower dose level or for a repeat-evaluation of the same dose level based on safety, tolerability, and PK data reported in the previous cohorts.

	The highest dose level tested will be based on an ongoing review of cumulative safety, tolerability and PK data from completed preceding dose-level cohorts, with due respect to the maximum permitted limits for human systemic exposure.
Study Population:	Healthy male or female adult subjects
Planned Number of Subjects:	Up to 48 subjects will be randomized (12 subjects per cohort).
Pharmacokinetic Analysis:	Plasma and urine samples for determination of AZD4041 concentration will be collected at various timepoints throughout the study. PK analyses will be performed by non-compartmental analysis and will be further detailed in the statistical analysis plan (SAP). The following PK parameters in plasma will be estimated for AZD4041, data permitting: Day 1: C_{max}, T_{max}, AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2,z}$, and CL/F Days 3 to 13: C_{trough} Day 14: C_{trough}, C_{max}, T_{max}, C_{τ}, AUC_{τ}, AUC_{0-t}, $t_{1/2,z}$, $t_{1/2Eff}$, CL/F_{ss}, , $R_{AC}(C_{max})$, $R_{AC}(AUC)$ The following PK parameters in urine will be estimated for AZD4041, data permitting: Day 1 and Day 14: Ae, fe, and CL_R AZD4041 CSF concentrations on Day 14 will be calculated as a percentage of the total and free plasma concentration (For cohorts 2 and 3 only) Additionally, the following biomarker will be measured prior to dosing on Day 1 and Day 14: plasma 4-β-hydroxy-cholesterol. CCI [redacted]
CCI [redacted]	CCI [redacted]
CCI [redacted]	CCI [redacted]
Statistical Analysis:	Descriptive statistics will be used to summarize the safety endpoints. Descriptive statistics will be calculated for AZD4041 concentrations in plasma, urine, CSF, and safety parameters (dECG intervals), at each individual timepoint (or time interval, for urine) and for all PK parameters; individual and mean concentration-time profiles will also be presented. The appropriate PK parameters (C_{max}, AUCs) will be assessed statistically for dose proportionality. Proportionality analysis will be done using a power model. C_{trough} will be displayed graphically and summarized descriptively to assess for steady state.

	The statistical analyses will be further detailed in the SAP.

STUDY ADMINISTRATIVE STRUCTURE

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C QTc Analysis	AstraZeneca Aaron Klug Building, Granta Park Cambridge CB21 6GH United Kingdom
CCI [redacted]	CCI [redacted]
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1. INTRODUCTION

1.1. Background

Opioid use disorder (OUD) is a public health crisis responsible for significant morbidity, mortality, and productivity loss. In 2019, approximately 70% of deaths from drug overdose involved opioids (Centers for Disease Control and Prevention [CDC], National Center for Health Statistics, 2020), and seven out of 1000 babies (~90 babies each day) were born with neonatal abstinence syndrome because their mothers had OUD (Healthcare Cost and Utilization Project 2016). OUD presents an enormous economic burden in the US estimated to be $170-215 billion per year (Society of Actuaries, 2019), with Medicaid billed OUD treatment alone costing over $8 billion in 2013 (Leslie et al, 2019). These economic burdens and costs to health, quality of life, and public assistance programs are all directly or indirectly related to OUD. There are only 3 Food and Drug Administration (FDA) approved treatments for OUD, all of which target μ opioid receptors, which are the major targets for opioid actions in the brain. Methadone, buprenorphine, and naltrexone act as an agonist, partial agonist, and antagonist, respectively, at μ opioid receptors. While each is effective with supervised use and accessory support services, such as counselling, they have limited clinical efficacy and utility (Leslie et al, 2019, Mancher et al, 2019). Limitations include intrinsic rewarding properties that lead to misuse/diversion (Mancher et al, 2019; Hall et al, 2008; Leslie et al, 2019), unpleasant side effects (Chen and Ashburn, 2015) that reduce compliance, including withdrawal symptoms of withdrawal (Mancher et al, 2019), and high residual rates of relapse to drug use (Leslie et al, 2019, Wakeman et al, 2020). Since OUD and its deleterious consequences are a dire national health crisis, the National Institute of Drug Abuse (NIDA) has identified several high priority drug targets for rapid therapeutic development (Rasmussen et al, 2019). Orexin OX1 receptor antagonists are high on this list and are considered one of the most promising targets for the development of novel OUD treatments (Rasmussen et al, 2019). The neuropeptides orexin A and orexin B (also known as hypocretin 1 and hypocretin 2, respectively) are hypothalamic neuropeptides that act through 2 closely related G protein coupled receptors (GPCRs), the OX1 and OX2 receptors. Orexin A has high affinity for both receptors whereas orexin B has higher affinity for OX2 over OX1 receptors. Orexin transmission has been implicated in a diverse range of physiological functions, including feeding and energy homeostasis (Sakurai et al, 1998), the sleep/wake cycle (Gotter et al, 2016), neuroendocrine homeostasis, cardiovascular functions (Samson et al, 2007), and motivated behaviours (Kodadek and Cai, 2010). CCI [redacted] Indeed, pharmacological blockade of OX1 receptors or genetic deletion of the gene encoding this receptor markedly decreases the consumption of opioids and other addictive drugs such as nicotine and cocaine in laboratory animals without altering their willingness to engage in and seek natural reinforces such as food or sex (Kohlmeier, et al, 2013). Based on these findings, it has been proposed that excessive orexin transmission at OX1 receptors may play a fundamental role in the development and maintenance of OUD. Recent human brain studies provide compelling support for this hypothesis. Post-mortem human brain studies have revealed that the numbers of orexin neurons detected in the lateral hypothalamus of those suffering from OUD are markedly upregulated compared with non-OUD patients (Pantazis

et al, 2020). Rodents treated with opioids also show increased numbers of orexin neurons in the brain (Thannickal et al, 2018). This response is thought to reflect the de novo production of orexin peptides by neurons in the hypothalamus in response to opioid use, which contributes to the excessive orexin transmission hypothesized to drive the pathologically elevated motivation to seek and consume opioids (Thannickal et al, 2018). Based on compelling preclinical studies and clinical literature, OX1 receptor antagonists are considered one of the most promising novel therapeutic strategies to treat OUD (Rasmussen et al, 2019). Importantly, OX1 receptors play a similar role in regulating the addiction-related actions of nicotine, psychomotor stimulants, and alcohol, raising the possibility that OX1 receptor antagonists may have utility for the treatment of addiction across different classes of abused drugs.

1.2. Study Rationale

CCI

[redacted]

1.3. Risk/Benefit Assessment

1.3.1. Known Potential Benefits

As the study treatment is not being given to subjects to treat any symptoms or illness, there will be no direct medical benefit from participation in this trial.

CCI

[redacted]. Supporting the feasibility of developing a safe and potent OX1 receptor antagonist for OUD is the fact that two

dual OX1/OX2 receptor antagonists (so-called DORAs) have already received FDA approval as sleep-promoting agents. The sleep-promoting actions of DORAs primarily reflect their ability to block the OX2 receptors. Neither of these DORAs show any evidence of concerning side-effects in the general population despite their wide-spread use. This suggests that novel OX1 receptor-selective antagonists, devoid of the sleep-promoting effects that limit the utility of DORAs for non-sleep-related disorders such as OUD, are unlikely to demonstrate mechanism-related toxicity in humans O'Connor et al, 2010. It therefore appears that AZD4041 could represent a novel strategy in the treatment of addictive disorders such as OUD, where it has the potential to address a significant unmet need.

1.3.2. Important Potential Risks

CCI

CCI

[redacted]

CCI

[redacted]

[redacted] (see Section 7.1).

1.3.2.1. Justification for Dose selection

For the purposes of predicting human exposures following multiple dosing with AZD4041 over a [redacted] oral dose range, steady-state profiles were simulated using nonparametric superposition in Phoenix-WinNonlin® using data achieved in the single-dose study over the dose range [redacted]. Results from steady-state profiles were used to estimate the

geometric mean for Cmax and AUC_{0-24} using noncompartmental analysis for the proposed MAD study. Comparable results were obtained from physiologically-based PK (PBPK) modelling using simCYP simulation software after dosing for a 2-week period, every 24 hours. The current PBPK model fitted dose ranges CCI in the single-dose study (see AZD4041 Investigator's Brochure for details).

CCI

Table 1-1. CCI

CCI

Table 1-2. CCI

CCI

The MAD study is designed to enable an exploration of safety and systemic exposure relationships over a dose-range that is predicted to be relevant to expected therapeutic exposures

for CCI opioid-use disorder. The rationale for the planned AZD4041 starting dose level CCI and higher provisional dose levels CCI is based on an understanding of data accumulated across non-clinical studies and clinical experience to-date summarised as follows:

- CCI
-
-
-
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1.3.3. Risk/Benefit Summary and Conclusion

CCI [redacted]. Dosing of subjects will be staggered appropriately and includes sentinel subjects for each cohort.

CCI [redacted]. The requirement for healthy volunteer subjects to use highly effective methods of contraception (APPENDIX 7) has been implemented into the study protocol.

Based on the considerations regarding the potential risks of AZD4041, as well as the risk-mitigation precautions included in the clinical study protocol, exposure of healthy volunteer subjects to repeat doses of AZD4041 is considered justifiable in relation to the significant unmet need for patients in opioid-use disorder. It is expected that the safety, tolerability and PK data acquired in the current repeat dose study in health volunteer subjects will provide a scientific basis and rationale for the subsequent evaluation of AZD4041 in patients with OUD.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of AZD4041 may be found in the AZD4041 Investigator's Brochure.

2. STUDY OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
To evaluate the safety and tolerability of orally administered AZD4041 in healthy subjects following daily doses for 14 days (or to steady state)	• AEs • Vital signs • Clinical laboratory tests • 12-lead ECG (dECG and safety ECG) and telemetry (bedside cardiac monitoring) • C-SSRS questionnaire • Physical and neurological examination • Measurement of male hormone levels: testosterone, LH, FSH, and inhibin B
Secondary	
• To characterize the multiple dose PK of AZD4041 and assess the time required to reach steady state, the degree of accumulation, and the time dependency of its PK • CCI [redacted] • To assess the CNS penetration potential of AZD4041 by quantification of AZD4041 concentration in the CSF at plasma steady state	The following AZD4041 PK endpoints will be estimated, data permitting: • Plasma: C_{max}, T_{max}, AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, AUC_{τ}, C_{trough}, C_{τ}, $t_{1/2,z}$, $t_{1/2Eff}$, CL/F, V_z/F, $R_{AC}(C_{max})$, and $R_{AC}(AUC)$, λ_z • Urine: $Ae_{\tau}fe$/F, and CL_R • CSF (Cohorts 2 and 3 only): CSF concentration (ng/ml) reported as a percentage of total and free plasma concentration Additional PK parameters (as noted in Section 8.4) may be estimated as appropriate to support the main PK endpoints. CCI [redacted] • [redacted]
Exploratory	
CCI [redacted]	

OBJECTIVES	ENDPOINTS
CCI	

Abbreviations: AEs= adverse events; CNS =central nervous system C-SSRS = Columbia Suicide Severity Rating Scale; CSF = cerebrospinal Fluid; CSR = Clinical Study Report; dECG = digital electrocardiogram; ECG = electrocardiogram; CCI FSH = follicle stimulating hormone; LH = luteinizing hormone; CCI ; CCI ; CCI ; PK = Pharmacokinetics; CCI ; CCI ; CCI

3. STUDY DESIGN

3.1. Overall Study Design

This is a Phase 1, single-centre, randomized, double-blind, placebo-controlled, MAD study in healthy male and female adult subjects.

The study will include up to 48 subjects (12 subjects per cohort) who will be randomized 9:3 to active drug or placebo. Each cohort will receive AZD4041 or placebo in a MAD study. However, the need for an additional cohort will be based primarily on the scientific need to explore a range of doses in Phase 1 that are anticipated to span a therapeutic exposure level that is predicted to be relevant to any subsequent Phase 2 efficacy evaluation of AZD4041 in patients with OUD.

Screening will occur within 28 days prior to the first study drug administration. Screening data will be reviewed to determine subject eligibility prior to the first study drug administration. Subjects who meet all inclusion criteria and none of the exclusion criteria and who consent to participation will be admitted to the study site for baseline evaluations before dosing (Day -2). All baseline safety evaluation results should be available prior to the first study drug administration and continued eligibility confirmed.

The protocol makes provision for the evaluation of AZD4041 across 3 cohorts of subjects (N=12 subjects per cohort). For each cohort, 9 subjects will be randomly assigned to receive AZD4041 and 3 subjects will be assigned to receive placebo (see Table 3-1 below). Within each cohort, 2 subjects will be randomized initially to AZD4041 or placebo (1:1 ratio) to allow a sentinel dosing approach. Providing no clinically significant issues have been noted after the first 3 doses of the initial 2 (sentinel) subjects in a cohort and provided the Day 2 safety laboratory tests for the 2 subjects have been reviewed, the remaining 10 subjects will be randomised to AZD4041 or placebo in an 8:2 ratio and will be dosed in 2 separate groups with at least 24 hours between groups to monitor for safety and tolerability. All subjects will receive either AZD4041 or placebo administered CCI for 14 days.

Table 3-1 Proposed Dose Levels

Cohort[a]	N[b]	Dose[c]	Regimen
1	9:3	Adaptive	Oral solution of AZD4041 or placebo CCI for 14 consecutive days (Day 1 to Day 14)
2		Adaptive	
3		Adaptive	

a. An additional cohort may be added if the maximum tolerated dose has not been defined and the maximum allowed exposure has not been reached after 3 cohorts
b. Active:Placebo
c. Or until maximum tolerated dose is defined

A sequential cohort MAD design will be employed to assure that higher doses are administered to healthy subjects only after lower doses have demonstrated an acceptable safety profile as assessed by physical and neurological examination, vital signs, ECG (dECG and safety ECG), telemetry, C-SSRS questionnaires, clinical laboratory testing, and AE monitoring.

CCI [redacted]. In addition, subjects from Cohorts 2 and 3 will have lumbar punctures performed to evaluate CSF levels of AZD4041. CCI [redacted]

Subjects will be confined to the clinical site from 36 hours prior to the first study drug administration until 72 hours after the last study drug administration (Days -2 to 17). A follow-up visit will occur 10 to 14 days after discharge (Days 27 to 31).

Subjects who terminate the study early will perform the Day 17 scheduled procedures at the time of Early Termination.

The total study duration will be up to 59 days (including Screening).

The SRC will review the safety, tolerability, and PK data of the previous cohort prior to initiating dosing of the subsequent cohort. Data sets from at least 10 subjects from the previous cohort must be reviewed during any safety meeting. Based on observed AZD4041 safety, tolerability, and available PK data, the dose escalation will be approved prior to dosing the next cohort. The study makes provision for dose-adjustment, with all due respect to exposure limits, such that each prescheduled cohort may be conducted at the dose outlined in the protocol or may be adjusted, at the discretion of the SRC, to allow for an evaluation of a lower dose level or for a repeat-evaluation of the same dose level an intermediate (lower) or equivalent dose, based on safety, tolerability, and PK data.

The study schema is presented in Figure 3-1.

The schedule of activities is presented in Table 6-1.

3.2. Adaptive Features and Risk Management of Study Design

The study design may be adapted as described in Table 3-2.

Table 3-2 Adaptive Features and Boundaries

Adaptive study design category	Adaptive Features	Boundaries
Dose levels	Doses will be determined/adapted in accordance with available safety, tolerability, and PK data collected in previous cohorts.	The planned AZD4041 starting dose level will be CCI [redacted], oral, CCI [redacted]. Exploration of subsequent dose levels will be based on a review of available safety, tolerability, and PK data from the previous cohorts. Complete data sets from at least

Adaptive study design category	Adaptive Features	Boundaries
		10 subjects in the previous cohort must be reviewed during the safety meetings. Doses are intended to escalate; however, they may be adjusted to an intermediate (lower) or equivalent dose, based on safety, tolerability, and PK data reported in the previous cohort(s), at the discretion of the SRC. Human exposure limits in the MAD will be based on those which have been achieved in the SAD study.
The number of MAD cohorts	The number of MAD cohorts may be adapted.	Three cohorts of 12 subjects each are planned. However, if the MTD has not been defined and the maximum allowed exposure has not been reached after 3 cohorts, up to 1 additional MAD cohort may be added, at the discretion of the SRC

Abbreviations: GLP = Good Laboratory Practices; MAD = multiple ascending dose; MTD = maximum tolerated dose; NOAEL = no-observed-adverse-effect level; PK = pharmacokinetics; SAD = single ascending dose; SRC= Safety Review Committee

The decision-making process for the above adaptive study categories will be as follows:

- Interim review of safety, tolerability, and PK data of AZD4041 from previous cohorts in a blinded fashion by the SRC. Complete data sets from at least 10 subjects in the previous cohort must be reviewed before dose escalation can take place.
- Outcome on the adaptive study (cohort by cohort) category will be documented by the SRC.

The SRC will be comprised of, at a minimum, the Principal Investigator at the Investigational site, the Study Manager, Medical Monitor, and the Sponsor's Study Physician.

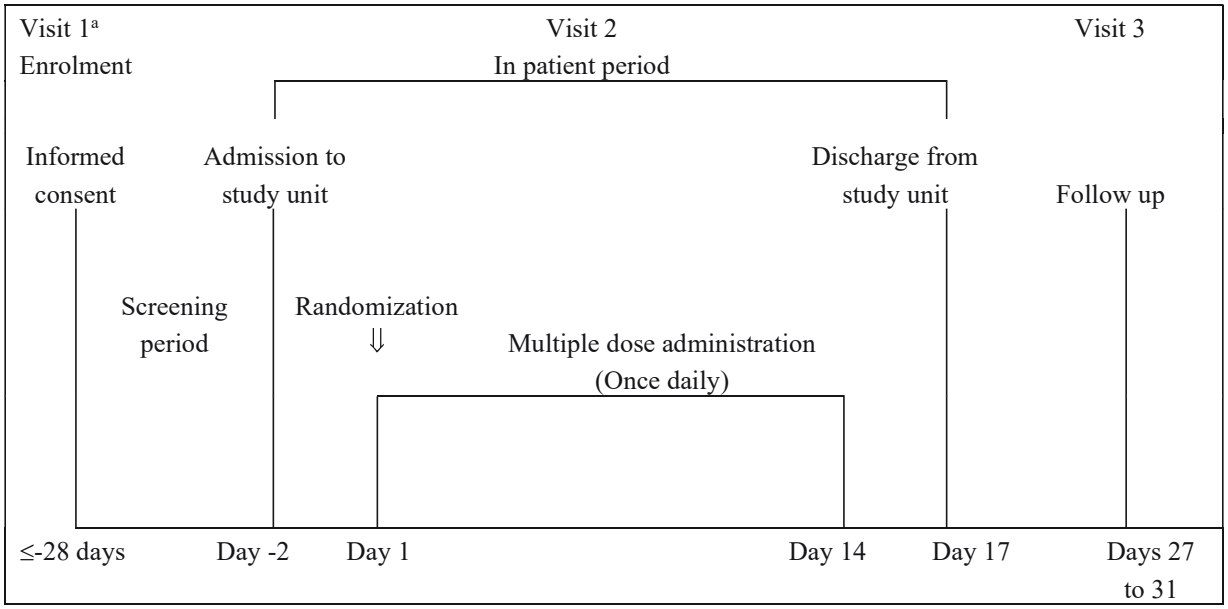
Based on the above, progression to the next dose cohort may be performed without delay (ie, without prior approval from the Research Ethic Committee or regulatory authorities), unless the adaptive features are outside of the prespecified boundaries.

3.2.1. Maximum Tolerated Dose

The MTD definition of transient, mild, or moderate AEs occurring at a given dose level will be evaluated by the SRC prior to dose escalation. If such AEs occurred in ≥ 3 subjects at a given dose level, dose escalation may be ceased and this dose level or the next lower one will be considered the MTD, or an intermediate dose level can be investigated. If such AEs occurred in ≤ 2 subjects depending on the particular adverse effects observed, the current dose level may be considered the MTD, or dose escalation may proceed.

If the MTD has not been defined and the maximum allowed exposure has not been reached after 3 cohorts, up to 1 additional MAD cohort may be added.

Figure 3-1. Study Schema



[a] Visit 1 may be conducted over one or more days during the screening period

4. SUBJECT POPULATION

Subjects meeting all the inclusion criteria and none of the exclusion criteria at Screening may be eligible for participation in this study. Continued eligibility will be assessed upon admission to the clinical site, prior to the first study drug administration.

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study.

4.1. Inclusion Criteria

1. Provision of signed and dated written informed consent form (ICF) prior to any study-specific procedures
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Healthy adult male or female subjects. Female subjects must be of non-childbearing potential (postmenopausal and/or surgically sterile)
4. If female, meets one of the following criteria:

   (1) Physiological postmenopausal status, defined as the following:
   a) absence of menses for at least 12 months following cessation of all exogenous hormonal treatments (without an alternative medical condition) at Screening and prior to the first study drug administration; and
   b) follicle stimulating hormone (FSH) levels ≥ 40 mIU/mL at Screening; and
   c) must have a negative pregnancy test result at screening and check-in.

   and/or

   (2) Surgical sterile, defined as those who have had:
   hysterectomy, bilateral oophorectomy and/or bilateral salpingectomy, or bilateral tubal ligation. Women who are surgically sterile must provide documentation of the procedure by an operative report, ultrasound, or other verifiable documentation; and must have a negative pregnancy test result at screening and check-in.

   If postmenopausal and has an FSH of < 40 mIU/mL, but meets all other criteria in (1) or (2) above as well as all the other inclusion criteria, screening oestradiol serum level must be equal to or below 150 pmol/L.
5. Men who are biologically capable of fathering children must agree and commit to use an adequate form of contraception (APPENDIX 7) for the duration of the treatment period and for no less than 120 days (4 months) after the last administration of study intervention. A male subject is considered capable of fathering children even if his sexual partner is sterile or using contraceptives.
6. Men who are biologically capable of fathering children must also agree to refrain from sperm donation for the duration of the treatment period and for at least 90 days after the last administration of study intervention.
7. Aged at least 18 years but not older than 55 years on the day of randomization

8. Body mass index (BMI) within 18.0 kg/m^2 to 30.0 kg/m^2, inclusive
9. Body weight of within 50 kg to 100 kg, inclusive
10. Non- or ex-smoker (*An ex-smoker is defined as someone who completely stopped using nicotine products for at least 180 days prior to the first study drug administration*)
11. Have no clinically significant diseases captured in the medical history or evidence of clinically significant findings on the physical or neurological examination (including vital signs) and/or ECG and/or safety laboratory tests, as determined by an Investigator
12. Suitable veins for cannulation or repeated venepuncture

4.2. Exclusion Criteria

1. Female who is lactating
2. Female who is pregnant according to the pregnancy test at Screening or prior to the first study drug administration
3. Male subjects with a history of oligospermia or azoospermia or any other disorder of the reproductive system
4. Male subjects who are undergoing treatment or evaluation for infertility.
5. History of significant allergy/ hypersensitivity to AZD4041 or products related to AZD4041 (including excipients of the formulation) as well as severe allergy/hypersensitivity reactions (like angioedema) to any drugs
6. Presence or history of significant gastrointestinal, liver or kidney disease, or any other condition that is known to interfere with drug absorption, distribution, metabolism, or excretion, or known to potentiate or predispose to undesired effects
7. History of any significant disease, including [but not necessarily limited to] significant cardiovascular, pulmonary, hematologic, neurological, psychiatric, endocrine, immunologic, or dermatologic disease
8. Maintenance therapy with any drug or significant history of drug dependency or alcohol abuse (> 21 units/week or > 3 units/day for men; > 14 units/week or > 2 units/day for women; intake of excessive alcohol, acute or chronic)
9. History of any significant psychiatric disorder according to the criteria of the Diagnostic and Statistical manual of Mental Disorders, 5th Edition (American Psychiatric Association 2013) which, in the opinion of the Investigator, could be detrimental to subject safety or could compromise study data interpretation.
10. History of substance use disorder, other than nicotine or caffeine (as per DSM-5 criteria)
11. Use of any prescription drugs, including hormone replacement therapy in the 28 days prior to the first study drug administration, that in the opinion of an Investigator would put into question the status of the participant as healthy
12. Use of St. John's wort in the 28 days prior to the first study drug administration

13. Positive test result for alcohol and/or drugs of abuse at Screening or prior to the first study drug administration
14. Any clinically significant illness, medical/surgical procedure or trauma within the 28 days prior to the first study drug administration
15. Any abnormal or clinically significant findings in laboratory test results at Screening that would, in the opinion of an Investigator, increase the subject's risk of participation, jeopardize complete participation in the study, or compromise interpretation of study data
16. Positive screening results to HIV Ag/Ab combo, hepatitis B surface antigen, or hepatitis C virus tests
17. Showing suicidal tendency as per the C-SSRS questionnaire administered at Screening (APPENDIX 9)
18. Any abnormal vital signs, after 10 minutes supine rest, as defined in the list below, at the Screening Visit/or Day -2 Out of range tests may be repeated once for each visit at the discretion of an Investigator.
    a. Systolic BP < 90 mmHg or >140 mmHg
    b. Diastolic BP < 50 mmHg or > 90 mmHg
    c. Heart Rate <45 or >85 beats per minute (bpm)
19. Any clinically important abnormalities in rhythm, conduction, or morphology of the resting ECG and any clinically important abnormalities in the 12-lead ECG which, in an Investigator's opinion, may interfere with the interpretation of QTc interval changes, including abnormal ST-T-wave morphology, particularly in the protocol-defined primary lead or left ventricular hypertrophy at Screening or prior to the first study drug administration

    (*Out of range test results may be repeated once for each visit at the discretion of an Investigator*)
20. Prolonged QT interval corrected for HR using Fridericia's formula (QTcF) > 440 ms at Screening or prior to the first study drug administration
21. Shortened QTcF < 340 ms at Screening or prior to first study drug administration
22. Known family history of long QT syndrome
23. ECG interval measured from the onset of the P wave to the onset of the complex between Q and S waves (QRS complex) (PR [PQ]) interval shortening < 120 ms (PR > 110 ms but < 120 ms is acceptable if there is no evidence of ventricular preexcitation) at Screening or prior to the first study drug administration
24. PR (PQ) interval prolongation (> 220 ms), persistent or intermittent second (Wenckebach block while asleep is not exclusive), or third degree atrioventricular (AV) block, or AV dissociation at Screening or prior to the first study drug administration
25. Persistent or intermittent complete bundle branch block, incomplete bundle branch block, or intraventricular conduction delay (IVCD) with ECG interval measured from the onset

of the QRS complex to the J point (QRS) > 110 ms. Subjects with QRS > 110 ms but < 115 ms are acceptable if there is no evidence of ventricular hypertrophy or preexcitation at Screening or prior to the first study drug administration

26. In the pre-dose 24 hour telemetry, presence of ≥ 10 ventricular premature contractions (VPCs) during 1 hour, or ≥ 100 VPCs during 24–hours of telemetry, or any occurrence of paired VPC (ventricular couplets) or other repetitive ventricular rhythms, including non-sustained or sustained (> 30 second duration), slow (< 100bpm), or fast (≥ 100 bpm) ventricular tachycardias.
27. Vaccination with the Coronavirus disease 2019 (COVID-19) vaccine less than 14 days prior to first study dose administration
28. Scheduled immunization with a COVID-19 vaccine (first or second dose) during the study that, in the opinion of an Investigator, could potentially interfere with subject participation, subject safety, study results, or any other reason
29. Use of any prescribed or nonprescribed oral and topical inhibitors/inducers of CYP3A4 (including shampoo).
30. Excessive intake of caffeine-containing drinks or food (eg, coffee, tea, chocolate) as judged by an Investigator
31. Subjects who have previously received AZD4041
32. Any history of tuberculosis
33. Involvement of any AstraZeneca or study site employee or their close relatives
34. Judgment by an Investigator that the subject should not participate in the study if they have any ongoing or recent (ie, during the Screening period) minor medical complaints that may interfere with the interpretation of study data or are considered unlikely to comply with study procedures, restrictions, and requirements
35. Presence of any tongue piercings or history of any tongue piercings in the last 90 days prior to the first study drug administration
36. Subjects who have medical dietary restrictions
37. Subjects who cannot communicate reliably with the Investigator
38. Inclusion in a previous group for this clinical study
39. Intake of an investigational product (IP) within at least 28 days or 5 half-lives; whichever is longer, prior to the first study drug administration
40. Donation of 50 mL or more of blood in the 28 days prior to the first study drug administration
41. Donation of 500 mL or more of blood (Canadian Blood Services, Hema-Quebec, clinical studies, etc.) in the 56 days prior to the first study drug administration

4.3. Rescreening Criteria

Subjects may be required to be re-screened as part of this study. Subject who are screen failures, due to transient exclusion criteria (ie, blood donations, consumption of products within last 28 days, etc), may be rescreened at the discretion of the Investigator, in consultation with the Sponsor.

4.3.1. Subjects within the Original Screening Window

A subject within the original screening window may enrol in any open cohort as long as he/she meets all eligibility criteria with the following conditions

- All Day -1 procedures (Table 6-1) must be repeated
- The Subject will retain his/her original subject identification number.

4.3.2. Subjects Outside the Original Screening Window

Subjects outside of the original screening window may be randomized for the next available cohort if the following conditions are met and he/she is deemed eligible:

The following screening procedures must be repeated to determine eligibility:

- Signed ICF (applicable only if there has been a revision to the original ICF)
- Recent medical/surgical history
- C-SSRS assessment
- Concomitant medication
- Physical examination
- Neurological examination
- Laboratory tests (between Day -14 and Day -3) expect for HIV, Hepatitis B and C, and FSH (female subjects ≤ 60 years of age only)
- Vital Signs
- 12-lead ECG

All Day -1 procedures (Table 6-1) must be repeated.

Subjects who are outside of initial screening window will have a new subject identification number issued.

4.4. Withdrawal Criteria

4.4.1. Before First Treatment Administration

Before the first treatment administration, inclusion/exclusion criteria will govern the eligibility of subjects to be enrolled into the study and dosed. Subjects withdrawn before first treatment administration will not be followed up and will not undergo Early Termination assessments. Other safety assessments may be performed if required.

Subjects are free to withdraw their consent to participate in the study at any time, without prejudice. The reason for their withdrawal or for deciding to end their participation will be documented.

4.4.2. After First Treatment Administration

Subjects may, at any time, voluntarily withdraw from the study or be removed from the study at the discretion of an Investigator or the Sponsor. An Investigator may withdraw a subject at any time if it is determined that continuing the study would result in a significant safety risk to the subject or if the subject's behaviour is deleterious to the study environment. If such withdrawal occurs, or if the subject fails to return for visits, an Investigator should determine the primary reason for a subject's premature withdrawal from the study and record the reason in the subject's study documents.

An Investigator may remove a subject from the study on the recommendation of the PK facility and/or Sponsor due to an unanticipated event that could result in an inadequately characterized PK profile (eg, a missed blood draw, an AE, meal deviation, concomitant medication intake).

In the case of a clinically significant illness detected during the trial (including COVID-19 diagnosis), the Principal Investigator (or delegate) will, in concert with the Sponsor, determine the most appropriate course of action on an individual basis. Evaluations will include but are not limited to:

- The safety of the subject and other study participants
- The possible effect the illness would have on the results gathered during the trial, and their ability to be appropriately analysed or interpreted
- The possibility of suspending participation then re-initiating it after recovery
- The implication of any inclusion or exclusion criteria that would contradict possible actions
- The implication of any adherence to regulatory guidelines that may be affected by actions decided; for example group effect analysis
- The sample size calculation, current number of subjects, and possibility of replacement subjects

Evaluations and decision-making for subject removal will be documented in the study file, reported to the Sponsor, and discussed where appropriate in the Clinical Study Report (CSR).

Attempts should be made to have such subjects complete the Early Termination assessments. Early Termination assessments (assessments from Day 17 in Table 6-1) should be performed as soon as possible after the last study treatment administration.

For subjects lost to follow-up (ie, those subjects whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), an Investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject, (eg, dates of telephone calls, registered letters, etc.).

4.4.2.1. Stopping Rules

4.4.2.1.1. Individual Subject Stopping Rules

Participation in the clinical study may be discontinued by the physician in charge of the study or by the Sponsor for any of the following reasons, but not limited to:

- AEs (including if a subject develops any significant illness or needs to undergo any major surgery during course of the study)
- Subject noncompliance (including any violation of protocol requirements which may affect the study outcome)

Subjects will be discontinued from the clinical study for any of the following reasons, but not limited to:

- Total serum bilirubin > 2 × ULN
- Aspartate aminotransferase (AST) > 3 x ULN
- Alanine aminotransferase (ALT) > 3 × ULN
- Creatinine > 1.5 × ULN
- Meets either of the following criteria:

  QTc prolongation defined as QTcF > 500 ms or a prolongation from baseline of >60 ms (persisting for at least 5 minutes) confirmed and determined post dose either during continuous 12-lead ECG monitoring or on a repeat 12-lead ECG.

4.4.2.1.2. Cohort Stopping Rules

If any of the following safety concerns are observed, dosing of all subjects at the given dose level or higher will be suspended/halted and all available data will be evaluated by the SRC. Dose continuation (remaining subjects within a cohort or remaining dosing for a subject) or escalation should not proceed for any of, but not limited to, the following reasons:

- If 1 AZD4041 related serious adverse event (SAE) (based on Investigator or Sponsor assessment) (Grade 3, 4, or 5) occurs in a cohort
- Moderate or severe AEs in 50% or more of the subjects in a cohort
- Two or more subjects that received AZD4041, have QTc prolongation defined as QTcF >500 ms or a prolongation from baseline of > 60 ms confirmed (persistent for at least 5 minutes) and determined postdose either during continuous 12-lead ECG monitoring or on a repeated 12-lead ECG
- Two or more subjects, who received AZD4041, have tachycardia defined as resting supine heart rate > 125 bpm persisting for at least 10 minutes
- Two or more subjects, who received AZD4041, have a non-sustained ≥5 beat if ‘slow’ (<100 bpm), or ‘fast’ (≥100 bpm) ventricular tachycardia.

- One or more subjects, who received AZD4041, have a sustained ventricular tachycardia (ie, >30 second duration, or leading to haemodynamic consequences requiring immediate intervention to terminate the arrhythmia)
- Two or more subjects, who receive AZD4041, have symptomatic bradycardia defined as resting supine heart rate < 40 bpm or asymptomatic bradycardia defined as resting supine heart rate < 30 bpm while awake and persisting for at least 10 minutes
- Two or more subjects, who receive AZD4041, develop hypertension defined as an increase in resting supine systolic BP > 40 mmHg to above 180 mmHg and persisting for at least 10 minutes
- Two or more subjects, who receive AZD4041, develop hypotension defined as an asymptomatic fall in systolic blood pressure (SBP) > 20 mmHg to below 70 mmHg persisting for at least 10 minutes, or a symptomatic fall in resting supine SBP > 20 mmHg (excluding vasovagal reaction).
- Clinically significant laboratory abnormalities including, but not necessarily limited to:
    - One or more subjects, who receive AZD4041, fulfil Hy's Law defined as "An increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥3x upper limit of normal (ULN) and total bilirubin (TBL) ≥2× ULN, where no other reason can be found to explain the combination of increases; eg, elevated serum alkaline phosphatase (ALP) indicating cholestasis, viral hepatitis, or another drug." The elevations do not have to be at the same time or within a specified time frame.
    - Two or more subjects, who receive AZD4041, have >3× ULN of either ALT or AST, or >2× ULN for TBL or ALP.

Dose escalation will be discussed during the SRC meeting and may be stopped depending on the Principal Investigator's (or delegate) or Sponsor's decision, based on but not limited to the following:

- New clinically significant abnormalities in physical examination; including neurological examination, 12-lead ECG, or vital signs in 2 or more subjects
- Overall pattern of clinical changes or symptoms that may have appeared minor in terms of an individual AE or subject, but which collectively present a safety concern
- Clinically significant changes in organ-specific laboratory parameters (eg, liver function enzymes, renal function studies in 1 or more subjects)
- Pattern of laboratory changes (eg, a consistent increase or decrease within 2 or more subjects or within or across dosing groups) which might indicate an overall safety concern

4.4.2.1.3. Trial Stopping Rules

Clinical trial stopping rules:

- If 2 AZD4041-related SAEs (based on Investigator or Sponsor assessment) (Grade 3, 4, or 5) occur in a cohort
- Occurrence of 1 death attributable to the study treatment

4.5. Lifestyle and/or Dietary Requirements

- Subjects will be prohibited from consuming food or beverages containing grapefruit and/or pomelo for 7 days prior to the first study drug administration and during the study.
- Subjects will be prohibited from consuming alcohol for 48 hours prior to the first study drug administration and during the study. Throughout the study (including the follow-up visit), in case of any doubt about alcohol consumption, a test for alcohol may be performed if requested by the physician.
- Subjects will be prohibited from consuming food or beverages containing xanthines (ie, tea, coffee, cola drinks, energy drinks, or chocolate) for 48 hours prior to the first study drug administration and during the study.
- Subjects will eat only the food provided by the study site during confinement at the clinical research unit (CRU).

4.6. Concomitant Treatment

In addition to the drugs prohibited as per the exclusion criteria (Section 4.2), subjects will also be prohibited from taking any over-the-counter (OTC) products for 14 days prior to the first study drug administration and during the study. Specifically restricted and prohibited medications are detailed in APPENDIX 8.

Except for medication which may be required to treat AEs (eg, paracetamol/acetaminophen), no other treatment or medication other than the study drugs will be allowed from the first study drug administration until all study activities and evaluations have been completed.

Subjects will be instructed to notify the study site about any new medications taken after the start of the study treatment. All medications and significant nondrug therapies (including physical therapy and blood transfusions) administered after the subject has received the study treatment must be listed in the subject's case report form (CRF). The drug name and dose taken will be noted. An Investigator (or delegate) and/or the Sponsor will decide whether the subject will be permitted to remain in the study, depending on the drug used, the time of drug intake, etc.

Medication other than those described above, which is considered necessary for the subject's safety and wellbeing, may be given at the discretion of the Investigator and recorded in the appropriate section of the eCRF.

4.7. Contraceptive Guidance

Contraception guidance for male and female subjects can be found in APPENDIX 7.

5. STUDY TREATMENTS

5.1. Investigational Products

All IPs will be provided by the Sponsor. The study treatments are presented in Table 5-1.

Table 5-1 CCI

CCI

5.2. Investigational Product Management

5.2.1. Packaging, Labelling, and Dispensing

The Sponsor will be responsible for ensuring that the IP is prepared (where applicable) in accordance with applicable current Good Manufacturing Practice regulations and requirements.

The IPs will be labelled according to the requirements of local law and legislation. The IPs will be dispensed by the CRU's pharmacy, unless the Sponsor supplies the pharmacy with prelabelled individual dosing samples.

5.2.2. Storage and Handling

All study drugs will be shipped from the client or client resources to the CRU's pharmacy.

AZD4041 should be stored under conditions as described on the label. The products should not be used if expired and should not be frozen.

The CRU's pharmacy will maintain an inventory record of the IPs received, stored (in a secure restricted area), and dispensed. Investigational products will be provided to study subjects only.

5.2.3. Method of Assigning Subjects to Treatment Groups

The CRU will generate the randomization code with a computer program according to the study design, the number of subjects, and the number of treatments. Eligible subjects within each cohort will be randomly assigned in a ratio of 9:3 to Active: Placebo. Once generated, the randomization code will be final and will not be modified.

Subjects who sign the ICF, are randomized, receive the study treatment, and subsequently withdraw, or are withdrawn or discontinued from the study, should not generally be replaced. However, consideration may be given to the replacement of such subjects in order to enable the completion of at least 10 subjects for the purposes of Safety Review Committee and dose escalation decisions. Replacement of subjects under these circumstances will be at the discretion of the Principal Investigator in consultation and agreement with the Medical Monitor and Sponsor Physician. Any decision to replace subjects should not conflict with the pre-defined individual subject or cohort stopping rules (Section 4.4.2.1) or otherwise jeopardise the safe conduct of the study.

5.2.4. Blinding

The treatment assignment will not be known by the study participants; however, the taste between the IP and placebo is distinguishable and unable to be masked. Therefore, it is possible that subjects will know the treatment to which they have been assigned.

The randomization code will not be available to the personnel of the bioanalytical facility until the bioanalytical phase of the study has been completed. Furthermore, the randomization code will not be available to the physician and clinical staff involved in the collection, monitoring, revision, or evaluation of AEs, as well as clinical staff who could have an impact on the outcome of the study, including the pharmacokineticist(s) and statistician(s) (or delegate), until all the CRFs have been approved and signed and the bioanalytical phase of the study has been completed.

The preparation and/or administration of the products will be done by designated personnel that are not directly involved in the clinical aspects of the trial.

The randomization code must not be broken except in emergency situations where the identification of a subject's study treatment is required by an Investigator for further treatment to the subject or to complete a SAE report. Randomization information will be held by designated individual(s). In the event of a medical emergency requiring identification of the study drug administered to an individual subject, an Investigator will make every attempt to contact the Sponsor's Study Physician to explain the need for breaking the blind within 24 hours of doing so. The date and reason for breaking the blind must be recorded.

The results of the PK and safety analyses will be made available only to the personnel responsible for evaluating the safety data before proceeding with the next dose level. The bioanalytical facility will preserve the blind by reassigning alternative subject numbers to the interim data before they are made available to the PK facility and Sponsor; these alternative subject numbers will be assigned by the lab at the time of sample analysis.

5.2.5. Study Drug Accountability

Complete and accurate inventory records of all study drugs will be maintained. This includes acknowledgment of receipt of each shipment of study product (quantity and condition), subject dispensing records, and returned or destroyed study product.

The labelling and storage conditions shall comply with the current FDA rules and regulations. Drug accountability will be performed at the completion of the trial.

5.3. Administration of Study Drug

Study drug will be administered in the morning. The date and time of each dose will be recorded. For each subject, all scheduled postdose activities and assessments will be performed relative to the time of each study drug administration.

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5.3.1. Treatment Compliance

The IP will be dispensed only to eligible subjects and administered under the supervision of study personnel.

The following measures will be employed to ensure treatment compliance:

- All doses will be administered under the supervision of an Investigator (or delegate).
- Following study drug administration, subjects' mouths will be inspected to confirm that the study drug was swallowed.

5.4. Meals

Food intake will be controlled for the confinement period and for all subjects.

On Days 1 and 14, subjects will be required to fast (abstain from food) for at least 10 hours prior to dosing and for at least 4 hours following dosing.

No fasting requirements will be implemented for the study drug administrations on Days 2 to 13.

5.5. Fluids

Fluid intake other than water will be controlled for the confinement period and for all subjects.

On Days 1 and 14, water will be permitted as needed except from 1 hour pre-dose until 1 hour after dosing (with the exception of the water to be administered during drug administration)

No water restriction will be implemented for the study drug administrations on Days 2 to 13.

5.6. Other Protocol Restrictions

On Days 1 and 14, subjects will remain seated or kept in minimal ambulatory movement for the first 4 hours following study drug administration, avoiding both vigorous exertion and complete rest. On Days 2 to 13, subjects will remain seated or kept in minimal ambulatory movement for the first hour following study drug administration, avoiding both vigorous exertion and complete rest. However, should AEs occur at any time, subjects may be placed in an appropriate position. During these intervals, subjects will be permitted, under supervision, to get up (eg, to use the washroom facilities).

Subjects will not engage in strenuous activity at any time during the confinement periods.

6. STUDY PROCEDURES

An overview of the study activities for each participant is detailed in Table 6-1.

Unless otherwise stated in the protocol, the Standard Operating Procedures (SOPs) of the study facilities, which are available for all activities relevant to the quality of the study, will be followed during this study. When the nominal time for multiple events occurs simultaneously, the events will be staggered using their acceptable windows (acceptable windows for each assessment are specified in the following sections of this protocol), with priority given to those events related to primary study endpoints.

Any deviation from protocol procedures should be noted in the source documentation and compiled for reporting in the CSR.

Table 6-1 Schedule of Activities

Day	Screening -28 to -1	-2 to -1[1]	1	2	3-13	14	15	16	17[2]	Follow-up 27 to 31
Informed Consent[3]	X									
Eligibility Criteria Review	X	X	X							
Demographics	X									
Medical History, including Height	X									
Body Weight	X	X				X				
Admission		X								
Clinic Confinement		X	X	X	X	X	X	X	X	
Discharge									X[4]	
Study Drug Administration[5]			X	X	X	X				
Randomization			X							
C-SSRS Questionnaire[6]	X	X		X		X			X	
Vital Signs[7]	X	X	X	X	X	X	X	X	X	X
Physical Examination[8]	X	X				X				X

[1] Activities need to be completed only once and should be performed based on the order of assessments and timing defined for specific assessments.

[2] For subjects who terminate early from the study, the assessments scheduled on Day 17 will be performed as soon as possible upon termination.

[3] The latest version of the consent form must be signed prior to a subject's inclusion (prior to any study-related procedures).

[4] Subjects will be discharged from the clinical site approximately 72 hours following the last study drug administration.

[5] Subjects will be administered an oral dose of AZD4041 or placebo CCI for 14 consecutive days. Timing of clinical activities are relative to dose administration on Day 1.

[6] On dosing days, the C-SSRS questionnaire should be completed prior to AZD4041 administration and at approximately the same time each day (±2 hours).The Baseline/Screening version (APPENDIX 9) will be used at Screening and the 'Since Last Visit' version (APPENDIX 10) will be used at all other timepoints.

[7] Vital signs at Screening and prior to each study drug administration include blood pressure, pulse rate, and body temperature. Vital signs after each study drug administration and at the follow-up visit include blood pressure and pulse rate. Vital sign assessments timepoints are specified in Section 6.2.3.

[8] A complete physical examination and modified neurological examination will be performed on all instances except for Day -1, where a symptom-oriented physical examination will be performed.

Day	Screening -28 to -1	-2 to -1[1]	1	2	3-13	14	15	16	17[2]	Follow-up 27 to 31
General Biochemistry, Haematology, and Urinalysis[9]	X	X		X	X[10]	X			X	X
Endocrinology[11]	X	X	X			X				
Serology (HIV, HbsAg, and HCV)	X									
Serology (Severe Acute Respiratory Syndrome Coronavirus-2)[12]		X								
Safety 12-Lead Electrocardiogram[13]	X	X	X	X	X	X	X	X	X	X
12-Lead Digital ECG[14]			X	X	X	X	X	X	X	
CCI										
Telemetry[17]		X	X	X	X	X	X	X		
Alcohol and Drugs of Abuse Screen	X	X								
Serum Pregnancy Test (Females Only)	X	X								

[9] Clinical laboratory parameters are detailed in APPENDIX 6. On dosing days, the clinical laboratory tests will be performed prior to study drug administration.

[10] Day 7 only.

[11] Endocrinology parameters are detailed in APPENDIX 6. Male hormones (FSH, testosterone, LH, Inhibin B) are to be collected: Day-1 (1 sample preferably in the morning); Day 1 (2 samples: pre-dose and 1.5 hours post-dose); Day 14 (2 samples: pre-dose and 1.5 hours post-dose).

[12] May be done up to 72 hours prior to Day 1.

[13] The 12-lead electrocardiogram assessment timepoints are specified in Section 6.2.4. Assessments may be conducted at additional timepoints according to Principal Investigator judgment.

[14] Timepoints and extraction times for the 12-lead dECG (extracted from CCI) are specified in Section 6.2.5. CCI is used for dECG extraction on Day 17

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[17] The telemetry timepoints are specified in Section 6.2.6

Day	Screening -28 to -1	-2 to -1[1]	1	2	3-13	14	15	16	17[2]	Follow-up 27 to 31
Blood Sampling for Pharmacokinetics[18]			X	X	X	X	X	X	X	
CCI										
Lumbar Puncture for CSF Sample (Cohorts 2 and 3 only)						X[21]				
Adverse Event Monitoring	X	X	X	X	X	X	X	X	X	X[22]
Concomitant Medication Recording	X	X	X	X	X	X	X	X	X	X

Abbreviations: CSF = Cerebrospinal fluid; C-SSRS = Columbia Suicide Severity Rating Scale; dECG = digital electrocardiogram; ECG= electrocardiogram; HbsAg = Hepatitis B surface antigen; HCV = Hepatitis C virus; HIV = Human Immunodeficiency Virus

[18] The pharmacokinetic blood sample timepoints are specified in Section 6.3.

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21 Lumbar puncture for CSF sample timepoint is specified in Section 6.3.4.

22 Adverse events check must be done at the last scheduled study visit.

6.1. Order of Assessments

As PK and PD samples occur at the same time as other schedule assessments, the following sequence should be followed to ensure that PK and PD sampling occurs as close as possible to the scheduled timepoints.

The sequence of assessment at any particular timepoint is:

1. ECG – dECG/safety ECG
2. Vital Signs (systolic and diastolic blood pressures, pulse rate)
3. PK CCI blood sampling (to be drawn at the specified timepoints)
4. PD blood sampling (to be drawn at the specified timepoints)
5. For Cohorts 2 and 3, Day 14 Lumbar Puncture for CSF

6.2. Safety Assessments

Safety assessments will include physical examination, neurological examination, vital signs, ECG (dECG and safety ECG), telemetry, clinical laboratory tests, C-SSRS questionnaires, and AE monitoring. At the discretion of an Investigator, additional safety assessments may be performed as needed to ensure subject safety.

The physician in charge will be present at the clinical site for at least the first 4 hours following the first study drug administration on Day 1 and will remain available at all times throughout the study.

6.2.1. Medical History

The medical history at Screening will include all queries by the medical and clinical staff related to the subject's well-being and history of relevant past medical events/experiences. Medical history will include all demographic data (age, sex, race, body weight, height, and BMI) and baseline characteristics. Alcohol and smoking habits will also be recorded.

6.2.2. Physical Examination

A physical and neurological examination will be performed by a medically qualified and licensed individual as outlined in Table 6-1.

The physical examination will include a general review of the following body systems (at minimum): head and neck, cardiovascular, respiratory, gastrointestinal, neurological, and general appearance, unless a symptom-oriented physical exam is indicated. The sponsor provides worksheets to facilitate a standardised approach to the physical and neurological examination at the study site (APPENDIX 12 and APPENDIX 13).

6.2.3. Vital Signs

Vital signs will be measured as outlined in Table 6-1. Timepoints for vital sign measurements are presented in Table 6-2.

Table 6-2 Vital Sign Recording Schedule

Vital Sign Recording - Scheduled Timepoints
Screening
Day -2 to Day -1
Days 1 and 14: predose and 1, 2, 3, 4, 8, 12, 24, and 48 hours postdose
Days 3 to 13: predose
Day 17: prior to discharge
Follow-up visit

Vital signs measured:

- at Screening and prior to each dosing will include BP, pulse rate, and body temperature.
- at all other times will include BP and pulse rate.

The acceptable windows for vital sign assessments are presented in Table 6-3.

Table 6-3 Acceptable Windows for Vital Sign Assessments Procedures

Elapsed Time	Accepted Window
Predose	No window specified
> 0 hour and ≤ 24 hours	± 30 minutes
> 24 hours and ≤ 72 hours	± 1 hour

6.2.4. 12-Lead Safety Electrocardiogram

The 12-lead safety ECGs will be performed on study days as outlined in Table 6-1. The 12-lead ECGs will be obtained after the subject has been resting in the supine position for at least 10 minutes. All 12--lead safety ECGs will be evaluated for HR, and for PR, time elapsed between two successive R-waves of the QRS signal on the ECG (RR), QRS, QT, and QTcF intervals, and an Investigator will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided whether the abnormality is clinically significant or not clinically significant, and the reason for the abnormality will be recorded. The date/time, physician interpretation (normal, abnormal clinically significant, abnormal not clinically significant), and all evaluated parameters and intervals will be recorded in the electronic CRF (eCRF), and the paper printouts will be stored at the site.

An Investigator (or designee) will evaluate the printout of the 12-lead ECG in real time, and with particular attention to the effects of clinical importance on the PR, QRS, and QTcF intervals.

Timepoints for 12-lead Safety ECG measurements are specified in Table 6-4.

Table 6-4 12-Lead Safety ECG

ECG Recording - Scheduled Timepoints
Screening
Day -1: In the morning at the time of start of 24-hour continuous wired ECG monitoring
Day 1: Prior to dosing and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 36 postdose,
Days 3, 5, 7, 8, 11, and 13: Prior to dosing
Day 14: Prior to dosing and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours postdose
Day 17: Prior to discharge
Follow-up visit

The acceptable windows for 12-lead safety ECG assessments are presented in Table 6-5.

Table 6-5. Acceptable Windows for 12-Lead Safety Electrocardiogram Assessments Procedures

Elapsed Time	Accepted Window
Predose	No window specified
> 0 hour and ≤ 24 hours	± 30 minutes
> 24 hours and ≤ 72 hours	± 1 hour

6.2.5. Electronic Capture of 12-lead Continuous Digital Electrocardiogram

The AstraZeneca ECG Centre will perform the dECG analysis, using the EClysis© system, version 4.0, or higher. Lead V2 will be used as the primary analysis lead, with lead V5 as the primary backup lead and lead II as the secondary back-up lead for all time points when lead V2 is found to be unsuitable for analysis.

On clinical study protocol (CSP)-indicated time points in Table 6-6, 12-lead continuous dECG files will be recorded using the CCI according to AstraZeneca ECG Centre's standard procedures for settings/configuration, recording and transfer of dECGs. CCI will be sent to the AstraZeneca ECG Centre with an agreed courier. The same recording device will be used for each subject at all time points, when possible. Date and time settings must be checked on the CCI equipment at the start of each study day and aligned with an official timekeeper. The metadata of each file will be checked by the responsible personnel at the study site to ensure that the cards sent to the AstraZeneca central dECG files repository have correct metadata.

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Skin preparation must be thorough and electrode positions must be according to standard 12-lead ECG placement. Permanent electrodes will be applied at least 30 minutes before first study recording and left in place for the duration of each relevant study day. Electrode positions for dECG take precedence over those for telemetry. Subjects will rest in a supine position for at least 10 minutes before the start of each recording. A shorter resting period (at least 5 minutes) may be agreed in certain cases, depending on competing procedures in the study).
The subject should be in the same supine body position (maximum 30 degrees flexion of the hip and feet not in contact with the footboard) at each recording time point during the study. Detailed description of the dECG data collection, handling of the devices and transfer of the data for the study centre staff is available in the ECG Manual. Acceptable window of assessment for dECG are detailed in APPENDIX 11.

From the continuous dECG files received at the AstraZeneca ECG Centre, the EClysis© system will extract continuous files of at least 5 minutes in length at CSP -indicated time points in Table 6-6. The extraction window can be adjusted by the responsible ECG Scientific Advisor during the metadata approval procedure, based on the Clinical Logs received from the site. As standard, from each dECG extracted window, 10-second ECGs will then be extracted by the EClysis© system twice per minute and automatically analysed by the software. The ECG Scientific Advisor will perform all necessary manual corrections of the ECG annotations provided automatically by EClysis©. All dECGs from one subject will be analysed by a single reader in a blinded manner.

The AstraZeneca ECG Centre cardiologist will review the data and provide, when requested, the dECG safety report to support dose escalation decisions by the SRC.

The AstraZeneca ECG Centre cardiologist will review all data and perform all necessary adjustments before locking the data into a read-only state. From the locked data, the numerical values for the ECG intervals and amplitudes will then be made accessible on a secure file share of the AstraZeneca dECG central repository to accredited data management specialists for conversion into SAS® files.

The following dECG variables will be reported by the AstraZeneca ECG Centre: RR, PR, QRS, and QT intervals from the lead defined as the primary analysis lead, as well as potential T-wave morphology changes.

Derived parameters (QTcF, heart rate, and others, as applicable) are calculated by the study statistician or delegate.

Table 6-6 CCI [redacted] **:**

Study Day	Visit Number	ECG Number	Time: Start of the Extraction Window, Hour:Minute [a]Error! Reference source not found.[b]	Dose	Time: Stop of the Extraction Window, Hour:Minute	Duration of Extraction window from continuous files[b, c, d]	Other
1	2		-02:00		-01:30		Apply electrodes
1	2		-01:10		-01:00		Rest in bed [c]
1	2	1	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
1	2		00:00	IP administration			
1	2	2	00:25		00:30	5 min	**d**
1	2	3	00:55		01:00	5 min	**d**
1	2	4	01:25		01:30	5 min	**d**
1	2	5	01:55		02:00	5 min	**d**
1	2	6	02:55		03:00	5 min	**d**
1	2	7	03:55		04:00	5 min	**d**
1	2	8	05:55		06:00	5 min	**d**
1	2	9	07:55		08:00	5 min	**d**
1	2	10	11:55		12:00	5 min	**d**
2	2	11	23:55		24:00	5 min	**d**
2	2			IP administration			
2	2	12	35:55		36:00	5 min	**d**
3	2		-02:00		-01:30		Apply electrodes
3	2		-01:10		-01:00		Rest in bed [c]
3	2	13	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
3	2		00:00	IP administration			
5	2		-02:00		-01:30		Apply electrodes

Study Day	Visit Number	ECG Number	Time: Start of the Extraction Window, Hour:Minute [a]Error! Reference source not found.[b]	Dose	Time: Stop of the Extraction Window, Hour:Minute	Duration of Extraction window from continuous files[b, c, d]	Other
5	2		-01:10		-01:00		Rest in bed [c]
5	2	14	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
5	2		00:00	IP administration			
7	2		-02:00		-01:30		Apply electrodes
7	2		-01:10		-01:00		Rest in bed [c]
7	2	15	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
7	2		00:00	IP administration			
8	2		-02:00		-01:30		Apply electrodes
8	2		-01:10		-01:00		Rest in bed [c]
8	2	16	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
8	2		00:00	IP administration			
11	2		-02:00		-01:30		Apply electrodes
11	2		-01:10		-01:00		Rest in bed [c]
11	2	17	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
11	2		00:00	IP administration			
13	2		-02:00		-01:30		Apply electrodes
13	2		-01:10		-01:00		Rest in bed [c]

Study Day	Visit Number	ECG Number	Time: Start of the Extraction Window, Hour:Minute [a]Error! Reference source not found.[b]	Dose	Time: Stop of the Extraction Window, Hour:Minute	Duration of Extraction window from continuous files[b, c, d]	Other
13	2	18	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
13	2		00:00	IP administration			
14	2		-02:00		-01:30		Apply electrodes
14	2		-01:10		-01:00		Rest in bed [c]
14	2	19	-01:00 (within 60 min prior to dosing)	Pre-dose	-00:50	10 min	d
14	2		00:00	IP administration			
14	2	20	00:25		00:30	5 min	d
14	2	21	00:55		01:00	5 min	d
14	2	22	01:25		01:30	5 min	d
14	2	23	01:55		02:00	5 min	d
14	2	24	02:55		03:00	5 min	d
14	2	25	03:55		04:00	5 min	d
14	2	26	05:55		06:00	5 min	d
14	2	27	07:55		08:00	5 min	d
14	2	28	11:55		12:00	5 min	d
15	2	29	23:55		24:00	5 min	d
15	2	30	35:55		36:00	5 min	d
16	2	31	47:55		48:00	5 min	d
17	2	32	71:55		72:00	5 min	d

[a] Time points for Extraction window from continuous dECG files may be adjusted according to emerging PK data.

[b] Subjects must be in the same supine body position (maximum 30 degrees flexion in the hip) at each time point and at all visits with feet out of contact with footboard.

[c] Skin must be cleaned and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 min before the first continuous dECG recording.

[d] The subjects must rest in bed for at least 10 min prior to each Extraction window from the continuous file (dECG time point).

[e] The subject must remain awake.

Abbreviations: dECG: Digital electrocardiogram, ECG: Electrocardiogram; h: Hour, IP: Investigational product; min: Minute; PK: Pharmacokinetics.

6.2.6. Telemetry

Subjects will be monitored using a 5-lead bedside wired cardiac telemetry with real-time interpretation and safety feedback as outlined in Table 6-1. Subjects will be able to ambulate short distances during the scheduled intervals for telemetry specified in Table 6-7.

Table 6-7 Telemetry Schedule

Telemetry - Scheduled Intervals
Day -1: Full 24 hours (Start 24 hours prior to first dose)
Days 1 and 14: Start at least 30 minutes prior to dosing to at least 24 hours postdose and up to 48 hours postdose (Day 3 and Day 16)[1]
Day 7: Start at least 30 minutes prior to dosing to 24 hours postdose (Day 8)

[1] It is recommended that ECG telemetry on Day 1 and Day 14 should be continued for up to 48 hours post dose when subjects are at rest.

6.2.7. Laboratory Evaluations

Laboratory evaluations will be performed as outlined in Table 6-1.

The laboratory evaluations to be conducted for this study are presented in APPENDIX 6. Additional clinical laboratory tests may be performed by the medical laboratory as part of larger standard test panels (not required for subject safety).

The physician in charge (or delegate) will assess each abnormal value to determine if it is clinically significant. Postdose clinically significant laboratory values will be reported as AEs, if applicable, as judged by the physician in charge (or delegate). Verification of collection for all laboratory test panels will be saved in the clinical database.

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

The C-SSRS is a questionnaire designed for the assessment of suicidal ideation and behaviour in adolescents and adults.

The C-SSRS will assess suicidality over the month preceding screening as well as over the subject's lifetime. At screening, the "Baseline Screening" version of the scale will be used, and at subsequent visits the "Since Last Visit" version of the scale will be used. Versions of the instrument relevant to the subject's native language (ie, English or Spanish or French) should be used. C-SSRS evaluations will be undertaken at the timepoints indicated in Table 6-1.

The questionnaire must be administered by an Investigator or other individual that is suitably qualified by education or training. See APPENDIX 9 for a sample C-SSRS – Baseline/Screening version assessment and APPENDIX 10 for a sample postdose C-SSRS – Since Last Visit version assessment.

If there is a positive result for suicidality on the C-SSRS after Screening (defined by a subject answering "yes' to questions 4 or 5 on the suicidal ideation portion of the C-SSRS), the subject will be evaluated by an Investigator or medically-qualified Sub-investigator for continuation in the study.

If a subject becomes suicidal during the study, an Investigator should provide the appropriate treatment to the subject.

6.3. Pharmacokinetic and Pharmacodynamic Specimen Sampling

6.3.1. Pharmacokinetic Blood Sampling

The complete PK blood sampling schedule is presented in Table 6-8.

Table 6-8 Pharmacokinetic Blood Sampling Schedule

Pharmacokinetic Blood Sampling - Scheduled Timepoints[a]
Day 1: Predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose
Days 3 to 10: Predose
Day 14: Predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose

Blood samples will be collected by direct venepuncture into a labelled tube containing the appropriate anticoagulant as specified by the bioanalytical facility. As an option to the subject or if judged necessary by the clinical staff, blood samples may be collected from an indwelling cannula (stylet catheter that requires no flushing), which will be placed in the vein of the subject.

The time of PK blood sample collection will be calculated relative to the time of treatment administration. The actual time of all PK blood draws will be recorded and reported for all subjects.

Windows for timed PK blood sample collections are presented in Table 6-9. PK samples collected outside of the prespecified windows will be documented as protocol deviations. Since actual times are to be used for the PK analysis, deviations will be reflected in the analysis unless indicated otherwise upon review of the data.

Table 6-9 Acceptable Windows for Timed PK Blood Specimen Collection Procedures

Elapsed Time	Accepted Window
Predose	No window specified
> 0 hour to ≤ 30 minutes	± 1 minute
> 30 minutes to ≤ 4 hours	± 2 minutes
> 4 hours to ≤ 12 hours	± 5 minutes
> 12 hours to ≤ 24 hours	± 10 minutes
24 hours to 72 hours	± 2 hours

AZD4041 concentrations for PK assessments will be obtained through bioanalysis of the plasma derived from the blood samples drawn during this study, using a validated bioanalytical method.

6.3.2. CCI

CCI Table 6-10.

Table 6-10 CCI

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6.3.3. CCI

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Table 6-11 CCI

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Table 6-12 CCI

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6.3.4. Lumbar Puncture for CSF Sample

For cohorts 2 and 3 only, subjects will have a single lumbar puncture performed on Day 14 post dose (approximately 3 hours ± 1 hour). The samples will be collected by insertion of an atraumatic spinal needle (eg, Whitacre atraumatic, 22G 0.7 mm needle between vertebrae L3 and L5). Approximately 10 mL will be collected.

Samples will be processed, stored, and shipped according to the sample processing instructions supplied by the bioanalytical facility.

6.3.5. Pharmacodynamic Assessments

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Table 6-13.

Table 6-13 CCI

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Blood samples will be collected by direct venepuncture into a labelled tube containing the appropriate anticoagulant as specified by the bioanalytical facility. As an option to the subject or if judged necessary by the clinical staff, blood samples may be collected from an indwelling cannula (stylet catheter that requires no flushing), which will be placed in the vein of the subject.

CCI [redacted]

CCI [redacted]

6.3.6. Pharmacokinetics and Pharmacodynamic Sample Handling

Blood, urine and CSF samples for PK determination will be processed, stored and shipped according to the sample processing instructions supplied by the bioanalytic facilities.

Blood and CSF samples for PD determination will be processed to plasma and PBMCs according to the local lab SOP. Sample processing must start within 1 hour of blood draw. Samples should be stored at -70ºC or lower. Once EOS has been achieved, samples will be shipped on dry ice and shipped to the AZ biobank for biomarker analysis.

6.4. Total Specimen Collection

Total specimen collection volumes per subject for blood, urine, and CSF are detailed in Table 6-14.

Table 6-14 Specimen Collected and Total Amount per subject

	Per cohort sample volume (mL)	
	Male	**Female**
Screening clinical lab tests (including pregnancy and serology)	8	8
On study General Biochemistry and Haematology (6 samples x 4 mL)	24	24
On study Endocrinology (3 cohorts) (3 samples per subject × 3.5 mL)	10.5	0
PK blood samples (37 samples per cohort x 4 mL per sample)	148	148
Additional PK blood samples (13 samples per cohort x 4 mL per sample)	52	52
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End of study clinical lab tests	6.5	6.5
Total Blood donation	**313**	**313**
Total Urine donation	**200**	**200**
Total Cerebrospinal Fluid	**10**	**10**

7. ADVERSE EVENTS DOCUMENTATION

7.1. Definitions

An AE is defined as any untoward medical occurrence in a subject administered an IP and which does not necessarily have a causal relationship with the study drug. An AE can therefore be any unfavourable and unintended sign (including a clinically significant abnormal clinical laboratory finding, for example), symptom, or disease temporally associated with the use of an IP, whether or not related to the IP.

A suspected adverse reaction (SAR) is any AE for which there is a reasonable possibility the drug caused the AE. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the AE. A SAR implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

An AE may be:

- A new illness
- Worsening of a concomitant illness
- An effect of the study drug; it could be an abnormal clinical laboratory value as well as a significant shift from baseline within normal range which an Investigator considers to be clinically important

Surgical procedures themselves are not AEs. They are therapeutic measures for conditions that required surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study drug administration. In the latter case, the condition should be reported as medical history.

An AE of Special Interest (AESI) (serious or nonserious) is one of scientific and medical concern specific to the sponsor's study drug/device or program, which warrants ongoing monitoring and rapid communication by the investigator to the sponsor. Such an event might warrant further investigation in order to characterize and understand it.

The following AESI(s) have been identified for the study intervention(s) in this protocol:

• Cardiac arrythmia (including CCI)

Non-serious AESIs are to be recorded in EDC within 72 hours, and serious AESIs are to be reported to the sponsor within 24 hours.

A SAE or reaction is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity (defined as a substantial disruption of a person's ability to conduct normal life functions)
- Is a congenital anomaly or birth defect
- Is an important medical event that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above (according to medical judgment of an Investigator)

7.2. Severity Assessment

All AEs will be graded per the current version of the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE). Every effort will be made to obtain an adequate evaluation of the severity.

7.3. Causality Assessment

An Investigator will determine the relationship of any AE to the study drug using the guidelines presented in Table 7-1.

Table 7-1 Adverse Event Relationship to Study Drug

Relationship to Drug	Comment
Reasonable Possibility	A temporal relationship exists between the AE onset and administration of the IP that cannot be readily explained by the subject's clinical state or concomitant therapies. Furthermore, the AE appears with some degree of certainty to be related, based on the known therapeutic and pharmacologic actions or AE profile of the IP. In case of cessation or reduction of the dose the AE may abate or resolve and it may reappear upon rechallenge.
No Reasonable Possibility	Evidence exists that the AE has an aetiology other than the IP. For SAEs, an alternative causality must be provided (eg, preexisting condition, underlying disease, intercurrent illness, or concomitant medication).

AE = adverse event; IP = investigational product; SAE = serious adverse event

7.4. Adverse Event Monitoring

For the purposes of this study, the monitoring period for AEs extends from the pre-trial evaluation until the follow-up visit. Registration of new AEs will start from the moment of ICF signature and will stop 17 days after the last study drug administration (ie, stop registration on new AEs on follow-up visit Day 31 in case last AZD4041 dose was administered on Day 14).

Subjects will be questioned on their health status from the beginning of the study, before departure from the clinical site, and at each follow-up visit until Day 31. Open-ended questions will be asked.

During the study, all AEs reported by the subject, observed by the clinical staff, or elicited by general questioning will be recorded for all subjects and reported in the CRF.

From the signing of the ICF until the first study drug administration, AEs will be recorded as screening events or as part of on the medical history eCRF page, as applicable. AEs occurring after study drug administration will be recorded on AE eCRF page and indicated as TEAEs in the CSR, as well as non-serious AESI (defined in Section 7.1).

Any AE which remains unresolved as of the last study visit will require an evaluation and follow-up until the AE has been resolved, stabilized or a reasonable explanation for its persistence found, or is deemed mild and safely resolving.

In the case of AEs deemed related to the IP, every effort will be made to determine the final outcome.

If necessary, every effort will be made to obtain an adequate follow-up of the subjects. Should any subject choose to withdraw from the study, they will be advised of the safety precautions to be taken. For those subjects whose status is unclear because they fail to appear for study visits without stating an intention to withdraw, Investigator should show "due diligence" by

documenting in the source records steps taken to contact the subject, (eg, dates of at least three telephone call attempts, registered letters, etc).

It is an Investigator's responsibility to ensure subjects experiencing AEs receive appropriate follow-up, treatment where required, and that every action is well documented.

Classification of AEs will be performed by System Organ Class (SOC) and Preferred Term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA), version 24.1 or higher.

Concomitant medications will be coded using the World Health Organization Drug Dictionary Enhanced (WHO-DDE March 2021 or later).

7.5. Serious Adverse Event Reporting

The CRU will report all SAEs to the Sponsor, which includes the AstraZeneca study physician (or designee) and MMS Holdings, Inc., without regard to causality, within 24 hours after becoming aware of its occurrence using the SAE Report Form provided by MMS Holdings. The study-specific Safety Management Plan (SMP) will provide further details on SAE distribution.

Registration of new AEs will start from the moment of ICH signature and will stop 17 days after the last study drug administration (ie, stop registration on new AEs on follow-up visit Day 31 in case last AZD4041 dose was administered on Day 14). SAE with a suspected causal relationship to the AZD4041 should be registered even if occur after the end of the clinical trial. The investigator does not need to actively monitor subjects for AEs once the clinical trial has ended with regard to the subjects treated by him/her.

If, during follow-up, any non-serious AE worsens and eventually meets the criteria for an SAE, that AE should be recorded as a new SAE.

Any SAE which remains unresolved as of the last study visit will require an evaluation and follow-up until the SAE has been resolved, stabilized or a reasonable explanation for its persistence found, or is deemed mild and safely resolving.

The initial SAE report must be as complete as possible, including details of the current illness and SAE, and an assessment of the causal relationship between the event and the IP(s). Information not available at the time of the initial report (eg, an end date for the AE, laboratory values received after the report, or hospital discharge summary) must be documented. All follow-up information must be reported as soon as the relevant information is available.

The appropriately completed SAE Report Form should be directed to the Sponsor's designee:

- MMS Holdings, Inc.
- Email: CCI [redacted]
- Facsimile: CCI [redacted]

Any SAE reports should be made using the SAE Report Form provided by MMS Holdings. It is **not** acceptable for an Investigator to send photocopies of the subject's medical records to the Sponsor in lieu of completion of the SAE report forms.

There may be instances when copies of medical records for certain cases are requested by AZ. In this case, all subject identifiers, with the exception of protocol number, site number and the subject number, will be redacted on the copies of the medical records before submission to AZ.

A SAE will be considered “unexpected” if the AE is not listed in the reference safety information section of current IB or is not listed at the specificity or severity that has been observed. "Unexpected", as used in this definition, also refers to AEs that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the AZD4041.

If reports of any new suspected unexpected serious adverse reactions (SUSARs) become available to the Sponsor or their designee during the clinical portion of this study (related or not to the present study), the Sponsor or their designee has to advise the CRU, through its clinical Investigator, of those events.

The CRU will determine whether suspected unexpected serious adverse reactions (SUSARs) must be reported to the Institutional Review Board (IRB). If so, the event will be reported via fax or email according to the IRB’s reporting policy.

All details on SUSAR reporting to regulatory authorities will be specified in the SMP.

7.6. Reporting of Pregnancy

All pregnancies and the subsequent outcome of any conception (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality/birth defect) that occurs from the first study drug administration until 16 weeks after study drug administration will be recorded and should be reported to the Sponsor’s designee within 24 hours of knowledge:

- MMS Holdings, Inc.
- Email: CCI [redacted]
- Facsimile: CCI [redacted]

Pregnancy reports should be made using the MMS Pregnancy Notification and Outcome Form.

Pregnancies should be followed until resolution of pregnancy with any abnormal outcomes of the mother or the child being reported. Should a female study participant become pregnant during the study, outcomes can be categorized into the following (1. Normal Outcome before end of the Study, 2. Abnormal outcome before end of study, 3. Normal outcome after end of study, or 4. Abnormal outcome after end of study). Congenital abnormalities/birth defects, spontaneous miscarriages, and any other SAEs experienced during pregnancy should be recorded and reported as an SAE according to Section 7.5. New-borns with congenital abnormalities/birth defects will be followed up until 3-month age with follow-up SAE reports submitted monthly. No follow up is required for the healthy at birth new-borns (no reported anomaly).

7.6.1. Pregnancy in Female Subjects

Pregnancy in a female study subject shall be reported as specified above in Section 7.6 within 24 hours of the knowledge of its occurrence by an Investigator or delegate. Because of the possibility the foetus/embryo could have been exposed to the study drug through the parent and

for the subject's safety, the pregnancy will be followed up to determine its outcome, including spontaneous or voluntary termination, details of birth, presence or absence of any birth defects, congenital anomalies, or maternal and/or newborn complications.

7.6.2. Pregnancy in Female Partners of Male Subjects

Pregnancy in a female partner of a male study subject shall be reported as specified above in Section 7.6 within 24 hours of the knowledge of its occurrence by the clinical site. Because of the possibility that the foetus/embryo could have been exposed to the study drug through the parent and for the safety of the subject's female partner, the pregnancy will be followed up to determine its outcome, including spontaneous or voluntary termination, details of birth, presence or absence of any birth defects, congenital anomalies, or maternal and/or newborn complications

8. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

Unless otherwise specified below, listings and statistical summaries will be presented by AZD4041 dose level and pooled placebo, and by scheduled time, as appropriate. Continuous variables and endpoints will be summarized using descriptive statistics (n, mean, SD, min median, max). Categorical variables will be summarized in contingency tables (by frequency and proportion)

The number of subjects who were included, who discontinued, and who completed the study will be tabulated. The primary reasons for discontinuation will be provided.

8.1.1. Safety Population

The safety population will include all subjects who received at least 1 dose of the IP or placebo.

8.1.2. Pharmacokinetic Population

The PK population will include all subjects who have received at least 1 dose of AZD4041 and have at least 1 PK concentration after dosing.

8.1.3. Pharmacodynamic Population

The PD population will include all subjects who have received at least 1 dose of AZD4041 and have at least 1 plasma 4 β hydroxy cholesterol concentration after dosing.

8.2. Demographic Data and Other Baseline Characteristics

Listings and descriptive summary statistics of demographic (age, height, weight, and BMI) and baseline data will be presented.

Further details of the statistical presentations for demographic and baseline data will be given in the SAP.

8.3. Safety

8.3.1. Safety Endpoints

The safety endpoints include the incidence of AEs and the assessments of vital signs, clinical laboratory tests, ECG (dECG and safety ECG), telemetry, C-SSRS questionnaire, and physical and neurological examination.

These parameters will be used to perform the safety statistical analysis.

8.3.2. Safety Statistical Methodology

Simple descriptive statistics as described above will be used to summarize the safety endpoints. Further details of the statistical analysis of AEs and other safety results will be given in the SAP. In addition, AESIs as defined in Section 7.1 will also be summarized.

8.4. Pharmacokinetics

The PK analysis will be carried out according to Altasciences' SOPs. Pharmacokinetic data handling and analysis will be further detailed in the SAP.

CCI [redacted].

8.4.1. Missing Values

The lack of concentration values due to failure to collect the sample, a lost or compromised sample, or due to the subject's early termination from the study will be termed "missing" in the dataset, and no imputation will be done. For urine data, if a subject has failed to void over a particular collection interval, the amount excreted will be set to zero.

If the actual collection time of a postdose plasma PK sample is unknown, but a valid concentration value has been measured, the sample will be set to missing in the PK analysis and will be presented in listing excluded from descriptive statistics. Unknown predose collection times will be handled on a case-by-case basis.

8.4.2. Measurements Below the Lower Limit of Quantitation

Concentration values below the lower limit of quantitation (LLOQ) associated with predose and postdose collection times will be replaced with zero for the non-compartmental analyses (NCA).

Concentration values below the LLOQ will be replaced with zero for mean PK profile representations as well as for descriptive statistic calculations.

8.4.3. Actual Time

The plasma NCA analysis will be based on the actual sampling times, except for predose samples, which will always be reported as zero, regardless of time deviations.

The individual plasma concentration/time profiles will be presented using actual sampling times whereas the mean plasma concentration/time profiles and tables presenting summary statistics of concentration -time series will be presented using nominal sampling times.

Actual times for plasma PK sample collections will be listed in the report.

Urine PK analysis and data presentation will utilize the nominal collection interval times; CSF concentration and biomarker data will be presented based on nominal collection time.

8.4.4. Baseline Reference Timepoint

Unless otherwise specified, the baseline value will be defined as the last non-missing evaluation prior to the first study drug administration.

8.4.5. Non-Compartmental Analysis

The following configuration for the NCA analysis (with Phoenix® WinNonlin® version 8, or higher) will be used:

- Data: Serial sampled data
- Model/Dose options Type: Plasma (200 202) and Urine (210 – 212)/Extravascular
- AUC Calculation Method: Linear up/Log down (applicable to plasma only)
- Lambda $_Z$ (λ_z) calculation: Best fit method for λ_z Linear-Log regression

Reasons for excluding PK parameters will include the following:

- AUC: AUC parameters will not be estimated if less than 3 consecutive measurable concentrations are observed.
- PK parameters requiring λz estimation (eg, $AUC_{0-\infty}$ and $t_{1/2}$) will be set to Not Reported (NR) in the Tables and Listings if they meet one of the following:
  - $R^2 < 0.8$
  - Extrapolated area > 20%

The PK parameters for AZD4041 are presented in Table 8-1.

Table 8-1 Pharmacokinetic Parameters of AZD4041 in Plasma

PK Parameter	Definition
Plasma, Day 1	
C_{max}	Maximum observed concentration
T_{max}	Time of maximum observed concentration
AUC_{0-24}	Area under the concentration time curve from time 0 (dose administration) to 24 hours *(The **nominal time** will be used to estimate partial AUC using the NCA built-in tool in Phoenix® WinNonlin®. This means that actual times off by more than 1 minute from the partial end-hour timepoint will be extrapolated/interpolated as per Phoenix® WinNonlin®'s built-in formulas. If extrapolation/interpolation is not possible, then no value is reported by the software.)*

AUC_{0-t}	Area under the concentration time curve from time 0 (dose administration) to the time of last quantifiable concentration (t_{last})
AUC_{0-inf}	Area under the concentration time curve extrapolated to infinity, calculated as $AUC_{0-t} + C_{last}/\lambda_Z$, where C_{last} is the measured concentration at time t_{last}
$t_{1/2,z}$	Terminal elimination half-life, calculated as $\ln(2)/\lambda_Z$
CL/F	Apparent total clearance, calculated as $Dose/AUC_{0-inf}$
V_z/F	Apparent volume of distribution, calculated as $Dose/\lambda_Z * AUC_{0-inf}$
C_{max}/D	Dose-normalized C_{max}
AUC_{0-t}/D	Dose-normalized AUC_{0-t}
AUC_{0-inf}/D	Dose-normalized AUC_{0-inf}
Plasma, Day 14:	
C_{max}	Maximum observed concentration
T_{max}	Time of maximum observed concentration; if it occurs at more than one timepoint, t_{max} is defined as the first timepoint with this value
C_τ	Concentration at the end of the dosing interval. Observed concentration, otherwise the predicted concentration value will be calculated as per Phoenix® WinNonlin®'s built-in rules for interpolation/extrapolation, and subject to the criteria for PK parameters requiring λ_z estimation.
AUC_τ	Area under the concentration time curve over the dosing interval at steady state, calculated from 0 to 24 hours (dosing interval) *(The **nominal time** will be used to estimate AUC_τ using the NCA built-in tool in Phoenix® WinNonlin®. This means that actual times off by more than 1 minute from the dosing interval end-hour timepoint will be extrapolated/interpolated as per Phoenix® WinNonlin®'s built-in formulas. If extrapolation/interpolation is not possible, then no value is reported by the software.)*
AUC_{0-t}	Area under the concentration time curve from time 0 (dose administration) to the time of last quantifiable concentration (t_{last})
$t_{1/2,z}$	Terminal elimination half-life, calculated as $\ln(2)/\lambda_Z$
$t_{1/2Eeff}$	Effective half-life, calculated as $\tau * \ln2 / \ln(Rac_{(AUC)}/(Rac_{(AUC)}-1))$, where τ is 24 hours[1]
CL/F_{ss}	Apparent total clearance at steady state, calculated as $Dose/AUC_\tau$
Vz/F_{ss}	Apparent volume of distribution at steady state, calculated as $Dose/\lambda_Z * AUC_\tau$
C_{max}/D	Dose-normalized C_{max}
AUC_τ/D	Dose-normalized AUC_τ
AUC_{0-t}/D	Dose-normalized AUC_{0-T}
$R_{AC}(C_{max})$	Accumulation ratio evaluated by comparing Day 14 C_{max} to Day 1 C_{max}
$R_{AC}(AUC)$	Accumulation ratio evaluated by comparing Day 14 AUC_τ to Day 1 AUC_{0-24}*

	**Day 1 AUC_{0-t} may be used if AUC_{0-24} cannot be estimated, provided that the 24-hour sample was collected within 10% of nominal time.*
Plasma, Various Days:	
C_{trough}	Observed concentration at the end of the dosing interval (predose concentrations on Day 2 [Day 1, 24-hours] through Day 14)
The following plasma PK parameters will be used for PK calculation and presented in the PK listings only	
t_{last}	Time of last measurable observed concentration
C_{last}	Observed concentration corresponding to t_{last}
λ_Z	Apparent elimination rate constant, estimated by linear regression of the terminal linear portion of the log concentration *versus* time curve
$\lambda_{Z\ Upper}$	Upper limit on time for values included in the calculation of λ_z
$\lambda_{Z\ Lower}$	Lower limit on time for values included in the calculation of λ_z
Number of Points	Number of data points in computing λ_Z
R^2	Goodness of fit for the terminal phase
Residual area	Extrapolated area (ie, percentage of $AUC_{0-\infty}$ due to extrapolation from t_{last} to infinity: $AUC_{0-\infty}$ - AUC_{0-t}/ $AUC_{0-\infty}$*100)
Urine, Day 1, and Day 14:	
Ae	Amount of drug excreted in urine The Ae at each urine collection interval will be calculated as follows: Concentration * Volume of urine during that time interval (t1 to t2) Cumulative Ae will be calculated as the sum of all urine collection interval Aes *Note: The Ae at pre-dose will not be included in the calculation of cumulative Ae.*
fe	Cumulative fraction of unchanged drug excreted in urine over all time intervals, calculated as (Ae / Dose)*100 (expressed in %) *Note: The Ae at pre-dose will not be included in the calculation of fe.*
CLr	Apparent renal clearance, calculated as: $Ae_{(0-24)}$ / AUC_{0-24} on Day 1 and as $Ae_{(0-24)}$ / AUC_{τ} on Day 14 A different time interval may be used providing not all single intervals are usable. **Day 1 AUC_{0-t} may be used if AUC_{0-24} cannot be estimated, provided that the 24-hour sample was collected within 10% of nominal time.*

[1] Sources: Boxenbaum et al, 1995 and Gidal et al, 2017

8.4.6. Cerebrospinal Fluid

For cohorts 2 and 3 only, AZD4041 CSF concentrations (ng/ml) on Day 14 will be calculated as a percentage of plasma concentration.

8.4.7. CCI

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8.4.8. Data Handling

Precision for individual values will be display as follows:

- Raw data will be displayed with the same precision as received from the bioanalytical laboratory
- Concentration-related PK parameters (eg, C_{max}, AUCs) will be displayed with the same precision as the raw PK concentration data
- Parameters associated with time will be displayed with 2 decimal places
- Percentages will be displayed with 2 decimal places
- R^2 and λ_z will be displayed with 4 decimal places

8.4.9. Pharmacokinetic Statistical Methodology

All tables, figures, and listings, when appropriate, will be stratified by cohort (dose level) and by study day, as applicable.

8.4.9.1. Summary Statistics

Summary statistics of the individual AZD4041 plasma, urine, and CSF concentration data and derived parameters as well as biomarker data will be calculated for the PK population. Summary statistics will be calculated for concentration at each individual timepoint and for all PK parameters.

Concentration data will be summarized by group using the following statistics: number of observations (N), arithmetic mean (mean), standard deviation (SD), minimum (min), median, maximum (max), and coefficient of variation (CV). PK parameters will be summarized using these same statistics, as well as geometric mean and geometric mean CV.

Summary statistics will be displayed with the same precision as the individual values (Section 8.4.8), with the exception of N and CV which will be presented with 0 and 1 decimal places, respectively.

If PK parameters can only be estimated in a limited number of subjects, the following criteria will be applied for reporting descriptive statistics:

- If a PK parameter value is reportable in less than 3 subjects for a given study part and day, then SD, CV, and geometric mean CV will be considered not reportable

- If a PK parameter value is only reportable in 1 subject for a given study part and day, only minimum and maximum will be reported

8.4.9.2. Statistical Analysis

Dose Proportionality for Day 1 and Day 14

Inferential statistical analysis will be performed with SAS.

Appropriate dose–normalized PK parameters (C_{max} and appropriate AUCs) will be assessed graphically for dose-proportionality.

Natural log-transformed PK parameters (C_{max} and appropriate AUCs) will be assessed statistically for proportionality. Proportionality analysis will be done using a power model. The power model is defined as:

$$\ln(\text{PK parameter}) = \alpha + \beta \bullet \ln(\text{Dose}) + \varepsilon$$

where α is the intercept, β is the slope and ε is the normal error term. A linear model with ln-transformed dose as a continuous effect will be fitted. A point estimate and a 90% confidence interval (CI) will be derived for the slope (β).

Dose proportionality may be assessed within different dose ranges if deemed appropriate with at least three doses.

Steady State

C_{trough} will be displayed graphically and summarized descriptively by day to assess for steady state.

8.5. Planned Interim Pharmacokinetic Analyses

Interim PK analysis may be performed after each cohort to support dose selection and safety assessments.

The SAP will describe the planned interim analyses in greater detail.

8.6. 12-Lead Digital ECG Analysis

8.6.1. 12-Lead Digital ECG Statistical Methodology

From the dECG data, the following parameters will be derived:

- QTcF will be calculated as $QTcF = QT*RR^{-1/3}$, where the QT interval is in milliseconds and the RR interval is in seconds.
- Heart rate will be calculated, based on the RR interval as HR = 60/RR interval, where the RR interval is in seconds.

Calculation of derived parameters will be performed after smoothing of QT and RR data.

The dECG data will be smoothed on an individual basis before performing the derivations above and prior to calculation of any changes from baseline or descriptive statistics. For each subject it will be done as follows: the mean value of all the measurements will be taken provided that at

least 4 measurements are present and the time between the first and last is greater than 2.75 minutes or else, the smoothed value at the corresponding target time point will be set to missing.

Digital ECG results will be listed by treatment (dose level of AZD4041 and placebo) for each subject and time point and will include all individual and smoothed values of PR, RR, QRS, QT interval, and the derived values of QTcF and HR. All smoothed and derived parameters will have changes from baseline derived and presented.

Descriptive statistics will be presented by treatment (dose level of AZD4041 and pooled placebo) and time point for smoothed values and changes from baseline of smoothed values of PR, RR, QRS, QT; derived values and changes from baseline for QTcF and HR will also be included. The baseline for the dECG measurements will be the (smoothed) pre-dose assessment on Day 1.

Outliers with respect to QTcF will also be tabulated for the following categories:

- Absolute value > 450 ms and ≤ 480 ms
- Absolute value > 480 ms and ≤ 500 ms
- Absolute value > 500 ms
- Increase from baseline > 30 ms and ≤ 60 ms
- Increase from baseline > 60 ms

All calculations of dECG parameters and reporting described in this section will be performed by Altasciences.

8.6.2. CCI

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8.6.3. CCI

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8.7. Determination of Sample Size

No formal sample size analysis was performed. It is estimated that 12 subjects per cohort should be sufficient to meet the objectives of the study.

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10. APPENDIX 1: ETHICS

10.1. Institutional Review Board

This protocol and the ICF will be submitted to an IRB (or Independent Ethics Committee [IEC]) prior to initiation of the study and the study will not start until the Board has approved the documents. Notification of the Board's approval will be appended to the final report.

10.2. Ethical Conduct of the Study

This study will be conducted in compliance with the study protocol, the ethical principles that have their origins in the Declaration of Helsinki, the International Council for Harmonisation (ICH) Guideline E6 for Good Clinical Practice (GCP), the FDA GCP Code of Federal Regulations (CFR) Title 21 Part 56, the European Union (EU) Clinical Trial Directive (EC) No. 2001/20/EC, the European regulation EU 536/2014, and the Tri-Council Policy Statement (Canada).

10.3. Subject Information and Consent

Before Screening activities commence, each subject will be given a copy of the ICF to read, as well as a full explanation of the purpose of the study, the procedures to be carried out, and the potential AE(s). Once this essential information is provided to the subject and the physician in charge (or delegate) has the conviction the subject understands the implications of participating in the study, and if the subject chooses to continue the screening process, they will be requested to sign and date a properly executed ICF prior to enrolment. Subjects will be assured they may withdraw from the study at any time without jeopardizing their medical care or future study participation (for which they qualify).

Subjects will be given a signed copy of the ICF. If an amended or revised ICF is introduced during the study, each subject's further consent must be obtained.

10.4. Subject Confidentiality

Investigators and the Sponsor will preserve the confidentiality of all subjects taking part in the study, in accordance with GCP and local regulations. Subjects should be identified by a unique subject identifier on all study documents provided to the Sponsor. In compliance with Federal regulations/ICH GCP Guidelines, it is required an Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and IRB access to review the subject's original medical records for verification of study-related procedures and data. An Investigator is obligated to inform the subject that his/her study-related records will be reviewed by the above-named representatives without violating the subject's confidentiality.

10.5. Safety Oversight

Safety oversight will be under the direction of a SRC composed of individuals with the appropriate expertise, including the Principal Investigator and the Sponsor's Medical Monitor. Additional reviewers with expertise in clinical pharmacology, PK, statistics, or other clinical disciplines may be included as deemed appropriate. The SRC will review the safety, tolerability, and PK data prior to proceeding to the next dose level in a blinded fashion. After each cohort, the

SRC will assess available data to decide on the next dose level. Data from at least 10 subjects in the previous cohort must be reviewed during the safety meeting.

11. APPENDIX 2: DATA COLLECTION, RETENTION, AND MONITORING

11.1. Case Report Forms

The subject level data is entered by the site from the source document into a study specific 21 CFR Part 11 compliant electronic clinical database to accurately collect data for each subject included in a clinical trial. Screen failure data may be entered into the database at the discretion of the Sponsor, when included in the contracted scope of work.

11.2. Data Management and Processing

Data management develops documentation to define activities performed during the data management conduct of the study trial. The electronic data capture (EDC) system is the tool used to conduct all data management data cleaning activities for monitoring, data review, and queries. Data management will use a combination of automated programmed edits and manual data review listings to issue queries for nonconforming or discrepant data. Data management activities are performed in accordance with the SOPs and study-specific data management documents.

Database locking is guided by the Data Management Locking Checklist based on the concept that all site activities are complete, data are considered clean and without errors, and CRF signoff by the Principal Investigator or delegate has been completed. User access is removed as part of the locking process.

Data from the clinical database will be output as SAS® datasets. All data will be included with the final report provided to the Sponsor.

11.3. Quality Control and Quality Assurance

Designated personnel from the Quality Assurance (QA) unit(s) of each corresponding operation unit will be responsible for maintaining QA to ensure that the trial is conducted and data are generated, documented, and reported in compliance with the protocol, ICH Guideline E6 for GCP, applicable requirements as outlined in the FDA and OECD Principles of GLP, and the European Medicines Agency's (EMA) *Reflection paper for laboratories that perform the analysis or evaluation of clinical trial samples* (EM/INS/GCP/532137/2010).

11.4. Record Retention

All essential documents and records will be maintained by the clinical site in accordance with and for the period specified in the applicable regulatory requirement(s) (FDA CFR 312.57 [C]).

11.5. Monitoring of the Study

The Sponsor or its representative may monitor the study in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation, and discussion of the conduct and progress of the study. The clinical site will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct and/or virtual access, where possible, to source data/documents.

12. APPENDIX 3: ADMINISTRATIVE PROCEDURES

12.1. Liabilities

It is the Sponsor's responsibility to guarantee sufficient insurance coverage should any serious events or deaths result, either directly or indirectly, from the execution of the present protocol.

12.2. Adherence to Protocol

Excluding an emergency in which proper treatment is required for the protection, safety, and wellbeing of the study subjects, the study will be conducted as described in the approved Protocol and performed according to ICH/GCP and the applicable regulatory requirements. Any deviation from the Protocol will be recorded and explained.

If amendments to the Protocol and/or amendments or revisions to the ICF are required, the modifications will be documented and submitted to an IRB for approval.

12.3. COVID-19 Response Plan

Regulatory authorities have recognized that the COVID-19 pandemic may impact the conduct of clinical trials of medical products. Challenges may arise, for example, from quarantines, site closures, travel limitations, interruptions to the supply chain for the IP(s), or other considerations if site personnel or study participants become infected with COVID-19. These challenges may lead to difficulties in meeting protocol-specified procedures, including administering or using the IP(s) or adhering to protocol-mandated visits and laboratory/diagnostic testing. To accommodate these challenges and mitigate safety risks associated with COVID-19, protocol modifications may be required which include (and are not limited to):

- Conducting the study in multiple (smaller) subject groups
- Altering the timing of study procedures and subject confinement
- Modification of standard inclusion or exclusion criteria

The exact mitigations will be documented in the study Risk Assessment and Mitigation Plan.

Additional health checks (eg, COVID-19 testing, body temperature monitoring) may be performed during the trial, even if not planned within the protocol.

12.4. Statement of Investigator

The form "Qualified Investigator Undertaking" will be signed by an Investigator responsible for the medical decisions and care provided to the subjects (being also referred to as the "Qualified Investigator") prior to the commencement of his/her responsibilities with respect to the clinical trial, as required by FDA regulations. The undertaking form will be maintained with the trial records and will be made available upon request.

12.5. Delegation of Investigator Duties

An Investigator will ensure all personnel involved in the trial are adequately qualified and informed about the protocol, any amendments to the protocol, the study treatment, and their trial-related duties and functions.

An Investigator will maintain a list of Sub-investigator(s) and other appropriately-qualified persons to whom he/she delegates significant trial-related duties.

Should an Investigator delegate the supervision of the IP administration to a designated person, this individual must have the appropriate professional-legal qualifications and certifications. An Investigator should also ensure key staff and personnel have the appropriate medical qualifications to effectively conduct or supervise any potential resuscitation procedures.

12.6. Premature Termination or Suspension of a Study

The Sponsor or its representative may terminate the study at any time for scientific or corporate reasons.

If the trial is prematurely terminated or suspended for any reason, the clinical site or an Investigator (or delegate) should promptly inform the trial subjects, assure appropriate therapy and follow-up for the subjects, and inform the regulatory authority(ies) when required.

13. APPENDIX 4: PROTOCOL REVIEW AND APPROVALS

TITLE: A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, and Pharmacokinetics of Multiple Ascending Doses of AZD4041 in Healthy Adult Subjects

I have carefully read this study protocol and agree it contains all necessary information required to conduct this study. I agree to conduct the study according to this protocol and in accordance with GCP and the applicable regulatory requirements.

Eric Sicard

Principal Investigator Name (Please Print)

CCI [redacted]

Principal Investigator Signature
Altasciences Company Inc.

CCI [redacted]

Date (yyyy/mm/dd)

TITLE: A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Safety, Tolerability, and Pharmacokinetics of Multiple Ascending Doses of AZD4041 in Healthy Adult Subjects

On behalf of the Sponsor, I am aware of, and agree to comply with, all the procedures contained within this protocol.

PPD

PPD

Date (yyyy/mm/dd)

PPD
PPD
PPD, Clinical Development, Neuroscience
Neuroscience Biopharmaceuticals Research and Development
Sponsor's Representative
AstraZeneca

14. APPENDIX 5: LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
AV	Atrioventricular
BMI	Body Mass Index
BP	Blood Pressure
BPM	Beats per Minute
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CI	Confidence Interval
CNS	Central Nervous System
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRU	Clinical Research Unit
CSF	Cerebrospinal Fluid
CSP	Clinical Study Protocol
CSR	Clinical Study Report
C-SSRS	Columbia Suicide Severity Rating Scale
CV	Coefficient Of Variation
CCI	[redacted]
DECG	Digital Electrocardiogram
DORA	Dual Orexin Receptor Antagonist
DSM	American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ECLYSIS©	User-Interactive, Modular Computer-Based System for dECG Data Processing, Analysis and Measurement of ECG Intervals and Wave Amplitudes, Exports and Reports, used by the AstraZeneca ECG Centre
EDC	Electronic Data Capture
EEG	Electroencephalogram

EMA	European Medicines Agency
CCI	CCI
EU	European Union
FDA	Food and Drug Administration
FIH	First-in-Human
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GPCR	G Protein Coupled Receptors
HBSAG	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HERG	Human Ether-A-Go-Go-Related Gene
HIV	Human Immunodeficiency Virus
HR	Heart Rate
CCI	CCI
IC_{50}	Half Maximal Inhibitory Concentration
IC_{90}	Ninety Percent (90%) Inhibitory Concentration
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IP	Investigational Product
IRB	Institutional Review Board
IU	International Unit
IVCD	Intraventricular Conduction Delay
KG	Kilogram
L	Litre
LH	luteinizing Hormone
LLOQ	Lower Limit of Quantitation
LN	Napierian Log Transformation
MAD	Multiple Ascending Dose
MEDDRA	Medical Dictionary for Regulatory Activities
MG	Milligram

MIN	Minute
ML	Millilitres
MMHG	Millimetre of Mercury
MTD	Maximum Tolerated Dose
N/A	Not Applicable
NCA	Non-Compartmental Analysis
NCI-CTCAE	National Cancer Institute's Common Terminology Criteria for Adverse Events
NG	Nanograms
NIDA	National Institutes on Drug Abuse
NOAEL	No-Observed-Adverse-Effect Level
NREM	Non-Rapid Eye Movement
CCI	[redacted]
OECD	Organization for Economic Co-Operation and Development
OTC	Over-The-Counter
OUD	Opioid Use Disorder
OX1/2	Orexin Receptor
PBMC	Peripheral blood mononuclear cells
PBPK	Physiologically based PK
PD	Pharmacodynamics
PH	The Logarithm, On the Base 10, of the Reciprocal of the Hydrogen Ion Concentration
PK	Pharmacokinetic
PR	Time Between P and R Wave
PR (PQ)	Prolongation of the PR (PQ) interval
PT	Preferred Term
QA	Quality Assurance
QD	Daily Dosing
QRS	ECG Interval Measured from the onset of QRS Complex to the J-Point
QT	QT Interval is the time from the start of the Q Wave to the end of the T Wave, Time Taken for Ventricular Depolarization and Repolarization
QTC	Qt Interval Corrected for Heart Rate
QTCF	Qt Interval Corrected for Heart Rate Using Fridericia's Correction Formula
RR	Time Elapsed Between Two Successive R-Waves of the QRS Signal on the ECG
SAD	Single Ascending Dose
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SMP	Safety Management Plan
SRC	Safety Review Committee
ST	ST Segment of the ECG
SUSAR	Suspected Unexpected Serious Adverse Reaction
T	T Wave of the ECG
TBL	Total Bilirubin
TEAE	Treatment-Emergent Adverse Event
CCI	CCI
ULN	Upper Limit of Normal
VPC	Ventricular Premature Contractions
WHO-DDE	World Health Organization Drug Dictionary Enhanced

15. APPENDIX 6: CLINICAL LABORATORY EVALUATIONS

Clinical Laboratory Test Panel	Description
General biochemistry:	Alanine aminotransferase, albumin, alkaline phosphatase, bilirubin total and unconjugated, C-reactive protein, chloride, creatinine, creatine kinase, gamma glutamyl transferase, protein total, glucose (fasting), calcium, potassium, magnesium[a], and sodium
Endocrinology:	Follicle stimulating hormone[de], oestradiol[a,b], thyroid stimulating hormone[a], T4[a,c], luteinising hormone[d], testosterone[d], and inhibin B[d]
Haematology:	White cell count with differential (absolute values of neutrophil, lymphocyte, monocyte, eosinophil, and basophil), red cell count, haemoglobin, haematocrit, mean corpuscular volume, and platelet count
Serology:	Human immunodeficiency virus Ag/Ab Combo, hepatitis B surface antigen, hepatitis C virus, and Severe Acute Respiratory Syndrome Coronavirus-2
Urinalysis:	Colour, clarity, specific gravity, pH, leukocyte, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen. Microscopic examination will only be performed if the dipstick test is outside of the reference range for leukocyte, blood, nitrite, or protein
Urine drug screen:	Amphetamines, barbiturates, cannabinoids, cocaine, cotinine, opiates, and phencyclidine
Pregnancy test (females only):	Serum pregnancy test
Alcohol screen:	Alcohol breathalyser or urine screen

a. At Screening only
b. Postmenopausal women only
c. Reflex only (if TSH is abnormal)
d. Males only at Day -1, Day 1 and Day 14
e. FSH for females at screening only

16. APPENDIX 7: CONTRACEPTION GUIDANCE

Females

Only females of non-childbearing potential will be considered for eligibility in this study. Females of non-childbearing potential are defined as those who are surgically sterile (ie, women who have had a hysterectomy, bilateral ovariectomy [oophorectomy], bilateral salpingectomy, or bilateral tubal ligation) or those who are postmenopausal (defined as 12 months or more with no menses without an alternative medical cause). (See Inclusion Criterion# 4, Section 4.1)

Males

Non-sterilized males who are sexually active with a female partner of childbearing potential must use a condom for the duration of the treatment period and for no less than 120 days after the last administration of study drug. As a male condom is not considered to constitute a highly effective contraception method, it is recommended that female partners of a male study subject also use a highly effective method of contraception throughout the study, including the protocol-specified follow-up period. A highly effective method of contraception is defined as one that results in a low failure rate (ie, less than 1% per year) when used consistently and correctly. The acceptable methods of contraception are described in Table 16-1.

Table 16-1. Highly Effective Methods of Contraception

Barrier methods	Hormonal methods
Intrauterine device Intrauterine hormone-releasing system (UIS)[a] Bilateral tubal occlusion Vasectomized partner[b] Sexual abstinence[c]	Combined (oestrogen and progestogen containing hormonal contraception) • Oral (combined pill) • Injectable • Transdermal (patch) Progestogen-only hormonal contraception associated with inhibition of ovulation[d] • Injectable • Implantable • Intravaginal

[a] This is also considered a hormonal method.
[b] With appropriate evidence of post-vasectomy surgical success.
[c] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of the study and if it is the preferred and usual lifestyle of the subject.
[d] Progestogen-only hormonal contraception, where inhibition of ovulation is not the primary mode of action (eg, minipill), is not accepted as a highly effective method).

17. APPENDIX 8: STUDY SPECIFIC RESTRICTIONS

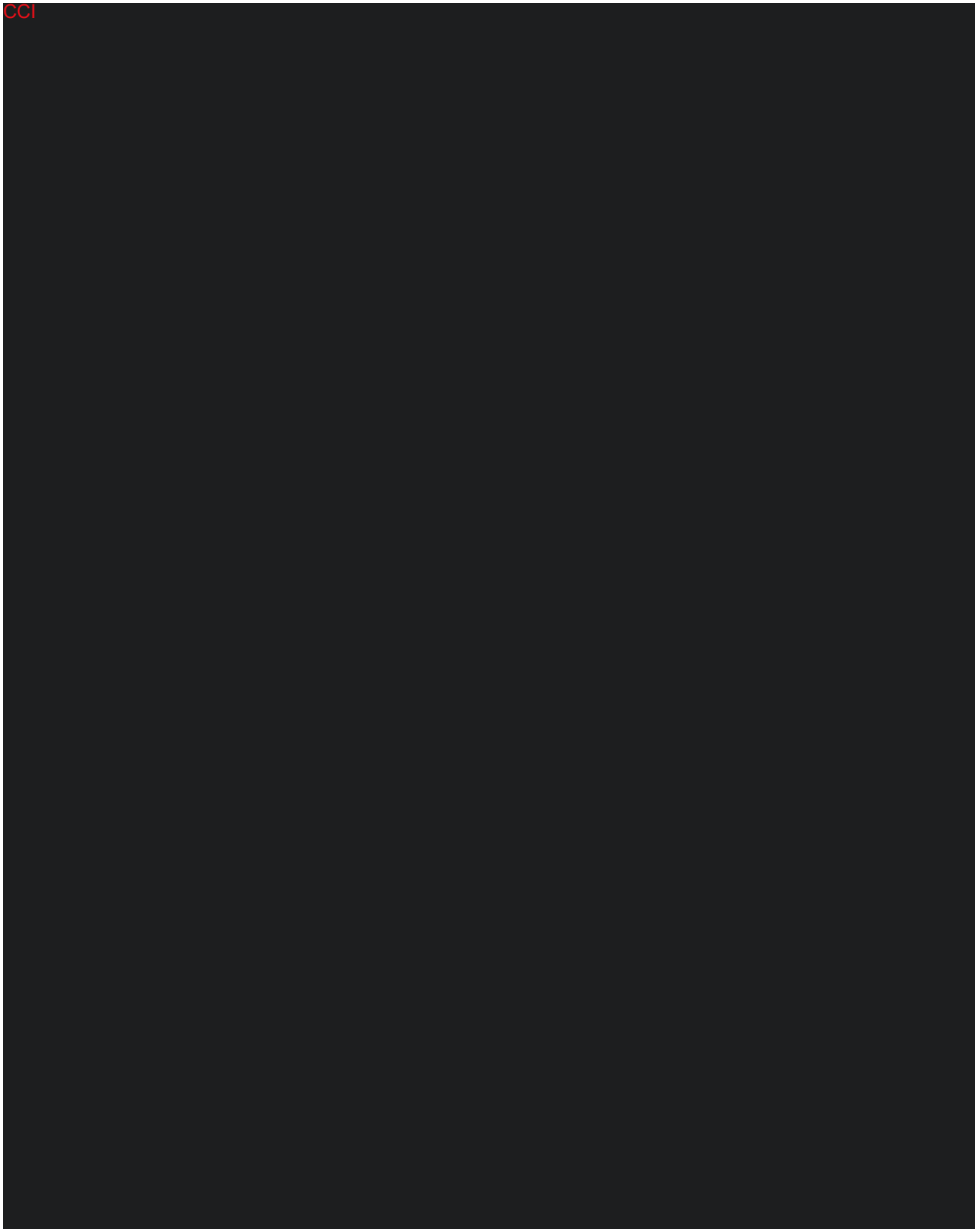
Table 17-1. Restricted medications/products

Medication/class of drug:	Usage (including limits for duration permitted and special situation in which it is allowed)
Antacids Analgesics other than paracetamol/acetaminophen Herbal remedies Megadose (20 to 600 times recommended daily dose) vitamins Megadose minerals	In the 2 weeks prior to administration of IP, or longer if the medication has a long half-life, use of any of these medications constitutes a basis for exclusion from the study.

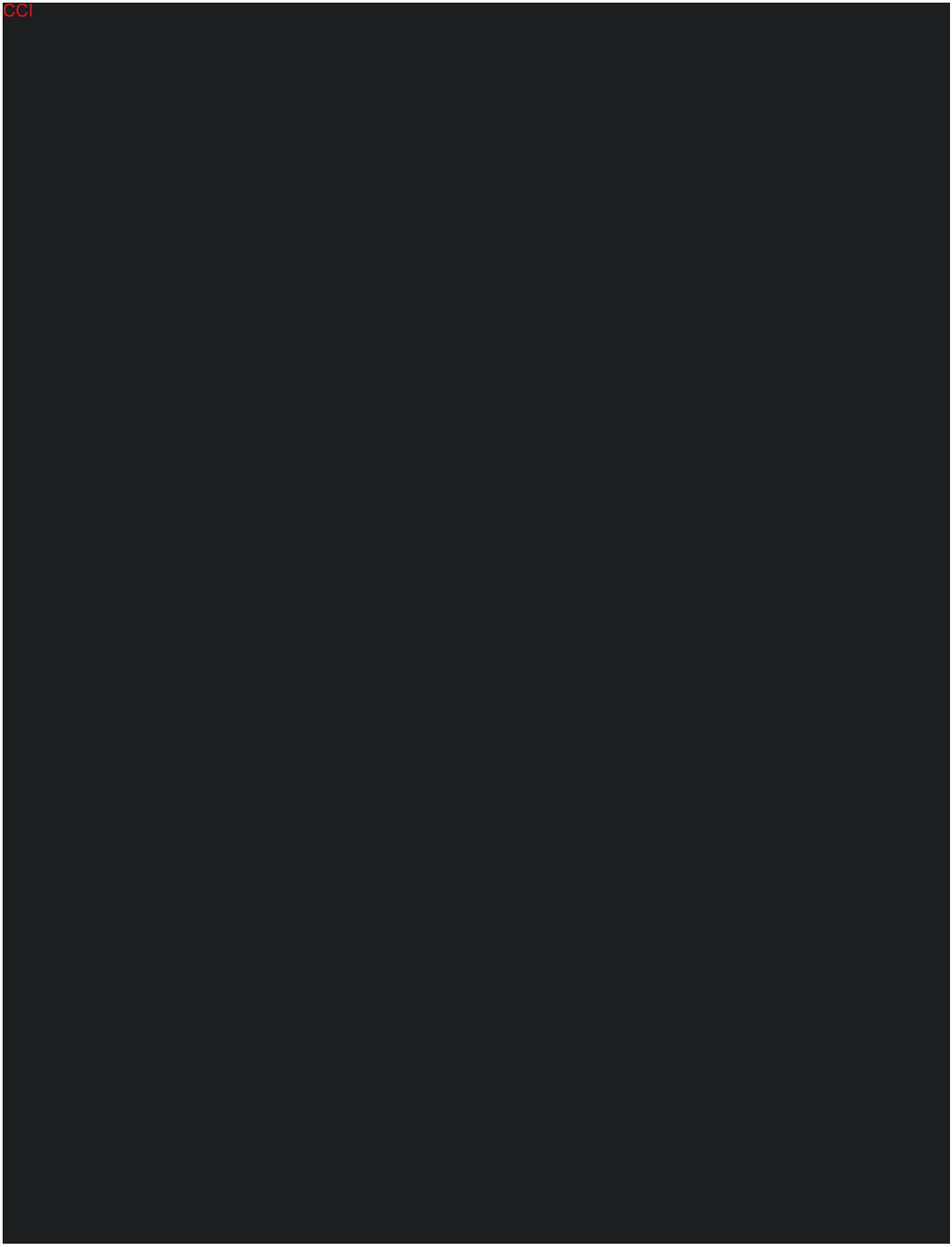
Table 17-2. Prohibited medications

Prohibited medication/class of drug:
Any other investigational product (other than provided in this study)
Any medication with enzyme inducing properties such as St John's Wort
Hormone replacement therapy
CCI [redacted]
Vaccination with COVID-19 vaccine less than 14 days prior to the proposed date of randomization

18. APPENDIX 9: COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) – BASELINE/SCREENING VERSION



Source Document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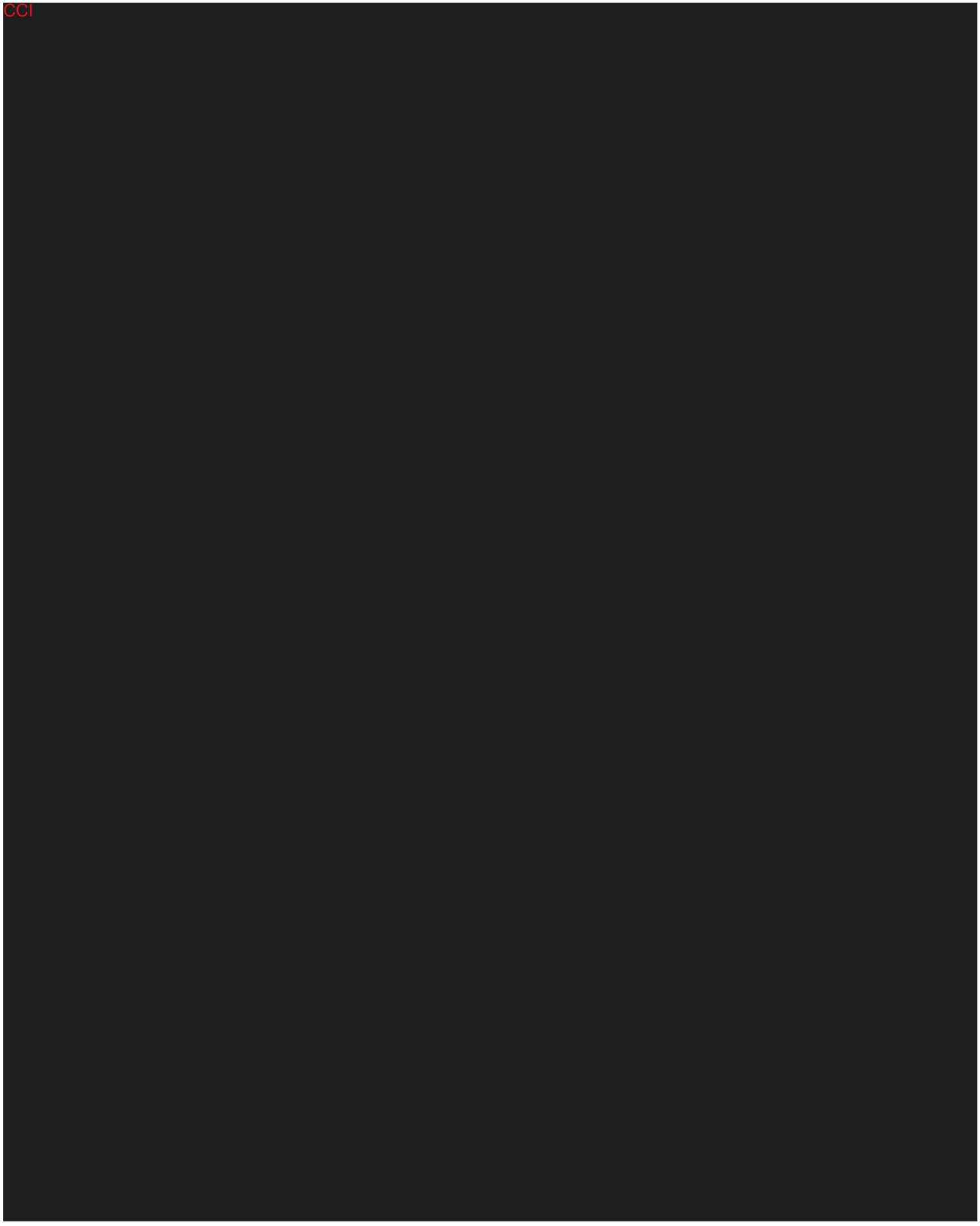
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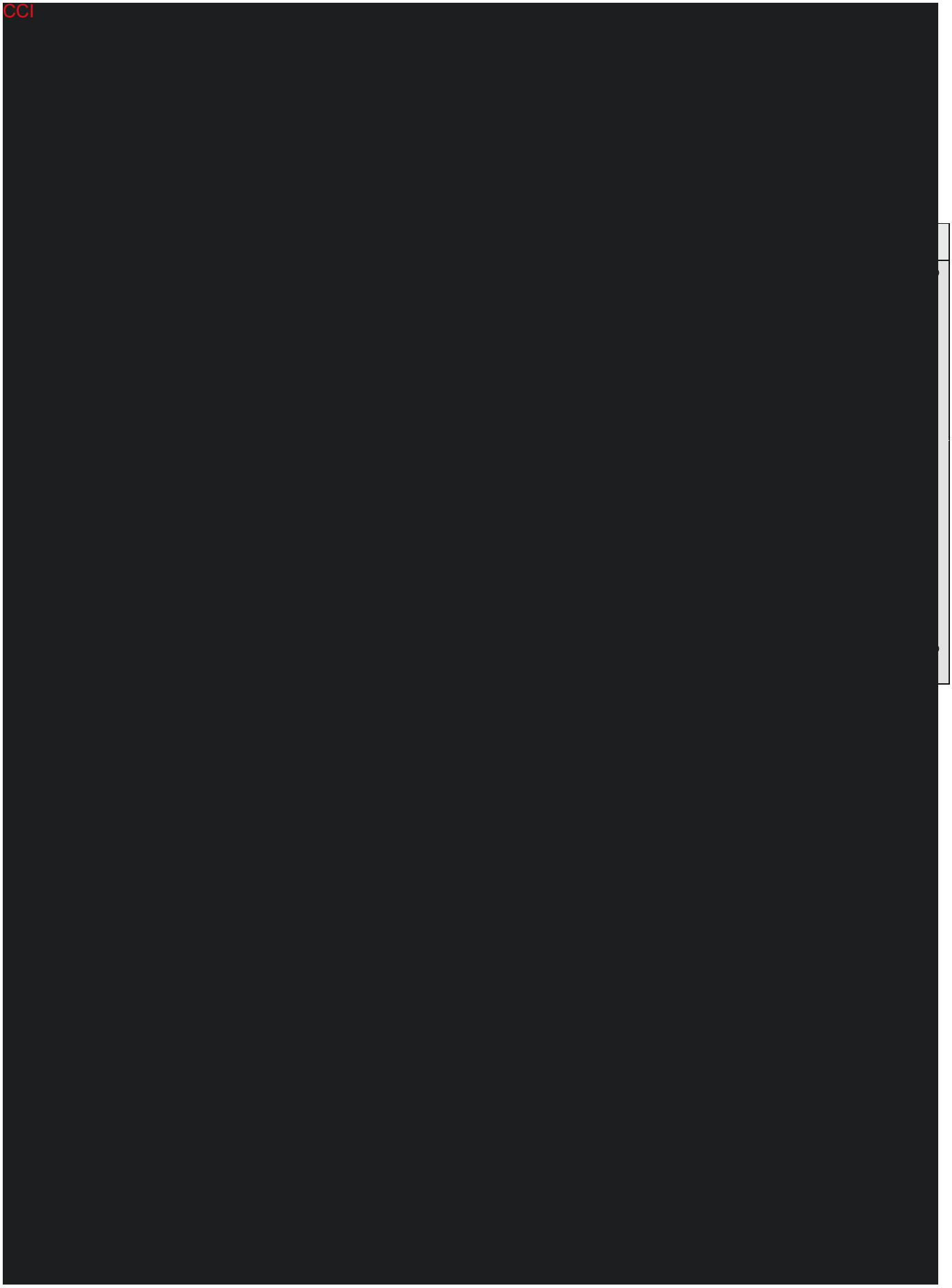
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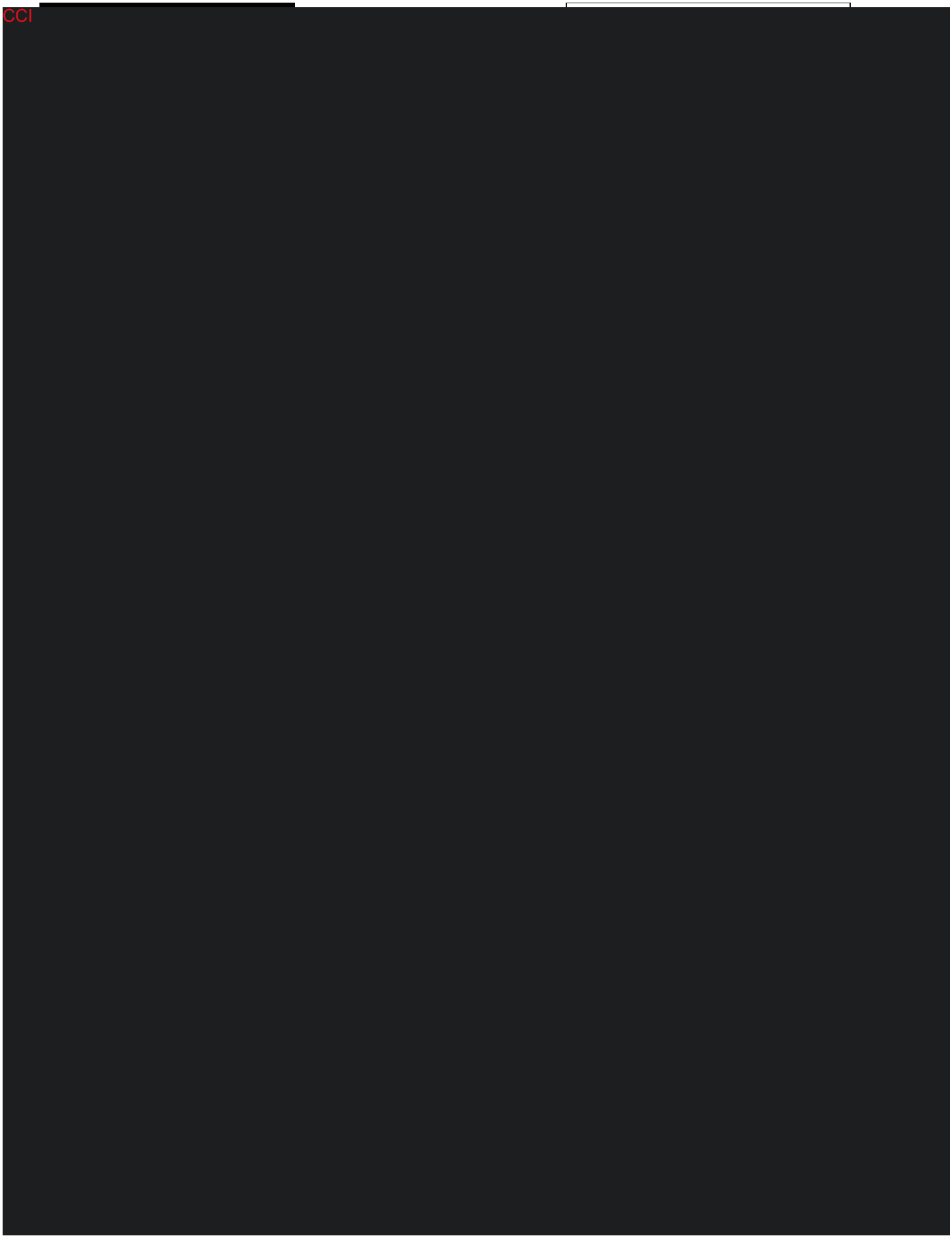
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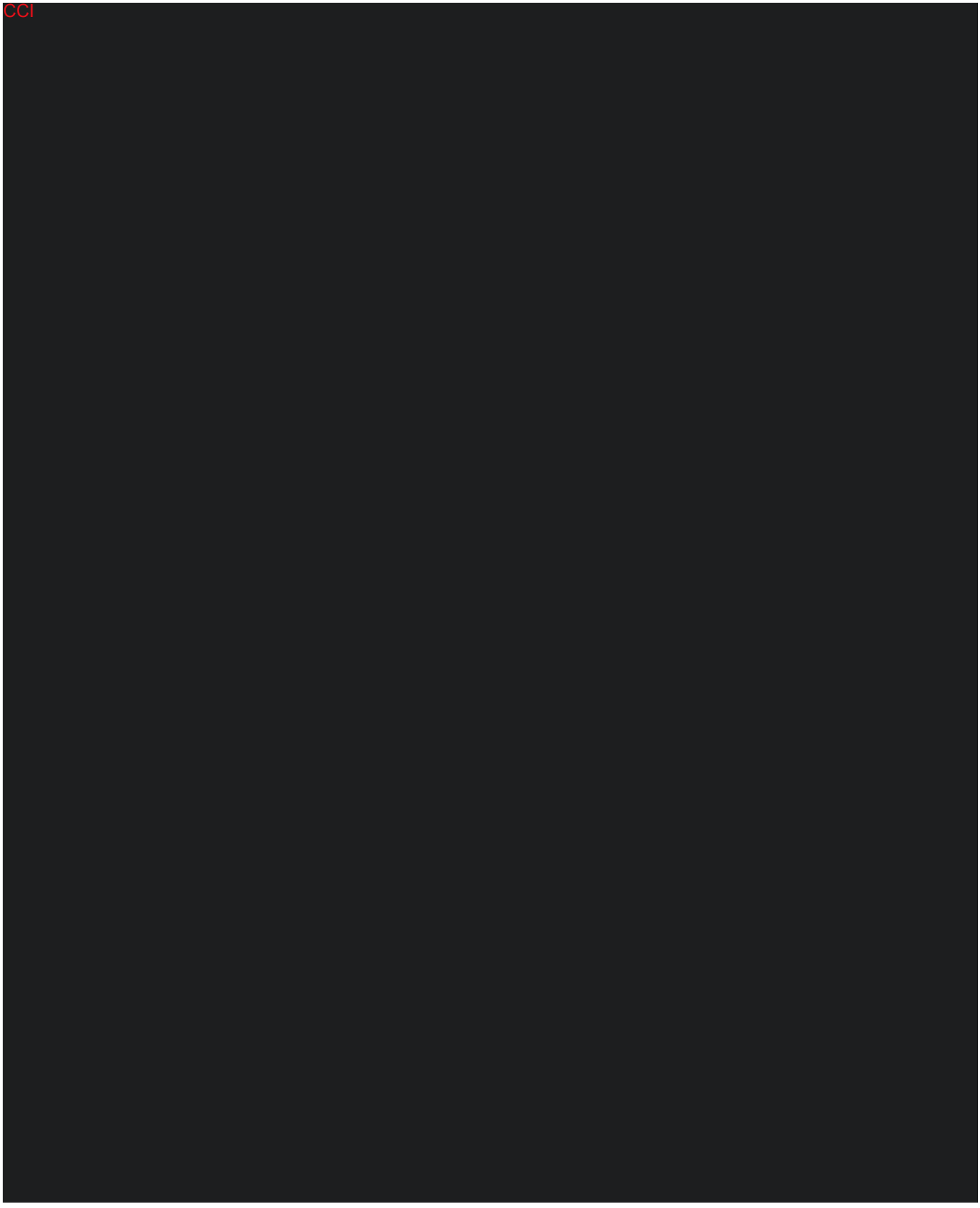
19. APPENDIX 10: COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) – SINCE LAST VISIT VERSION



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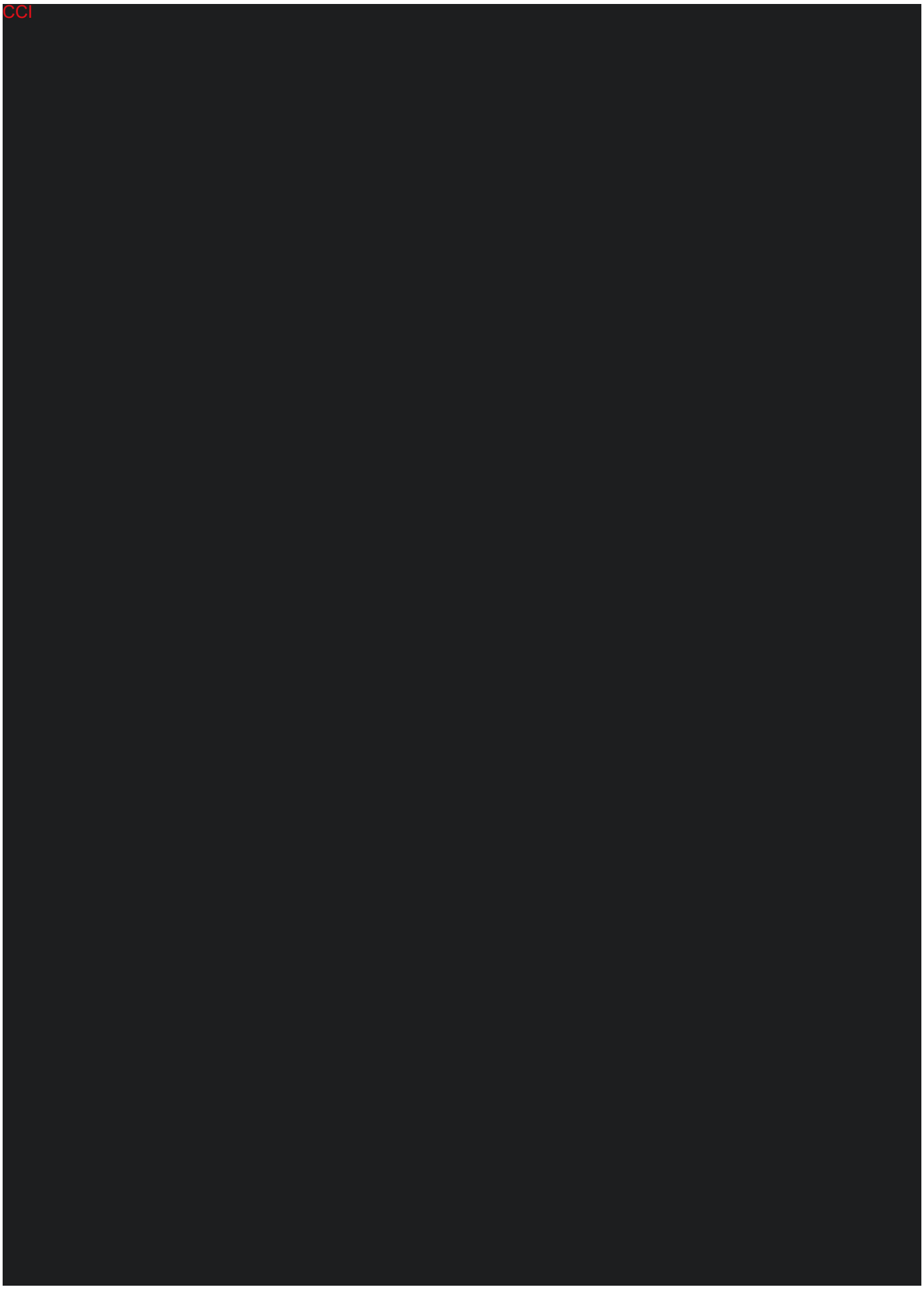
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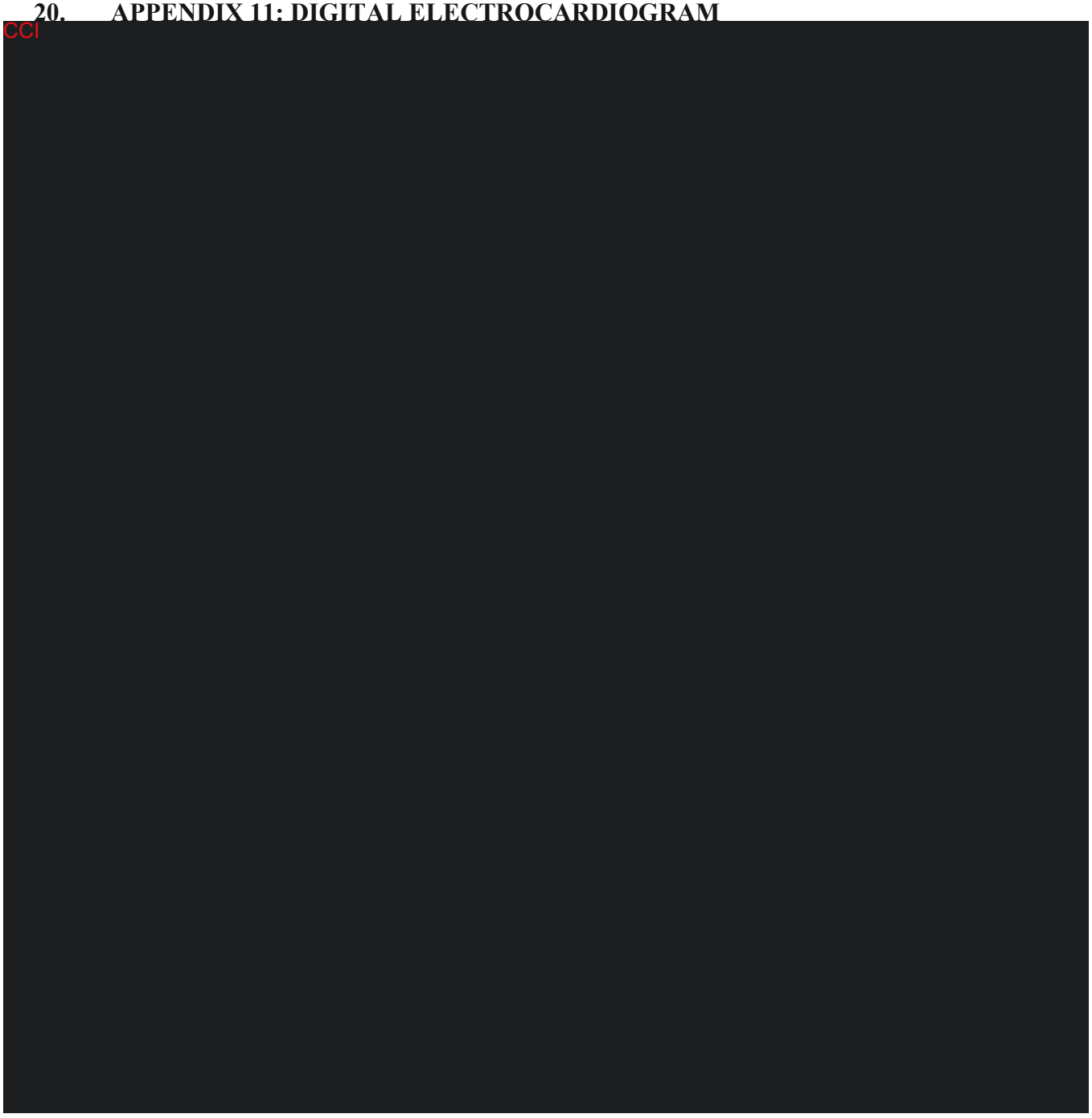
20. APPENDIX 11: DIGITAL ELECTROCARDIOGRAM

CCI



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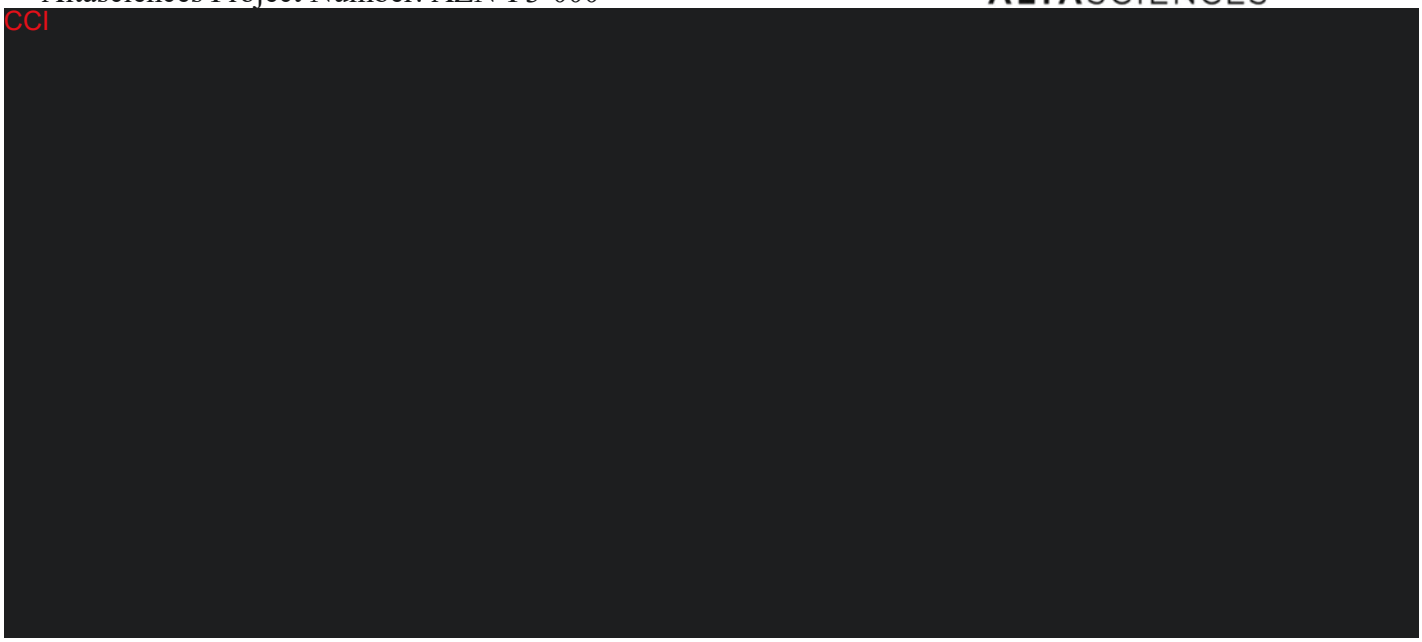


21. APPENDIX 12: PHYSICAL EXAMINATION

Physical Examination Form

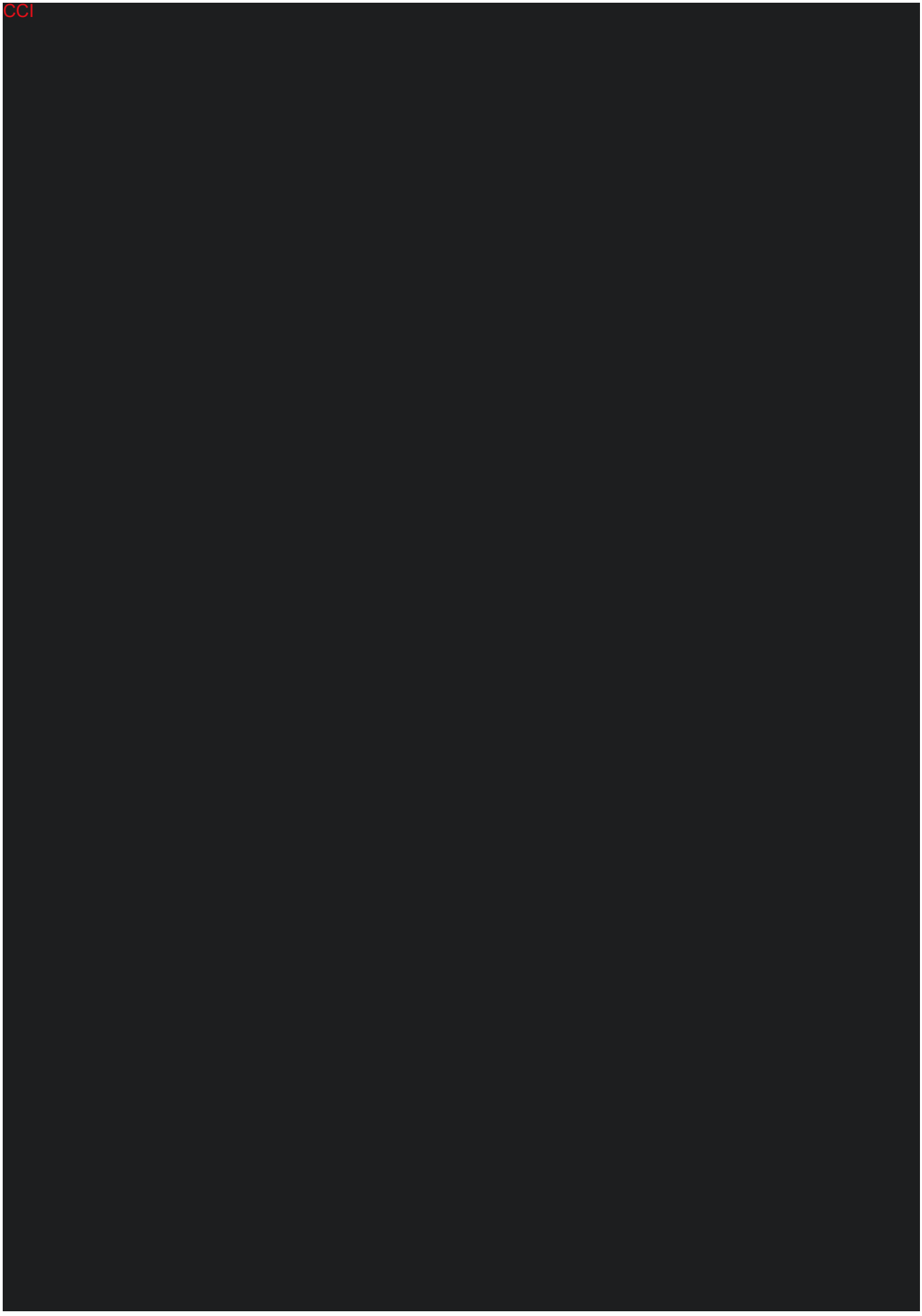
CCI

[redacted]

22. APPENDIX 13: NEUROLOGICAL EXAMINATION

CCI

[redacted]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