CLINICAL STUDY PROTOCOL

Randomized, double-blind, placebo-controlled, two way crossover, single centre study evaluating the acute and chronic effect of clonazepam on cognitive tests and patient-reported outcome measures in patients with ARID1B-related intellectual disability

Short Title:	Clonazepam in ARID1B Evaluation (CARE study)
Version:	6
Date:	21-January 2022
CHDR number:	CHDR1939
Toetsing Online number:	NL71395.056.19
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PROTOCOL VERSION 6, AMENDMENT 5, 11-Jan-2022

Change	Rationale	Justification & Classification	Changed Document(s), Section
Update window between COVID- vaccination and study days to 3 days	Clonazepam is a registered drug, AEs between clonazepam and COVID-vaccination can easily be differentiated, and no interaction is expected	Substantial	- Protocol - ICD
Removed PPI assessment	PPI assessment is considered too invasive for this patient population	Substantial	- Protocol - ICD
Removed inclusion criteria regarding necessity in being able to perform 5 out of 6 NeuroCart assessments	Update after removal of PPI. Valuable data is still obtained if a subject can perform less than 5 NeuroCart assessments	Substantial	- Protocol
Update study team	Addition of Cecile Berends as project leader to the study team	Non-substantial	- Protocol - ICD

PROTOCOL VERSION 5, AMENDMENT 4, 09-Sep-2021

Change	Rationale	Justification & Classification	Changed Document(s), Section
Description of placebo description	Update for incorrect description of placebo study medication	Substantial	- Protocol

PROTOCOL VERSION 4, AMENDMENT 3, 01-Jul- 2021

Change	Rationale	Justification & Classification	Changed Document(s), Section
Change LUMC pharmacist	Personnel change	Non-substantial	- Protocol
Change LUMC contact person	Personnel changes	Non-substantial	- Protocol
Change project leader /co-investigators	Personnel change	Substantial	- Protocol, ICD
Change is independent physician	Update with CHDR policy	Substantial	- Protocol, ICD
Included pre-pulse inhibition assessment in part B	Additional translational endpoint based on preclinical evidence	Substantial	- Protocol, ICD
Removed early PK assessments in part B	Futile timepoints based on interim analysis part A	Substantial	- Protocol
Changed dosing regimen during first 6 days, including dose escalation decision	Safety concerns raised by pediatric neurologist	Substantial	-Protocol, ICD
COVID risk assessment part B	To protect trial participants and trial staff during the SARS-CoV-2 outbreak	Substantial	- Protocol

PROTOCOL VERSION 3, AMENDMENT 2, 22-Jul-2020

Change	Rationale	Justification & Classification	Changed Document(s), Section
Additional objective part A: Direct comparison linear- and nonlinear regression	Additional scientific value	Substantial	- Protocol
Interim analysis part A removed from protocol.	Change in study objective part A.	Substantial	- Protocol

PROTOCOL VERSION 2, AMENDMENT 1, 24-Jun-2020

Change	Rationale	Justification & Classification	Changed Document(s), Section
Abbreviations added to List of Abbreviations	To clarify abbreviations used in this amendment	Non-substantial	- Protocol List of Abbreviations
COVID-19 risk assessment added per CCMO guidance	To protect trial participants and trial staff during the SARS-CoV-2 outbreak	Substantial	- Protocol Appendix 1 - ICF
COVID-19 risk mitigation strategies added per CCMO guidance	To protect trial participants and trial staff during the SARS-CoV-2 outbreak	Substantial	- Protocol Appendix 1 - ICF
Upper limit of inclusion criterion age and BMI are temporarily set to <70 years and ≤ 30 kg/m2.	To temporarily not enroll subjects with risk factors for a COVID-19 infection	Substantial	- Protocol Appendix 1 - E3. Advertising document
Change to medication labels	Wrong lay-out used during initial submission	Non-substantial	- D3. Medication labels
Replaced Ria Kroon and Emilie Jonxis with Ard Vink	Personnel change compared to initial admission.	Non-substantial	- Protocol contact details and signature page

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Study Title

Randomized, double-blind, placebo-controlled, two way crossover, single centre study evaluating the acute and chronic effect of clonazepam on cognitive tests and patient-reported outcome measures in patients with ARID1B-related intellectual disability

I acknowledge accountability for this protocol in accordance with CHDR's current procedures.

This is an investigator-initiated trial, CHDR acts as both the investigator as well as the sponsor of the study, with all applicable responsibilities.

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Principal investigator

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I acknowledge responsibility for this protocol in accordance with CHDR's current procedures.

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LIST OF ABBREVIATIONS

ABC	Aberrant Behaviour Checklist
ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee; in Dutch, ABR = Algemene Beoordeling en Registratie
AE	Adverse Event
ANOVA	Analysis of Variance
aPTT	activated partial thromboplastin time
ARID1B	AT-rich interactive domain-containing protein 1B
AUC	Area under the concentration – time curve
AUC_{inf}	Area under the concentration – time curve from time zero to infinity
AUC_{last}	Area under the concentration – time curve from time zero to time of last measurable concentration
AUC_{tau}	Area under the concentration – time curve between consecutive dosing
AVG	Algemene Verordening Gegevensbescherming
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
bpm	beats per minute
CA	Competent authority (also CCMO)
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CHDR	Centre for Human Drug Research
CGI-I	Clinician's Global Impression of Improvement
CGI-S	Clinician's Global Impression of Severity
CI	Confidence Interval
CLR	Renal clearance
C_{max}	Maximum concentration
C_{trough}	Concentration immediately prior to dosing at multiple dosing
CRF	Case Report Form
CV	Coefficient of variation
DSMB	Data Safety and Monitoring Board
EC	Ethics Committee (also Medical Research Ethics Committee (MREC); in Dutch: Medisch Ethische Toetsing Commissie (METC).
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbant assay
EMA	European Medicines Agency
EU	European Union
GABA	Gamma-Aminobutyric acid
GCP	Good Clinical Practice
GGD	Municipal health service
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee

IMPD	Investigational Medicinal Product Dossier
INR	International Normalized Ratio
i.v.	Intravenous(ly)
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
LUMC	Leiden University Medical Center
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NONMEM	Nonlinear Mixed Effects Modeling
PD	Pharmacodynamic
PK	Pharmacokinetic
RIVM	Dutch Centre for Infectious Disease Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-COV-2	Coronavirus
SD	Standard Deviation
SEM	Standard Error of the Mean
SOC	System Organ Class
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
t.i.d.	ter in diem / three times a day
$t_{½}$	Terminal Elimination Half-life
t_{lag}	Absorption lag time
t_{max}	Time to attain C_{max}
ULN	Upper Limit of Normal
V_z/F	Apparent volume of distribution during the terminal elimination phase after extravascular administration
WHO	World Health Organization
WBP	Personal Data Protection Act; in Dutch: Wet Bescherming Persoonsgevens
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen.

PROTOCOL SYNOPSIS

Title

Randomized, double-blind, placebo-controlled, two way crossover, single centre study evaluating the acute and chronic effect of clonazepam on cognitive tests and patient-reported outcome measures in patients with ARID1B-related intellectual disability.

Short Title

Clonazepam in ARID1B Evaluation (CARE study)

Principal investigator & Trial Site

R. (Rob) Zuiker, MD, PhD, Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands

Background & Rationale

Intellectual disability (ID) is one of the most frequent neurodevelopmental disorders and affects up to 2-3% of the population [1, 2]. Heterozygous truncating mutations in ARID1B, leading to haplo-insufficiency, are found to be the most frequent cause of ID, explaining about 1% of the patients [3, 4]. Besides non-specific ID, ARID1B mutations have been identified in a high proportion (>50%) of patients with a clinical diagnosis of Coffin-Siris syndrome [5-7]. This syndrome was first described in 1970 as a combination of facial features, hypertrichosis, and fifth digit nail hypoplasia. Other symptoms that have been reported are epilepsy, feeding disorders, congenital heart disease, speech impairment and agenesis of the corpus callosum [8, 9]. Although there are no good estimates of incidence and prevalence, ~50 patients with ARID1B mutations are currently known in the LUMC's expertise center. Worldwide the largest group of described patients consists of 143 [8]. Except for supporting therapies such as physiotherapy and speech therapy, no treatment options are available for ARID1B patients.

In the past two years three Arid1b-haploinsufficient ($Arid1b^{+/-}$) mice models have been generated and investigated. $Arid1b^{+/-}$ mice display increased anxiety, reduced memory, learning and social interaction [10-13]. A study with mouse brain tissue revealed that the amount of GABAergic inhibitory interneurons, was reduced [11]. This prompted investigation of the ratio of inhibitory and excitatory synapses, which indeed showed a reduction of GABAergic inhibitory synapses [11]. Further analysis showed increased apoptosis in the progenitor population in the medial ganglionic eminence, which is the likely cause of the reduced numbers of inhibitory interneurons since it is the origin of 60-80% of cortical interneurons. Subsequently investigators decided to administer a single treatment of 0.0625 mg/kg i.p. clonazepam in adult $Arid1b^{+/-}$ mice, leading to restored recognition and social memory, and reduced anxiety-like behavior, but not depression-like behavior symptoms 30-60 min after treatment [11]. To provide further support for the involvement of the GABAergic system, the LUMC department of Clinical Genetics, generated ARID1B haplo-insufficient ($ARID1B^{+/-}$) and ARID1B knockout ($ARID1B^{-/-}$) induced pluripotent stem cells and noticed that the GABAergic interneurons cell number sharply decreased with decreasing functioning copies of ARID1B (unpublished data). This all has led to the hypothesis that haploinsufficiency of ARID1B leads to fewer interneuron progenitors and GABAergic inhibitory interneurons, and an imbalance in excitatory/inhibitory synapses, whilst providing stimulation to the GABAergic system restores this balance and at least partially restores the consequences on behavior and cognition.

Clonazepam (Rivotril) is a nonselective benzodiazepine with a half-life of 20-40 hours, and a tmax of 1–4 hours after oral dosing, and is currently used for treatment of seizures in children and adults

and occasionally for behavioral problems. Recently an exploratory study (CHDR1828) was conducted to investigate the neurocognitive phenotype in 12 ARID1B patients aged 2-31 years and 12 age-matched controls. Based on previous experience and literature we selected a subset of tests of the NeuroCart® (CHDR1828). Important results of these study were that tests for animal fluency, eye movements (saccadic peak velocity and smooth pursuit), body sway, finger tapping and adaptive tracking could be performed adequately by ARID1B patients. Most of these biomarkers have previously been evaluated in benzodiazepines [14, 15]

Plasma sampling is not desirable in the ARID1B patient population, therefore we will first establish the relations between clonazepam plasma concentrations and saliva in healthy volunteers**, Part A**. Such correlation was previously established for diazepam [16]. The study will continue with **Part B**, a two way crossover study evaluating the effect of clonazepam on cognitive tests in 20 patients with ARID1B syndrome.

Study objectives

- To test the hypothesis that clonazepam administration has acute beneficial effects compared to placebo on neurocognitive tests.
- To test the hypothesis that multiple-doses clonazepam has beneficial effects compared to placebo on behaviour and cognitive function in ARID1B patients as measured by the ABC, and CGI-I scale.
- Assess safety and tolerability of clonazepam in ARID1B-patients.
- To explore the feasibility of using saliva PK to predict plasma PK
- To assess the potential of at-home neurocognitive tests for the evaluation of treatment effects in children with neurodevelopmental disorders.
- To assess and compare the difference in predictive capability between linear and nonlinear (NONMEM) regression of the saliva:plasma relationship.

Study design

Part A. Open label study in 20 healthy volunteers where pharmacokinetics of clonazepam will be measured in paired plasma and saliva samples. Detailed scheduling can be found in **Table 1**.

Part B. Two-way cross over, placebo-controlled randomized study in patients with ARID1B-related intellectual disability. Each period will be 22 days and periods will be separated by a three-week washout. Patients will be monitored in the clinic for 5 hours for safety, PK, and biomarker effects on day 1 and 22 in both periods. Between those days, patients remain at home and fill in questionnaires and wear digital technologies. Detailed scheduling can be found in **Table 2**.

Investigational drug

Part A

- Clonazepam (Rivotril) droplets for oral use, solution 2.5 mg/ml, dissolved in lemonade. Dose: 0.5 mg or 1 mg, both administered to 50% of subjects.

Part B

- Active medication. Clonazepam (Rivotril) droplets for oral use, solution 2.5 mg/ml, dissolved in lemonade, tea or juice.
- Placebo: dissolved in lemonade, tea or juice

Study drug or placebo during part B will be administered to the subjects as follows:

- Day 1-3: Starting dose is 0.005 mg/kg, twice daily (max 0.5 mg, twice daily)
- Day 4-6: 0.01 mg/kg, twice daily (max 0.5 mg, twice daily)
- Day 7-22: 0.015 mg/kg, twice daily (max 0.5 mg, twice daily)

Patients will receive a single starting dose at their first visit of 0.005 mg/kg (max 0.5 mg). Patients will be monitored for 5 hours for safety, PK, and biomarker effect. If the dose on day 1 is well tolerated, patients will take the same dose before bedtime, and continue this regimen as described.

After three days, the (adverse) effects will be evaluated by phone. If the dose on day 1 is well tolerated, the dose will be increased to 0.01 mg/kg/day. After another three days, the effects will be evaluated again, after which the dose will be increased to 0.015 mg/kg/day. During these dosing adjustments, the maximum dose will not exceed 0.5 mg, twice daily. In case of relevant adverse events during any of the dose evaluations, the daily dose will be halved. Then, after three days without relevant adverse events, the dose will be increased to the dose that was prescribed at the time adverse events appeared. When relevant adverse events re-appear, subjects will continue at the lowest tolerable dose.

At the end of the study period, clonazepam will be tapered by decreasing the daily dose by 0.01 mg/kg/day every three days.

Subjects / Groups

Part A: healthy volunteers (n = 10-20) will be recruited.

Part B. Patients with ARID1B-related ID (n = 20) will be drawn from the LUMC's expertise centre for Coffin-Siris syndrome which currently has about 60 patients. If required, additional patients will be identified through the 8 clinical genetic laboratories who may have diagnosed ARID1B patients.

Inclusion criteria

Part A: healthy volunteers

1. Healthy male or female volunteers aged 18-30 years.
2. Informed consent provided by volunteer.

Part B: ARID1B patients.

1. Informed consent provided by both parents, or the legal guardian prior to any study mandated procedure.
2. Known mutation in ARID1B.
3. Assent provided by the participant.
4. Aged 6 years or older.

Exclusion criteria

Part A: healthy volunteers

1. Disorder that could interfere with saliva production.
2. Known hypersensitivity to clonazepam, other benzodiazepines or other excipients of the study medication.
3. Treatment with another investigational drug within 3 months prior to screening or more than 4 times a year.
4. History or clinical evidence of any disease and/or existence of a surgical or medical condition which might interfere with the absorption, distribution, metabolism or excretion of the study drug.
5. History of severe respiratory problems or severe liver- or renal insufficiency.
6. Other medical or psychosocial condition or history making the participant unsuitable for participation.
7. History or clinical evidence of alcoholism within the 3-year period prior to screening (i.e. regular use of more than 21 units of alcohol/week).

8. Clinically significant findings on physical examination.
9. Medications with a strong influence on CYP3A4 metabolism.
10. Clinically meaningful blood loss (including blood donation), or a transfusion of any blood product within 12 weeks before screening.

Part B: ARID1B patients.

1. Clear indication of not wanting to participate during the study.
2. Use of benzodiazepines or any other medication or drug with the potential to influence study related endpoints in the investigator's opinion (including e.g. CYP3A4-related drugs).
3. Known hypersensitivity to clonazepam, other benzodiazepines or other excipients of the study medication.
4. History of severe respiratory problems or severe liver- or renal insufficiency.
5. Other medical or psychosocial condition or history making the participant unsuitable for participation as determined by the treating physician or general practitioner.

Concomitant medications

All prescription medications taken within 30 days of study screening will be recorded. Medication known to influence CNS-activity will be assessed for each patient. Subjects will be allowed to participate when they have been on a stable dose for at least 3 months prior to the first study day. Medications with a strong influence on CYP3A4 metabolism are an exclusion criterion.

Tolerability / safety endpoints

- Adverse events
- Vital signs measurements
- General physical examination findings (only performed when clinically indicated)

Pharmacokinetic endpoints

Part A: serum and saliva. Part B: saliva only.

- The maximum serum concentration, Cmax
- The time to reach maximum serum concentration, tmax
- The terminal disposition rate constant (λz) with the respective half-life, t½
- The area under the serum concentration-time curve from zero to infinity, AUC0-inf
- The area under the serum concentration-time curve from zero to t of the last measured concentration above the limit of quantification, AUC0-last
- Clearance, Cl
- Volume of distribution, Vz

Trial@home endpoints

- Physical activity
- Sleep (duration, %light sleep, amount of times woken up)
- Heart rate
- Daily symptom scores
- Tapping frequency, adaptive tracking, animal fluency (twice-weekly)

Pharmacodynamic endpoints

- **NeuroCart**
    - Adaptive Tracking
    - Animal fluency test
    - Body Sway

- Saccadic Eye Movements
- Smooth Pursuit Eye Movements
- Tapping frequency

- **Questionnaires**
  - ABC (Aberrant Behaviour Checklist) questionnaire (parents, teacher)
  - Clinician's Global Impression of Improvement and Severity (CGI-I, CGI-S)

Sample Size Justification and statistical methodology

For part A, Linear and non-linear relationships between plasma and saliva concentrations will be explored. In the case of a nonlinear relationship, which could be expected due to delayed penetration in the salivary compartment, it is widely accepted that around 20 subjects are necessary to develop a reliable model. The predictive capability of the two regression equations will be compared to determine which method is superior.

For part B, the sample size is based on practical considerations (the total ARID1B population in the Netherlands) and based on the previous study (CHDR1828). We consider 20 patients to be a feasible amount of subjects to recruit, while obtaining sufficient power to detect clinically relevant treatment effects. To establish treatment effects the repeatedly measured cross-over PD endpoints will be analyzed by mixed model analyses of variance with treatment, period, time, and treatment by time as fixed effects, with subject, subject by treatment and subject by time as random effect, and with the (average) baseline value as covariate. The PK-PD relationship between clonazepam concentration and PD endpoints will be assessed by calculating the estimated plasma concentration using the derived formula in part A of the study.

TABLE 1. ASSESSMENT SCHEDULE PART A (HEALTHY VOLUNTEERS)

	SCR	**Study day**									
Time / Assessments	-28 days	-1 h	0 h	0.5h	1h	2h	4h	6h	8h	24h	48h
Informed consent	X										
Demography	X										
Inclusion and exclusion criteria	X										
Medical history	X										
Height / weight	X										
Blood chemistry, coagulation, hematology and virology	X										
Alcohol breath test	X										
Urine drug screening, urinalysis	X										
Urine pregnancy (females)	X										
Vital signs (HR, BP)	X	X									
Vital signs (temp)		X								X	X
COVID-19-related questions		X								X	X
Symptoms	X	X			X		X		X	X	X
Arrival in clinic		X								X	X
Drug administration (oral)			X								
Rinse mouth			X	X	X	X	X	X	X	X	X
Blood and Saliva sample PK			X	X	X	X	X	X	X	X	X
Discharge									X	X	X

SCR = screening; BS = blood sample; PK = pharmacokinetic; HR = Heart rate; BP = Blood pressure

TABLE 2. ASSESSMENT SCHEDULE PARTS B (ARID1B SUBJECTS)

	SCR		Study period 1 & 2					
			Day 1 & 22					Day 2-21
Time / Assessments	-60 days[4]	-1 h	0 h	1h	3h	4.5h	5h	
Informed consent	X							
Demography	X							
Inclusion and exclusion criteria	X							
Medical history	X							
Symptoms	X	X	X	X	X		X	
Arrival in clinic		X						
Explanation of NeuroCart® and study procedures	X	X						
Vital signs (Weight, length, HR, BP & temp)		X						
Adverse events			X	X	X		X	X[7]
Questionnaires[1]		X						
Drug administration (oral)			X					X[8]
Cognitive test battery[3]		X		X	X		X	
Rinse mouth		X				X	X	
Saliva sample PK		X[6]				X	X	
Discharge							X	
trial@home device explanation to parents							X	
trial@home[2]								X
Teacher/caregiver questionnaire								X[5]

SCR = screening; PK = pharmacokinetic; HR = Heart rate; BP = Blood pressure

1. Questionnaires consists of: ABC questionnaire, and the Clinician's Global Impression (CGI-S at day 1, CGI-I at day 22).

2. Continuous physical activity, heart rate and sleep monitoring, as well as twice-weekly finger tapping, adaptive tracking and animal fluency.

3. NeuroCart tests consist of: animal fluency test, adaptive tracking, body sway, saccadic eye movements, smooth pursuit and tapping frequency.

4. Screening by phone.

5. ABC questionnaire on paper. Interview by phone for CGI-S (> day 1) and CGI-I (> day 21), conducted as soon as possible after the study-day.

6. Only on day 22

7. Evaluation adverse events by phone by study physician at Day 3 and Day 6 and when needed

8. Starting on the evening of Day 22, clonazepam will be tapered by decreasing the daily dose by 0.01 mg/kg/day every three days.

1 BACKGROUND AND RATIONALE

1.1 Context

Intellectual disability (ID) is one of the most frequent neurodevelopmental disorders and affects up to 2-3% of the population [1, 2]. Large-scale genetic studies have shown that in many patients with ID genetic causes can be identified [3, 17]. Heterozygous truncating mutations in ARID1B, leading to haploinsufficiency, are continuously found as the most frequent cause of ID, explaining about 1% of the patients [3, 4]. Besides non-specific ID, ARID1B mutations have been identified in a high proportion (>50%) of patients with a clinical diagnosis of Coffin-Siris syndrome [5-7]. This syndrome was first described in 1970 as a combination of course facial features, hypertrichosis, and fifth digit nail hypoplasia. Other symptoms that have been reported are epilepsy, feeding disorders, congenital heart disease, speech impairment and agenesis of the corpus callosum [8, 9]. Almost all adults with ARID1B mutations are incapacitated. Although there are no good estimates of incidence and prevalence, ~50 patients with ARID1B mutations are currently known in the LUMC's expertise center. Worldwide the largest group of described patients consists of 143, reflecting the accessibility of clinical genetics in the Netherlands. Currently, no treatment options are available for ARID1B patients, except for supporting therapies such as physiotherapy and speech therapy.

There is little knowledge on the pathophysiology of ARID1B mutations. However, ARID1B is one of the members of a chromatin remodeling complex (BAF complex). The subunit composition of this complex plays a key role in neural differentiation. Since mutations in other components of the BAF complex have also been identified in Coffin-Siris syndrome, it is likely that these mutations lead to impaired neural differentiation and ID.

In the past two years three Arid1b-haploinsufficient ($Arid1b^{+/-}$) mice models have been generated and investigated. Although different tests have been performed in these studies, and results are not always in agreement, the emerging picture is that $Arid1b^{+/-}$ mice display increased anxiety, reduced memory, learning and social interaction [12]. One study identified growth hormone (GH) deficiency in $Arid1b^{+/-}$ mice, and showed that GH treatment does not have an effect on behavioral tests. Interestingly however, ARID1B patients are shorter than their sibs, and GH deficiency has been observed in patients, indicating that the mouse model does have validity.

In another study mouse brain tissue was extensively investigated, and the amount of GABAergic inhibitory interneurons, especially of the parvalbumin-positive subtype, was reduced. This prompted investigation of the ratio of inhibitory and excitatory synapses, which indeed showed a reduction of GABAergic inhibitory synapses. Further analysis showed increased apoptosis in the progenitor population in the medial ganglionic eminence, which is the likely cause of the reduced numbers of inhibitory interneurons since it is the origin of 60-80% of cortical interneurons.

Based on these findings, the investigators decided to administer a single treatment of 0.0625 mg/kg i.p. clonazepam in adult mice. This treatment led to restored impaired recognition memory, social memory and heightened anxiety-like behavior in adult Arid1b heterozygous mice, but not depression-like behavior symptoms 30-60 min after treatment [11].

Currently, this is the only study to directly implicate a key role of GABAergic interneurons in ARID1B pathophysiology. However, the GABAergic system has been previously linked to various other syndromes which feature ID, such as Fragile-X, Rett, and Down syndromes. To provide further support for the involvement of the GABAergic system, the Leiden University Medical Center has generated ARID1B haploinsufficient ($ARID1B^{+/-}$) and ARID1B knockout ($ARID1B^{-/-}$) induced

pluripotent stem cells by using CRISPR-Cas technology. Upon differentiation to GABAergic interneurons, we noticed that the cell number sharply decreased with decreasing functioning copies of ARID1B.

Together, this data leads to the hypothesis that haploinsufficiency of ARID1B leads to fewer interneuron progenitors and GABAergic inhibitory interneurons, and an imbalance in excitatory/inhibitory synapses, whilst providing stimulation to the GABAergic system restores this balance and at least partially restores the consequences on behaviour and cognition.

1.2 Endpoints and biomarkers in ID.

Thus far, no detailed characterization of ARID1B patients has been performed, and there are no widely accepted biomarkers in ID. For an extensive discussion on biomarkers we refer to Cohen *et al*. [18]. Ideally, a biomarker shows a clear difference between patients and controls, has little within-patient variability, correlates well to a relevant endpoint, and reflects treatment response. Since no treatment is currently available, the last criterion cannot be established. Therefore, Centre for Human Drug Research recently performed a first exploratory study to investigate the neurocognitive phenotype in 12 ARID1B patients aged 2-31 years and 12 age-matched controls. Patients and controls had 2 visits at the CHDR, and all measurements were performed 2-4 times. The goals of this study were to (1) establish which tests could be performed by ARID1B patients, (2) the variability of these tests within each patient, (3) the variability of the tests between patients, and (4) the difference between patients and age-matched controls. Based on previous experience and literature we selected a subset of tests of the NeuroCart® (CHDR1828).

Important results of these study were that the vast majority of the tests could be performed adequately by ARID1B patients. The burden (2x 3h visits) to the patients was acceptable, given that most patients indicated they would consider participating in future studies. We did notice however that especially EEG-related tests were difficult to perform, and that the placement of EEG caps was a lengthy and burdensome process in the patient population.

Analysis of the results focused on desirable characteristics for biomarkers for future trials, such as a clear difference between patients and controls, and limited variability within and between patients. Several tests satisfied these criteria, most notably adaptive tracking, smooth pursuit, body sway, tapping frequency, and the animal fluency test. We have also correlated these markers to disease severity as measured by IQ and the Aberrant Behavior Checklist, but the small sample size does not allow definitive conclusions.

Most of these biomarkers have previously been evaluated in benzodiazepines. Sedative effects of benzodiazepines can be readily identified by their effect on saccadic eye movements. As expected, CHD1828 did not identify any differences between ARID1B patients and controls in saccadic eye movement.

Another important aspect of biomarker selection is whether they could potentially be measured in the home environment. Since ARID1B syndrome is a rare disease, patients have to come from all over the country. Especially for multiple dose studies, measuring endpoints in the home environment offers substantial advantages. CHDR has developed a trial@home platform called CHDR MORE®. It offers multiple study assessments for use in a home-setting. It includes a wearable platform using devices produced by Withings, such as the Withings Steel HR, a smartwatch capable of measuring physical activity, heart rate and sleep characteristics. Furthermore, the platform consists of the Promasys

ePRO application, enabling study participants to answer digital questionnaires and allowing investigators to send phone notifications when it is time for a study assessment.

1.3 Clinical information about the drug

GABA-agonists reduce excitability by indirectly opening chlorine channels which leads to depolarization. The best-known class of GABA-agonists are the benzodiazepines, of which > 10 have been synthesized. More selective GABA-agonists have also been developed. Clonazepam (Rivotril) is a nonselective benzodiazepine with a half-life of 20-40 hours, a tmax of 1–4 hours after oral dosing, and is currently used for treatment of seizures in children and adults and psychoses. For detailed information about pharmacology, pharmacokinetics, metabolism and safety we refer to the summary of product characteristics (SmPC).

To the best of our knowledge, clonazepam has been prescribed to one 12-year old ARID1B patient by his treating psychiatrist, because of severe behavioral issues. Previous treatment response with methylphenidate, risperidone, clonidine, pipamperone, nortriptyline, oxazepam and aripiprazole was unsatisfactory. Whilst still on nortriptyline and aripiprazole clonazepam treatment was initiated in November 2018 (0.25 mg 2dd, since January 2019 on 0.5 mg 2dd). He has since been on this treatment without any side-effects, and with good clinical response on behavior (personal communication, unpublished data).

1.4 Study rationale

1.4.1 Benefit and risk assessment

This is an interventional trial with the aim of benefiting patients. The NeuroCart® assessments have been previously evaluated in the study population (study CHDR1828). The measurements were well tolerated, and most patients indicated they would consider participating again in a trial when asked. The most disliked assessment (EEG measurements) are not included in this trial. Therefore, we estimate the assessment burden for subjects is relatively lower than during CHDR1828.

The actual burden for subjects consists of the time spend at CHDR and the potential side-effects from clonazepam treatment. The 4 study days will last approximately 6.5 hours, but include 5 hours of break time. In this time, subjects can roam freely and sufficient entertainment will be arranged together with parents. The study does not include blood draws or other invasive measurements. Clonazepam is a registered drug and approved for use in pediatric patients. Possible adverse events include fatigue, diminished concentration, dizziness and headache. Furthermore, extremely rare paradoxal reactions have been reported: irritability, aggression, agitation, nervousness, hostility, anxiety, sleep disturbances, nightmares, abnormal dreams, hallucinations. Patients will be monitored during each study day and the investigator will be continuously available for parents and caregivers. Dose-adjustment or study discontinuation will follow in the case of clinically relevant adverse events.

Study subjects will have potential benefit from study participation. Pre-clinical data suggests an effect on behavior and cognition. The additional value of this research is in gaining the first insights into the neurocognitive effects of clonazepam treatment on ARID1B-related ID subjects and possible validation of novel noninvasive endpoints obtained via the NeuroCart®. The proposed research can be considered group-related because it is only feasible by including ARID1B-related ID patients themselves.

1.4.2 Medical and regulatory background

The investigator will follow the code of conduct 'The expression of objection by people with mental disabilities in the context of the WMO', originally published as TVAZ 17: 3, 1999, and the code of conduct regarding conducting research with minors ('Gedragscode bij verzet van minderjarigen die deelnemen aan medisch wetenschappelijk'onderzoek'). If a patient objects to the Neurocart® tests, the test will be skipped and a 10-minute break will be provided before the subject will conduct the next test. If the patient still objects no further tests will be performed and the patient will either drop out of the study or continue the study at the next study day, depending on the amount of completed tests and the opinion of parents and caregivers. Objection of the patient will be interpreted considering the usual behavioural pattern of the patient. As the parents and caregivers are best informed about the usual behaviour of the patient, they will be mostly responsible to decide whether or not the patient objects.

1.4.3 Study population

This research can only be carried out in ARID1B patients. Based on the results of our exploratory study (CHDR1828) we have chosen to include patients aged 6 and older, since these children were able to execute the NeuroCart®-related tasks. Considering the rarity of the disease, it is not possible to conduct the complete study in a subgroup, e.g. relatively competent or adult patients. All adults known to date are incapacitated. In addition, based on the hypothesized mechanism of action and brain plasticity it is possible that the treatment will only work in younger children. However, we will start this study with a cohort of 5 subjects aged 12+.

1.4.4 Study design

Rationale for part A (healthy volunteer study). Clonazepam PK is variable due to variations in CYP3A4 activity. Although plasma sampling is not desirable in the ARID1B patient population, knowledge of PK is required to establish concentration-effect relationship and the development of PK/PD models. Therefore, we will first conduct a limited study in healthy volunteers where plasma PK and saliva PK are correlated.

De Gier et al show that there is a close correlation between salivary and plasma levels of diazepam [19]. In a similar study Hallstrom et al confirmed this correlation although the correlation coefficient was lower (0.81 versus 0.96) [20]. No plasma-saliva PK studies have been performed with clonazepam, but urine and saliva PK has been investigated in subjects undergoing withdrawal which show that clonazepam detection in saliva is extremely sensitive [21]. Considering the nature of this part of the study, we will only include healthy adult volunteers.

Rationale for part B (cross-over study). Since the ARID1B patient population is small, with significant variability between patients, but limited within patients as demonstrated by our study CHDR1828, and the disease itself is stable, a cross-over design has been selected for the current study. In order to establish the acute and chronic administration of clonazepam whilst taking into account the development of tolerance to some of the effects of benzodiazepines, there is a 3-week treatment period with detailed measurements on the first and last days. By combining measurement of biomarkers with clinically relevant endpoints (such as cognition and behaviour) we can establish clonazepam effect on both, and test whether responses to acute and chronic treatment correlate.

1.4.5 Comparative drug(s) and/or placebo

Since there is currently no approved or effective pharmacological treatment available for ARID1B related intellectual disability, the choice was made for a placebo-controlled design.

1.4.6 Safety margin calculations, dose selection, dose escalation, and stopping criteria

The optimal dose of clonazepam to restore the excitation/inhibition imbalance is not known. The dose used pre-clinically (0.0625 mg/kg) [11] is comparable to the doses used in mouse seizures models (0.08-0.5 mg/kg). The HED is 0.0625 mg/kg * scaling factor 0.08 = 0.005 mg/kg, thus 0.35 mg for an adult male, in the same order as the regular starting dose (0.5 mg twice daily), but much lower than the regular maintenance dose (4-8 mg/day). Although generally considered safe, paradoxical reactions have been described in children and elderly. Usually these occur at higher doses. Other reported side effects include dizziness and drowsiness, which are also dose-related. Tolerance usually develops in 1-2 weeks [22, 23].

The dose used in this study is based on the following rationale:

1. The pre-clinically effective dose is similar to the starting dose in preclinical seizure models, and the human equivalent dose is in the range of the recommended starting dose for seizures in humans.
2. The dose is identical to the dose given to the case of the 12 year old ARID1B patient currently on clonazepam with good clinical effect and absence of toxicity.
3. Clonazepam is a registered drug. The recommended starting dose is the same as given in this study.

Therefore, the patients will receive a single starting dose at their first visit of 0.005 mg/kg (max 0.5 mg). Patients will be monitored for 5 hours for safety, PK, and biomarker effect. If the dose on day 1 is well tolerated, patients will take the same dose before bedtime, and continue this regimen as described.

After three days, the (adverse) effects will be evaluated by phone. If the dose on day 1 is well tolerated, the dose will be increased to 0.01 mg/kg/day. After another three days, the effects will be evaluated again, after which the dose will be increased to 0.015 mg/kg/day. During these dosing adjustments, the maximum dose will not exceed 0.5 mg, twice daily. In case of relevant adverse events during any of the dose evaluations, the daily dose will be halved. Then, after three days without relevant adverse events, the dose will be increased to the dose that was prescribed at the time adverse events appeared. When relevant adverse events re-appear, subjects will continue at the lowest tolerable dose.

At the end of the study period, clonazepam will be tapered by decreasing the daily dose by 0.01 mg/kg/day every three days.

Stopping criteria

Dosing will be stopped in case of an unacceptable tolerability profile based on the nature, frequency, and intensity of observed AEs judged by the investigators.

1.4.7 Treatment duration

Since clonazepam has been shown safe and effective in children, and treatment of ARID1B-related intellectual disability would require chronic administration, we have chosen a multiple-dose trial design. The choice for the duration of treatment is based partly on practical considerations and on data on the development of tolerance. It is known that tolerance to different effects of benzodiazepines develops on different time scales. Development of tolerance for sedative effects of benzodiazepines varies from 7 days to 2 weeks in most studies [22, 23], whilst development of tolerance to anti-epileptic

and anxiolytic effects needs much more time to develop. Therefore, we have chosen to treat patients for 3 weeks, to ensure we can establish the effects of clonazepam in absence of the potential sedative side-effects

1.4.8 Primary endpoint

This study is exploratory and therefore, there is no primary endpoint.

1.4.9 Statistical hypotheses and sample size

This is an exploratory study; therefore, the sample size is not only based on statistical considerations, but also on practical considerations such as the amount of subjects available and willing to participate in the Netherlands. Motivation to participate in clinical studies in the population is high, but the absolute numbers are limited (around 60 patients in the Netherlands). We expect to be able to recruit 20 subjects within a feasible timeline.

Still, we are able to calculate the power of the chosen exploratory endpoints and sample size. The CGI-I has a standard deviation of 1.5 points. This study has an 80% power to detect a 1 point difference in CGI-I. Assuming a sample size of 20 subjects and a drop-out rate of 20%, we can detect the following effect sizes for NeuroCart tests:

Test	Minimum detectable effect size (MDES)	Corresponding proportion of the difference between ARID1B and control subjects
Animal fluency test	3.3 animals	24%
Finger tapping	2.2 taps	21%
Adaptive tracking	1.35%	8%
Smooth pursuit eye movement	5.32%	25%
Saccadic eye movements - Peak velocity - Reaction time	 13.95 degrees/s 0.0286 sec	 NA NA
Body sway	301 mm	37%

2 STUDY OBJECTIVES

- To test the hypothesis that clonazepam administration has acute beneficial effects compared to placebo on neurocognitive tests.
- To test the hypothesis that multiple-doses clonazepam has beneficial effects compared to placebo on behaviour and cognitive function in ARID1B patients as measured by the ABC, and CGI-I scale.
- Assess safety and tolerability of clonazepam in ARID1B patients.
- To assess the potential of at-home neurocognitive tests for the evaluation of treatment effects in children with neurodevelopmental disorders.
- To assess and compare the difference in predictive capability between linear and nonlinear (NONMEM) regression of the saliva:plasma relationship.

3 STUDY DESIGN

3.1 Overall study design and plan

Part A. Open label study in healthy volunteers where pharmacokinetics of clonazepam will be measured in paired plasma and saliva samples. Detailed scheduling can be found in **Table 1**.

For part A, the total duration of the study for each subject will be up to 2 days divided as follows:

- Screening: Up to 28 days before dosing.
- Saliva PK study day: Day 1.

Part B. Two-way cross over, placebo-controlled randomized study. Each period will be 22 days and periods will be separated by approximately a three-week washout. Patients will be monitored in the clinic for 5 hours for safety, PK, and biomarker effects on day 1 and 22 in each period. Within those days, patients remain at home and fill in questionnaires, wear a smartwatch and perform home-based assessments. Detailed scheduling can be found in **Table 2**.

For part B, the total duration of the study for each subject is divided as follows:

- Screening: By telephone, up to 60 days before dosing;
- In clinic study days study period 1: Day 1 - 22 plus a down titration period of ±3 days;
- In clinic study days study period 2: Day 1 - 22 plus a down titration period of ±3 days;
- Trial@Home periods: Days 2-21
- Follow-up phone call: approximately 14 days after the last dose of each study period.

4 STUDY POPULATION

4.1 Subject population

For part A, subjects will be recruited via media advertisement or from the subjects' database of the Centre for Human Drug Research, Leiden, the Netherlands. For part B, ARID1B-related ID patients will be recruited via the LUMC's expertise centre for Coffin-Siris syndrome. If required, additional patients will be identified through the 8 clinical genetic laboratories who may have diagnosed ARID1B patients.

4.2 Inclusion criteria

Part A, healthy volunteers.

1. Healthy male or female volunteers aged 18-30 years
2. Informed consent provided by volunteer

Part B, ARID1B patients.

1. Informed consent provided by both parents, or the legal guardian prior to any study mandated procedure.
2. Known mutation in ARID1B
3. Assent provided by the participant.
4. Aged 6 years or older

4.3 Exclusion criteria

Part A, healthy volunteers

1. Disorder that could interfere with saliva production.
2. Known hypersensitivity to clonazepam, other benzodiazepines or other excipients of the study medication.
3. Treatment with another investigational drug within 3 months prior to screening or more than 4 times a year.
4. History or clinical evidence of any disease and/or existence of a surgical or medical condition which might interfere with the absorption, distribution, metabolism or excretion of the study drug.
5. History of severe respiratory problems or severe liver- or renal insufficiency.
6. Other medical or psychosocial condition or history making the participant unsuitable for participation.
7. History or clinical evidence of alcoholism within the 3-year period prior to screening (i.e. regular use of more than 21 units of alcohol/week).
8. Clinically significant findings on physical examination.
9. Medications with a strong influence on CYP3A4 metabolism
10. Clinically meaningful blood loss (including blood donation), or a transfusion of any blood product within 12 weeks before screening.

Part B, ARID1B patients.

1. Clear indication of not wanting to participate during the study
2. Use of benzodiazepines or any other medication or drug with the potential to influence study related endpoints in the investigator's opinion (including e.g. CYP3A4-related drugs).
3. Known hypersensitivity to clonazepam, other benzodiazepines or other excipients of the study medication.
4. History of severe respiratory problems or severe liver- or renal insufficiency.

5. Other medical or psychosocial history making the participant unsuitable for participation as determined by the treating physician or general practitioner.

4.4 Concomitant medications

All prescription medications taken within 30 days of study screening will be recorded. Medication known to influence CNS-activity will be assessed for each patient. Subjects will be allowed to participate when they have been on a stable dose for at least 3 months prior to the first study day. Medications with a strong influence on CYP3A4 metabolism are an exclusion criterion.

4.5 Lifestyle restrictions

Alcohol will not be allowed from at least 24 hours before each scheduled visit, and whilst in the study unit. At other times throughout the study, subjects should not consume more than 2 units of alcohol daily on average (one unit is 10 grams of alcohol).

Subjects will not be allowed to have excessive caffeine consumption, defined as >800 mg per day from 7 days prior to the first study day. Subjects will abstain from caffeine-containing products for 24 hours prior to the start of the study. Caffeine quantities defined as: one cup of coffee contains 100 mg of caffeine; one cup of tea, or one glass of cola, or potion of chocolate (dark:100 g, milk 200 g) contains approximately 40 mg of caffeine; one bottle of Red Bull contains approximately 80 mg of caffeine.

Any nutrients known to modulate CYP enzymes activity (e.g., grapefruit or Seville orange containing products or quinine containing drinks (tonic water or bitter lemon)) will not be permitted from 3 days before dosing until 24 hours after the final dose.

The use of (illicit) drugs including cannabis can influence the measurements. Therefore, using 'drugs' is not permitted from 3 days before dosing and until the end of the study. Subjects will abstain from the use of tobacco-or nicotine-containing products (including e-cigarettes and patches) for 24 hours prior to dosing until the end of the study.

Strenuous physical activity (e.g., heavy lifting, weight or fitness training) is not allowed from 48 hours prior to each study day until collection of the final pharmacokinetic blood sample discharge from the study unit. Light ambulatory activities (e.g. walking at normal pace) will be permitted, with the level of activities kept as similar as possible on all days in the study unit.

4.5.1 Contraception requirements

All women of child bearing potential during part A of the study must practice effective contraception during the study and be willing and able to continue contraception for at least 90 days after their last dose of study treatment. Women of child bearing potential are defined as all women physiologically capable of becoming pregnant, unless they meet one of the following conditions:

- Postmenopausal: 12 months of natural (spontaneous) amenorrhea or 6 weeks after surgical bilateral oophorectomy with or without hysterectomy;
- Posthysterectomy.

For the purposes of the study, effective contraception is defined as follows:

- Females: Using 1 or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation), intrauterine contraception/device, hormonal contraception, or any 2 barrier methods (a combination of male or female condom with diaphragm, sponge or cervical cap).

Abstinence can be considered an acceptable method of contraception at the discretion of the investigator. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post ovulation methods) and withdrawal are not considered acceptable methods of contraception.

4.6 Study drug discontinuation and withdrawal

4.6.1 Study drug interruption or discontinuation

Before trial medication is administered, changes in the subject health status will be checked. The investigator must temporally interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject. The interruption or premature discontinuation of study drug might be triggered by an Adverse Event (AE), a diagnostic or therapeutic procedure, an abnormal assessment (e.g., ECG or laboratory abnormalities), or for administrative reasons in particular withdrawal of the subject's consent. The reason for study drug interruption or premature discontinuation must be documented.

4.6.2 Subject withdrawal

Subjects have the right to withdraw from the study at any time for any reason. Should a subject decide to withdraw from the study, all efforts should be made to complete and report the observations, particularly the follow-up examinations, as thoroughly as possible. When subjects show signs of resistance or show signs of not wanting to participate during the study, study assessments will be stopped. Signs of resistance include the expression of fear, sadness or anxiety, and will be evaluated in close collaboration with the parents.

4.6.3 Replacement policy

Subjects can be replaced if they withdraw from the study on day 1. At most, 2 subjects will be replaced.

5 INVESTIGATIONAL DRUG

5.1 Investigational drug and matching placebo

Part A:

- Clonazepam (Rivotril) droplets for oral use, solution 2.5 mg/ml, dissolved in lemonade. Dose: 0.5 mg or 1 mg, both administered to 50% of subjects.

Part B:

The following substances will be administered:

- Active medication. Clonazepam (Rivotril) droplets for oral use, solution 2.5 mg/ml, dissolved in lemonade, tea or juice.
- Placebo: dissolved in lemonade, tea or juice

Study drug or placebo during part B will be administered to the subjects as follows:

- Day 1-3: Starting dose is 0.005 mg/kg, twice daily (max 0.5 mg, twice daily)
- Day 4-6: 0.01 mg/kg, twice daily (max 0.5 mg, twice daily)
- Day 7-22: 0.015 mg/kg, twice daily (max 0.5 mg, twice daily)

Patients will receive a single starting dose at their first visit of 0.005 mg/kg (max 0.5 mg). Patients will be monitored for 5 hours for safety, PK, and biomarker effect. If the dose on day 1 is well tolerated, patients will take the same dose before bedtime, and continue this regimen as described.

5.2 Study drug up- and down-titration

After three days, the (adverse) effects will be evaluated by phone. In the absence of adverse events, dose will be increased to 0.01 mg/kg/day. After another three days, the effects will be evaluated again, after which the dose will be increased to 0.015 mg/kg/day. During these dosing adjustments, the maximum dose will not be allowed to exceed 0.5 mg twice daily. In case of reported adverse events during any of the dose evaluations, the daily dose will be halved. Then, after three days without adverse events, the dose will be increased to the dose that was prescribed at the time adverse events appeared. When adverse events re-appear, subjects will continue at the lowest tolerable dose.

At the end of the study period, starting in the evening of Day 22, clonazepam will be tapered by decreasing the daily dose by 0.01 mg/kg/day every three days.

5.3 Study drug packaging and labelling

Study drug will be ordered at the pharmacy of Leiden University Medical Center. Upon arrival at the pharmacy, the investigational products should check them for damage and verify proper identity, quantity, integrity of seals and temperature conditions, and report any deviations or product complaints upon discovery. The dispensing of the study drug will be performed by the pharmacy. Study drug will be dispensed for each subject according to the randomization list. Study drug packaging will be overseen by the Leiden University Medical Centre Pharmacy and bearing a label with the identification required by local law, the protocol number, drug identification, and dosage.

5.4 Drug accountability

Drug accountability will be maintained by the Leiden University Medical Centre Pharmacy and assessed by maintaining adequate study drug dispensing records. The investigator is responsible for ensuring that dosing is administered in compliance with the protocol. Delegation of this task must be clearly documented and approved by the investigator. All in-house study drug administration will occur under medical supervision.

5.5 Treatment assignment and blinding

5.5.1 Randomization and treatment assignment

Subjects must be randomized in a consecutive order starting with the lowest number.

Subject numbers consist of 3 digits. Subject numbers for part A will consist of 101-120 and subject numbers for part B of 201-220. Stratification for gender takes place in Promasys, by adding key value “F” to subject numbers for females and “M” to subject numbers for males. There will be no reserve subjects. The randomization code will be generated by a study-independent CHDR statistician. The randomization code will be unblinded/broken and made available for data analysis only after study closure, i.e., when the study has been completed, the protocol deviations determined, and the clinical database declared complete, accurate and locked. The randomization code will be kept strictly confidential. Sealed individual randomization codes, per subject and per treatment, will be placed in a sealed envelope containing the labelled 'emergency decoding envelopes' will be kept in a safe cabinet at CHDR.

5.5.2 Blinding

This study will be performed in a double-blind fashion. The investigator, study staff, subjects, and monitor will remain blinded to the treatment until study closure. The investigational drug and its matching placebo are indistinguishable and will be packaged in the same way.

The investigator will receive a set of sealed emergency codes to be broken in case of emergency situations. If the identity of the study drug administered needs to be known in order to manage the subject’s condition i.e., in case of a medical emergency or in the case a SUSAR occurs, the treatment emergency code for that subject may be broken and the study drug identified. All such occurrences should be documented in the study file. Treatment emergency codes should not be broken except in emergency situations and, if possible, the investigator should be contacted before the emergency code is opened. Just prior to database lock, the unused emergency code labels will be checked and a statement to the effect that all are intact (or not as the case may be) will be made on the database lock form.

6 STUDY ENDPOINTS

6.1 Safety and tolerability endpoints

- Treatment-emergent (serious) adverse events ((S)AEs).
- Concomitant medication

6.2 Pharmacokinetic endpoints

Part A: serum and saliva. Part B: saliva only.

- The maximum serum concentration, Cmax
- The time to reach maximum serum concentration, tmax
- The terminal disposition rate constant (λz) with the respective half-life, t½
- The area under the serum concentration-time curve from zero to infinity, AUC0-inf
- The area under the serum concentration-time curve from zero to t of the last measured concentration above the limit of quantification, AUC0-last
- Clearance, Cl
- Volume of distribution, Vz

6.3 Pharmacodynamic endpoints

Questionnaire endpoints

- ABC questionnaire (parents, teacher)
  - Irritability subscale
  - Social Withdrawal subscale
  - Stereotypic Behavior subscale
  - Hyperactive/Noncompliance subscale
  - Inappropriate Speech subscale
- Clinician's Global Impression (CGI-I and CGI-S)
  - Score at the start (CGE-S) and at the end (CGE-I)of the treatment period.

NeuroCart endpoints

- Saccadic eye movements:
  - saccadic reaction time (second),
  - saccadic peak velocity (degrees/second), and
  - saccadic inaccuracy (%);
- Smooth pursuit eye movements:
  - percentage of time the eyes of the subjects are in smooth pursuit of the target (%);
- Body sway:
  - antero-posterior sway (mm);
- Adaptive tracking:
  - average performance (%);
- Animal fluency:
  - Number of correctly named animals in one minute;
- Finger tapping
  - Amount of taps in 10 seconds;

Trial@home endpoints

- Steel HR watch
  - Physical activity
  - Sleep (duration, %light sleep, amount of times woken up)
  - Heart rate
- Adapted NeuroCart endpoints for home use:
  - Tapping frequency
  - Adaptive tracking average performance
  - Animal fluency

7 STUDY ASSESSMENTS

See Table 1 and 2 for the time points of the assessments.

7.1 Safety and tolerability assessments

The definitions, reporting and follow-up of AEs, SAEs and potential pregnancies are described in section 8.

7.1.1 Vital signs

Evaluations of systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature will be performed throughout the study. Pulse and blood pressure will be taken after 5 minutes in the supine position. Automated oscillometric blood pressures and pulse rate will be measured using a Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400.

7.1.2 Weight and height

Weight (kg) will be recorded at screening (part A) or the first visit (part B). Height (cm) will be recorded and body mass index (BMI) calculated.

7.1.3 Physical examination

Physical examination (i.e., inspection, percussion, palpation and auscultation) will be performed on indication during the course of the study. Clinically relevant findings that are present prior to study drug initiation must be recorded with the subject's Medical History. Clinically relevant findings found after study drug initiation and meeting the definition of an AE (new AE or worsening of previously existing condition) must be recorded.

7.2 Baseline variables

The following baseline variables will be recorded: age, gender, diagnosis, specific ARID1B mutation, clinical phenotype, IQ (if available), dyslexia.

7.3 Questionnaire assessments

The Dutch translation of the Aberrant Behaviour Checklist (ABC) will be used [24]. The ABC is a validated questionnaire used for patients with intellectual disability. It consists of 58 symptoms of which the severity of symptoms during the last 2 weeks is scored by parents on a scale of 0-3 (0 = no problem, 3 = the problem is very severe). Parents of ARID1B subjects will complete the questionnaire on day 1 and day 22 of the two treatment periods. Teachers or primary caregivers (in the case of institutionalized care) will complete the questionnaire at the earliest opportunity after day 1 and day 22.

The Clinician Global Impression Score (CGI) is a well-validated clinician-rated measure commonly used in drug studies because it allows the clinician to integrate all sources of information, including the parent/caregiver history, observations in the clinic, and reports from other sources, into a single rating of severity (CGI-S) and improvement (CGI-I) during treatment. The CGI-S will be assessed on day 1 of every treatment period.

For this assessment, the investigator considers all aspects of the subject's neurobehavioral function, including but not limited to internalizing problems, externalizing problems, and social engagement, and rates a scale employing a 7-point Likert scale. The CGI-I monitors improvement and is scored on a 7-point Likert scale: very much improved, much improved, minimally improved, no change, minimally worse, much worse, very much worse. The score will be determined via a semi-structured interview with parents on day 22 of both treatment periods. Furthermore, an additional score will be

determined via a semi-structured phone interview with teachers or primary caregivers at the earliest opportunity after day 22 of both treatment periods.
Conversations made for the CGI assessment will be recorded for review by an independent physician.

7.4 Pharmacodynamic assessments

7.4.1 Saccadic eye movements

Saccadic peak velocity is one of the most sensitive parameters for measuring sedation. Recording of eye movements will be performed in a quiet room with dimmed illumination. There will be only one subject per session in the same room. Recording and analysis of saccadic eye movements is conducted with a microcomputer-based system for sampling and analysis of eye movements. The program for signal collection and the AD-converter from Cambridge Electronic Design (CED Ltd, Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, Massachusetts, US) and the sampling and analysis scripts have been developed at CHDR (Leiden, the Netherlands). Disposable silver-silver chloride electrodes (Ambu Blue Sensor N) will be applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance is reduced to less than 5 kOhm before measurements. Head movements are restrained using a fixed head support. The target consists of a moving dot that is displayed on a computer screen. This screen is fixed at 58 cm in front of the head support. Saccadic eye movements are recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades are recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of saccadic peak velocity (expressed as degrees/second) of all correct saccades will be used as parameter and recorded. The measurements of saccadic eye movements will take approximately 3 min.

7.4.2 Smoot pursuit eye movements

For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5' eyeball rotation to both sides. Four cycles will be recorded for each stimulus frequency. The time during which the eyes are in smooth pursuit of the target will be calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies will be used as a parameter. This parameter can be used as an accurate biomarker for oculomotor function and attention. The smooth pursuit eye movements will take approximately 3 min.

7.4.3 Adaptive tracking

The adaptive tracking test will be performed as originally described by Borland and Nicholson [25], using customized equipment and software (Hobbs 2004, Hertfordshire, UK). The assessment includes a run-in time of 0.5 min, during which data are not recorded. Adaptive tracking is a pursuit-tracking task. A circle moves pseudo-randomly on a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average tracking performance (%) will be used as variable and recorded into the subject's eCRF. The adaptive tracking test is more sensitive to impairment of eye-hand coordination by drugs than compensatory pursuit tasks or other pursuit tracking tasks, such as the pursuit rotor. The adaptive tracking test has proven to be useful for measurement of central nervous system effects of alcohol, various other psychoactive drugs, and sleep deprivation. The tracking performance test will last approximately 4 min in total.

7.4.4 Body sway

Body sway will be assessed using a body sway meter and with eyes closed. The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway is measured with a pot string meter (celesco) based on a Wright ataxiameter. At CHDR, the method has been used to demonstrate effects of sleep deprivation, ethanol, benzodiazepines, and other psychoactive agents (data on file). With a string attached to the waist, all body movements over a period of time are integrated and expressed as sway (measured in mm). Subjects will be instructed to wear a pair of comfortable, low-heeled shoes on each session. Before starting a measurement, subjects will be asked to stand still and comfortable, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body and eyes closed. Subjects may not talk during the measurement. The total period of body-sway measurement will be 2 min. All body movements over a 2-min period are integrated and expressed as millimeters of sway, which will be recorded.

7.4.5 Finger tapping

The test has been adapted from the Halstead Reitan Test Battery [26], and evaluates motor activation and fluency. Speed of finger tapping is measured for the index finger for the dominant hand; a session contains five performances of 10 seconds (e.g. 5 x 10 seconds). Feedback on performance is given by a counter in the center of the screen, while the amount of taps of each 10 second trial is shown on the screen in between the trials. The space bar is used as tapping device. The volunteer is instructed to tap as quickly as possible with the index finger of the dominant hand and to rest the wrist on the table. The mean tapping rate and the standard deviations for the dominant hand are used for statistical analysis.

7.4.6 Animal Fluency test

Animal fluency test. Naming items from a specified category has long been used as a test of verbal fluency and ability to access semantic memory. Tasks such as animal naming have proved useful as cognitive screening tools. Subjects that perform the Animal Fluency test are asked to verbally produce as many different animals as they can sum up within sixty seconds. Animals that are named twice or more do not count towards the total amount of animals named. The administrator of the test will write down all the animals named by the subject on the ‘Animal fluency scoring form’ or as a note to the activity. Also an audio recording will be made of the one minute of animal name calling, so that afterwards the scoring can be checked for exactness.

7.5 Trial at home assessments

7.5.1 Nokia Steel HR

All subjects will wear the Nokia Steel HR. The Nokia Steel HR can track daily physical activity (step count) and is able to measure heart rate every 10 minutes. Also, the device can determine sleep duration and sleep pattern. Patients will be required to wear the device as a watch on their wrist day and night for duration of the study periods. The device will be required to be paired with a smartphone which will collect the data. The device needs charging every 21 days. The watch is waterproof according to the manufacturer.

7.5.2 Digital questionnaire

Subjects will, together with their parents, answer questions on their smartphone via an ePRO application. They will provide several behavior scores. This will take approximately 3 minutes.

7.5.3 Adapted NeuroCart assessments

Of the included NeuroCart tests, finger tapping, animal fluency and adaptive tracking have been adapted for use at home. Parents will loan a laptop to perform the tests twice-weekly with their children. Written and oral instructions will be provided on day 1.

7.6 Pharmacokinetic assessments

During part A of the study, approximately 50 mL blood in total will be collected via an i.v. catheter placed in an antecubital vein in the arm in 4ml EDTA tubes. The indwelling catheter will be kept patent by saline flush after each blood sampling. Immediately following collection of the required blood volume, the tubes will be slowly tilted backwards and forwards (no shaking) to bring the anti-coagulant into solution. Within 30 minutes of collection, the tubes will be centrifuged at approximately 2000 g for 10 minutes at 2 to 8 °C. The plasma will be transferred into two labelled polypropylene tube with approximately 0.9 mL plasma each, avoiding carryover of erythrocytes. Volume will be equally divided between the two tubes, but each sample shall have a minimum of 0.4 mL. All samples will be stored in an upright position at - 20 °C or lower. The exact actual clock time of withdrawal of the blood sample will be recorded.

Saliva samples will be obtained during part A and part B using the SalivaBio Infant's Swab (Salimetrics, Carlsbad, CA, USA). Originally designed for infants, the swabs are quite long and therefore suitable to use in subjects that are relatively incapacitated. The swabs can absorb 200 to 1000 µL in 60 - 90 seconds (7). For determination of clonazepam in saliva, a minimal volume of 20 µL is required for injection. The swab will be placed in the cheek of the participant until the pad is saturated. After collection, the saliva will be extracted from the pads by 5 minutes of centrifugation at 1500-2500 G. The saliva will be transferred into labelled polypropylene tubes. At least 20 microliter is necessary for analysis. Thereafter, the samples are stored at -80 ºC until analysis. The exact actual clock time of withdrawal of the saliva sample will be recorded.

7.6.1 Labelling

Pre-printed, waterproof labels will be used to identify the tubes used during sample collection and for storage of separated plasma or saliva. Each label will contain the following information:

- CHDR Protocol number
- Subject Number
- Occasion number (date)
- Protocol (delta) time
- Activity: Sample type (blood) & purpose (PK)

7.6.2 Shipping Procedures

CHDR will arrange shipment of the samples. The samples must be packed securely together with completed shipment forms in polystyrene insulated shipping containers together with enough dry ice to last for 48 hours.

7.6.3 Bioanalysis

Plasma and saliva concentrations will be determined via a validated LC/MS/MS method by the UMC Utrecht pharmacy.

7.7 Laboratory parameters

Blood and other biological samples will be collected for the following clinical laboratory tests during Part A of the study only (screening of healthy volunteers):

Lab	Tests	Collection & Analysis
Haematology	Haemoglobin [including Mean Corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC)], haematocrit, red cell count (RBC), total white cell count (WBC)and Platelet count. Differential blood count, including: basophils, eosinophils, neutrophils, lymphocytes, and monocytes.	2 mL of venous blood in a BD Vacutainer® K2EDTA tube. Samples will be analysed by the Clinical Chemistry Laboratory (AKCL) of Leiden University Medical Center (LUMC).
Chemistry and electrolytes	Sodium, potassium, calcium, inorganic phosphate, total protein, albumin, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin[2], alkaline phosphatase, AST, ALT gamma-GT and LDH.	3.5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the AKCL of LUMC
Coagulation	International Normalised Ratio (INR), prothrombin time (PT), activated partial thromboplastin time (APTT), Fibrinogen	2,7 mL of venous blood in a BD Vacutainer® Sodium Citrate (99NC BD) tube. Samples will be analysed by the AKCL of LUMC
Serology	HIV1 and HIV2 antigen and antibodies, Hepatitis B surface antigen, Hepatitis B antibodies and Hepatitis C antibodies	5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the Microbiology Laboratory (CKML) of the LUMC
Urinalysis	Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose. If there is a clinically significant positive result, urine will be sent to the AKCL for microscopy and/or culture.	A midstream, clean-catch urine specimen will be analysed by dipstick (Multistix® 10 SG, Siemens Healthcare Diagnostics, Frimley, UK).
Pregnancy[3]	hCG. If there is a clinically significant, positive result, urine will be sent to the AKCL for confirmation.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
Alcohol	Alcohol Breath Test	The hand-held Alco-Sensor IV meter (Honac, Apeldoorn, the Netherlands) will be used to

		measure the breath ethanol concentrations.
Urine drug screen	Cocaine, amphetamines, opiates (morphine), benzodiazepines and cannabinoids.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
[2]Conjugated bilirubin will be reported only when total bilirubin is outside the reference range. [3]Pregnancy test for women of childbearing potential will be performed at screening and if pregnancy is suspected during the study.		

7.8 Total blood sample amount (Healthy volunteers only)

Sample	**Samples taken**			**Sample Volume***			**Volume**	
Haematology	1		x	2	mL	=	2	mL
Chemisty	1		x	3.5	mL	=	3.5	mL
Coagulation	1		x	2.7	mL	=	2.7	mL
Serology	1		x	5	mL	=	5	mL
Pharmacokinetics	9		x	4	mL	=	36	mL
* exclusive discarded volume				**Total blood volume/subject**			**49.2**	**mL**

7.9 Sequence of assessments and time windows

When a PK assessment is scheduled for the same nominal time as another scheduled assessment, the PK sample will take precedence. The deviations of actual time points from the expected time points will be within ten percent, calculated from the zero point (time of drug administration) or the last relevant activity. The expected time points are defined as the time points in Promasys. Deviations of more than 10% will be explained in a note. Pre-dose assessments are given in indicative expected times.

8 SAFETY REPORTING

8.1 Definitions of adverse events

An Adverse Event (AE) is any untoward medical occurrence in a subject who is participating in a clinical study performed. The AE does not necessarily have to follow the administration of a study drug, or to have a causal relationship with the study drug. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory or vital sign finding), symptom, or disease temporally associated with the study participation, whether or not it is related to the study drug.

8.1.1 Intensity of adverse events

The intensity of clinical AEs is graded three-point scale as defined below:

- Mild: discomfort noticed but no disruption of normal daily activity;
- Moderate: discomfort sufficient to reduce or affect normal daily activity;
- Severe: inability to work or perform daily activity.

8.1.2 Relationship to study drug

For each AE the relationship to drug as judged by the investigator:

- Probable;
- Possible;
- Unlikely;
- Unrelated.

8.1.3 Chronicity of adverse events

The chronicity of the AE will be classified by the investigator on a three-item scale as defined below:

- Single occasion: single event with limited duration;
- Intermittent: several episodes of an event, each of limited duration;
- Persistent: event which remained indefinitely.

8.1.4 Action

Eventual actions taken will be recorded.

8.1.5 Serious adverse events

A Serious Adverse Event (SAE) is defined by the International Conference on Harmonization (ICH) guidelines as any AE fulfilling at least one of the following criteria:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a SAE.

8.1.6 Suspected unexpected serious adverse reactions

A SUSAR (Suspected Unexpected Serious Adverse Reaction) is a SAE that is unexpected, (nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product)) and suspected (a reasonable possibility of causal relationship with investigational drug, regardless of the administered dose).

8.1.7 Reporting of serious adverse events

SAEs and SUSAR's will be reported according to the following procedure.

The investigator will report the SAEs through the web portal ToetsingOnline (see https://toetsingonline.nl/) to the accredited EC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the SAE.

SUSARS must be reported to the EC that approved the study, the CA and the Dutch Medicines Evaluation Board (College ter Beoordeling van Geneesmiddelen).

The investigator will report expedited the following SUSARs through the web portal ToetsingOnline to the EC:

- SUSARs that have arisen in the clinical trial that was assessed by the EC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the EC.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the EC, CA and the Dutch Medicines Evaluation Board, a separate notification is not necessary. To prevent a double notification, it must be indicated in ToetsingOnline if the SUSAR is reported in the European Medicines Agency (EMA) EudraVigilance database, this will prevent the notification of the CA and the Dutch Medicines Evaluation Board through the web portal ToetsingOnline.

The expedited reporting will occur not later than 15 days after the first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.1.8 Follow-up of adverse events

All AEs will be followed until they have abated, returned to baseline status or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

8.2 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the investigator will inform the subjects and the EC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the EC, except insofar as suspension would jeopardise the subjects' health. The investigator will ensure that all subjects are kept informed.

8.3 Annual safety report or development safety update report

In addition to the expedited reporting of SUSARs, the investigator will submit, once a year throughout the clinical trial, a safety report to the EC and CA.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Pregnancy

8.4.1 Teratogenicity

If a woman becomes pregnant when on study drug, permanent discontinuation of study drug should be considered as appropriate. The investigator must counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the foetus. Monitoring of the subject should continue until the outcome of the pregnancy is known.

9 STATISTICAL METHODOLOGY AND ANALYSES

9.1 Statistical analysis plan

All safety and statistical programming is conducted with SAS 9.4 for Windows or newer (SAS Institute Inc., Cary, NC, USA). PK variable programming is conducted with R 3.6.1 for Windows or newer (R Foundation for Statistical Computing/R Development Core Team, Vienna, Austria, 2010.

9.2 Protocol violations/deviations

Protocol deviations will be identified based on conditions related to the categories below:

- Protocol entry criteria
- Forbidden concomitant medications
- Missing evaluations for relevant endpoints
- Other protocol deviations occurring during study conduct.

Major protocol deviations will be identified before the study closure, and listed where appropriate.

9.3 Power calculation

This study is exploratory and no formal power calculation for a primary endpoint has been performed. However, we expect the calculated MDES of the NeuroCart tests represent reasonable improvements to expect for an effective treatment.

9.4 Missing, unused and spurious data

All missing or incomplete safety and PD data, including dates and times, are treated as such. Missing test results or assessments will not be imputed. Missing PD data, indicated as ‘M’ in the data listing, will be estimated within the statistical mixed model using SAS PROC MIXED.

For graphical and summary purposes PD and safety values below the limit of quantification will be set to half (½) of the limit of quantification. For analysis no undetermined values will be replaced.

If single data points for PK concentrations are missing, the AUC parameters will be derived by interpolating with regard to the two neighboring non-missing concentrations. For calculation of PK parameters, all PK concentration values below the quantification limit (BLQ) occurring prior to Cmax will be replaced by 0, except for embedded BLQ values (between two measurable time points) which will be treated as “missing”. All BLQ values after Cmax will be treated as “missing”. The handling of missing, unused and spurious data will be documented in the study report.

9.5 Analysis sets

Data of all subjects participating in the study will be included in the analyses if the data can meaningfully contribute to the objectives of the study.

9.5.1 Safety set

The safety population will be defined as all subjects who were validated (randomised) and received at least 1 dose of study treatment.

9.5.2 Pharmacokinetic analysis set

The PK analysis population is defined as all subjects who were validated (randomised), received at least one dose of study treatment, and have at least one measurable drug concentration in samples collected.

9.5.3 Pharmacodynamic analysis set

The analysis population for pharmacodynamics is defined as all subjects who were validated (randomised), received at least one dose of study treatment, and have at least one post-baseline assessment of the parameter being analysed.

9.6 Subject disposition

Subject disposition will be listed by subject.

The following subject data will be summarized:

- number and percentage of subjects screened,
- number and percentage of subjects enrolled,
- number and percentage of subjects completed,
- number and percentage of subjects included in safety population and
- number and percentage of subjects included in the PD analysis population.

A subject who completed the study is defined as a subject where the last study visit has taken placd

9.7 Baseline parameters and concomitant medications

9.7.1 Demographics and baseline variables

Continuous demographic variables (e.g., age, height, weight, BMI) will be summarized by descriptive statistics (n, mean, SD, median, Min, Max).

Qualitative demographic characteristics (sex, race/ethnicity) will be summarized by counts and percentages.

9.7.2 Medical history

Relevant medical history will be listed.

9.7.3 Treatment compliance/exposure

Exposure to study treatment is described in terms of duration of treatment and diary records of medication.

9.8 Safety and tolerability endpoints

The safety set is used to perform all safety analyses. Baseline is defined as the last value prior to dosing. Change from baseline will be calculated for all continuous safety parameters.

9.8.1 Adverse events

The AE coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarize AEs by primary system organ class (SOC) and preferred term (PT). All adverse events will be displayed in listings. A treatment-emergent adverse event (TEAE) is defined as an adverse event observed after starting administration of the specific treatment, and up to 5 days (120 hours) after study drug administration. If a subject experiences an event both prior to and after starting administration of a treatment, the event will be considered a TEAE (of the treatment) only if it has worsened in severity (i.e., it is reported with a new start date) after starting administration of the specific treatment, and prior to the start of another treatment, if any. All TEAEs collected during the investigational period will be summarized.

The number of subjects with treatment emergent AEs will be summarized by:

1. treatment, MedDRA SOC and PT;
2. treatment, MedDRA SOC, PT and severity;
3. treatment, MedDRA SOC, PT and drug relatedness.

9.9 Pharmacokinetic and pharmacodynamic endpoints

9.9.1 Pharmacokinetics

The individual plasma and saliva drug concentrations will be listed by treatment, subject, visit and time. Individual plasma and saliva drug concentrations versus time will be plotted in panel plots for each treatment using both a linear and log y-axis.

The individual plasma and saliva drug concentrations will be summarised (n, mean, SD, %CV, median, Min and Max values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars.

When an actual sampling time of a drug concentration sample differs from the protocol time by more than 20% and at least 10 minutes the concentration will be excluded from calculation of descriptive statistics and a note will be added to the sample in the listing.

The individual PK parameters (except t_{max} and t_{lag}) will be summarized (n, mean, SD, %CV, geometric mean, geometric %CV, median, Min and Max) and will be presented graphically as boxplots. For t_{max} and t_{lag},the n, median, Min and Max statistics will be reported.

During part A, linear correlations will be assessed between saliva and plasma concentrations of clonazepam. When a linear correlation is found, the resulting formula will be used to estimate plasma concentrations in part B of the study. When there is no linear correlation, a population pharmacokinetic model of plasma and saliva concentrations will be constructed using nonlinear mixed effects modelling via NONMEM. Based on the PK model of plasma clonazepam concentrations in plasma, linear and/or exponential relationships will be explored to predict salivary clonazepam. Exploratory analysis of covariates will be performed on the models concerning the relationships between salivary and plasma clonazepam. The resulting optimal model will then be used to predict plasma concentrations from saliva concentrations in part B of the study.

9.9.2 Pharmacodynamics

The final analysis will be preceded by a blind data review which consists of individual graphs per visit by time of all pharmacodynamic measurements by time. The graphs will be used to detect outliers and measurements unsuitable for analysis.
The PD parameters will be listed by treatment, subject, visit and time. Individual graphs by time will be generated.

All PD endpoints will be summarised (n, mean, SD, SEM, median, Min and Max values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars.

Parameters will initially be analyzed without transformation, but if the data suggest otherwise, log-transformation may be applied. Log-transformed parameters will be back-transformed after analysis where the results may be interpreted as percentage change.

To establish whether significant treatment effects can be detected with the parent-reported questionnaires (ABC) and physician-reported questionnaires (CGI-I), a paired T-test shall be conducted. For the ABC test, the difference between the baseline and end-of-treatment period subscale scores shall be calculated and used for statistical analysis.

To establish whether significant treatment effects can be detected on the NeuroCart® PD parameters (animal fluency, smooth pursuit eye movement, saccadic eye movements, adaptive tracking and finger tapping) and Withings Steel HR (physical activity, average heart rate, sleep duration, amount of times to wake up) parameters, each parameter will be analyzed with a mixed model analysis of covariance (ANCOVA) with treatment, time, and treatment by time as fixed factors and subject, subject by treatment and subject by time as random factors and the (average) baseline measurement as covariate.

The Kenward-Roger approximation will be used to estimate denominator degrees of freedom and model parameters will be estimated using the restricted maximum likelihood method.

The general treatment effect and specific contrasts will be reported with the estimated difference and the 95% confidence interval, the least square mean estimates and the p-value. Graphs of the Least Squares Means(LSM) estimates over time by treatment will be presented with 95% confidence intervals as error bars, as well as change from baseline LSM estimates.

The following contrasts will be calculated within the model:

- Treatment - Placebo

9.9.3 Inferential methods

The study is exploratory. No adjustments for multiple comparisons will be applied.

9.9.4 PK/PD modelling

Population PK and PK/PD models will be developed to address objectives that require an integrative interpretation of the study results. The pharmacokinetic model developed during part A will be used to calculated plasma concentrations and drug exposure. Clonazepam metabolism is quite variable. The model will be used to investigate whether drug exposure is related to the pharmacodynamics effects. A separate Pharmacometric Analysis Plan will be written before initiation of the PK/PD model development..

9.10 Exploratory analyses and deviations

Exploratory data-driven analyses can be performed with the caveat that any statistical inference will not have any confirmatory value. Deviations from the original statistical plan will be documented in the clinical study report.

9.11 Interim analyses

There are no planned interim analyses.

10 GOOD CLINICAL PRACTICE, ETHICS AND ADMINISTRATIVE PROCEDURES

10.1 Good clinical practice

10.1.1 Ethics and good clinical practice

The investigator will ensure that this study is conducted in full compliance with the protocol, the principles of the Declaration of Helsinki (www.wma.net), ICH GCP guidelines (http://www.ich.org/products/guidelines.html), and with the laws and regulations of the country in which the clinical research is conducted.

10.1.2 Ethics committee / institutional review board

The investigator will submit this protocol and any related documents to an Ethics Committee (EC) and the Competent Authority (CA). Approval from the EC and the statement of no objection from the CA must be obtained before starting the study, and should be documented in a dated letter/email to the investigator, clearly identifying the trial, the documents reviewed and the date of approval. A list of EC members must be provided, including the functions of these members. If study staff were present, it must be clear that none of these persons voted.

Modifications made to the protocol after receipt of the EC approval must also be submitted as amendments by the investigator to the EC in accordance with local procedures and regulations.

10.1.3 Informed consent

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The investigator must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time for any reason.

The Informed Consent and Subject Information will be provided in Dutch.

10.1.4 Insurance

The investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO. The investigator (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23rd June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

- € 650,000.- (i.e., six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 5,000,000.- (i.e., five million Euro) for death or injury for all subjects who participate in the Research;
- € 7,500,000.- (i.e., seven million and five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.2 Study funding

CHDR is the sponsor of the study. Additional study funding is requested via the ZonMW Goed Gebruik Geneesmiddelen Rediscovery Grant.

10.3 Data handling and record keeping

10.3.1 Data collection

Data will be recorded on electronic data collection forms in Promasys for subsequent tabulation and statistical analysis. The data will be handled confidentially. A Subject Screening and Enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.
If applicable, data recorded on paper data collection forms and will be entered after quality control in a Promasys database for tabulation and statistical analysis. The data will be handled confidentially. Data collection will comply with the General Data Protection Act (GDPR, Dutch: Algemene Verordening Gegevensbescherming (AVG)).

10.3.2 Database management and quality control

All data from paper source will be entered into the Promasys database twice, by two different individuals. A quality control check will be done by CHDR staff on all data entered in the Promasys database, using data entry progress checks and database listings (blind data review). Errors with obvious corrections will be corrected before database lock.
Results of computer (NeuroCart/trial@home) tests and electronically captured questionnaires, clinical laboratory and pharmacokinetic analyses will be sent electronically to CHDR and loaded into the database.
After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the investigator and the statistician.

10.4 Access to source data and documents

All study data will be handled as confidential. The investigator will retain the originals of all source documents generated at CHDR for a period of 2 years after the report of the study has been finalised, after which all study-related documents will be archived (at a minimum) on micro-film which will be kept according to GCP regulations. The investigator will permit trial-related monitoring, audits, EC review and regulatory inspections, providing direct access to source data and documents.

10.5 Quality control and quality assurance

This study will be conducted according to applicable Standard Operating Procedures (SOPs). Quality assurance will be performed under the responsibility of CHDR's Quality Assurance manager.

10.5.1 Monitoring

An initiation visit will be performed before the first subject is included. Monitoring visits and contacts will occur at regular intervals thereafter, according to a frequency defined in the study-specific monitoring plan. A close-out visit will be performed after study closure.

10.6 Protocol amendments

Any change to a protocol has to be considered as an amendment.

10.6.1 Substantial amendment

Significant changes that affect subject safety and/or the scientific value of a trial require a substantial amendment. Examples of significant changes are given in EU guidelines on the request to the competent authorities for authorisation of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1, 2010/C 82/01). The need for submitting a substantial amendment is the responsibility of the sponsor.

Substantial amendments are to be approved by the appropriate EC and the CA will need to provide a 'no grounds for non-acceptance' notification prior to the implementation of the substantial amendment.

10.6.2 Non-substantial amendment

Non substantial amendments do not affect subject safety or the scientific integrity of the trial. Non-substantial amendments will be approved (signed) by the investigator(s) and will be recorded and filed by the investigator/sponsor. Non-substantial amendments will be submitted to the EC for information only. The CA will only be notified by changes in Eudract form and ABR form (if applicable) at toetsingonline. The implementation of a non-substantial amendment can be done immediately.

The EU guideline CT-1 2010/C 82/01 stipulates the importance of preventing over-reporting. Therefore the following changes are by definition non-substantial in this study:

- changes in assay-type and / or institution where an assay will be performed, provided that validated assays will be used;
- editorial changes to documents in the submission dossier including the volunteer information sheets and the protocol. An editorial change is defined as a modification in the documents of typographical errors and other modifications that in no way alter the meaning or content of the document
- other statistical analyses than described in the protocol.
- A change in clinical staff, including the principal investigator, when this concerns regular staff members of CHDR who comply with internal regulations for training and authorisation.

10.6.3 Urgent amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the investigators in the best interests of the subjects. Therefore, if deemed necessary, an investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by the EC(s) and CA.

10.7 End of study report

The investigator will notify the EC and the CA of the end of the study within a period of 90 days. The end of the study is defined as the last subject's last visit. In case the study is ended prematurely, the investigator/sponsor will notify the EC and the CA within 15 days, including the reasons for the premature termination. The sponsor will notify the EC immediately of a temporary halt of the study, including the reason of such an action. Within one year after the end of the study, the investigator and/or sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the EC and the CA. The principal investigator will be one of the signatories for the study report.

10.8 1.1 Public disclosure and publication policy

In accordance with standard editorial and ethical practice, the results of the study will be published. The authorship guidelines of the Vancouver Protocol will be followed regarding co-authorship.

http://www.icmje.org/

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APPENDIX 1 COVID-19 RISK ASSESMENT AND MITIGATION STRATEGIES

The current SARS-CoV-2 pandemic can pose a challenge to integrity of the trials, protection of participants' rights, safety and wellbeing and the safety of clinical trial staff. Therefore, risk mitigation strategies will be put in place for this trial following the CCMO guidance *Recommendations for the conduct of clinical research at the time of restrictive measures due to the coronavirus*. These mitigation strategies will be kept in place and evaluated on an ongoing basis for the duration of this trial, or until there is a consensus that the period of the SARS-CoV-2 outbreak in the Netherlands has passed. If the dynamics of the SARS-CoV-2 outbreak change in such a way that the safety of the trial participants and clinical trial staff or integrity of the data collected during this clinical trial cannot be guaranteed the trial will be halted.

COVID-19 RISK ASSESSMENT - Part A

Risk for Trial Participants and Trial Staff

Healthy subjects in the current study fall in a low risk category for complications of COVID-19, the disease caused by the SARS-CoV-2 virus. To prevent SARS-CoV-2 infections among trial participants, measures and procedures based on the advice issued by the Dutch Centre for Infectious Disease Control (RIVM) and COVID-19 measures declared by the Dutch government will be adhered to as outlined in CHDR SOP GGECOVID. Site trial staff in direct contact and/or within 1.5 m distance of study subjects will receive additional protection via the use of Personal Protective Equipment (PPE). Trials subjects do no stay overnight during this study and therefore will not be tested on COVID-19 in the absence of symptoms. Healthy subjects will be excluded from the study when tested positive for SARS-CoV-2.

Protection of Trial Integrity

Adherence to the protocol and CHDR SOP GGECOVID protects the integrity of the data collected during this clinical trial, as well as participants' data protection rights.

Impact of Investigational Drug on COVID-19 disease

Based on the mechanism of action of the investigational drug and the available information in the Investigators Brochure, there is currently no reason to believe that the investigational drug could 1) increase the susceptibility of trial participants to the SARS-CoV-2 virus, or 2) worsen or mask any COVID-19 signs, symptoms or complications.

COVID-19 Contingency Plan

Any subject that presents with COVID-19-related symptoms and/or has a positive SARS-CoV-2 PCR will be excluded from (further) participation in the trial and will receive follow-up medical attention per CHDR SOP GGECOVID.

COVID-19 RISK MITIGATION MEASURES

SARS-CoV-2 Screening

Subjects that test positive for a SARS-CoV-2 infection prior to the first dose will be withdrawn from the study and will be replaced. Subjects that test positive for a SARS-CoV-2 infection after the first study dose will be withdrawn from the study and may be replaced. Subjects with a SARS-CoV-2 infection will be followed-up according to SOP GGGECOVID.

COVID-19 Arrival Checklist and Temperature Measurement

Trial participants are requested to come to the clinic only if they have no symptoms that could indicate a COVID-19 infection and if they have not been in contact with a COVID-19 patient for at least 14 days. A standard checklist and temperature measurement are used upon arrival at the clinic.

Temporary Exclusion of Subjects with a Risk Factor for a COVID-19 Infection

During the period of the SARS-CoV-2 outbreak in the Netherlands only subjects without underlying conditions comprising a risk factor for a COVID-19 infection will be recruited into the study. Therefore subjects ≥ 70 years, subjects with a BMI > 30 and/or cardiovascular, respiratory or immune system disorders will be excluded, even where protocol inclusion and exclusion criteria would allow enrollment of these subjects. Exclusion of these subjects will be safeguarded via COVID-19 specific validation rules in the Promasys study database.

COVID-19 Lifestyle Restrictions
Trial participants will be required to adhere to the measures and procedures outlined in CHDR SOP GGECOVID, based on the advice issued by the Dutch Centre for Infectious Disease Control (RIVM) and COVID-19 measures declared by the Dutch government, to prevent SARS-CoV-2 infections among trial participants and clinical site staff.

COVID-19 RISK ASSESSMENT - Part B
Risk for Trial Participants and Trial Staff
To the best of current knowledge, subjects with ARID1B-related ID in the current study do not fall in a high-risk category for complications of COVID-19, the disease caused by the SARS-CoV-2 virus.

To prevent SARS-CoV-2 infections among trial participants, measures and procedures based on the advice issued by the Dutch Centre for Infectious Disease Control (RIVM) and COVID-19 measures declared by the Dutch government will be adhered to as outlined in CHDR SOP GGECOVID. Site trial staff in direct contact and/or within 1.5 m distance of study subjects will receive additional protection via the use of Personal Protective Equipment (PPE). Trials subjects do not stay overnight during this study and therefore will not be tested on COVID-19 in the absence of symptoms. Subjects will be excluded from the study when tested positive for SARS-CoV-2 during the trial.

Protection of Trial Integrity
Adherence to the protocol and CHDR SOP GGECOVID protects the integrity of the data collected during this clinical trial, as well as participants' data protection rights.

Impact of Study Drug on COVID-19 disease
Based on the mechanism of action of the study drug and the available information in the Summary of Product Characteristics, there is currently no reason to believe that the study drug could 1) increase the susceptibility of trial participants to the SARS-CoV-2 virus, or 2) worsen or mask any COVID-19 signs, symptoms or complications.

COVID-19 Contingency Plan
Any subject that presents with COVID-19-related symptoms and/or has a positive SARS-CoV-2 will be excluded from (further) participation in the trial and will receive follow-up medical attention per CHDR SOP GGECOVID.

SARS-CoV-2 Vaccination
No SARS-CoV-2 vaccination (initial and/or follow-up injections) should be administered three days prior to any of the four study days.

COVID-19 RISK MITIGATION MEASURES
SARS-CoV-2 Screening

Decisions on study continuation/discontinuation of individuals that test positive for SARS-CoV-2 after the first dose will be made by the principal investigator on a case-by-case basis. Subjects with a SARS-CoV-2 infection will be followed-up according to SOP GGECOVID

COVID-19 Arrival Checklist and Temperature Measurement

Trial participants are requested to come to the clinic only if they have no symptoms that could indicate a COVID-19 infection and if they have not been in recent contact with a COVID-19 patient as per current RIVM guidance. A standard checklist and temperature measurement test are used upon arrival at the clinic.

COVID-19 Lifestyle Restrictions

Trial participants will be required to adhere to the measures and procedures outlined in CHDR SOP GGECOVID, based on the advice issued by the Dutch Centre for Infectious Disease Control (RIVM) and COVID-19 measures declared by the Dutch government, to prevent SARS-CoV-2 infections among trial participants and clinical site staff.