I.R.I.S.

Institut de Recherches Internationales Servier

Document title	**AMENDED CLINICAL STUDY PROTOCOL**
Study official title	Efficacy and safety of bumetanide oral liquid formulation in children aged from 2 to less than 7 years old with Autism Spectrum Disorder.
Study brief title	*A 6-month randomised, double-blind, placebo controlled multicentre parallel group study to evaluate efficacy and safety of bumetanide 0.5mg twice a day followed by an open label active 6-month treatment period with bumetanide (0.5mg twice a day) and a 6 weeks discontinuation period after treatment stop.*
Test drug code	Bumetanide – S95008
Indication	Autism Spectrum Disorder
Development phase	Phase III
Protocol code	CL3-95008-002
EudraCT Number	2017-004420-30
ClinicalTrial.gov Number	NCT03715153
Universal Trial Number	Not applicable
Sponsor	Institut de Recherches Internationales Servier (I.R.I.S.) 50 Rue Carnot 92284 Suresnes Cedex – France
International Coordinator	Not applicable
Date of the document	25 March 2021
Version of the document *Substantial Amendment integrated*	Final version

No	Final version date	Countries concerned
1	16/05/2018	ALL
2	12/12/2018	ALL
3	12/08/2019	ALL
4	12/02/2020	FRA
5	14/02/2020	POL
6	03/08/2020	FRA
7	10/09/2020	POL
8	30/11/2020	ALL
9	25/03/2021	CZE

CONFIDENTIAL

FOLLOW-UP OF VERSIONS

	Substantial amendment No	Final version date	Countries concerned	Nature of modifications
Initial protocol	NA	*27/02/2018*	ALL	Not Applicable
Amended protocol	1	16/05/2018	ALL	Sections 5.2. and 6.3.: - Typo errors Section 8.2. : Update of the Potassium supplementation recommendations in case of hypokalaemia
	NA	07/09/2018	FRA	See appendix 19
	NA	17/09/2018	DEU	See appendix 20
	NA	03/10/2018	PRT	See appendix 21
	2	12/12/2018	ALL	• Study summary sheet: increase of the total planned number of countries (from 10 to 12) and suppression of safety measurements [drug screening (urine), βHCG] • Section 2: addition of information about risks, benefits and burdens in the study • Section 4.2.1: clarification of study plan and that 'safety follow up visit' is 'follow up visit' • Section 4.2.2: addition of a legend in Table (4.2.2) 1 about IMP dispensation at W026 and clarification about blood electrolytes analysis correction of Table (4.2.2)2: suppression of βHCG

				addition of instructions on how to perform study evaluations and non-compulsory visits on site for the patient addition of the Table (4.2.2) 3 about the conduct in case of definitive discontinuation of IMP during the double-blind period • Section 4.3: additional information about analysis to minimise bias • Section 4.4.1: clarification on the way of administration of the oral solution • Section 4.6: clarification on VABS II scale for source data • Section 5.2: Non-selection criteria modification of criterion 15 modification of the non-selection criterion 24 to further describe stabilised epilepsy modification of non-selection criterion 25 with an information about alternative interventions modification of the non-selection criterion 27 with a clarification for the use of laxatives • Section 5.4: Exclusion criteria modification of the exclusion criterion 34 (*i.e.*value of QT interval and value of hypercalciuria)

				modification of exclusion criteria 35 (nephropathy) • Section 5.6.1: modification of withdrawal criteria for: Occurrence of seizures Proteinuria/creatininuria Value of hypercalciuria Value of QT prolongation Addition of absence of 2 consecutive planned urinary monitoring analysis • Section 5.6.2.Procedure Addition of information on withdrawal visit • Section 6.2: correction of two errors of IMPs dispensing (at W026) during the double blind period and the open label safety extension period • Section 6.3: addition of recommendations about the use of laxatives and use of light gas sedation • Section 7.2: information on who will be authorized to complete the VABS II • Section 8.2: Methods and measurement times information on training by the investigators on the C-SSRS-C and the PAERS, information on ECG reading process (centralised and locally), clarification on biological safety measurements clarification on the recommendation that investigators should encourage patients to eat foods containing potassium as soon as the patient is included in the study • Section 8.8: addition of

				explanation about causal causal relationship • Section 8.9.2: addition of information about responsibility of the investigator • Section 8.9.3: addition of information about new safety information • Section 12.4: clarification of the section on Supervisory committee • Sections 13.2 and 13.3: clarification about assent/consent • Section 14.1: addition of information for the investigator after the final data base lock about participant data from his/her centre for archiving in the study file • Section 14.2: additional information about data collected on paper form (VABSII) on data entry and data validation • Section 14.3: Archiving: further clarification about archiving process • Section18: REFERENCES: updating of references
	NA	12/12/2018	BRA	See appendix 22
	NA	13/03/2019	IRL	See appendix 23
	NA	26/03/2019	BRA	See appendix 24
	NA	27/03/2019	IRL	See appendix 25
	NA	26/04/2019	BRA	See updated appendix 24
	NA	30/07/2019	USA	See appendix 26
	3	12/08/2019	ALL	• Section 5.4. Exclusion criteria: updating of criterion 34 about abnormal urinary calcium/creatinine ratio (>0.85mmol/mmol of

				creatinuria) and calciuria (≥3mmol/l) • Section5.6.1. Withdrawal criteria: updating of criterion about abnormal urinary calcium/creatinine ratio (>0.85mmol/mmol of creatinuria) and calciuria (≥3mmol/l)
	4	12/02/2020	FRA	See appendix 27
	5	14/02/2020	POL	See appendix 28
	6	03/08/2020	FRA	See updated appendix 27
	7	10/09/2020	POL	See updated appendix 28
	8	30/11/2020	ALL	• Study summary sheet: update of Study completion date • Addition of the signature of the Biostatistics Head • Section 4.2.1. Study plan: update of the definition of the End of trial • Section 4.2.2. Investigation schedule: update of the Table (4.2.2)1 and addition of the mention of CARS QPC questionnaire for the parents questionnaires (SRS2 and quality of life questionnaires) and update of table (4.2.2) 2 • Update of the section 4.4.3. Management of the blinding systems • Update of the section 5.6.1. for Special conduct in case of gastrointestinal

				symptoms and weight loss • Section 8.2. Update of part about renal ultrasound • Section 9.1. Measurement of drug concentration: with cancellation of the sentence 'Thus, the exact PK sampling times cannot be specified' • Section 16. Ownership of the results – Data sharing policy and publication policy: update of the paragraph • Section 17.1.3. Final study report: addition of a primary clinical report after the 6-month double-blind period, a main clinical report after the 6-month open label period with the 6-week follow-up period and addition of an addendum to the report for the optional 6-month extension period • Addition of the appendix 29: Instruction to investigators for handling data rights requests
	9	25/03/2021	CZE	See appendix 30

STUDY SUMMARY SHEET

Name of the sponsor: I.R.I.S. 50 Rue Carnot 92284 Suresnes Cedex - France	Individual Study Table Referring to Part of the Dossier	*(For National Authority Use only)*
Name of Finished Product:	**Volume:**	
Name of Active Ingredient: Bumetanide (S95008)	**Page:**	

Title of study:
Efficacy and safety of bumetanide oral liquid formulation in children aged from 2 to less than 7 years old with Autism Spectrum Disorder.
A 6-month randomised, double-blind, placebo controlled multicentre parallel group study to evaluate efficacy and safety of bumetanide 0.5mg twice a day followed by an open label active 6-month treatment period with bumetanide (0.5mg twice a day) and a 6 weeks discontinuation period after treatment stop.
Protocol No.: CL3-95008-002

Coordinator(s) *or* **Investigator:**
National coordinators and investigators: listed in a separate document

Study centre(s):
International multicentre study
Total planned number of centres: Around 50
Total planned number of countries: 12

Study period:	**Study development phase: III**
- Study duration for the participant: up to 66 weeks - Study initiation date (planned date of first visit first participant): Q3 2018 - Study completion date: (planned date of last visit last participant in the open label period or in the extension period, whichever occurs the latest) : Q4 2022	

Objective(s):
The primary objective is to demonstrate the superiority of bumetanide (0.5mg BID) oral liquid formulation compared to placebo in the improvement of ASD core symptoms using CARS-2 after 6 months of treatment in children aged from 2 to less than 7 years old presenting with a confirmed ASD diagnosis.
The secondary objectives are:
- To assess the effect of bumetanide on the secondary efficacy endpoints
- To assess the safety of bumetanide
- To confirm the acceptability and palatability of the oral liquid formulation
- To describe the bumetanide effects on patients quality of life
- To improve existing pharmacokinetic model of bumetanide in this population

The exploratory objective is to describe the Bumetanide effect on utility index scores

Methodology:
Study design: 6-month, randomized, double-blind, placebo-controlled, parallel groups followed by an open label active 6-month treatment period with bumetanide.

Number of participants:
Planned: 200 patients included
Total for each treatment (approximately): 100 in bumetanide group, 100 in the placebo group

Diagnosis and main criteria for inclusion:
- Male and female patients from 2 to less than 7
- Primary diagnosis of ASD as per DSM-5 criteria
- Criteria met for ASD on Autism Diagnostic Observation Schedule-Generic (ADOS-2) and Autism Diagnosis Interview Revised (ADI-R)
- CGI (Clinical Global Impression) – Severity rating Score ≥ 4
- Childhood Autism Rating Scale second edition (CARS2-ST or HF) total raw score ≥ 34
- Social responsiveness Scale second edition (SRS-2) total score ≥ 66 T-Score
- Absence of diagnosis of Fragile X or Rett Syndrome
- Absence of any clinically significant abnormality likely to interfere with the conduct of the study according to the judgment of the investigator.

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Test drug:
BUMETANIDE oral solution dosed at 0.5 mg/mL
- For patients with a weight < 25 kg: bumetanide 0.02mg/kg corresponding to 0.04ml/kg oral liquid formulation taken twice daily (in the morning at wake up and in the afternoon 3 hours before going to bed at the latest)
- For patients with a weight ≥ 25kg: bumetanide 0.5mg corresponding to 1mL oral liquid formulation taken twice daily (in the morning at wake up and in the afternoon 3 hours before going to bed at the latest)

Comparator:
PLACEBO
- For patients with a weight < 25 kg: 0.04ml/kg oral liquid formulation taken twice daily (in the morning at wake up and in the afternoon 3 hours before going to bed at the latest)
- For patients with a weight ≥ 25kg: 1mL oral liquid formulation taken twice daily (in the morning at wake up and in the afternoon 3 hours before going to bed at the latest)

Duration of treatment:
Run-in period: up to 4 weeks (without IMP)
Study treatment period (with IMP administration): 52 weeks (+/- 28 days)
Follow up period: 6 weeks after discontinuation of IMP

Criteria for evaluation:
Efficacy measurements:
Primary efficacy endpoint: CARS2 total Raw score, expressed mainly in terms of change from baseline to 6 month
Secondary efficacy endpoints:
- The change in each individual CARS2 domain from baseline to 6 month
- The change in SRS-2 total raw score from baseline to 6 month
- CGI-I score at 6 month
- The change in Vineland Adaptative Behaviour Scale II (VABS II) from baseline to 6 month

Safety measurements:
- Adverse events, Pediatric Adverse Event Rating Scale (PAERS)
- Clinical laboratory evaluation: biochemistry including electrolytes monitoring, haematology, , calciuria, creatininuria, proteinuria,
- Vital signs and clinical examination: weight, height, Body Mass Index, heart rate, blood pressure
- Electrocardiogram
- Renal ultrasound
- Suicidality: Columbia-Suicide severity scale Children's version (C-SSRS-C)

Other measurements:
- Acceptability and palatability assessments
- Paediatric Quality of Life Inventory (PedsQL)
- WHOQOL-Bref questionnaire
- Utility index score on the EQ-5D-3L

Pharmacokinetic measurements:
- Concentrations of bumetanide will be determined in plasma by Cephac, using a validated bioanalytical method based on a protein precipitation followed by reverse phase liquid chromatography with tandem mass spectrometric detection. Procedures will be described in a separate bioanalytical protocol established by the assay centre.

Statistical methods:
Efficacy analysis:

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All efficacy analyses will be performed in all randomized patients.
The primary endpoint is defined as the change from baseline to 6 month in CARS2 total score.
Primary analysis: bumetanide will be compared to placebo using a general linear model with baseline CARS2 and stratification factors as covariates.
Missing data handling: CARS values post treatment discontinuation due to adverse event or lack of efficacy will be imputed using a reference based multiple imputation with a jump-to-reference approach. This approach assumes treatment benefit in patients who discontinue the Bumetanide arm disappears immediately upon discontinuation. Treatment effect estimation will take into account the situations when a patient can no longer tolerate or benefit from the treatment (occurrence of AE, lack of efficacy). CARS values post treatment discontinuation due to others reasons will be imputed using a classic multiple imputation approach assuming these patients could have theoretically continued to be treated without being put at undue risk.
Sensitivity analysis: to assess the robustness of the results of the primary analysis, sensitivity analyses to the method of handling missing data will be performed including a mixed model for repeated measurement (MMRM).

Study participants (disposition, baseline characteristics and follow-up) and Safety analysis:
Safety analysis:
Safety analyses will be performed in the Safety Set defined as all included patients having taken at least one dose of study treatment.
For every safety measurements, descriptive statistics will be provided by treatment groups.

Pharmacokinetic analysis:
A population pharmacokinetic analysis will be performed to assess PK profiles of bumetanide in children. The population PK analysis will provide population PK parameters and their associated variability. In this analysis, the potential influence of covariates will be also investigated. Individual secondary PK parameters (such as AUC12,ss, C_{min}) will be derived from the model for each patient. The population PK analysis will be detailed in a separate Data Analysis Plan.
Exploratory assessment of the relationship between exposure and pharmacodynamics (efficacy and potentially safety) will be performed and if applicable, population PK/PD models will be developed and a Data Analysis Plan will be set up.

Contractual signatories

I, the undersigned, have read the foregoing protocol and the "Participant information and consent form" document attached to the protocol and agree to conduct the study in compliance with such documents, Good Clinical Practice (GCP) and the applicable regulatory requirements.

NAME **DATE** **SIGNATURE**

COORDINATOR / INVESTIGATOR :

CENTER NUMBER	

[redacted]

Other sponsor's signatories	
BIOSTATISTICS HEAD OR DESIGNEE:	
NAME	[redacted]
DATE	[redacted]
SIGNATURE	[redacted]

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List of abbreviations

µmol	:	micromole
ADI-R	:	Autism Diagnostic Interview - Revised
ADOS-2	:	Autism Diagnostic Observation Schedule, second edition
AE	:	Adverse Event
AEOSI	:	Adverse Event Of Special Interest
ALP	:	ALkaline Phosphatase
ALT	:	ALanineaminoTransferase
am	:	ante meridiem
ASD	:	Autism Spectrum Disorders
AST	:	ASpartateaminoTransferase
bid	:	bis in die (twice a day)
BMI	:	Body Mass Index
BP	:	Blood Pressure
bpm	:	beats per minute (heart rate unit)
CARS2	:	Childhood Autism Rating Scale, Second Edition
CARS2-HF	:	Childhood Autism Rating Scale, Second Edition – High functioning clinical tool
CARS2-QPC	:	Childhood Autism Rating Scale, Second Edition – Questionnaire for parents and caregivers
CARS2-ST	:	Childhood Autism Rating Scale, Second Edition – Standard Tool
CGI	:	Clinical Global Impression
CGI-I	:	Clinical Global Impression – Global Improvement
CGI-S	:	Clinical Global Impressions Scale
CHMP	:	Committee for Medicinal Products for Human Use
cm	:	Centimetre
CMP	:	Clinical Monitoring Plan
CRF	:	Case Report Form
CRO	:	Contract Research Organisation
C-SSRS-C	:	Columbia Suicide Severity Rating Scale Children's Version
CV	:	Curriculum Vitae
DBP	:	Diastolic Blood Pressure
DMC	:	Data Monitoring Committee
DSM-5	:	Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition – Text revision
DMC	:	Data Safety Monitoring Board
e.g.	:	Exempli gratia (for example)
E2	:	Estradiol
ECG	:	ElectroCardioGram
e-CRF	:	Electronic Case Report Form
ENT	:	Ear, Nose and Throat
ERIN	:	Event Requiring Immediate Notification
g	:	gram
GABA	:	Gamma-aminobutyric acid
gamma GT	:	Gamma-Glutamyltransferase
GCP	:	Good Clinical Practice

GI	:	GastroIntestinal
h	:	hour
HAV	:	Hepatitis A Virus
HBs	:	Surface antigen of Hepatitis B virus
HBV	:	Hepatitis B Virus
HCV	:	Hepatitis C Virus
HDL	:	High-Density Lipoprotein
HIV	:	Human Immunodeficiency Virus
HR	:	Heart Rate
HRT	:	Hormone Replacement Therapy
i.e.	:	id est (that is)
I.R.I.S.	:	Institut de Recherches Internationales Servier
ICH	:	International Conference on Harmonisation
ICPM	:	International Classification of Procedures in Medicine
ICTR	:	International Centre for Therapeutic Research
IEC	:	Independent Ethics Committee
IMP	:	Investigational Medicinal Product: a pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial (test drug / placebo)
IQ	:	Intellectual Coefficient
IRB	:	Institutional Review Board
IRS	:	Interactive Response System
IU	:	International Unit
IUD	:	IntraUterine (contraceptive) Device
IV	:	IntraVenous (route)
IVRS	:	Interactive Voice Response System
IWRS	:	Interactive Web Response System
K	:	Kalium
kg	:	kilogram
L	:	Litre
LDL	:	Low-Density Lipoprotein
MCV	:	Mean Corpuscular Volume
MedDRA	:	Medical Dictionary for Regulatory Activities
mg	:	milligram
min	:	minute
mL	:	Millilitre
mm	:	Millimetre
mmHG	:	Millimetre of mercury
MMRM	:	Mixed Model for Repeated Measurement
NA	:	Not Applicable
Na	:	Natrium
ng	:	nanogram
NKCC1	:	Na+/K+/2Cl- cotransporter
NS	:	Not statistically Significant
OCD	:	Obsessive Compulsive Disorder
PAERS	:	Paediatric Adverse Event Rating Scale
PD	:	PharmacoDynamics
PED QL	:	Paediatric Quality of Life Inventory
PIP	:	Paediatric Investigation Plan
PK	:	PharmacoKinetics

pm	:	post meridiem
po	:	per os (orally)
PT	:	Prothrombin Time
QC	:	Quality Control
QTc	:	QT interval corrected for heart rate
SAE	:	Serious Adverse Event
SBP	:	Systolic Blood Pressure
SD	:	Standard Deviation
SE	:	Standard Error
sec	:	second
SmPC	:	Summary of Product Characteristics
SRS2	:	Social Responsiveness Scale, Second Edition
SSRI	:	Selective serotonin reuptake inhibitor
SUSAR	:	Suspected Unexpected Serious Adverse Reaction
test drug	:	Drug substance in a given dosage form, tested in a clinical trial (S95008)
V	:	Visit
VABS II	:	Vineland Adaptative Behaviour Sales, Second Edition
WHO	:	World Health Organization
WHO-DD	:	World Health Organization, Drug Dictionary
yo	:	Years old

1. ADMINISTRATIVE STRUCTURE OF THE STUDY

Non sponsors parties, sponsors parties and CRO responsible for local management of the study are described in a separate document entitled Administrative part of clinical study protocol.

The list of investigators for each country is given in separate documents attached to the protocol and entitled "List of investigators for [name of the country]".

The composition and role of the Data Monitoring Committee are described in sections 8.10 and 12.4.

2. BACKGROUND INFORMATION

Autism Spectrum Disorders (ASD) is a group of complex neurodevelopmental disorders characterized by the presence of multiple and persistent deficits in social communication and social interaction associated with restricted interests and stereotyped and repetitive behaviours. Aetiology of ASD is still poorly understood, but the disease appears highly genetically heritable with identified genetic mutations – despite not fully penetrant - in about 15% of the cases. Nonspecific environmental risks factors, as parental age, foetal exposure to alcohol or valproate and premature birth/ low birth weight, have also been identified.

The most frequent comorbidities and associated symptoms are: learning disabilities, sleep disturbances, epilepsies, anxiety, depression, attention problem, hyperacusis, irritability (including symptoms of aggression toward others, deliberate self-injuriousness, temper tantrums, and quickly changing moods), conduct disorder, and Obsessive Compulsive Disorder (OCD). The presence and the severity of these symptoms are very heterogeneous among patients and depend on their age.

Usual cares include global care, often in specialized institutions, and psychosocial interventions as educational measures, patients and families support and/or specific psychotherapies (mainly behavioural therapies). Associated symptoms frequently lead to drug prescription, however only second-generation antipsychotics have demonstrated clinical efficacy in behavioural disorders. Frequently prescribed off-label drugs include methylphenidate and atomoxetine for attention disorders, melatonin for sleep disorders, and selective serotonin reuptake inhibitors (SSRIs) for anxio-depressive disorders, however there is presently no approved treatment targeting core symptoms of autism.

Recent studies (Cellot, 2014) (Coghlan, 2012) suggest that GABAergic neurons and circuits may be altered in ASD. The conversion of GABA-mediated neuronal excitation into inhibition during maturation of specific neuronal populations has been reported to be altered in neurodevelopmental diseases such as autism. This lack of “GABA switch” is due to persistent high level of expression of Na+/K+/2Cl- co-transporter (NKCC1) *vs* K-Cl co-transporters (KCC2). This in turn may lead to abnormal cell migration and differentiation, immature and imbalanced neuronal network development and thus to clinically diagnosed deficits observed in this pathology.

Levels of intracellular chloride determine the levels of neuronal inhibition and have been shown to be elevated in immature neurons and being progressively reduced within neuronal development. These observations suggest that drugs reducing intracellular chloride levels may

be helpful in normalising chloride levels and thereby restore inhibitory GABAergic function and neuronal network maturation.

Bumetanide (Burinex®) is a sulfonamide-derived loop diuretic used for the management in adult patients of oedema associated with congestive heart failure, hepatic cirrhosis and renal disease including nephropatic syndrome.

Acting centrally as a NKCC1 inhibitor, bumetanide provokes reduction of intracellular chloride, switching the aberrant excitatory action of GABA into an inhibitory action.

Up to now, the efficacy and safety of bumetanide in ASD children and adolescents have been studied in a clinical development programme consisting of 5 clinical studies (Please refer to Clinical Investigator's Brochure latest version, on section 5 for further details).

The first use of Bumetanide in ASD was a pilot study conducted in 5 children with ASD age 3 to 11 years of age (Lemonnier, 2010). This study was followed by a placebo controlled randomized phase II study in 54 ASD children (BUMEA study [NCT01078714]:(Lemonnier, 2012). Of note as well, an fMRI study (academic study), carried out in open label conditions in 7 young autistic patients showed an improvement of facial processing after 1 month of bumetanide treatment (Lemonnier, 2012; Hadjikhani, 2015).

In parallel, Neurochlore sponsored a retrospective, observational study evaluating the long-term safety of Bumetanide in children and adolescents with Autism Spectrum Disorders (ASD) treated with Bumetanide on an off-label basis in the CHRU of Brest (France).

In June 2012 Neurochlore submitted a Paediatric Investigation Plan (PIP) for the condition 'Autistic Spectrum Disorder' under Article 30 of the Paediatric Regulation. It was agreed by EMA on 30 April 2013 (EMEA-001303-PIP01-12).

Neurochlore carried out the phase IIb dose ranging study assessing the efficacy and safety of Bumetanide 0.5, 1.0 and 2.0mg BID doses (oral liquid formulation) in male and female patients aged ≥2 to ≤18 years of age diagnosed with ASD according to DSM-IV-TR. The main objective of the study was to determine the optimal dose of bumetanide for the pivotal phase III study. The primary criterion was the CARS scale. Three bumetanide oral solution doses, 0.5-1-2mg administered twice a day, were tested over a 3 month-treatment period. Eighty eight patients were included in the study. Using a general linear model including baseline CARS value as covariate, the difference between bumetanide 0.5 mg BID and placebo was statistically significant in the FAS -3.06 (95% CI =[-5.63 ; -0.49], p = 0.015). When the CARS score was evaluated for 73 out of 88 patients who had a CARS value at D90 (CARS completers analysis), the results were confirmed using the general linear model for the comparison between Bumetanide 0.5 mg BID and placebo with an estimated difference at -2.93 (95% CI=[-5.57;-0.29], p= 0.026). Bumetanide also produced significant improvements over placebo on the secondary efficacy endpoints (SRS scale, CGI-scale). On a qualitative point of view, clinicians describe that children with bumetanide are more present, more open to the world, more attentive, more participative, more willing to exchange, less hyperactive, less aggressive, less agitated and less stereotyped. The children also look more at their interlocutor. The safety profile of Bumetanide was dose-related. The best tolerability profile was observed with the Bumetanide 0.5 mg twice a day, whereas an important number of adverse events and study withdrawals were observed with Bumetanide 1.0 and 2.0 mg twice a day. Considering the 0.5mg dose is effective and presented a better safety profile than 1mg and 2mg: this dose was retained for the phase III.

Taking into account these elements, bumetanide was proposed as the first candidate allowing treatment of ASD core symptoms in paediatric population.

On the 24th February 2017, Neurochlore and Les Laboratoires Servier' entered into an agreement for development and commercialisation of the bumetanide paediatric formulation in Europe. 'Les Laboratoires Servier' takes over the phase III development program.

Following the completion of the phase II, a Committee for Medicinal Products for Human Use (CHMP) Scientific Advice procedure was performed beginning 2017 to discuss the bumetanide phase III development plan in ASD paediatric patients. A randomised, placebo controlled study design with a 6-month double-blind treatment period followed by a 6-month open labelled extension period was considered by the CHMP as the most appropriate to have a comprehensive bumetanide efficacy and safety assessment. A modification to the agreed PIP has been submitted to the Paediatric Committee (PDCO) of the European Medicine Agency (EMA) and has received a positive decision (European Medicines Agency decision EMA-001303-PIP01-12-M02 dated 10th November 2017).

The present study (CL3-95008-002) will be performed in children from 2 to less than 7 years old presenting with ASD. A 6-month double-blind treatment period will be performed in which efficacy and safety of bumetanide 0.5mg BID will be assessed *versus* placebo. This double-blind period will be followed by a 6-month open label treatment period of bumetanide 0.5mg BID in which long term safety will be evaluated and a 6 weeks discontinuation phase. Bumetanide dose will be adapted on body-weight.

The present phase III bumetanide paediatric study has been developed based on the European Guideline and validated by the Paediatric Committee in the European Medicinal Agency (European Medicines Agency decision, EMA-001303-PIP01-12-M02 dated 10th November 2017). In this development program all children (even the placebo patients) will have at least 6 months active treatment by bumetanide.

The main burden of this study for the patient is related to the outpatient centre visits to follow up the safety of the diuretic effects, which requires physical examination [including blood pressure (SBP, DBP), heart rate, body weight, height], blood and urine tests, ECG and renal ultrasound, which are of low and known risks, as well as adverse event collection. Additional burden corresponds to cognitive test evaluations which are necessary for bumetanide efficacy assessment.

The Sponsor notes that these clinical studies will be carried out in a vulnerable population and has implemented several measures in order to minimize any potential burden:

- In the framework of this Paediatric Investigational Plan endorsed by the EMA, Servier has chosen the use of scales and questionnaires to be completed as much as possible by the parents/legal representative/caregiver instead of participants. This will significantly reduce the length of a visit where the presence of the child is compulsory.
- Based on the family environment and for organizational reasons, safety evaluations required for the most time consuming visits (Selection, inclusion, visit Week 12, visit Week 26, visit Week 38 and Visit 52) could be splitted in several days.
- Regarding the management of blood sampling, all the centres selected for this study are very familiar with working with children with Autism Spectrum Disorder (ASD) and their families. In the previous studies with bumetanide in ASD paediatric population, blood samplings were found quite easily manageable by experienced

teams, and field returns reported that only the very first samplings were challenging, further ones becoming rituals. For these new studies, all efforts will be made to make the blood samplings as stress-free as possible, coordinated with daily activities as far as possible and the experience of previous trials centres will be shared with the new ones. The investigator and/or centre staff will discuss with the parents/carergivers which strategies are most likely to be successful taking into account positive or negative past experiences of participants with doctors or blood sample taking. The site will always refer to parents/Legal Representative (LR)/caregiver for behavior management, communication and child's preferences. The study team will aim to have the same experienced staff involved for each visit. The study nurse and investigator will explain what will happen before starting the venepuncture and will check the understanding when applicable. It's up to the clinical team to decide how much time is necessary to explain, distract or comfort the patient. The investigator can refer to tools used in normal practice or available at its site such as visual supports or social story boards to prepare a child for having a blood sample, with the parent/LR/caregiver support. Local anaesthesia creams or vapo-coolant spray can be used to minimise discomfort. Then, the blood samplings should be performed quickly and efficiently to avoid escalation of potential anxiety or irritability.

- All those measures contribute to the installation of a ritual much appreciated by patients with ASD.
- Sponsor highly recommends that the same person in charge of study evaluations follows a given child during the study in order to minimize any potential bias in study assessments and to reduce the burden linked to the potential stress suffered by the child.
- Paediatric liquid formulation has been specially developed for this paediatric clinical program. This formulation showed a good acceptability and palatability profile in the phase II.

For the benefit of the study, bumetanide could be the first pharmacological candidate allowing the treatment of ASD core symptoms in paediatric population, acting on a key component of the disease pathophysiology (alteration of GABAergic neurons and circuits, the conversion of GABA-mediated neuronal excitation into inhibition during maturation of specific neuronal populations being reported to be altered in neurodevelopmental diseases such as autism).
Acting centrally as a NKCC1 inhibitor, bumetanide provokes reduction of intracellular chloride, switching the aberrant excitatory action of GABA into an inhibitory action, and therefore may restore important developmental capacities.
Consequently, as there is no drug treatment for core symptoms of ASD, we set up a study with bumetanide in young developing patients with ASD in order to reduce the ASD core symptoms and functional impairment and to improve quality of life. Studies are needed in paediatric population, childhood being a time when brain plasticity is at the greatest and where behavioural and educative interventions could have the most benefit.

For the risk for the patient, the safety profile of bumetanide is similar to that reported for other loop diuretics. The target organ is the kidney and the safety profile is related to the potent diuretic activity of the compound. Based on the safety experience gained at this stage of drug development in ASD (5 clinical studies described above), the safety profile of bumetanide in ASD children and adolescents seems comparable to the one observed in adult patients treated for oedema. Despite the penetration of bumetanide into brain, no toxic effects on the Central Nervous System have been observed.

The emergent bumetanide safety profile observed in paediatric population with ASD was mainly related to bumetanide diuretic effect (enuresis, pollakiuria, polyuria, electrolyte disturbances and dehydration) and was dose-related. It did not differ from that of bumetanide tablets. Hypokalaemia is an identified risk and therefore blood potassium level will be regularly monitored during the phase III bumetanide paediatric program (blood potassium sampling schedule validated with the EMA). It has to be highlighted that all hypokalaemia reported during the phase II NeuroClin02 dose-range study were asymptomatic (and without any cardiac impact), and thus it is crucial to perform all planned samplings. Lastly, the most favourable tolerability profile was observed with bumetanide 0.5 mg twice a day corresponding to the dose that will be given during the 2 phase III studies.

Considering the targeted population of patients, a Data Monitoring Committee (DMC) will be set up and will be responsible for periodic review of patient's safety data throughout the study. Details on the role and organisation of the DMC will be provided in a separate charter.
The study will be carried out in compliance with the protocol, GCP, the ethical principles that have their origin in the Declaration of Helsinki and the applicable regulatory requirements.

3. STUDY OBJECTIVES AND PURPOSE

3.1. Primary objective

The primary objective is to demonstrate the superiority of bumetanide (0.5mg BID) oral liquid formulation compared to placebo in the improvement of ASD core symptoms after 6 months of treatment in ASD children aged from 2 to less than 7 years old.

3.2. Secondary objectives

- To assess the effect of bumetanide on the other efficacy endpoints
- To assess the safety of bumetanide
- To confirm the acceptability and palatability of the oral liquid formulation
- To describe the bumetanide effects on patients quality of life
- To improve existing pharmacokinetic model of bumetanide in this population.

3.3. Exploratory objectives

To describe the Bumetanide effect on utility index scores

4. STUDY DESIGN

4.1. Endpoint(s)

Table (4.1) 1 - Endpoints

	Objectives	Endpoints
Primary	To demonstrate the superiority of bumetanide (0.5mg BID) oral liquid formulation as compared to placebo in the improvement of ASD core symptoms after 6 months of treatment in children aged from 2 to less than 7 years old with ASD	Childhood Autism Rating Scale, Second Edition (CARS2) total raw score. Main expression will be change from baseline to 6 month.

	Objectives	Endpoints
Secondary	To assess the effect of bumetanide on the other efficacy endpoints	- The change in SRS-2 total raw score from baseline to 6 month - CGI-I score at 6 month - The change in VABS II subscores from baseline to 6 month - The change in each individual CARS2 domain from baseline to 6 month
	To assess the safety of bumetanide	- Adverse events (AE), Paediatric Adverse Event Rating Scale (PAERS) - Clinical laboratory evaluation - Vital signs and clinical examination: weight (kg), height (m), Body Mass Index (BMI) (kg/m²), systolic blood pressure (mmHg), standing, sitting; Diastolic blood pressure (mmHg), standing, sitting, heart rate (bpm) - Electrocardiogram - Renal ultrasound - Assessment of suicidal ideation and suicidal behaviour using the Columbia Suicide Severity Rating Scale Children's version (C-SSRS-C)
	To confirm the acceptability and palatability of the oral liquid formulation	Acceptability and palatability questionnaire
	To describe the bumetanide effects on patients' quality of life	- Paediatric Quality of Life Inventory (PedsQL) expressed in term of change from baseline to month 6 - WHOQOL-Bref questionnaire summarized at each planned visit for each period using descriptive statistics
	To improve existing pharmacokinetic model of bumetanide in this population	PK points at W012 and W026
Exploratory	To describe the Bumetanide effect on utility index scores	Utility index score on the EQ-5D-3L

Refer to sections 6.1 and/or 7.1 for further details.

4.2. Experimental design

4.2.1. Study plan

This study is divided into the following periods:

1. A run-in period up to 4 weeks between selection (ASSE) and inclusion (week 0) visits:
   This period is without Investigational Medicinal Product (IMP) treatment to evaluate eligibility. Stable psychosocial intervention is maintained if any.
   This period allows investigators to perform ECG, renal ultrasound, laboratory examinations, and to complete, if needed, the wash-out period of forbidden treatments before inclusion.

2. Double-blind treatment period of 6 months between inclusion (week 0) and month 6 (week 26):
   At inclusion patients will be randomised to one of the two parallel groups: bumetanide 0.5mg BID or placebo BID. The treatment (bumetanide or placebo) will be assigned at inclusion by a balanced randomisation (ratio 1:1).
   - Patients randomised in the bumetanide arm will receive bumetanide up to 0.5mg BID. Bumetanide doses will be adapted on body-weight:
     - If their weight is < 25kg, they will receive bumetanide 0.02 mg/kg BID (oral solution 0.5 mg/mL) corresponding to a volume of solution of 0.04 ml/kg BID from inclusion to month 6
     - If their weight is ≥ 25kg, they will receive bumetanide 0.5mg BID (1mL oral solution BID) from inclusion to month 6
   - Patients randomised in the placebo arm will receive placebo BID
     The volume of the oral solution will be calculated on body-weight:
     - If their weight is < 25kg, they will receive placebo BID (corresponding to a volume of placebo solution of 0.04 ml/kg BID) from inclusion to month 6
     - If their weight is ≥ 25kg, they will receive placebo BID (1mL placebo BID oral solution) from inclusion to month 6

   Stable psychosocial intervention is maintained if any.

3. Open label active treatment period of 6 months between month 6 (week 26) and month 12 (week 52):
   - Patients randomised in the bumetanide arm during the double blind period will continue to be treated by bumetanide up to 0.5mg BID during the whole open label period. Bumetanide doses will be adapted on a body-weight as in the double blind period.
   - Patients randomised in the placebo arm during the double blind period will receive bumetanide up to 0.5mg BID during the whole open label period. Bumetanide doses will be adapted on a body-weight as in the double blind period.

4. A follow-up visit (Wend), 6 weeks after treatment discontinuation:
   This period is without any IMP and it will be completed at the end of the study or in case of premature discontinuation from the study at any moment.

The study plan is shown in Figure (4.2.1) 1.

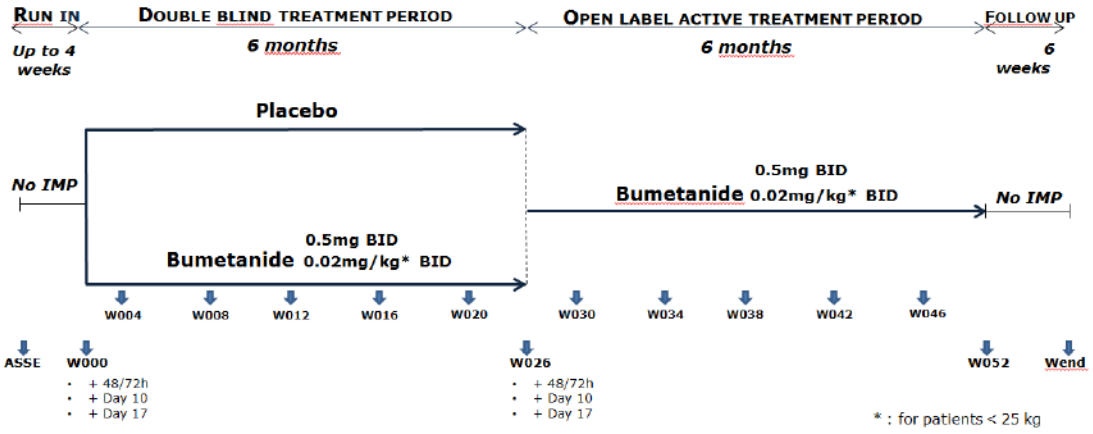
In case of non-inclusion of a participant, it is the investigator's responsibility to ensure, in accordance with the local standards of care and medical practices that:
- The reason of non-inclusion is explained to the participant and to his/her parents/legal representative,
- Any event associated with any procedure/condition required by the study protocol (e.g. an event occurring following the discontinuation of a forbidden treatment) is collected,
- Adequate medical care is proposed to the participant.

A non-inclusion visit is not mandatorily carried out, provided these requirements are met and documented in the medical file of the participant.

The following variation can be accepted:
- Variation of maximum ± 1 day between visits is allowed for W000+Day10, W000+Day17 and W004 visits
- Variation of maximum ± 2 days between visits is allowed for , W008, W012, W016, W020 and W026 visits

- Variation of maximum ± 1 day between visits is allowed for W026+Day10, W026+Day17 and W030 visits
- Variation of maximum ± 2 days between visits is allowed for W034, W038, W042, W046 and W052 visits

Figure (4.2.1) 1 – Study plan



The study start is defined as the date of the first visit of the first participant (selection visit - ASSE).

A 6-month extension period in open-label is proposed in some countries where it is not possible to provide the treatment at the end of the main periods *via* a Named Patient Basis (NPB) access or a Post-Access Study Program.

Therefore, the End of Trial is defined as the date of the last follow-up visit of the last participant (including a phone contact) in the open label active treatment period or in the extension period whichever occurs the latest, or the date of the last contact attempt if the last participant is declared lost to follow-up.

4.2.2. Investigation schedule

Table (4.2.2) 1 describes the measurement of efficacy and safety assessed during the study.

Table (4.2.2) 1 - Investigation schedule

	Selection	Inclusion	Double-blind 26 week treatment period									Visits to complete in case of IMP discontinuation during the double blind period BUT not withdrawal from the study	
	ASSE	W000	W000 48/72h	W000 Day 10	W000 Day 17	W004	W008	W012	W016	W020	W026	W012	W026
Informed consents/assents	X[1]												
Demography	X												
IQ test[2]	X												
DSM-5	X												
ADOS-2[3]	X												
ADI-R[4]	X												
Selection / Non selection criteria	X												
Inclusion / Non-inclusion criteria		X											
Autism diagnostic history	X												
Medical / surgical history	X												
Previous treatments[5]	X												
Concomitant treatments[5]	X	X	X	X	X	X	X	X	X	X	X	X	X
IRS Patient number Randomisation IMP allocation	X	 X X				 X	 X	 X	 X	 X	 X		
IMP dispensation Oral solution volume adaptation to patient weight if needed		X X				X	X	X X	X	X	X[6] X		
Compliance IMP						X	X	X	X	X	X		

(1) To obtain at the latest at ASSE but before any procedure related to the study
(2) Only to be performed if retrospective exam is not available in the 12 months prior to ASSE
(3) Should not be performed if an ADOS-2 evaluation has been done within the 12 months prior to ASSE and is documented in the site
(4) Only to be performed if retrospective ADI-R is not available after the 4 y.o. of the patient
(5) Including non-pharmacological therapies (psychotherapy social skills training behavioural interventions etc)
(6) First IMP dispensation of the open label 26 week active treatment period

Table (4.2.2) 1 – Investigation schedule (continued)

	Selection	Inclusion	Double-blind 26 week treatment period									Visits to complete in case of IMP discontinuation during the double blind period BUT not withdrawal from the study	
	ASSE	W000	W000 48/72h	W000 Day 10	W000 Day 17 D017	W004	W008	W012	W016	W020	W026	W012	W026
Efficacy measurements CARS2-HF/ CARS2-ST/ CARS2-QPC		X				X		X			X	X	X
CGI	X(1)	X(1)			X(2)	X(2)	X(2)	X	X(2)	X(2)	X	X	X
SRS-2		X				X		X			X	X	X
VABS II		X									X		
Safety measurements Suicidality (C-SSRS-C)		X						X			X		
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X
PAERS scale		X			X	X	X	X	X	X	X		
Laboratory tests (blood and urine)	X							X			X		
Blood electrolytes monitoring (K, Na) (performed locally except at ASSE)	X		X	X	X	X	X(3)	X	X(3)	X(3)	X		
ECG	X(6)					X(3)	X(3)	X(3)			X(5)		
Renal ultrasound	X										X(5)		
Sitting and standing blood pressure/Heart rate	X	X			X			X			X		
Body Weight and Height	X	X(4)			X(4)	X(4)	X(4)	X	X(4)	X(4)	X		
Pharmacokinetics (blood samples)								X			X		
Other measurements PedsQL		X				X		X			X		
WHOQOL-Bref		X				X	X	X			X		
EQ5D-3L		X						X			X		
Acceptability Questionnaire											X		

(1) CGI-S only
(2) CGI-I only
(3) Prescribed based on the clinical opinion of nephrologist or investigator
(4) Only body weight
(5) Results of the exam should be available at this visit prescription to be done at the previous visit
(6) ECG Triplicate only at ASSE

Table (4.2.2) 1 – Investigation schedule (continued)

	Open label 26 week active treatment period									FU	WD
	W026 48/72h	W026 Day 10	W026 Day 17 (D199)	W030	W034	W038	W042	W046	W052	Wend	Withdrawal visit to be undergone in case of IMP definitive discontinuation or in case of total withdrawal from the study whatever the period
Concomitant treatments(1)	X	X	X	X	X	X	X	X	X	X	X
IRS IMP allocation				X	X	X	X	X			
IMP definitive discontinuation											X
IMP dispensation Oral solution volume adaptation to patient weight				X	X	X X	X	X			
Compliance IMP				X	X	X	X	X	X		X
Efficacy measurements CARS2-HF/ CARS2-ST/CARS2-QPC						X			X	X	X(4) (7)
CGI						X(6)			X	X	X(7)
SRS-2						X			X		X(4) (7)
VABS II									X		X(4) (7)
Safety measurements Suicidality (C-SSRS-C)						X			X		X
Adverse events PAERS scale	X	X	X X	X X	X X	X X	X X	X X	X X	X	X X
Laboratory tests (blood and urine)						X			X		X
Blood electrolytes monitoring (K, Na) (performed locally except at WD)	X	X	X	X	X(2)	X	X(2)	X(2)	X		X
ECG				X(5)	X(5)	X( )			X(5)		X
Renal ultrasound Sitting and standing blood pressure/ Heart rate			X			X			X(5) X		X X
Body Weight and Height			X(3)	X(3)	X(3)	X	X(3)	X(3)	X		X
Other measurements PedsQL				X		X			X		X(4) (7)
WHOQOL-Bref				X	X	X			X		X(4) (7)
EQ5D-3L Acceptability Questionnaire						X			X		X(4) (7) X(7)

(1) Including non-pharmacological therapies (psychotherapy social skills training behavioural interventions etc)
(2) Prescribed based on the clinical opinion of nephrologist or investigator
(3) Only body weight
(4) Only to be undergone after the last IMP intake by patients discontinuing prematurely from the study after W000 Day10
(5) Results of the exam should be available at this visit prescription to be done at the previous visit
(6) CGI-I only
(7) Should not be done in case of study discontinuation for consent withdrawal

For further practical details, methods of measurement are provided in sections 7, 8 and 9.

For organisational reasons ASSE, W000, W012, W026, W038 and W052 visits can be split in several days, in order to be able to complete all the study evaluations.
The following recommendations should be followed:

For ASSE:

- ECG, renal ultrasound and laboratory tests could be performed after ASSE visit date but always during the run in period and results must be available for the inclusion visit

For W000, W012, W026, W038, W052:

- CARS2 and CGI study assessments planned at the corresponding visit should be performed on the same day
- efficacy evaluations should be always performed prior to any safety procedure in case both efficacy evaluations and safety procedures have been planned to be performed on the same day,
- parents questionnaires (CARS QPC, SRS2 and quality of life questionnaires) and Vineland scale could be completed on site within 3 days prior to the day of the visit,
- safety evaluations could be done within 7 days prior to the visit date.

Furthermore, taking into account local regulation, it is not compulsory for the patient to attend the visits W000+48h/72h, W000+Day 10, W000+Day17,W026+48h/72h, W026+Day 10 and W026+Day17 on site.
In such a case the following recommendations should be followed:

- The planned blood sampling for electrolytes (Na and K) at these visits will be performed locally.
- A phone contact must be established by the investigator with the patient (when possible) and the parent(s)/ legally representative or caregiver (when applicable) in order to collect any adverse event at these visits.
- vital signs will be collected at home by a study nurse when required.

Special conduct in the case of definitive discontinuation of IMP during the double blind period:

1. Patient will complete the study evaluations planned at WD visit.
2. If IMP definitive discontinuation occurs prior to W012:
    a. patient will attend W012 visit (if there is at least 2 months between WD and W012) and will be invited to complete CARS2, SRS-2, CGI and AE assessment at W012 as planned in the investigation schedule (Table (4.2.2) 1).
    b. patient will attend then W026 visit and will be invited then to complete CARS2, SRS-2, CGI and AE assessment at W026 visit as planned in the investigation schedule (Table (4.2.2) 1).
3. If IMP definitive discontinuation occurs between W012 and W026:
    a. patient will attend W026 (if there is at least 2 months between WD and W026) and will be invited to complete CARS2, SRS-2, CGI and AE assessment at W026 as planned in the investigation schedule (Table (4.2.2) 1).

In such a case, CARS2, SRS-2, CGI evaluations and AE assessments at W012 and W026 will be completed ONLY if the patient who has definitively discontinued the IMP during the

double blind period, does not require an important modification on his/her current background medical care (see section 5.6.1.).

The conduct in the case of definitive discontinuation of IMP during the double blind period is summarized in the Table (4.2.2) 2.

Table (4.2.2) 2 - Conduct in case of definitive discontinuation of IMP during the double blind period without study withdrawal

Visit *(when IMP is definitely discontinued)*	**WD** *(to be performed)*	**W012** *(to be performed)*	**W026** *(to be performed)*	**Wend** *(to be performed)*
D017	x	x	x	NA
W004	x	x	x	NA
W008	x	NA	x	NA
W012	x	NA	x	NA
W016	x	NA	x	NA
W020	x	NA	NA	X
W026	x	NA	NA	X

NA: Not applicable

The maximum total volume of blood collected per participant during the study will be 34 mL See Table (4.2.2) 3

Table (4.2.2) 3 – Volume (mL) of blood collected per participant during the study

	ASSE	W000 + 48/72h	W000 + Day 10	W000 + Day 17	W004	W012	W026
Haematology	1					1	1
Biochemistry	4					2.5	2.5
Serology							
Pharmacokinetic						1	1
Na/K monitoring	1	1	1	1	1	1	1
TOTAL volume (mL) per visit	6	1	1	1	1	5.5	5.5
	W026 + 48/72h	W026 + Day 10	W026 + Day 17	W030	W038	W052	
Biochemistry					2.5	2.5	
Haematology					1	1	
Na/K monitoring	1	1	1	1	1	1	
TOTAL volume (mL) per visit	1	1	1	1	4.5	4.5	
TOTAL volume (mL) per participant	**34**						

4.3. Measures to minimise bias

The following measures have been taken to avoid biases:

- This is a randomised, placebo-controlled study carried out in double-blind conditions. Active treatment (bumetanide) and placebo oral formulations will have the same appearance and the same taste.

- An Interactive Response System (IRS) will be used for the treatment allocation: bumetanide or placebo will be assigned at inclusion (W000) by balanced (ratio 1:1) randomisation, with stratification on the country and on the patient sex. The structure responsible for designing and constructing the randomisation list in blind will be the Center of Excellence Methodology and Valorisation of Data of I.R.I.S.

- Investigators in charge of diagnosis should have a deep knowledge on the Autism Diagnostic Observation Schedule, second edition (ADOS-2) and Autism Diagnostic Interview - Revised (ADI-R) diagnostic tools.

- Training sessions on assessment scales will be organized for the investigators prior to their involvement and their participation will be mandatory.

- In order to maintain the double-blind conditions, the main efficacy criterion (CARS2) will be assessed by an independent rater. This rater will not be involved with any aspect of medical management of the patient and will not have access to patient data. The independent rater will be instructed not to discuss what adverse event (if any) the patient is experiencing from his/her medication. Prior to being examined by the independent rater, patients and parent/legal representative(s) or caregiver (when applicable) will be instructed not to discuss what (if any) adverse event patient may be experiencing. As far as possible same independent rater will assess CARS2 for a given patient all along the study (from W000 till W052 and at least till W026) and, when possible, all the patients for a given site.

- The laboratory parameters will be performed by a central laboratory in order to centralize and harmonise the data, with the exception of Na^+/K^+ which should be monitored always locally when the patient is under treatment to quickly manage Potassium Supplementation when needed.

- In addition to immediate local safety reading for safety reasons, a centralized assessment will be performed for each ECG (central reading reports for ECG overrule local reading for inclusion/exclusion/withdrawal criteria).

- Samples for pharmacokinetic analysis will be sent to the central laboratory and transferred to the assay centre Cephac for analysis using a validated assay method. The assay centre in charge of bumetanide measurement will be provided with the treatment codes so that only samples from patients being treated with bumetanide will be assayed. The results of bumetanide blood concentration will be transferred from Cephac to the PK department of I.R.I.S. before the clinical database [associated to statistical analysis (ASSE W026 period)] is locked and blind is broken. In order to prevent a blind break, administration and sample times will only be recorded on the requisition form and not on the electronic Case report Form (e-CRF). The central laboratory will capture data of the requisition form and will calculate the time after dose. Real time after dose will be transferred to Cephac for analyses. The PK department will receive only recoded data (recoded patient number). PK results

from Cephac and requisition form data from the central laboratory will be transferred to the I.R.I.S. Data Management department only after blind is broken.

- The main analysis of the study concerns the double blind period from W000 to W026 visit. The blind will be broken as soon as all efficacy and safety data collected during this period are available and the mandatory steps defined in section 14.2 "Data Management" have been performed. Nevertheless, although the blind will be broken centrally before the end of the study, neither the investigators, nor the raters, nor the patients/legal representatives/caregivers, nor the parents, nor the local project managers, nor the monitors will be informed of the study treatment taken during the double-blind treatment period and of the study results of the first period before the database lock for the whole study period (W000-W052).

4.4. Study products and blinding systems

4.4.1. Products administered

In the frame of this protocol, IMPs are S 95008 (at the dose of 0.5 mg/bid) and placebo.

The IMP dispensed will be an oral formulation (solution of 0.5mg/mL of bumetanide or a placebo solution with the same appearance and taste). This solution will be administered with a 1 mL graduated pipette.

All the patients will take orally the study treatment twice a day:

- **in the morning at wake up**
- **in the afternoon, 3 hours before going to bed at the latest.**

In any case the study treatment must be taken around 15 minutes before any food intake.

The pipette containing the oral solution should be administered preferably into the mouth of the patient.

The volume of the oral solution will be adapted according to a body-weight basis for patients with a weight lower than 25Kg (See Appendix 1).

An adaptation of the oral solution volume according to the weight of the patients will be performed during the study at visits W012, W026 and W038.

Each treatment dispensed throughout the study will be similar so that it is not possible to differentiate the study treatments (bumetanide or placebo).

- **From selection to inclusion**: patients will not take IMP.
- **During the double-blind period of 6 months**: from W000 (inclusion) to W026 visits
  The first study treatment intake will be on the day of the inclusion visit in the afternoon, 3 hours before going to sleep at night at the latest.
  - Patients with a body weight < 25 kg:
    Patients randomised in the bumetanide arm: from W000 to W026 visits will take 0.02 mg/kg of bumetanide twice a day i.e.:

- volume of bumetanide oral solution (mL) = 0.04 x body weight (kg) in the morning at wake-up,
- volume of bumetanide oral solution (mL) = 0.04 x body weight (kg) in the afternoon, 3 hours before going to bed at the latest.

Patients randomised in the placebo arm: from W000 to W026 visits will take placebo solution twice a day i.e.:

- the volume of placebo oral solution (mL) = 0.04 x body weight (kg) in the morning at wake-up,
- the volume of placebo oral solution (mL) = 0.04 x body weight (kg) in the afternoon, 3 hours before going to bed at the latest.

➢ Patients with a weight ≥ 25kg:

Patients randomised in the bumetanide arm: from W000 to W026 visits will take 0.5mg of bumetanide twice a day i.e.:

- 1ml of bumetanide oral solution in the morning at wake-up,
- 1ml of bumetanide oral solution in the afternoon, 3 hours before going to bed at the latest.

Patients randomised in the placebo arm: from W000 to W026 visits will take placebo solution twice a day i.e.:

- 1ml of placebo oral solution in the morning at wake-up,
- 1ml of placebo oral solution in the in the afternoon, 3 hours before going to bed at the latest.

- **During the open label treatment period of 6 months:** from W026 to W052 visits

All patients will be treated by bumetanide.

➢ For patients with a weight < 25 kg:

From W026 to W052 visits, patients will take 0.02 mg/kg of bumetanide twice a day:

- the volume of bumetanide oral solution (mL) = 0.04 x body weight (kg) in the morning at wake-up,
- the volume of bumetanide oral solution (mL) = 0.04 x body weight (kg) in the afternoon, 3 hours before going to bed at the latest.

➢ For patients with a weight ≥ 25kg:

From W026 to W052 visits, the patients will take 0.5 mg of bumetanide twice a day, in the morning at wake-up and in the in the afternoon 3 hours before going to bed at the latest i.e.:

- 1ml of bumetanide oral solution in the morning at wake-up,
- 1ml of bumetanide oral solution in the afternoon, 3 hours before going to bed at the latest.

- **During the Follow-up period,** the patients will not be treated with IMP.

Table (4.4.1) 1provides a description of the IMP(s).

Table (4.4.1) 1 – Description of the IMPs

	S95008	Placebo
Pharmaceutical form	Oral solution	Oral solution
Unit dosage	0.5 mg/mL 1 mL corresponds to 0.5 mg of bumetanide	0
Appearance, colour	Colourless to pale yellow solution	Colourless to pale yellow solution
Composition	Bumetanide 0.5 mg Sodium methyl parahydroxybenzoate Sodium propyl parahydroxybenzoate	Sodium methyl parahydroxybenzoate Sodium propyl parahydroxybenzoate

Table (4.4.1) 2 and Table (4.4.1) 3 provide a description of the packaging of the IMPs.

Table (4.4.1) 2 – Description of packaging of the double-blind IMP

Number of units of the pharmaceutical form per primary packaging	60mL of S95008 or placebo oral solution in an amber glass bottle with polypropylene / low density polyethylene child-resistant and tamper-evident cap
Number of primary packaging per secondary packaging	2 bottles of S95008 or placebo per box with 2 graduated pipettes of 1mL
Number of secondary packaging per participant and per treatment period	1 box given per visit with allocation by the IRS

Table (4.4.1) 3 – Description of packaging of the open IMP

Number of units of the pharmaceutical form per primary packaging	60mL of S95008 oral solution in an amber glass bottle with polypropylene / low density polyethylene child-resistant and tamper-evident cap
Number of primary packaging per secondary packaging	2 bottles of S95008 per box with 2 graduated pipettes of 1mL
Number of secondary packaging per participant and per treatment period	1 box given per visit with allocation by the IRS

The labelling of packages complies with the regulatory requirements of each country involved in the study, as well as the recommendations in Appendix 18 of the European Guide to Good Manufacturing Practice.

4.4.2. IMP management

IMP will be supplied from the manufacturing site /Unité d'Appui Clinique (les Laboratories Servier Industrie, 905 route de Saran, 45220 GIDY, FRANCE). For some countries, an intermediate storage chosen by I.R.I.S will be involved in supplying the centres.

The Interactive Response System (IRS) will trigger the first shipments of the IMPs and the re-supplies.

The IMP will then be sent by the Clinical Supply Unit (CSU) either directly to the investigational centers or to intermediate storage centers or to local pharmacies depending on the geographic areas and the local regulatory requirements.

The IMPs will be sent to the investigator (or to the pharmacists) who will acknowledge the receipt by signing and returning the shipping form.

IMP receipt, dispensing according to the experimental design of the study (for the description of dispensing methods, refer to section 6.2), accountability and collection are the responsibility of the investigator and/or pharmacist of the medical institution.

Destruction of the IMP is the responsibility of the sponsor and/or the investigator and/or the pharmacist of the medical institution according to local organization.

Remaining treatments (used and unused IMPs) will subsequently be collected. An IMP recovery and destruction form (RDF) will be completed before shipment of IMP to the Intermediate storage site pending destruction or directly to the destruction body. Destruction of IMP may be possible (after drug accountability and sponsor authorization) when the product has been used, has expired or after the last visit of the last treated patient.

The IMP should be stored in a secure area with restricted access.

Specific storage conditions, if any, are mentioned on IMP labelling and are detailed in investigator's brochure.

IMP management will be verified on a regular basis by the study monitor.

The investigator and/or the pharmacist of the medical institution and/or a designated person from their study team must complete in real time all the documents provided by the sponsor concerning IMP management (therapeutic unit tracking form or an equivalent document). Therapeutic unit tracking form, or an equivalent document, is the source document to fulfil.

The investigator and/or the pharmacist of the medical institution should only use the IMP provided for the participants involved in the study.

All defects or deterioration of IMPs or their packaging are to be reported to the study monitor, and/or if applicable to the IRS. The investigator will notify the monitor of all complaints set out by a participant (change of taste, appearance…).

In the event of anticipated return of IMPs to the sponsor (batch recall), the sponsor will prepare an information letter intended for the investigator and/or pharmacist of the medical institution. This letter will be sent by the person locally responsible for the study to each study centre. On receipt of the letter, the investigator and/or the pharmacist will identify the participants in possession of the IMP at the moment the incident becomes known, by using, among other tools, the therapeutic unit tracking form, or an equivalent document, and will contact them immediately to arrange return and re-supply of IMP (if required).

4.4.3. Management of blinding systems

A centralised IWRS decoding system will be used in this study.

The first part of the study (from W000 to W026) is double-blinded in which the patient, the investigators, all other site personnel, and I.R.I.S. will not have knowledge of patient treatment.

The blind for any study participant should only be broken by the investigator or authorised person if it is absolutely necessary to ascertain the type of treatment given.

The circumstances under which the code may be broken are any serious adverse event and/or any severe medical condition **where the knowledge of the allocated treatment is necessary for the follow up of the patient**.

All information concerning adverse drug reactions, drug interactions and the procedure to be followed in the event of overdose is given in the Investigator's Brochure for bumetanide and the Summary of Product Characteristics (SmPC Bumetanide, November 2017) (provided with this protocol).

The procedure to be followed by the investigator or authorized person is detailed in the IRS manual. The system is available 24 hours a day, 7 days a week. If IWRS is not available, the helpdesk of the IWRS will be contacted by phone.

Additionally, decoding will be possible by calling the Emergency Phone Number of I.R.I.S. (+33 1 55 72 60 00) 7/7d and 24/24h).

The DMC may also ask for specific cases as far as the safety of patients is concerned.

4.5. Discontinuation of the study

4.5.1. Premature discontinuation of the study or temporary halt

This study may be temporarily halted or prematurely discontinued at any time for any sufficient reasonable cause such as DMC recommendation or occurrence of new scientific /clinical knowledge that may jeopardise the participants' safety.

After having informed the coordinator(s), the sponsor or the Data Monitoring Committee (DMC) or the Independent Ethics Committee (IEC) or the Competent Authorities may terminate the study before its scheduled term. Two copies of the written confirmation will be dated and signed by the coordinator(s). The IECs and Competent Authorities will be informed according to local regulations.

If the study is prematurely discontinued, the on-going participants should be seen as soon as possible and the same assessments as described in Section 4.2.2 should be performed.

Under some circumstances, the investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the participant's interests.

In case of study suspension (temporary halt), the study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the Sponsor, the Data Monitoring Committee (DMC), the Independent Ethics Committee (IEC) and Competent Authorities.

4.5.2. Discontinuation of the study in the event of objective reached

Not applicable.

4.6. Source data

The following data will be considered as source documents:

- the patient's medical file [including ECG tracing, renal ultrasound report, clinical laboratory reports, and all other patient's examinations results],
- Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) Appendix 2,
- ADOS2 paper modules
- ADI-R paper modules
- Intellectual Coefficient (IQ) to determine the CARS2 version to be used for a given patient. If an IQ test has been performed within the 12 months prior to selection and it is documented in the site, it is not necessary to repeat this evaluation at ASSE for a given patient. In the framework of this study no specific recommendation regarding the type of IQ test is given to the clinician. When needed, each site will assess the IQ of a given patient with the IQ/developmental questions tests which they use in their usual medical practice,
- Childhood Autism Rating Scale, Second Edition – Standard Tool (CARS2-ST) (Appendix 3) or Childhood Autism Rating Scale, Second Edition – High functioning clinical tool (CARS2-HF) (Appendix 4) and Childhood Autism Rating Scale, Second Edition – Questionnaire for parents and caregivers (CARS2-QPC) (Appendix 5),
- Social Responsiveness Scale, Second Edition (SRS-2) Pre-school Version (Appendix 6)
- Social Responsiveness Scale, Second Edition (SRS-2) School Version (Appendix 7)
- C-SSRS-C (Appendix 8) and PAERS Clinician form (Appendix 9),
- Vineland Adaptative Behaviour Sales, Second Edition (VABS II) (Appendix 10),
- Clinical Global Impression (CGI) (Appendix 11),
- Acceptability and palatability questionnaire (Appendix 12),
- PAERS Youth form (Appendix 13) and PAERS Parent form (Appendix 14),
- Quality of life questionnaires: PedsQL questionnaire (Appendix 15), WHOQOL-Bref. (Appendix 16), EQ5D-3L (Appendix 17),
- Patient's diary for study medication intake.

Source data and source documents of the centre should be clearly identified in a specific, detailed and signed document before the beginning of the study.

The diagnostic criteria of "Autism Spectrum Disorder" according to the DSM-5 should be directly recorded in the paper copy by investigator and should be kept in the patient's medical file, then the scores must be entered in the e-CRF and will be considered as source data. DSM-5 specifiers must be documented for all study subjects.

The ADOS-2 (Lord, 2012) is a semi-structured, standardized assessment of communication, social interaction, play/imaginative use of materials and restrictive and repetitive behaviours for individuals who have been referred because of possible autism spectrum disorders (ASD). The ADOS-2 is a revision of the ADOS, which have been referred to as the gold standard observational assessment for diagnosing ASD.

The ADOS-2 contains five assessment modules. Each module offers standard activities designed to elicit behaviours that are directly relevant to the diagnosis of ASD at different developmental levels and chronological ages. In the framework of this study only four modules will be used:

- Toddler Module: for use with toddlers who are 12 to 30 months of age and who do not consistently use phrase speech
- Module1: for use with children who are 31 months and older and who do not consistently use phrase speech
- Module 2: for use with children of any age who use phrase speech, but who are not verbally fluent
- Module 3: for use with verbally fluent children and young adolescents

Each ADOS-2 module has its own Protocol booklet, which provides order and structure to the administration, coding and scoring of the module.

The ADOS-2 is a highly structured observational assessment that requires specific training. Professionals using the ADOS-2 should have prior education, training and experience. When using the ADOS-2, examiners need to be sufficiently familiar with the activities and codes so that they can focus their attention on observation of the individual being assessed, rather than on administration rules. This requires practisce in observation and coding, as well as in administering the activities.

In the framework of this study the ADOS-2 should be completed by a Medical Doctor (child&adolescent psychiatrist, neuropaediatrist) or by a psychologist fulfilling the following requirements:

- o experienced on ASD and co-morbid diagnoses
- o knowledge on child development
- o playing a key role on the current site organization
- o being involved in the diagnosis of ASD patients on a regular basis
- o deeply knowledge of ADOS-2 (having performed an official training will be preferable)
- o used to perform ADOS-2 (or at least ADOS-1) on a regular basis

The paper modules of ADOS-2 should be kept in the patients' medical file, they will be considered as source data. Some data of the ADOS-2 will be entered in the e-CRF. If retrospective ADOS-2 has been performed within the 12 months prior to selection and it is documented in the site, it is not necessary to repeat ADOS-2 evaluation at ASSE for a given patient.

The ADI-R is an extended interview designed to elicit a full range of information needed to produce a diagnosis of autism and to assist in the assessment of related disorders here referred to as ASD. Use of the ADI-R involves an experienced clinical interviewer and an informant, someone who is familiar with the child's behaviour at that age. Administration of the ADI-R requires use of its Interview Protocol which focuses primarily on the three domains of functioning: language/communication; reciprocal social interactions and restricted, repetitive

and stereotyped behaviours and interest. Scoring is then conducted using the Comprehensive Algorithm Form.
In the framework of this study the ADI-R could be completed by a Medical Doctor (child&adolescent psychiatrist, neuropaediatrist) or by a psychologist fulfilling the same requirements as for ADOS-2 and trained in the use of (semi-) structured interviews.

The paper modules of ADI-R should be kept in the patients' medical file, they will be considered as source data. If previous ADI-R is available (done after the 4yo of the patient) and documented in the center, it is not necessary to repeat the ADI-R evaluation at ASSE for a given patient.

The paper copy of the CARS2-ST or CARS2-HF must be completed by independent rater with the adequate scores and rating justifications. Then scores will be entered in the e-CRF. Completed paper copies will be used as a source document and should be kept in the patient's medical file. The paper form of the CARS2-QPC must be completed by the parents/legal representative or caregiver (when applicable), they will be kept in the patients' medical file and will be considered as source data.

The paper form of the SRS-2 (preschool or school) must be completed by parent/legal representative or caregiver (when applicable) and will be considered as a source document. The answers to the questionnaire will be transferred in the e-CRF.

The paper copy of CGI must be assessed by investigator, should be kept in the patient's medical file and will be considered as source data. Then scores will be recorded in the e-CRF.

The C-SSRS-C will have to be completed by investigator on paper copies and then data entered in the e-CRF. The paper copies should be kept in the patient's medical file and will be considered as source data.

The papers forms of the PAERS will be completed by the patient (when possible) and the parent/legal representative or caregiver (when applicable) will be considered as source data. Data collected from PAERS Clinician paper form by the investigator should be reported in the e-CRF. The paper copy should be kept in the patient's medical file and be considered as source data.

The VABS II will have to be completed by investigator on paper copies. Then the paper copies will be sent to I.R.I.S. The duplicate paper copies should be kept in the patient's medical file and will be considered as source data.

The acceptability and palatability questionnaire will have to be completed by the patient (when possible) and the parents/legally authorized representative or caregiver (when applicable) on paper copies. The data will be transferred in the e-CRF. The paper copies should be kept in the patient's medical file and will be considered as source data.

PedsQL, WHOQOL-Bref and EQ5D-3L questionnaires will be completed by parents/legally authorized representative or caregiver (when applicable) on paper copies. The data will be transferred in the e-CRF. The paper copies should be kept in the patient's medical file and will be considered as source data.

5. SELECTION AND WITHDRAWAL OF PARTICIPANTS

5.1. Selection criteria

5.1.1. Demographic characteristics

1. Male or female
2. Aged from 2 to less than 7 years of age
3. Out patients (patients living in an institution can be selected)
4. Living with their parent/legal representative(s) or caregiver (when applicable)

   Those patients who do not live with their parent/legal representative(s) (i.e. internees patients living in an institution, patient living with other family member, etc.) can be selected in the study if there is an identified caregiver who could accompany the patient at each visit to the Research Center. In such a case, the caregiver will be in charge to complete the planned study evaluations intended for the patient parent/legal representative.

   Patient legal representative: An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.

   Patient caregiver: a person who has a daily contact / a personal contact for a minimum of 50% of the time per week with the participant and who knows him/her for at least one year. The caregiver needs to be the same person throughout the study and needs to be willing to attend study visits, oversee the patient's compliance and report on patient's status as well as sign an informed consent.

5.1.2. Medical and therapeutic criteria

5. Primary diagnosis of Autism Spectrum Disorder as per DSM-5 criteria.
6. Criteria met for ASD on Autism Diagnostic Observation Schedule (ADOS-2) and Autism Diagnostic Interview Revised (ADI-R).

   - *If retrospective ADOS-2 has been assessed within the 12 months prior to selection and documented in the center, it is not necessary to repeat ADOS-2 evaluation at ASSE.*
   - *If previous ADI-R is available (assessed after the 4yo of the patient) and documented in the center, it is not necessary to repeat the ADI-R evaluation at ASSE.*
7. CGI (Clinical Global Impression)-Severity rating score ≥ 4.
8. Absence of any clinically significant abnormality likely to interfere with the conduct of the study, based on the judgment of the investigator.

5.1.3. Informed consent

Obtained as described in Section 13 of the protocol.

5.2. Non-selection criteria

5.2.1. General criteria

9. Absence of written informed assent of the patient (for those patients able to give assent).

10. Absence of written consent of one or the two parents/legal representative(s) and caregiver (when applicable) according to local regulation.
11. Parents/legal representative(s) or caregiver (when applicable) unable to cope with the study constraints.
12. Patients not able to follow the study assessments defined by the protocol, with the exception of self-rating questionnaires which will be assessed by parent/legal representative or caregiver(when applicable) for those patients unable to complete them.
13. Patients participating in another study at the same time or having participated in another study with medicinal product within the 3 months prior to inclusion (patients participating to data collection register can be selected).
14. Patients having already been treated by bumetanide for ASD without clinical benefit according to investigator's opinion.
15. (A) Patients having already been included in the study.
16. Patients having a first degree relative living in the same home and already participating (already included) in the study.

5.2.2. Medical and therapeutic criteria

Related to the studied disease

17. Known monogenic syndrome (Fragile X, Rett Syndrome, list not exhaustive)

Related to other psychiatric conditions

18. Patients having a high suicidal risk according to the investigator judgement or having attempted a suicide in the previous 3 months based on information obtained during the investigator interview.
19. Any other associated psychiatric condition likely to interfere with the conduct of the study and/or requiring the use of a forbidden concomitant psychotropic medication.

Related to miscellaneous conditions

20. Chronic hepatic diseases as autoimmune hepatitis; chronic hepatitis A, B, C; cirrhosis; Wilson's disease; primary sclerosing cholangitis; non-alcoholic fatty liver disease; alpha1-antitrypsin deficiency (list not exhaustive).
21. Chronic renal dysfunction.
22. Chronic cardiac dysfunction.
23. Known hypersensitivity to sulphonamide, thiazides derivate, bumetanide and/or any of the excipients.
24. (B) Any other severe, uncontrolled or chronic condition incompatible with study treatment or likely to interfere with the conduct of the study [e.g. neoplasic, neurologic (such as uncontrolled epilepsy; epilepsy is considered as uncontrolled in case of epileptic seizure occurrence that requires any anti-epileptic treatment modifications during the screening period, pulmonary, metabolic and digestive disorders, known hyperuricemia), list not exhaustive].

Related to previous and concomitant treatments

25. (A) Patient with unstable psychotherapy, behavioural, cognitive or cognitive-behavioural therapy.

"unstable" means:

- started or stopped during selection period (i.e. in the month prior to inclusion)
- planned to be significantly modified during the double blind treatment period (in terms of type of therapy or in terms of therapy rhythm).
  - *Planned classic interruptions during the double blind period (eg summer vacations) are not considered as an exclusion criterion.*
  - *Patients having no psychotherapy, behavioural, cognitive or cognitive-behavioural therapy are not considered as an exclusion criterion but these patients won't be able to start one during selection period (in the month prior to inclusion) and during the double blind treatment period.*

Alternative interventions (hyperbaric oxygen, secretin, chelation, exclusion diets not justified by medical reasons) are forbidden all along the study.

26. Patient requiring concomitant psychotropic medication (exceptions reported, See Section 6.3).
27. (A) Patients requiring contraindicated medications (non-exhaustive list):

- Chronic administration of non-steroidal anti-inflammatory drugs: *risk of NSAIDs nephrotoxicity increased*
- non-repolarising neuromuscular blocking agents: *hypokaliemia increases the sensitivity to non-repolarising neuromuscular blocking agents*
- Digitalis glycosides: *Risk of digitalis toxicity secondary to hypokaliemia (and possibly hypomagnesemia)*
- Antiarrhythmics: *Risk of cardiotoxicity (QT prolongation, torsades de pointe, cardiac arrest) secondary to hypokaliemia (and possibly hypomagnesemia)*
- Antihypertensive agents (Angiotensin II receptors antagonists, Angiotensin Converting Enzyme Inhibitors) and medicinal products inducing postural hypotension (e.g. tricyclic antidepressants): *Increased risk of postural hypotension*
- Potassium depleting agents such as other diuretics, laxatives (refer to section 6.3 Previous and concomitant treatments), chronic high doses of mineralo and glucocorticoids, amphotericin B: *The potassium depleting effect of bumetanide may be increased by other potassium depleting agents (other diuretics, laxatives…)*
- Aminoglycosides: *Ototoxic and nephrotoxic effects of aminoglycosides may be increased by concomitant administration of bumetanide*
- Probenecid: *Decreased bumetanide effectiveness. Probenecid has been demonstrated to produce a dose related inhibition of bumetanide induced natriuresis*
- Iodinated contrast products: *Risk of renal insufficiency due to dehydration provoked by a concomitant administration of bumetanide*
- Lithium: *Bumetanide reduces lithium clearance resulting in high serum levels of lithium*
- High doses of Vitamin D (> 100 000 IU/3 months) received as a supplement: Risk of nephrocalcinosis
- Desmopressin: *Increased risk of severe hyponatremia*

5.3. Inclusion criteria

28. Patient still fulfilling all the selection criteria
29. CARS2 (ST or HF)- Total raw score ≥ 34
30. SRS-2 [parent report- pre-school or school age version] ≥ 66T-Score

Preschool version: A T-score of 66 corresponds to a Total Raw score of 83 to 84

School version: A T-score of 66 corresponds to a Total Raw score of 73 to 75 for male patients and a Total Raw score of 66 to 68 for female patients)

31. Patient respecting wash out periods for forbidden treatments, considering these treatments were not or poorly effective when applicable.

5.4. Exclusion criteria

32. Any non-selection criterion which could have appeared after the selection visit
33. Patients having already been treated by bumetanide for ASD (with clinical benefit) but stopped in less than 3 month prior to inclusion
34. (C) Any clinically significant abnormality detected during screening period that is likely to interfere with the study conduct or evaluation:
    - physical examination,
    - renal ultrasonography especially unique kidney, renal hypoplasia, renal dysplasia, nephrocalcinosis, renal hyperechogenicity, loss of corticomedullary differentiation (list not exhaustive)
    - ECG long QT interval ($QTC_F \geq 460$ms),
    - laboratory test especially:
        - ✓ hypokalaemia (K<3.5mEq/L),
        - ✓ Clinically relevant hypercalciuria.

          In case of abnormal urinary calcium/creatinine ratio that may be the expression of a clinically relevant hypercalciuria, a retest should be performed as soon as possible. Based on these results, the investigator should have a discussion with the local paediatric nephrologist in order to assess whether it corresponds to the presence of a clinically relevant hypercalciuria according to their clinical judgment (based on broader sources including risk factors, medical history, clinical examination and other biological parameters).
        - ✓ positive for hepatitis A or hepatitis B serology not explained by a vaccination or by a past resolved infection, positive for hepatitis C serology

35. (A) Clinically relevant nephropathy according to investigator's clinical judgment (based on broader sources including risk factors, medical history, clinical examination and biological parameters).
36. eGFR $\leq$ 90 mL/min/1.73m² (estimated glomerular filtration rate, Schwartz formula 2009).
37. Severe electrolyte imbalance that is likely to interfere with the study conduct or evaluation.
38. Patient who has a current suicide risk according to the investigator (based on the information obtained during the evaluation of the C-SSRS-C (Children version) Baseline/Screening: "suicidal ideation" part, item 4 or 5 is "yes" in "6 months" part).

5.5. Additional information recorded at the selection/inclusion visit

The following additional assessments will be recorded at the selection visit:

- Information on previous or concomitant psychological therapy (details on the type of therapy, number of hours per week), if any.
- Information on previous psychotropic treatment, if any.

5.6. Participant withdrawal

5.6.1. Withdrawal criteria

Premature discontinuation of IMP during the double blind treatment period (W000-W026) does not mean that the participant prematurely stops the participation in the study.

In the case of premature discontinuation of IMP during the double blind period and, if the patient does not require an important modification on his/her current background medical care, he/she will be invited to complete CARS2, SRS-2, CGI evaluations and AE assessment for W012 (if discontinuation occurs prior to W012) and W026 visit as planned in the investigation schedule (Section 4.2.2).

Important modification of current medical care is defined as follows:

- ***Newly initiated formal behavioural, cognitive or cognitive-behavioural therapy***
- ***Increase/decrease weekly prescribed hours >25% of behavioural, cognitive or cognitive-behavioural therapy***
- ***Newly initiated or recently changed pharmacotherapy:***
  - ***Introduction of a psychotropic medication (considered as forbidden in the study, section 6.3)***
  - ***Introduction or dose increase of an antipsychotic***
  - ***Introduction or dose increase of ADHD treatment***

The following criteria will lead to a premature discontinuation of IMP:

- Serum potassium levels < 3mEq/L
- Any suicide attempt, whatever its severity
- High suicidal risk, according to investigator's judgment or with a suicidal ideation of 4 or 5 on C-SSRS-C
- Nephrocalcinosis observed at the renal ultrasound
- Occurrence of seizure(s)
  - in a patient without any medical history of seizure.
  - or in a patient with a medical history of seizure if this require any modification of the on-going anti-epileptic treatment.
- Any symptoms or signs of potential renal function failure.
- eGFR ≤ 80mL/min/1.73m² (estimated glomerular filtration rate, Schwartz formula 2009).
- Clinically relevant nephropathy according to investigator's clinical judgment (based on broader sources including risk factors, medical history, clinical examination and biological parameters).
- Clinically relevant hypercalciuria.

  In case of abnormal urinary calcium/creatinine ratio that may be the expression of a clinically relevant hypercalciuria, a retest should be performed as soon as possible. Based on these results, the investigator should have a discussion with the local paediatric nephrologist in order to assess whether it corresponds to the presence of a clinically

relevant hypercalciuria according to their clinical judgment (based on broader sources including risk factors, medical history, clinical examination and other biological parameters).

Additional urinary retest(s) and other appropriate exams should be performed if they judge them as relevant for the patient's safety.

- Absence of 2 consecutive planned electrolytes monitoring analysis.
- Absence of 2 consecutive planned urinary monitoring analysis.
- Occurrence of QT prolongation ($QTC_F \geq 480ms$), torsade de pointe and ventricular arrhythmia. Even if a 12-lead ECG reading and interpretation will be centralised, a local assessment of ECG exams is necessary for detection of medical urgencies and must be enclosed in the source data. Central reading reports for ECG overrule local reading for inclusion/exclusion/withdrawal criteria.
- Occurrence of a new psychiatric or other medical conditions leading to necessary administration of forbidden concomitant medication or likely to interfere with the conduct of the study.
- Any event or circumstances related or unrelated to the treatment justifying the discontinuation of treatment in the investigator's opinion (including treatment failure i.e. lack of efficacy).
- Major deviation to protocol if it interferes with the study evaluations and/or if it jeopardises participant's safety, e.g. any medical event requiring administration of an unauthorised concomitant treatment (see section 6.3).

Other criteria for premature discontinuation of IMP and for premature withdrawal from the study:

- Lost-to-follow up.
- Any non-medical circumstances leading to patient withdrawal (e.g. assent/consent withdrawal).

Information to be collected during the last visit of these participants is given in Section 5.6.2. These follow-up modalities are used to ensure the efficacy and safety evaluation of all participants who received the IMP.

Special conduct in case of gastrointestinal symptoms and weight loss

If the patient experiences digestive symptoms (≥ 3 liquid stools per day and/or ≥ 3 vomits per day) or digestive symptoms with a weight loss greater than 5%, the study treatment should be temporarily interrupted. Such an interruption will last 48h/72hours.

In such a case a monitoring of K+, Na+ must be done locally prior to study treatment re-introduction. Further electrolytes follow up will be locally done according to the clinical opinion of the medical doctor.
If these symptoms are persistent following 72 hours, the study treatment should be definitely withdrawn.

5.6.2. Procedure

The investigator must notify the sponsor of study withdrawals by entering data of the withdrawal visit in the e-CRF. The investigator must record the reason and the exact date of the premature discontinuation from the study in the e-CRF. If more than one reason is given, the investigator must indicate the main reason.

In case of premature discontinuation from the study a withdrawal visit will be done as soon as possible after the last dose intake and within one week at the latest

In the case of premature withdrawal of IMP due to an adverse event (event requiring immediate notification or not), the investigator must make every effort to collect the information relating to the outcome of the event. If necessary, the information will be collected afterwards (see section 8.9). This information is recorded in that part of the e-CRF which concerns adverse events. If the investigator cannot collect the information from a visit, he/she must collect it from the doctor ensuring the follow-up of the participant.

If the study is stopped / treatment is discontinued as a result of an event requiring immediate notification, the procedure described in Section 8.9.2.5 is to be implemented.
The dispositions to be taken after the IMP discontinuation are described in Section 6.5.

5.6.3. Lost to follow-up

When the investigator has no news of the participant, he/she must make every effort to contact him/her and his/her parents/legal representative(s) and caregiver (when applicable) or a person around them (phone calls, letters including registered ones… etc.), to establish the reason for the discontinuation of IMP and to suggest the participant comes to an end-of-study visit. If all these attempts to contact the participant fail, the investigator can then declare the participant "lost to follow-up". The investigator should document all these attempts in the corresponding medical file.

6. TREATMENT OF PARTICIPANTS

6.1. IMP administered

During the run in and the follow up periods patients will not receive IMP.

During the treatment period (from W000 to W052), the patient will take orally twice a day:

- **in the morning at wake-up**

- **in the afternoon, 3 hours before going to bed at night at the latest**

the following volume of the oral solution:

For patients with a weight < 25kg:
- The volume (mL) = 0.04 x body weight (kg) twice per day from inclusion to W052

For patients with a weight ≥ 25kg:
- 1 ml twice per day from inclusion to W052

The study treatment should be taken at least 15 minutes before any food intake.

The first study treatment intake will take place in the afternoon at the latest 3 hours before going to bed at night of the inclusion visit day.
The last IMP intake will take place in the morning of the day of the scheduled visit.
The description of the IMP is detailed in Section 4.4.1.

6.2. IMPs dispensing

Double blind treatment period: IMP box dispensing will occur at W000 (1 Kit A), W004 (1 Kit A), W008 (1 Kit A), W012 (1 Kit A), W016 (1 Kit A), W020 (1 Kit A). The kit number of Kit A will start by 20XXXX and the label will be orange.

Open label safety extension period: IMP box dispensing will occur at **W026 (1 Kit B),** W030 (1 Kit B), W034 (1 Kit B), W038 (1 Kit B), W042 (1 Kit B), W046 (1 Kit B). The kit number of Kit B will start by 22XXXX and the label will be blue.

The Kit number(s) will be given to patient according to number(s) allocated by IRS (please see details in IRS manual).

The detachable portion of the label on the IMP box must be stuck by the investigator on an IMP label collection form or on the prescription form where the IMPs are dispensed by a pharmacist.

6.3. Previous and concomitant treatments

This is a paediatric study, treatments administrated to the patients should be in-label or recommended as per local care guidelines.

Forbidden and contraindicated medications

Contraindicated medications (due to safety reasons) were listed in non-selection criteria 27. When applicable, considering these treatments may not be or poorly be effective for the patient, the washout period before inclusion should be of **3 weeks**.

Psychotropic treatments and treatments likely to interfere with the CNS function may by their activity interfere with the study evaluations, these are forbidden.

When applicable, considering these treatments may not be or poorly be effective for the patient, the washout period before inclusion is the following:

- Benzodiazepines with medium or short half-life: **3 weeks**
- Benzodiazepines with long half-life: **4 weeks**
- Antipsychotics: **4 weeks**
- Stimulants: **3 weeks**

Exception: *Methylphenidate, Atomoxetine, Guanfacine stabilized for at least 4 weeks prior to the inclusion and not planned to be modified or stopped during the W000-W026 period.*

Treatments with special warning and precaution of use

- **NSAIDs** could be exceptionally administered at the lowest possible dose and during a period not exceeding 2 consecutive days for the patients who do not respond to paracetamol. During this period the study treatment should be temporarily interrupted.

- **Vitamin D supplementation**, when required, should not exceed 100 000 IU/3 months.

- Benefit risk assessment should be taken into account before prescribing a nephrotoxic and/or ototoxic drug concomitantly to bumetanide.

- **Laxatives** are forbidden except osmotic laxatives such as Polyethylene glycol. Dosage should be in accordance with the SmPc of the drug.

After having ruled out a constipation caused by organic conditions, education should be the first step in treatment (dietary changes: increased intake of fluids and fiber…).

Authorized medications
To relieve pain and fever during the study PARACETAMOL should be preferred (see NSAIDs special warning).
Melatonine is authorised for sleep aid.

Light gas sedation used to perform the required study safety exams is not considered as forbidden concomitant treatment.

6.4. IMP compliance

Study medication taken by each participant must be recorded together with corresponding times in the patient's diary for study medication intake provided by the investigator.

The number of oral solution formulation doses taken by the participant is to be counted by the investigator or a designated person from his/her team and recorded in the electronic case report (e-crf) form and therapeutic unit tracking form or equivalent document.

If the participant, parents/legal representative(s) or caregiver (when applicable) did not bring back the patient's diary for study medication and/or the liquid bottle/s dispensed at the previous visit, the investigator must estimate the number of IMP doses taken by the participant since the previous visit, by questioning him/her or his/her parents/legal representative(s) or caregiver (when applicable).

The compliance will be assessed from the method described above and from the questioning of the participant or parents/legal representative(s) or caregiver (when applicable).

6.5. Discontinuation of the IMP

In case of discontinuation of the IMP before W52, the participants' treatment is left to the physician's discretion.

Specific rules may be followed in some countries according to local regulation.

7. ASSESSMENT OF EFFICACY

7.1. Efficacy measurements

Efficacy measurements performed during the study are indicated in Table (4.2.2) 1.

The efficacy assessments are the following:
- Childhood Autism Ratings Scale, Second edition (CARS2)
- Clinical Global Impression (CGI)
- Social Responsiveness Scale, Second edition (SRS-2)
- Vineland Adaptative Behaviour Sales, Second Edition (VABS II)

7.2. Methods and measurement times

The **Childhood Autism Ratings Scale, Second edition (CARS2)** (Schopler, 2010) is the main efficacy criterion.

In order to maintain the double blind conditions, the CARS2 should be completed by an independent rater, e.g. an individual qualified to perform CARS2 evaluation who is masked from a subject's data in order to minimize bias.

In the framework of this study the independent rater could be:
- a medical doctor: child & adolescent psychiatrist or neuro-paediatrician
- a clinical psychologist / health professional with:
  - o a detailed knowledge and experience of ASD and child development
  - o who plays a key role in the current site organization of the team
  - o and is involved in the diagnosis of ASD patients on a regular basis
  - o and who has a detailed knowledge and experience of ADOS-2 (certification preferable)
  - o who performs ADOS-2 (or at least ADOS-1) on a regular basis

A mandatory training for CARS2 will be performed for independent raters before their involvement in the study. Only raters who have successfully completed this training will be involved in the trial. The aim is to provide an homogeneous training and common rating rules on this tool in order to minimize bias related to individual rating variability.

The CARS2 is a 15 item rated instrument that will be assessed:
- At Inclusion
- During the Double Blind treatment period at: W004, W012, W026 visits
- During the Open Label treatment period at: W038, W052 visits
- At Wend visit or at the withdrawal visit in case of premature withdrawal after visit W000+Day10

As far as possible same independent rater will assess CARS2 for a given patient all along the study (from W000 till W052 and at least till W026) and, when possible, all the patients for a given site.

The CARS2 includes three forms:
- Childhood Autism Rating Scale, Second Edition-Standard Version (CARS2-ST) which should be used for assessing patients with overall IQs ≤ 79, who have notably impaired communication, or who are younger than 6 years of age regardless of their estimated IQ.
- Childhood Autism Rating Scale, Second Edition-High Functioning Version (CARS2-HF) is used to assess patients with estimated overall IQs ≥ 80, who have relatively good verbal skills and who are aged 6 or older.
- Questionnaire for Parents or Caregivers (CARS2-QPC) is an unscored questionnaire provided for acquiring information from parents/legal representative(s) or caregiver (when applicable) for subsequent use by professionals making CARS2-ST or CARS2-HF ratings.

The choice of the CARS2 form (standard version or high functioning version) applicable for a given patient will be based on:
- the age of the patient
- the IQ of the patient: If a IQ has been performed within the 12 months prior to selection, it is not necessary to repeat the IQ test at ASSE.

In making ratings, independent rater should compare the patient's behaviour with that of a typically developing individual of the same age. When behaviours are observed that are not typical for an individual of the same age, then the peculiarity, frequency, intensity and

duration of these behaviours should be considered. All behaviour should be rated without recourse to causal explanation.

Rating values for each of the 15 CARS2-ST/CARS2-HF items range from 1 to 4. Generally, a rating value of 1 indicates that an individual behaviour is within normal limits for an individual of that age, whereas a value of 4 indicates that the individual's behaviour is severely abnormal for someone of that age. For each item, the independent rater will record notes which justify the ratings. The rating values are summed to produce a Total score.

The **Clinical Global Impression Scale (CGI)** (Guy, 1976) will be assessed by the medical doctor;
The CGI scale rates:
- The severity of illness (Clinical Global Impressions – Severity (CGI-S)): at Selection, Inclusion, W012, W026, W052, Wend visits or at the withdrawal visit in case of premature withdrawal
- The Global Improvement (Clinical Global Impression – Global Improvement (CGI-I)) in comparison with
    - patient's condition at inclusion for W000+Day 17, W004, W008, W012, W016, W020, W026, W038, W052 or at the withdrawal visit (in case of premature withdrawal during the double blind or withdrawal during open label treatment period)
    - patient's condition at W026 for W038, W052 or at the withdrawal visit (in case of premature withdrawal during the open label treatment period)
    - for Wend visit:
        - patient's condition compared to W052 for patients completing the study
        - patient's condition compared to Withdrawal visit for patients having prematurely withdrawn from the study

The **Social Responsiveness Scale, Second Edition (SRS-2)** (Costantino, 2012) is a 65 items, Likert scale, objective measure of symptoms associated with autism. The SRS-2 generates a Total score that serves as an index of severity of social deficits in the autism spectrum.

It will be completed by the parents/legal representative or caregiver (when applicable):
- At Inclusion
- During the Double Blind treatment period at: W004, W012, W026 visits
- During the Open Label treatment period at: W038, W052 visits
- At the withdrawal visit in case of premature withdrawal after visit W000+Day10

It is highly recommended same parent/legal representative or caregiver (when applicable) completes the SRS-2 all along the study.

In the framework of this study the following forms will be completed:
- SRS-2 Preschool form (ages 2 to $4^1/_2$)
- SRS-2 School form (ages 4 to 18 yo).

For younger children, some overlap in form age has been provided so that an older pre-schooler can be rated and scored on either Preschool or School age forms. Children in this overlapping period are generally rated on the School-Age Form, excepted for those children with lower functioning, with behaviour more similar to that described on the Preschool Form.

The **Vineland Adaptative Behaviour Scales-Second Edition (VABS II)** is designated to measure adaptative behaviour. It explores 5 domains: Communication, Daily Living Skills, Socialization, Motor Skills and Malaptative Behaviour.
It will be completed by a Medical Doctor or a Psychologist/ Health professional with:

- o a detailed knowledge and experience of ASD and child development
- o who plays a key role in the current site organization of the team
- o is involved in the diagnosis of ASD patients on a regular basis
- o and has been trained on VABSII with the sponsor training

- At Inclusion
- During the Double Blind treatment period at: W026 visit
- During the Open Label treatment period at: W052 visit
- At the withdrawal visit in case of premature withdrawal after visit W000+Day10

It is highly recommended the same rater with the same parent/legal representative or caregiver (when applicable) completes the VABS II all along the study.

8. SAFETY MEASUREMENTS

All adverse events and other situations relevant to the safety of the participants must be followed up and fully and precisely documented in order to ensure that the sponsor has the necessary information to continuously assess the benefit-risk balance of the clinical trial.

8.1. Specification of safety parameters

Safety measurements performed during the study are indicated in Table (4.2.2) 1.

Assessment of suicidal ideation and suicidal behaviour using the Columbia Suicide Severity Rating Scale Children's version (C-SSRS-C)

- Physical examination: blood pressure (SBP, DBP), heart rate, body weight, height, BMI,
- 12-lead ECG
- Renal ultrasound
- Laboratory parameters
- Adverse events
- PAERS

The investigator may also conduct any other assessments he/she deems necessary during the study to ensure the safety of the patient.

8.2. Methods and measurement times

The **Columbia Suicide Severity rating Scale Children's version (C-SSRS-C)** (Posner, 2007) (Posner, 2011) assesses suicidal ideation and suicidal behaviour. Investigator will complete an e-training on this tool prior to the study start.

The C-SSRS-C includes two forms:

- Baseline/Screening version: completed at W000

- Since Last Visit version: to be completed at W012, W026, W038, W052 or at the withdrawal visit in case of premature withdrawal

The **Paediatric Adverse Event Rating Scale (PAERS)** (Shapiro, 2009) is an inventory report designed to identify signs/symptoms experienced by the patient since the study treatment initiation. Investigator will complete an e-training on this tool prior to the study start.

The PAERS will be completed:

- During the Double Blind period at: W000, W000+Day 17, W004, W008, W012, W016, W020, W026 visits
- During the Open Label period at: W026+Day17, W030, W034, W038, W042, W046, W052 visits
- At the withdrawal visit in case of premature withdrawal

PAERS includes three forms:

- Youth form, to be completed when possible by the patient,
- Parent form, to be completed by a parent/legal representative or caregiver (when applicable).
  - As far as possible same person should complete the form during the study
- Clinician form filled after assessment of youth form and parent forms

Physical examinations

- DBP, SBP and heart rate must be always assessed. Appropriate conditions are assessment in a sitting or lying position after 5-minute rest and at 1mn after standing position (orthostatic hypotension: known effect of bumetanide):
  - At Selection
  - During the Double Blind period at: W000, W002, W012, W026 visits
  - During the Open Label period at: W028, W038, W052 visits
  - At the withdrawal visit in case of premature discontinuation.

  DBP and SBP will be measured with the specific device provided by the Sponsor for the study.
- Body weight must be assessed:
  - At Selection
  - During the Double Blind period at: W000, W000+Day17, W004, W008, W012, W016, W020, W026 visits
  - During the Open Label period at: W026+Day17, W030, W034, W038,W042, W046, W052 visits
  - At the withdrawal visit in case of premature withdrawal.
- Height must be assessed:
  - At Selection
  - During the Double Blind period at: W012, W026 visits
  - During the Open Label period at: W038, W052 visits
  - At the withdrawal visit in case of premature withdrawal

12-lead ECG

The following 12-lead ECGs will be performed during the study:

- A first 12-lead ECG will be prescribed at the selection visit and the assessment of the recording must be available before Inclusion visit. An ECG in triplicate (3 ECG in 10 minutes) is expected.
- During the Double Blind treatment period 4 ECGS will be performed and its assessment by local cardiologist/qualified person should be available at W004, W008, W012 and W026 visits
- During the Open Label treatment period 4 ECGS will be performed and its assessment by local cardiologist/qualified person should be available at W030, W034, W038 and W052 visits
  - At the withdrawal visit in case of premature withdrawal

ECG device will be the same for all centres and will be supplied by the CRO in charge of ECG central reading. The ECG device should be used only for the present study and for the sister study (CL3-95008-001). The recording should be done under the supervision of the investigator or a cardiologist or a specialized person associated with the centre. An ECG in triplicate (3 ECG in 10 minutes) is only expected for selection visit.

Even if the 12-lead ECG reading and interpretation will be centralised, a local assessment of ECG exams is necessary for detection of medical urgencies and must be enclosed in the source data. Central reading reports for ECG overrule local reading for inclusion/exclusion/withdrawal criteria.

The ECG central reading centre will be in charge of the coding of the abnormalities of the EGC using the chart issued from MINESOTA dictionary.

For each ECG time-point, the following parameters will be assessed: heart rate (bpm), RR interval (msec), PR interval (msec), QRS complex (msec), QT and QTcF interval (msec) (Fridericia's correction formula interval), sinus rhythm.

A qualitative assessment of the ECG received will also be performed by a central reading structure: Identified abnormalities, overall assessment and comparison versus baseline.

During the study, all findings on laboratory examinations, ECG or vital signs having considered as clinically significant by the investigator will be followed-up until the patient's value returns to normal or is stabilized.

Renal ultrasound

A renal ultrasound will be prescribed:

- At the selection visit and the assessment of the recording must be available before Inclusion visit.
- During the Double blind treatment period it will be prescribed at W020 and the assessment of the recording must be available for/during at W026 visit
- During the Open label treatment period it will be prescribed at W046 and the assessment of the recording must be available for/during at W052 visit
- At the withdrawal visit in case of premature withdrawal.

This exam could be done within 7 days prior to the day of the visit.

This exam should be assessed by a nephrologist or by a qualified specialist who will transmit the renal assessment to the investigator. As far as possible the same nephrologist/qualified specialist will assess renal ultrasound exams for a given patient all along the study.

Biological Safety measurements

To ensure homogeneity of the results, all biological assessments (except Na^+/K^+ parameters under treatment) will be carried out by the central laboratory chosen by I.R.I.S. The reference values of measured parameters will be mentioned in each laboratory report.

Biological results will be sent to site from central laboratory via a laboratory report, with the exception for Na^+/K^+ parameters which will be assessed locally (except at the selection visit before the first IMP intake and at the withdrawal visit (WD) after the last IMP intake. All laboratory reports (central and local) should be assessed by investigator and kept in the patient's medical file.

The sampling process and storage conditions on site will be described in the laboratory manual provided by the central laboratory.

The laboratory tests will be performed:
- Selection visit (laboratory tests will be prescribed at), so that the results are available for the inclusion visit
- During the double blind treatment period at W000+48/72h (only Na^+/K^+), W000+Day10 (only Na^+/K^+), W000+Day17 (only Na^+/K^+), W004 (only Na^+/K^+), W012 and W026 visits
- During the open-label extension period, at W026+48/72h (only Na^+/K^+), W026+Day10 (only Na^+/K^+), W026+Day17 (only Na^+/K^+), W030 (only Na^+/K^+), W038 and W052 visits
- At the withdrawal visit in case of premature withdrawal

The results should be checked carefully by investigator once received, and the patient/ parent/legal representative(s) or caregiver (when applicable) should be contacted again to inform them about results and any abnormality that necessitates a specific follow-up.

Laboratory tests at Selection visit

At selection visit, blood and urinary test samplings will be prescribed. All the results of these examinations must be available for the inclusion visit.

The following parameters will be analysed on blood samplings:
- **Biochemistry parameters**: sodium, potassium, calcium, chloride, magnesium, bicarbonates, phosphor, urea, uric acid, creatinine, creatinine clearance-eGFR (Schwartz formule 2009), AST, ALT, total bilirubin, free bilirubin, conjugated bilirubin, (ALP), Gamma-Glutamyltransferase (gamma GT), total cholesterol, HDL and LDL cholesterol, triglycerides, glucose, protein (Total, Albumin)
- **Haematology parameters:** haemoglobin, haematocrit, erythrocytes, white blood cells count, platelets, MCV.
- **Screening for hepatitis A, B and C** serologic markers.

The following parameters will be analysed on urinary samplings:
- **Calciuria, proteinuria and creatininuria**

Laboratory tests during the study:

The following parameters will be analysed on blood samplings:

- **Biochemistry parameters**: sodium, potassium, calcium, chloride, magnesium, bicarbonates, phosphor, urea, uric acid, creatinine, creatinine clearance (Schwartz formule 2009), AST, ALT, total bilirubin, free bilirubin, conjugated bilirubin, (ALP), Gamma-Glutamyltransferase (gamma GT), total cholesterol, HDL and LDL cholesterol, triglycerides, glucose, protein (Total, Albumin).
- **Haematology parameters:** haemoglobin, haematocrit, erythrocytes, white blood cells count, platelets, MCV.

The following parameters will be analysed on urinary samplings:

- **Calciuria, proteinuria and creatininuria**

		BLOOD ANALYSIS			URINE ANALYSIS
		Biochemistry excluding Na/K	Na/K monitoring via local laboratory	Haematology	Calciuria, Creatininuria Proteinuria
Double blind treatment period	W000 + 48/72h		X		
	W000 + Day 10		X		
	W000 + Day 17		X		
	W004		X		
	W012	X	X	X	X
	W026	X	X	X	X
Open label treatment period	W026 + 48/72h		X		
	W026 + Day 10		X		
	W026 + Day 17		X		
	W030		X		
	W038	X	X	X	X
	W052	X	X	X	X

Additional laboratory tests could be performed during the study if the medical doctor deems them as necessary.

ELECTROLYTES MONITORING

In the framework of this study the monitoring of electrolytes under treatment will be assessed locally. This will allow saving time in the delivery of laboratory results and to manage the Potassium supplementation when needed.

Investigators should always encourage patients to eat foods containing potassium as soon as they know that they will be included in the study. See Table (8.2) 1

Table (8.2) 1 - Foods that have a higher potassium contents

Fruit and nuts	Fruit in particular - bananas, apricots, avocados, blackcurrants, rhubarb, fresh fruit juices Dried fruits eg currants, raisins, prunes, figs, sultanas, are rich sources of potassium All varieties of nut eg almonds, peanuts and walnuts
Potatoes	Potatoes are very rich sources of potassium if they are not boiled Jacket potatoes Ordinary fried chips Instant potato products eg waffles, potato croquettes Potato crisps
Vegetables and pulses	In particular - sprouts, mushrooms, parsnips, spinach, baked beans, kidney beans, lentils
Cereals	Cereals which are high in bran eg bran flakes, all bran, muesli
Biscuits, cakes and sweets	Fruit cakes, fig rolls, muesli bars Chocolate, fudge, liquorice
Milk and dairy products	Drinking milk or eating yoghurts

The first electrolytes monitoring under treatment, in both double and open label periods, should be performed locally no later than 72h after W000 and W026 respectively.

In case of any abnormality, electrolytes will be monitored until return to normal values.

In case of **Mild Hypokalaemia (3mEq/L ≤ K^+< 3.5 mEq/L)** arising under study treatment:
→ Foods containing Potassium will be recommended such as leafy green vegetables, tomatoes, citrus fruits, oranges or bananas. An adequate care should be implemented for the hypokalaemia follow up.
→ Patient will be supplied with oral potassium according to investigator's opinion which can be based on local guidelines or local medical practice. The dose could be adapted to hypokalaemia severity or patients' weight.
The following Potassium Chloride doses are recorded only for indicative purposes:

- <20 kg= 400 mg/day (200 bid)
- 20-40 kg = 600 mg/day (300 bid)
- 40 kg = 1200 mg/day (600 bid)

In case of **Moderate Hypokalaemia (2.2mEq/L ≤ K+< 3 mEq/L)** arising under study treatment:
→ The study treatment should be immediately discontinued.
→ An adequate care should be implemented for the hypokalemia follow up.
→ Cardiac monitoring will be performed and level of potassium should be monitored until return within normal range
→ Patient will be supplied with oral potassium. According to investigator's opinion which can be based on local guidelines or local medical practice, the dose could be adapted to hypokalaemia severity or patients' weight.
The following Potassium Chloride doses are recorded only for indicative purposes:

- <20 kg= 400 mg/day (200 bid)
- 20-40 kg = 600 mg/day (300 bid)

- 40 kg = 1200 mg/day (600 bid)

In case of **Severe Hypokalaemia (< 2.2mEq/L),** arising under study treatment:
→ The study treatment should be immediately discontinued.
→ The patient should be immediately hospitalized, an adequate care should be implemented for the hypokalaemia follow up (eg intravenous KCl and/or oral high K dose according to the hospital usual practice). In any case the level of potassium should be monitored until return within normal range.

Potassium supplementation will not be provided by the Sponsor.

8.3. Definition of Adverse event

An adverse event is defined as any untoward medical occurrence in a subject participating in a clinical study, whether or not there is a causal relationship with the IMP and/or experimental procedures, occurring or detected from the date the participant signs the information and consent form, irrespective of the period of the study (periods without administration of the IMP (e.g. run-in period and after IMP discontinuation) are also concerned).

An adverse event can therefore be:
- any unfavourable and unintended sign (including an abnormal finding from an additional examination such as lab tests, X-rays, ECG, …) which is deemed clinically relevant by the investigator,
- any symptom or disease,
- any worsening during the study of a symptom or a disease already present when the participant entered the study (increase in frequency and/or intensity), including the studied pathology,

and detected during a study visit or at an additional examination or occurred since the previous study visit (including relevant event reported in participant's diary or safety evaluation scale).

Of note:
- Any **hospitalisation for social reasons, ASD diagnosis, educational purpose** (e.g. learning of diabetes management by the participant) or routine check-up should not be considered as an adverse event and should not be reported in the eCRF.
- The following procedures, whether planned before the study or not, whether leading to a hospitalisation or not, **should not be reported in the eCRF and kept in the source data (or patient file)**:
  - therapeutic procedures related to a non-aggravated medical history (e.g. plaque and screw removal following an osteosynthesis performed before the study),
  - prophylactic procedures (e.g. wisdom teeth removal),
  - comfort procedures (e.g. cosmetic surgery),
  - control procedures of a pre-existing condition without aggravation (e.g. gastroscopy to control the evolution of an ancient, previously-diagnosed hiatal hernia).

8.4. Definition of Serious adverse events

Any adverse event that at, any dose:
- results in death,
- is life-threatening[(1)],

- requires inpatient hospitalization or prolongation of existing hospitalization,
- is medically significant[2],
- results in persistent or significant disability/incapacity[3],
- is a congenital anomaly/birth defect[4].

[1] Life-threatening in this context refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

[2] Any event that might not be immediately life-threatening or result in death or hospitalisation, but might jeopardise the participant or might require intervention to prevent one of these outcomes (for example: oedema or allergic bronchospasm that required intensive treatment at home, blood dyscrasia, convulsions that do not result in hospitalisation, or development of drug dependence or drug abuse). The investigator should exercise his/her scientific and medical judgement to decide whether or not such an event requires expedited reporting to sponsor.

[3] Disability/incapacity in this context refers to any event that seriously disrupts the ability of the participant to lead a normal life, in other words leads to a persistent or permanent significant change, deterioration, injury or perturbation of the participant's body functions or structure, physical activity and/or quality of life.

[4] Congenital anomaly or birth defect refers to the exposure to the IMP before conception (in men or women) or during pregnancy that resulted in an adverse outcome in the child.

8.5. Definition of Overdose

This refers to any intake of a quantity of IMP which is above the maximum dose recommended in the study protocol, independently of the occurrence of any adverse event.

The quantity should be considered per administration or cumulatively regarding the maximum dose recommended in the study protocol.

In the framework of this study a daily dose administration greater than 2mL (or the maximum daily dose recommended in appendix 1 for those patients with a weight < 25kg) should be considered as overdose.

8.6. Definition of Adverse event of special interest

An adverse event of special interest (AEOSI) is one of scientific and medical interest or concern regarding the IMP for which recording rules, special documentation such as hospital records and/or adjudication committee could be appropriate. It may be a serious or non-serious AE that may require further investigation in order to characterize and understand.

In the framework of this study protocol AEOSI include any biological Potassium electrolyte abnormality < 3.5 mEq/L.

8.7. Definition of Events requiring an immediate notification (ERIN)

An event must be **notified immediately** (i.e. without delay and within 24 hours at the latest) to the sponsor if it is:

- a serious adverse event (as defined in Section 8.4),
- an adverse event of special interest (as defined in Section 8.6),

- an overdose of the IMP even if asymptomatic,
- any intake of the IMP by a person around the participant,
    - if there are signs or symptoms,
    - if there are no signs or symptoms, in the following cases:
        - if the person is a minor subject,
        - if the intake of treatment is intentional (including suicide attempt).

8.8. Classification of an adverse event (seriousness, severity, causality, expectedness)

It is important that the investigator gives his/her own opinion regarding the **seriousness**, the **intensity** of the event as well as the **cause-effect relationship** between an adverse event and the test drug. This evaluation must be assessed by the investigator and reported in the AE form. In addition, the sponsor will be responsible for the evaluation the **expectedness** of the event (See Section 8.9.3).

The Seriousness should be evaluated according to international guidances (see definition Section 8.4, in accordance with ICH Topic E2A and DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April).

The Intensity should be evaluated according to the following rule:
- mild: signs or symptoms, easily tolerated, relieved with symptomatic treatment,
- moderate: enough discomfort to cause interference with usual activity, only partially relieved with symptomatic treatment,
- severe: incapacity in some regular activities, not easily relieved with symptomatic treatment.

The causal relationship to the test drug or to the experimental procedures must be assessed when reporting the AE in the AE form. Cases ticked "related" by the investigator, or judged by the sponsor as having a reasonable suspected causal relationship to the test drug (AE linked to the mechanism of action of the test drug…), will be considered as suspected Adverse Drug Reaction. In general, **if a relationship between AE and drug is at least reasonably possible (i.e. the relationship cannot be ruled out) it is to be considered as "related".**

8.9. Reporting procedures

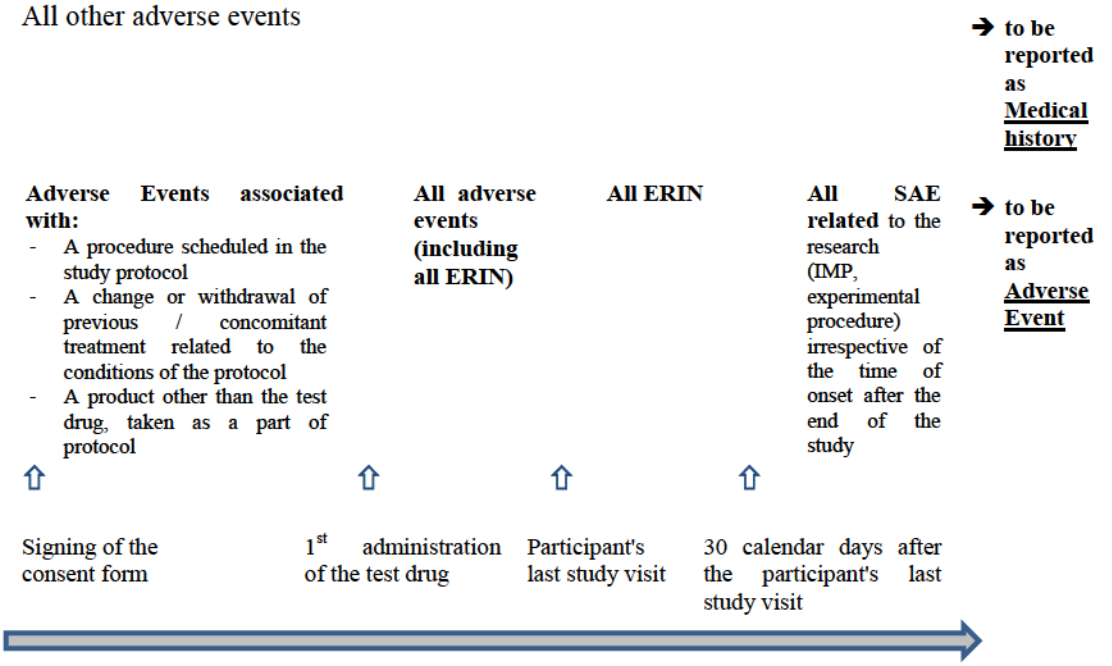
8.9.1. Time frame for AE reporting

Any event meeting the above mentioned definitions (see Sections 8.3 to 8.7) must be reported to the sponsor on an adverse event form if it occurred:
- before the first intake of the test drug, **for event associated with any procedure/condition required by the study protocol**: procedure (blood sampling, renal echography, etc.), change or withdrawal of previous/concomitant treatment relating to the conditions of the protocol
- at any time after the first intake of the **test drug** up to the participant's last study visit for all events,
- after the participant's last study visit:
    - up to 30 calendar days after the participant's last follow up study visit (WEND) for all ERIN, regardless of the supposed role of the research (IMP or experimental procedure).

- irrespective of the time of onset after the end of the study in case of serious adverse event related to the research (IMP or experimental procedure).

Of note, events occurring between the signature of the informed consent and the first administration of the test drug for which the investigator does not consider an association with any procedure/condition required by the study protocol must be reported as **medical history** in the dedicated form of the e-CRF.

These rules apply also to patients who prematurely stop the test drug during the double blind period, but who still participate in the study.



8.9.2. Responsibilities of the investigator

For any adverse event and special situation mentioned above the investigator must:

- **Note in the participant's medical file** the date on which he/she learned of the event (at a follow-up visit or a telephone contact with the participant or a third person,…) and any other relevant information which he/she has learned of the event,
- **Assess** the event in terms of seriousness, intensity and causality,
- Based on his/her clinical judgment, the investigator could decide **to interrupt or stop the treatment without resuming it, should an adverse reaction occur.**
- **Report the event to the sponsor** using the AE form (in case of ERIN, the reporting should be done immediately),
- **Document** the event with additional useful information,
- Ensure the **follow-up** of the event,
- **Fulfil his/her regulatory obligations** to the Competent Authorities and/or to the IRB/IEC, in accordance with local regulations.

Moreover, the investigator must report to the sponsor and/or to the IRB/IEC and/or to the Competent Authorities in accordance with the local regulation, any new information that might materially influence the benefit-risk assessment of the test drug or that would be sufficient to consider changes in the test drug administration or in the overall conduct of the clinical investigation.

8.9.2.1. Documentation of the event

The investigator must ensure that all events are well documented. In particular for ERIN, he/she should provide the sponsor, as they become available, with anonymized copies of the documents which provide additional useful information, such as hospital admission reports, reports of further consultations, laboratory test reports, reports of other examinations aiding diagnosis (where possible, the results from pre-test drug assessments should be appended for comparison with the results obtained under test drug), or the autopsy report, if autopsy is performed.

8.9.2.2. Follow-up of adverse events

The investigator must ensure that follow-up of the participant is appropriate to the nature of the event, and that it continues until resolution if deemed necessary.

Any change in terms of diagnosis, intensity, seriousness, measures taken, causality or outcome regarding an adverse event already reported must be written up in a new complete evaluation of the event documented on the "Adverse event" page previously created for the event.

If the adverse event has not resolved at the participant's final visit in the study, the participant must be followed up suitably and any information on the outcome of the event will be noted on the « Adverse Event » page previously created for the event.

If the follow-up of the participant is not done by the investigator him/herself (hospitalisation, followed by a specialist or the participant's general practitioner,…), the investigator will do everything to establish/maintain contact with the person/department in charge of follow-up of the participant.

8.9.2.3. Special situations (overdoses, intake of IMP by a person around the participant)

Overdose of IMP

- In case of overdose, the investigator should report it on an "Adverse Event" page to be notified immediately (ERIN).
- Overdose should be followed-up to ensure that the information is as complete as possible with regards to:
  - dose details (number of units, duration,…) and, if multiple overdose, details regarding other medicinal products or substance,
  - context of occurrence, i.e. intentional (suicide attempt, other reason) or accidental (error in prescription, administration, dispensing, dosage),
  - related signs and symptoms ("No related adverse events" to be reported otherwise),
  - outcome.
- Insofar as possible, a blood sample should be collected for assay of the IMP taken.

Intake of IMP by a person around the participant This event should not be reported in the e-CRF. The investigator should immediately contact the sponsor (contact details provided in the investigator's study file) who will inform him/her about the procedure to be followed.

8.9.2.4. Recording Methods in the e-CRF

Adverse events must be documented on the « Adverse Event » page of the e-CRF.

In case of chronic disease:
- if the disease is known when the participant enters in the study, only worsening (increased frequency and/or intensity of the episodes/attacks) will be documented as an adverse event,
- if the disease is detected during the study and if repeated episodes enable diagnosis of a chronic disease, the episodes will be grouped on the « Adverse Event » page previously created for the event which will clearly describe the diagnosis.

8.9.2.5. Procedure for an event requiring an immediate notification

In case of an event requiring an immediate notification, the investigator must:
- **Immediately** after being informed of this event, **fill in** the **participant's medical file** as well as the **« Adverse Event » page** of the e-CRF according to the general instructions available in the e-CRF, without waiting for the results of the clinical outcome or of additional investigations. When data will be submitted into Inform, an e-mail will be immediately and automatically sent to the sponsor.
- Provide the sponsor (person designated in the contact details provided in the investigator's study file), as they become available, with anonymized copies of the documents which provide additional useful information,
- Fulfil his/her regulatory obligations to the Competent Authorities and/or to the IRB/IEC, in accordance with local regulations.

If an adverse event initially non-serious worsens and becomes serious (ERIN), this must be reported **immediately** on an "Adverse event" page of the e-CRF.

In case the e-CRF is unavailable when the investigator was informed of the ERIN, he/she should:
- **Immediately** fill in a paper "Adverse event" page:
  - For serious event on a paper "Adverse event – Initial information" page,
  - For event initially non-serious on a paper "Adverse event – Initial information" page, and the worsening leading to seriousness on a paper "Adverse event – Additional information" page,
- Immediately send them by fax or e-mail them to the person(s) designated in the contact details provided in the investigator's study file or outside working hours, the 24-hour phone line(01.55.72.60.00 for a call from France, or international prefix followed by +33.1.55.72.60.00 for a call from outside France) or the local phone hotline (contact details are provided in the investigator's study file)
- As soon as the e-CRF becomes available, the investigator should enter these data in the « Adverse Event » page of the e-CRF.

8.9.3. Responsibilities of the sponsor

In accordance with international guidances, the assessment of the seriousness and the causality of adverse events are usually made by the investigator but falls also under sponsor's duties, who is responsible for ensuring that all suspected unexpected serious adverse reactions are reported to Competent Authorities and Ethics Committees.

The sponsor will review the seriousness of the adverse events and the causality of (at least) the serious adverse events, whether reported by the investigator or upgraded by the sponsor. The causality and the seriousness may be upgraded (but never downgraded). Anonymized copies of documents providing useful information such as reports of further consultations, laboratory tests reports, reports of other examination aiding diagnosis may be asked for the event assessment. If the assessments of the investigator and the sponsor are different, both will be reported in the clinical study report.

In addition, the sponsor is responsible for determining whether an AE is **expected or unexpected**. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the IMP.

Independently of the regulatory obligations of the investigator, the sponsor must report the pharmacovigilance data to the appropriate Authorities and to all the investigators involved, according to the requirements stated in ICH Good Clinical Practice guidelines and local regulations.

In case of any new safety information (likely to affect the Benefit/Risk balance of the product), the sponsor will inform all investigators involved in any study with the same drug that this new safety information has been reported.

As a result, study participants will be informed via his/her respective investigator of this new safety information and will receive a revised informed consent to be signed as described in section 13.3 of the protocol ''Modification of the information and consent form''.

The concerned Authorities will be notified as soon as possible by the sponsor of the Data Monitoring Committee (DMC) recommendations if any, where relevant for the safety of subjects (i.e. modification or termination of the study).

8.10. Responsibilities of Data Monitoring Committee

In accordance with the DMC charter and the rules for DMC functioning, the DMC is responsible for reviewing the safety data on a regular basis, and providing written recommendations to the Sponsor regarding the conduct of the study (modification or termination).

9. OTHER ASSESSMENTS NOT SPECIFICALLY RELATED TO EFFICACY OR SAFETY

9.1. Measurement of drug concentration

The concentrations of bumetanide will be determined in plasma by Cephac, using a validated bioanalytical method based on a protein precipitation followed by reverse phase liquid

chromatography with tandem mass spectrometric detection. Procedures will be described in a separate bioanalytical protocol established by the assay centre.

For each patient, 2 PK samples will be collected at W012 and W026, during the biological assessment.

All samples will be shipped to the central laboratory and then the first samples (aliquots 1) will be transferred from the central laboratory to the assay centre (Cephac) for analysis using a validated assay method. The assay centre in charge of bumetanide measurement will be provided with the treatment codes so that only samples from patients being treated with bumetanide will be assayed

The second samples (aliquots 2) will be stored by the central laboratory until the end of the study when they will be destroyed upon request from I.R.I.S, unless needed during the course of the study. If needed, the study samples will be sent to the assay center upon special request from I.R.I.S.

At the end of the study, upon request of I.R.I.S., the assay centre will be responsible for the destruction of the samples received.

In order to prevent a blind break, administration and sampling times will only be recorded on the requisition form and not on the electronic Case report Form (e-CRF). The central laboratory will capture data of the requisition form and will calculate the time after dose. Real time after dose will be transferred to Cephac for analyses. The PK department will receive only recoded data (recoded patient number). Bioanalytical results from Cephac and requisition form data from the central laboratory will be transferred to the I.R.I.S. Data Management department only after blind is broken.

9.2. Quality of life questionnaires

PedsQL (Varni, 1999) is a brief, standardized, generic assessment instrument that systematically assesses patients' and parent/legal representative or caregiver (when applicable) perceptions of health related quality of life. Four different domains are explored: physical, emotional, social and school functioning.

In the framework of this study, PedsQoL proxy-reported versions intended for parent/legal representative or caregiver (when applicable) will be completed:

- at Inclusion,
- during the double blind treatment period: W004, W012 and W026 visits
- during the open label period: W030, W038 and W052 visits
- in case of premature withdrawal after visit W000+Day10.

WHOQOL-Bref (WHOQOL Group, 1994) (WHOQOL, 1998a) (WHOQOL Group, 1998b) is a Health-Related Quality of life questionnaire intended to document the quality of life of parents/legal representatives or caregivers (when applicable) of children with ASD. Different domains are explored: physical, psychological, social relationships, environment and general health.

The WHOQOL-Bref will be completed by parent/legal representative or caregiver (when applicable):

- at Inclusion,

- during the double blind treatment period: W004, W008, W012 and W026 visits,
- during the open label period: W030, W034, W038 and W052 visits,
- in case of premature withdrawal after visit W000+Day10.

QUALITY OF LIFE UTILITY INSTRUMENTS

EQ-5D-3L (Khanna, 2013) is a standardised measure of health status developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical and economic appraisal. It explores different domains such us mobility, anxiety/depression, pain/discomfort, self-care and usual activities.
In the framework of this study, EQ-5D-3L will be completed by parent/legal representative or caregiver (when applicable) to collect their utility:

- at Inclusion,
- during the double blind treatment period: W012 and W026 visits,
- during the open label period: W038 and W052 visits,
- in case of premature withdrawal after visit W000+Day10.

It is highly recommended same parents/legal representative or caregiver (when applicable) completes the questionnaires as far as possible all along the study.

9.3. Acceptability and palatability questionnaire

It is a questionnaire mainly for parent/legal representative(s) or caregiver (when applicable) regarding the ease of use to use the dosing device. It also includes a 5-point hedonic scale (pictorial scale of facial expressions) for patients.

It will be completed at W026 or in case of premature withdrawal.

10. STATISTICS

10.1. Determination of sample size

The sample size was estimated to meet the primary endpoint defined as the change in CARS from baseline to 6 months using a two-sided Student's test.
With a total of 170 patients, statistical significance should be established with a power of 90% and a type-one error of 0.05 for an effect size of 0.5 between groups.
Assuming around 15% prematurely withdrawal rate, the total number of patients to be included is 200.
Missing data will be imputed using a reference based multiple imputation with a jump-to-reference approach or a standard multiple imputation depending on the reasons of withdrawal. Simulations were carried out to evaluate the impact of this procedure on power under various scenarios. The results on 1000 simulations have shown that power does not drop under 80% in the worst case scenario.

10.2. Statistical analysis

Statistical analysis will be performed by the I.R.I.S. Center of Expertise Methodology and Valorisation of Data.

A Statistical Analysis Plan, and associated templates for Tables, Listings and Graphs, will be written and completed before study unblinding. These specifications will detail the

implementation of all the planned statistical analyses in accordance with the main characteristics stated in the protocol.

Two sets of statistical analysis will be performed: a first analysis at the end of the double blind treatment period and a second analysis at the end of the open label active treatment period. The main analysis will be the first one based on the double blind treatment period.

10.2.1. Analysis sets / Treatment groups

10.2.1.1. Analysis sets

Randomised Set (RS):
All patients to whom a therapeutic unit was randomly assigned using IRS at W0.

Safety Set (SS):
All patients having received at least one dose of IMP.

10.2.1.2. Treatment groups

For the 6-months double-blind period, treatment groups will be defined as:
- Bumetanide group
- Placebo group

It will correspond to allocated treatment by IRS except for safety analyses for which treatment received at W0 will be considered.

For the open-label active treatment period, treatment groups will be defined as:
- Bumetanide group : patients with Bumetanide 0.5 mg BID received at W0
- Placebo / Bumetanide group: patients with placebo received at W0 and treated by Bumetanide 0.5 mg BID in the open label period.

10.2.2. Statistical methods

10.2.2.1. General considerations

The following descriptive statistics will be provided depending on the nature of considered data:
- **Qualitative data**: number of observed values, number and percentage of patients per class.

- **Quantitative data**: number of observed values, mean and standard deviation, median, first and third quartiles, minimum and maximum.

10.2.2.2. Disposition and baseline characteristics

Demographic data and other baseline characteristics will be summarized using descriptive statistics by treatment group, to assess their comparability, and overall in the RS.

Disposition of patients, including reasons for withdrawal and protocol deviations will be summarized overall and by visit in the RS.

10.2.2.3. Treatments of patients

Extent of exposure and treatment compliance, as well as prior and concomitant treatments will be summarized by treatment group in the SS.

10.2.2.4. Efficacy analysis

All efficacy analyses will be carried out on the RS.

10.2.2.4.1. Statistical hypotheses

The hypotheses to be tested are:
Let μ_0 and μ_1 be the population means of the change from baseline in CARS total raw score at 6 month (primary endpoint) under placebo and Bumetanide, respectively. The statistical hypotheses that will be tested are:

$$H_0: \mu_0 = \mu_1 \textit{(no difference between Bumetanide and placebo)}$$
$$\textit{versus}$$
$$H_1: \mu_0 \neq \mu_1 \textit{(difference between Bumetanide and placebo)}$$

The type I error of the statistical tests will be set at 5% (bilateral situation), which is consistent with the objective of demonstrating the superiority versus placebo (unilateral situation at 2.5%).

Similar hypotheses will also be tested for the secondary efficacy parameters.

10.2.2.4.2. Primary efficacy endpoint

The primary efficacy endpoint is the CARS total raw score, expressed in term of change from baseline to 6 month.

Primary analysis: Bumetanide will be compared to placebo using a general linear model with baseline CARS and stratification factors as covariates.

Missing data handling: CARS values post treatment discontinuation due to adverse event or lack of efficacy will be imputed using a reference based multiple imputation with a jump-to-reference approach. This approach assumes treatment benefit in patients who discontinue the Bumetanide arm disappears immediately upon discontinuation. Treatment effect estimation will take into account the situations when a patient can no longer tolerate or benefit from the treatment (occurrence of AE, lack of efficacy). CARS values post treatment discontinuation due to others reasons will be imputed using a multiple imputation approach assuming these patients could have theoretically continued to be treated without being put at undue risk.

Sensitivity analysis: sensitivity analyses detailed in the SAP to assess robustness to the method for handling missing data will be performed including a mixed model for repeated measurement.

Supplementary analysis:
The primary analysis will be repeated for the CARS total score expressed in term of change from baseline to 3 month.

The CARS total score and subscores will be summarized by treatment group at each planned visit for each period (double-blind treatment period and open-label active treatment period) using descriptive statistics.

10.2.2.4.3. Secondary efficacy endpoints

SRS total raw score:

The change in SRS total score from baseline to 6 month will be compared between Bumetanide group and placebo group using a general linear model with baseline SRS and stratification factors as covariates.

For missing data handling and sensitivity analysis, the same approach as for the primary endpoint will be performed.

Supplementary analysis:
The main analysis will be repeated for the SRS total score expressed in term of change from baseline to 3 month.

The SRS total score and subscores will be summarized by treatment group at each planned visit for each period (double-blind treatment period and open-label active treatment period) using descriptive statistics.

CGI-I:

The CGI-I at 6 months will be analyzed using a Robust General Linear Model using a rank-based analysis (Wilcoxon scores). Analysis will include the fixed, categorical effects of treatment, stratification factors and no interaction.

Vineland II:

The change in each Vineland II subscore from baseline to 6 month will be compared between Bumetanide group and placebo group using a general linear model with baseline subscore and stratification factors as covariates.

The Vineland II subscores will be summarized by treatment group at each planned visit for each period (double-blind treatment period and open-label active treatment period) using descriptive statistics.

PedsQL:

The PedsQL questionnaire will be summarized by treatment group at each planned visit for each period (double-blind treatment period and open-label active treatment period) using descriptive statistics.

WHOQOL-Bref:

The WHOQOL-Bref questionnaire will be summarized by treatment group at each planned visit for each period (double-blind treatment period and open-label active treatment period) using descriptive statistics.

10.2.2.4.4. Subgroup analyses

Additional descriptive analyses could be provided in subpopulation of interest.

10.2.2.5. Exploratory analysis

EQ-5D-3L:

Each index utility derived from the EQ-5D-3L will be summarized by treatment group at each planned visit for each period (double-blind treatment period and open-label active treatment period) using descriptive statistics.

10.2.2.6. Safety analysis

10.2.2.6.1. Adverse events

Double-blind treatment period:
Number of events, number and percentage of patients reporting at least one event, presented by primary system organ class, and preferred term will be provided for serious adverse events and emergent adverse events under treatment during the double-blind treatment period in the Safety Set.

Emergent adverse events will be described according to the seriousness, the intensity, the relationship, the action taken regarding the IMP, the requirement of added therapy, and the outcome.

Of note, the seriousness and the relationship to the IMP of the adverse event correspond to the investigator opinion or, in case of events upgraded by the sponsor for seriousness or for causality in case of SAE, to the sponsor opinion.

Open-label active treatment period:
The same analysis will be performed for adverse events occurred or worsened during the open-label active treatment period in the Safety Set.

Post-treatment period:
All adverse events reported after the last study drug intake will be listed.

10.2.2.6.2. Clinical laboratory evaluation

For clinical laboratory parameters, the following analyses will be performed by treatment period (double-blind treatment period and open-label active treatment period):

- Number and percentage of patients with at least one high/low emergent abnormal value under treatment, according to the laboratory reference ranges and to the cut-offs for PCSA values.
- Laboratory parameters classified (number and percentage of patients in each class) according to these reference ranges and cut-offs, and using shift tables from baseline to the worst (high and/or low) values under treatment.

10.2.2.6.3. Vital signs, clinical examination and other observations related to safety

10.2.2.6.3.1. Vital signs and clinical examination

Vital signs and clinical examination will be described by treatment period (double-blind treatment period and open-label active treatment period), in terms of value at baseline, value at each post-baseline visit under treatment; as well as in terms of change from baseline to each post baseline visit under treatment.

10.2.2.6.3.2. Electrocardiogram

Presence of clinically significant ECG abnormalities (yes/no) will be listed for each individual patients.

10.2.2.7. Planned interim analysis

No formal efficacy interim analysis is planned.

10.2.3. Other endpoints analysis

10.2.3.1. Pharmacokinetics

Pharmacokinetic interpretation

To assess PK profiles of bumetanide in children, a population pharmacokinetic model will be built using the sparse concentrations time data of bumetanide collected in the phase III studies as well as the data from phase IIb study. The population PK analysis will provide population PK parameters and their associated variability. In this analysis, the potential influence of covariates will be also investigated. Individual secondary PK parameters (such as $AUC_{12,ss}$, C_{min}) will be derived from the model for each patient. The population PK analysis will be detailed in a separate Data Analysis Plan.

Exploratory assessment of the relationship between exposure and pharmacodynamics (efficacy and potentially safety) will be performed and if applicable, population PK/PD models will be developed and a Data Analysis Plan will be set up.

11. DIRECT ACCESS TO SOURCE DATA / DOCUMENTS

The investigator will allow the monitors, the persons responsible for the audit, the representatives of the IRB/IEC, and of the Competent Authorities to have direct access to source data / documents.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1. Study monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the structure mentioned in Section 1.
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independant audit may be conducted by IRIS to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

12.1.1. Before the study

The investigator will allow the monitor to visit the site and facilities where the study will take place in order to ensure compliance with the protocol requirements.

Training sessions may be organised for the investigators and/or instruction manuals may be given to the investigators.

12.1.2. During the study

The investigator will allow the monitor to:
- review of the study site's processes and procedures,
- verify appropriate clinical investigator supervision of study site staff and third party vendors,
- inspect the site, the facilities and the material used for the study,
- meet all members of his/her team involved in the study,
- consult the documents relevant to the study,
- have access to the electronic case report forms (i.e. access to an analogic phone line or his/her computer)
- check that the electronic case report formshave been filled in correctly,
- directly access source documents for comparison of data therein with the data in the electronic case report forms,
- verify that the study is carried out in compliance with the protocol and local regulatory requirements.

The study monitoring will be carried out at regular intervals, depending on the recruitment rate and / or the investigation schedule, and arranged between the investigator and monitor.

All information dealt with during these visits will be treated as strictly confidential.

12.2. Computerised medical file

If computerised medical files are used, and if the computer system allows, no change made in the medical files by the investigator should obscure the original information. The record must clearly indicate that a change was made and clearly provide a means to locate and read the prior information (i.e. audit trail). The investigator will save data at regular intervals.

The investigator must guarantee the integrity of the study data in the medical files by implementing security measures to prevent unauthorised access to the data and to the computer system.

If the computerised medical files are considered as not validated by the sponsor, the investigator undertakes:
- at the start of the study, to print the medical files of all participants allowing a reliable verification of the study criteria (e.g. medical history/previous treatments/ characteristics of the studied disease documented within the period of time defined by the study protocol),
- during the study, to print in real time each data entry and each data change.

The investigator will personally sign, date and give the number of pages on the first or last page of each print-out. At each visit by the monitor, the investigator will provide all the print-outs of the medical files of the participants. The monitor will personally sign and date the first (or last) page then initial all pages in each paper print-out.

If the computer system allows the tracking of the changes made to the medical files, the investigator will supply the monitor, at each visit, with a print-out of the medical files of the participants and the records of the changes made. Each print-out will be personally dated and signed, by the investigator and the monitor on the first page. The number of pages will also be indicated by the investigator and the monitor on the first page.

If the computerised medical files are considered as validated by the sponsor, the investigator undertakes to give access to the monitor to the computerised medical files of all participants. If the monitor cannot access to the tracking of the changes made to the medical files, the investigator will supply the monitor, at each visit, with a print-out of the records of the changes made to the medical files of the participants. Each print-out will be personally dated and signed, by the investigator and the monitor on the first page. The number of pages will also be indicated by the investigator and the monitor on the first page.

The investigator undertakes to keep:
- all medical file print-outs signed and dated by him/her and by the monitor when the computer system is considered as not validated by the sponsor,
- if the computer system used allows changes to be made, the print-outs of the audit trail when the computer system is considered as not validated by the sponsor or when the monitor cannot access to the audit trail in the computer system,
- all original source-documents (originals of specific examinations, informed consent forms, therapeutic unit tracking form...).

12.3. Audit - Inspection

The investigator should be informed that an audit may be carried out during or after the end of the study.

The investigator should be informed that the Competent Authorities may also carry out an inspection in the facilities of the sponsor and/or the study centre(s). The sponsor will inform the investigators concerned immediately upon notification of a pending study centres inspection. Likewise, the investigator will inform the sponsor of any pending inspection.

The investigator must allow the representatives of the Competent Authorities and persons responsible for the audit:
- to inspect the site, facilities and material used for the study,
- to meet all members of his/her team involved in the study,
- to have direct access to study data and source documents,
- to consult all of the documents relevant to the study.

If the computerised medical file is considered as not validated, the investigator undertakes to provide all the source-documents and the print-outs of the medical files of the participants and, if the computer system used allows, the record of the changes made during the study.

If the computerised medical file is considered as validated, the investigator undertakes to:

- give access to the representatives of the Competent Authorities and persons responsible for the audit to the computerised medical files of all participants,
- provide the print-outs of the changes made during the study, if the tracking of the changes made to the medical files cannot be accessed in the computer.

12.4. Supervisory committees

In the frame of this study, a Data Monitoring Committee (DMC) will be set up.

According to the "Guideline on data monitoring committees" (Guideline CHMP/EWP/5872/03 Corr., 27 July 2005), the decision to set up of a DMC should take into account the study population as well as the study duration. The present study takes place in participants aged from 2 to 7 with ASD. As this vulnerable population will be exposed to study treatment for up to 56 weeks followed by a 6 week discontinuation period, the set-up of a DMC is justified in order to detect any potential harm to patients as early as possible.

All along the study, in order to ensure patients' safety, the DMC will be responsible for a follow up of the patients by a periodical review of safety data including all adverse events with all hypokalaemia. The DMC will treat all data as strictly confidential and will not disclose them to anyone else than members of the DMC.

DMC recommendations will be forwarded to the IEC/Competent Authorities only if relevant for the safety of participants. Details of the role and organisation of the DMC are detailed in a separate DMC charter.

13. ETHICS

13.1. Institutional Review Board(s)/Independent Ethics Committee(s)

The study protocol, the "Participant information and assent/consent form" document, *the* "Legal representative information and consent form" document (adapt, if applicable), the list of investigators document, the insurance documents, the Investigator's Brochure of administered IMPs will be submitted to IEC(s) by the the national coordinator(s) or the sponsor in accordance with local regulations.

The study will not start in a centre before written approval by corresponding IRB/IEC(s) has been obtained, the local regulatory requirements have been complied with, and the signature of the clinical study protocol of each contractual party involved has been obtained.

13.2. Study conduct

The study will be performed in accordance with the ethical principles stated in the Declaration of Helsinki 1964, as revised in Fortaleza, 2013 Participant information and informed consent

13.3. Participant information and informed consent

In any case, the participant and his/her parent/legal representative must be informed that he/she is entitled to be informed about the outcome of the study by the investigator.

The investigator or a person designated by him/her is to collect written assent from each participant (as much as possible), when of appropriate intellectual maturity and written

consent form(s) from his/her parent(s) (according to local regulations)/legal representative and his/her caregiver (when applicable) before his/her participation in the study.

Prior to this, each participant in the presence of his/her parent(s)/legal representative should be informed by the investigator or his/her delegate to the fullest extent possible about the study in language and terms he/she is able to understand. The investigator should also inform the parent(s)/legal representative of the objectives, benefits, risks and requirements imposed by the study, as well as the nature of IMPs. The participant, his/her parent(s)/legal representative and his/her caregiver (when applicable) must be informed that they have the possibility not to participate in the study and that they are free to reconsider assent/consent at any time.

The parent(s)/legal representative and the caregiver (when applicable) of the participant and the participant* will be provided with an information and assent/consent form in clear, simple language. The participant and his/her parent(s)/legal representative and his/her caregiver (when applicable) must be allowed ample time to inquire about details of the study and to decide whether or not to participate in the study.

Two original legal representative information and consent forms must be completed, co-signed and dated personally by participant's parent(s)/legal representative and by the person responsible for collecting the informed consent.

When applicable two original patient caregiver information and consent forms must be completed, co-signed and dated personally by participant's caregiver and by the person responsible for collecting the informed consent. Two original participant information and assent forms must be completed, co-signed and dated personally by the participant if capable and by the person responsible for collecting the informed consent.

The participant, his/her parent(s)/legal representative and the caregiver (when applicable) will be given one signed original information and assent/consent form, signed by the investigator and by them, the second originals will be kept by the investigator.

A participant entering in a new age set (7-8yo; 9-11yo; 12-18yo; patient becoming major) must sign a new assent form/ICF, depending on patient's level of understanding according to investigator's judgement, corresponding to his/her new age set.

In case of any unforeseen condition that necessitates either an interim or permanent replacement of the caregiver, the person replacing the caregiver must sign an informed consent.

A copy of the information and assent/consent forms for the participant, his/her parent(s)/legal representative and his/her caregiver (when applicable) in the language(s) of the country is or are given in the "Participant information and consent form" document, in the "Legal representative information and consent form" document and in the "Informant information and consent form" attached to the protocol

** Depending on the age of the minor, two different written information and consent forms are be prepared: one for the participant and one for his/her parent(s)/legal representative*

13.4. Modification of the information and consent form

Any change to the information and assent/consent form constitutes an amendment to this document and must be submitted for approval to the IRB/IEC(s), and if applicable to the Competent Authorities.

A copy of the new version of the information and assent/consent form in the language(s) of the country will be given in the amendment to the "Participant Information and consent form".

Such amendments may only be implemented after written approval of the IRB/IEC has been obtained and compliance with the local regulatory requirements, with the exception of an amendment required to eliminate an immediate risk to the study participants.

Each participant affected by the amendment and his/her parents/legal representatives and his/her caregiver (when applicable) must complete, date and sign two originals of the new version of the information and assent/consent form together with the person who conducted the informed consent discussion. He/she will receive one signed original amendment to the information and assent/consent form.

14. DATA HANDLING AND RECORD KEEPING

14.1. Study data

A 21 CRF Part 11-compliant electronic data capture system is going to be used for this study. An electronic case report form (e-CRF) is designed to record the data required by the protocol and collected by the investigator.

The e-CRF will be produced by I.R.I.S. in compliance with its specifications. The investigator or a designated person from his/her team will be trained for the use of the e-CRF by the sponsor.

Data entry at the investigator's site will be performed by the investigator or by the designated person from his/her team after completion of the participant's Medical File.

Upon entry, data will be transmitted via the Internet from the study centre to the study database.

The investigator or the designated person from his/her team agrees to complete the e-CRF, at each participant visit, and all other documents provided by the sponsor (e.g. documents relating to the IMP management…).
The eCRF should be completed within 5 days after the visit of participant and before the next schedule visit.
Data recorded directly on e-CRF and considered as source data (see Section 4.6) must be collected immediately in the e-CRF. The other e-CRF forms must be completed as soon as possible following each visit.

All corrections of data on the e-CRF must be made by the investigator or by the designated person from his/her team using electronic data clarifications according to the provided instructions. All data modification will be recorded using the audit trail feature of Inform

software, including date, reason for modification and identification of the person who has made the change.

In order to ensure confidentiality and security of the data, usernames and passwords will be used to restrict system access to authorised personnel only, whether resident within the investigator's sites, the sponsor or third parties.
Data will be verified in accordance with the monitoring strategy defined for the study. After comparing these data to the source documents, the monitor will request correction / clarification from the investigator using electronic data clarifications that should be answered and closed as quickly as possible.

Data can be frozen during the study after their validation. However the investigator has the possibility to modify a data if deemed via a request to the sponsor.

After the last visit of the participant at the double-blind 24 week treatment period and at the open label 28 active treatment period, the investigator or co-investigator must attest the authenticity of the data collected in the e-CRF by entering his/her user name and password.

After the final data base lock, the investigator, or an authorised member of their team, will have to download from the e-CRF an electronic file containing participant data from his/her centre for archiving in the study file (see Section 14.3).

14.2. Data management

Data are collected via a eCRF and stored in a secured database.

For data collected on the e-CRF, Clinical Data Management Department of I.R.I.S.is responsible for data processing including data validation performed according to a specification manual describing the checks to be carried out. As a result of data validation, data may require some changes. An electronic data clarification form is sent to the investigator who is required to respond to the query and make any necessary changes to the data.

For data collected on paper form (VABSII), the Clinical Data Management Department of I.R.I.S. is responsible for data processing including:

- Data entry: independent, blind, double data entry with a third person resolving any discrepancy between first and second entry,

- Data validation performed according to a specification manual describing the checks to be carried out. As a result of data validation, data may require some changes. A data clarification form is sent to the investigator for confirmation or correction and signature. In some cases, mentioned in the specification manual, changes (obvious errors) are not subject to the investigator's approval. A record of these data changes is provided to the investigator when the study is completed.

For data transferred from CROs (Central laboratory, ECG, IRS, PK), the Data & Clinical Logistics of I.R.I.S. *i*s responsible for data transfer: CROs provide electronic transfer of computerised data to the Clinical Data Management Department of I.R.I.S. Data are transferred according to a transfer protocol issued by the I.R.I.S. data manager.

The Medical Data Department of I.R.I.S. is responsible for data coding including:
- medical / surgical history, adverse events and procedures related to adverse events using MedDRA,
- medications using WHO-DD.

The coding process is described in a specification manual.

The investigator ascertains he/she will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact the sponsor or its representatives monitoring the study, if any, to request approval of a protocol deviation, as no deviations are permitted. If the investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the sponsor and approved by the IRB/IEC it cannot be implemented. All important protocol deviations will be recorded and reported in the clinical study report.

At the end of the double-blind 26 week treatment period, when data validation is achieved, a blind review of the data is performed according to the sponsor standard operating procedure. When the database has been declared to be complete and accurate, it will be locked and the IMP codes will be unblinded and made available for data analysis.

At the open label 26 week active treatment period, when data validation is achieved, a review of the data is performed according to the sponsor standard operating procedure. When the database has been declared to be complete and accurate, it will be locked and made available for data analysis.

14.3. Archiving

The investigator will keep all information relevant to the study for at least 25 years after the end of the study, or more if specified by the local regulation.

At the end of the study, the investigator, or an authorised member of their team, will download an electronic copy of each participant's data from the eCRF and should keep it in a reliable, secure and durable location. The file must include appropriate restrictions (password protection) and adequate protection from loss, physical damage or deterioration for the duration of the archiving period. These data include all data and comments reported in the e-CRF, the history of all queries and signatures and the full audit trail reports.

15. INSURANCE

I.R.I.S., or any parent company of SERVIER GROUP in charge of the management of clinical trials, is insured under the liability insurance program subscribed by LES LABORATOIRES SERVIER to cover its liability as sponsor of clinical trials on a worldwide basis.

Where an indemnification system and/or a mandatory policy are in place, I.R.I.S. or any parent company of SERVIER GROUP will be insured under a local and specific policy in strict accordance with any applicable law.

All relevant insurance documentation are included in the file submitted to any authorities' approval of which is required.

16. OWNERSHIP OF THE RESULTS –DATA SHARING POLICY AND PUBLICATION POLICY

I.R.I.S., acting as the study sponsor, assumes full responsibilities relating to this function and retains exclusive property rights over the results of the study, which it may use as it deems fit.

I.R.I.S. will ensure that the key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov. In addition, upon study completion and finalization of the study report, the results of this study will be either submitted for publication and/or posted in a publicly accessible database of clinical study results.

Any project of publication and/or communication relative to the study and/or relative to the obtained results during the study or after the study end shall be submitted to the sponsor in accordance with the guidelines set forth in the applicable publication policy or financial agreement.

The investigator, who submitted the project, shall take the sponsor's comments into due consideration.

Servier's Data Sharing Policy is available at https://clinicaltrials.servier.com/data-request-portal/. Researchers can ask for a study protocol, patient-level and/or study-level clinical trial data including clinical study reports (CSRs).

They can ask for all interventional clinical studies:

- submitted for new medicines and new indications approved after 1 January 2014 in the European Economic Area (EEA) or the United States (US).
- Where Servier or an affiliate are the Marketing Authorization Holders (MAH). The date of the first Marketing Authorization of the new medicine (or the new indication) in one of the EEA Member States will be considered within this scope.

In addition, Servier's data sharing policy includes all interventional clinical studies in patients:

- sponsored by Servier,
- with a first patient enrolled as of 1 January 2004 onwards,
- for New Chemical Entity or New Biological Entity (new pharmaceutical form excluded) for which development has been terminated before any Marketing authorization (MA) approval.

The datasets generated and/or analysed during the current study will be available upon request from www.clinicaltrials.servier.com after the Marketing Authorisation has been granted.

As the study is a multicentre one, the first publication must be performed only with data collected from several centres and analysed under the responsibility of I.R.I.S. The investigator commits himself not to publishing or communicating data collected in only one centre or part of the centres before the publication of the complete results of the study, unless prior written agreement from the other investigators and I.R.I.S. has been provided.

Authors and signatory(ies) of the publication(s) shall be defined as per the publication policy.

17. ADMINISTRATIVE CLAUSES

17.1. Concerning the sponsor and the investigator

17.1.1. Persons to inform

In accordance with local regulations, the investigator and/or the sponsor will inform, the Director of the medical institution, the pharmacist involved in the study and the Director of the analysis laboratory.

With the agreement of the participant and his parents/legal representatives, the investigator will inform the participant's general practitioner about his/her patient's participation in the clinical study.

17.1.2. Substantial protocol amendment and amended protocol

If the protocol must be altered after it has been signed, the modification or substantial amendment must be discussed and approved by the coordinator(s) and the sponsor.

The substantial protocol amendment must be drafted in accordance with the sponsor standard operating procedure and an amended protocol must be signed by both parties. Both documents must be kept with the initial protocol.

All substantial amendments and corresponding amended protocols must be sent by the investigator(s) or the coordinator(s) or the sponsor, in accordance with local regulations, to the IRB/IEC that examined the initial protocol. They can only be implemented after a favourable opinion of the IRB/IEC has been obtained, local regulatory requirements have been complied with, and the amended protocol has been signed, with the exception of a measure required to eliminate an immediate risk to the study participants.

When the submission is performed by the investigator or the coordinator, the latter must transmit a copy of IRB/IEC's new written opinion to the sponsor, immediately upon receipt.

Furthermore, the substantial amendment and amended protocol are to be submitted to the Competent Authorities in accordance with local regulations.

17.1.3. Final study report

The study report will be drafted by Medical Writing Department in compliance with I.R.I.S. standard operating procedure as follows:
- a primary clinical report after the 6-month double-blind period,
- a main clinical report after the 6-month open label period with the 6-week follow-up period,
- an addendum to the report for the optional 6-month extension period in open label.

The sponsor's representative and the coordinator(*s)* must mutually agree on the final version. One copy of the final report, must be dated and signed by the coordinator(s) and the Director of the Center for Therapeutic Innovation.

17.2. Concerning the sponsor

The sponsor undertakes to:
- supply the investigator with adequate and sufficient information concerning the IMPadministered during the study to enable him/her to carry out the study,
- supply the investigator with investigator's brochure if the test drug is not marketed,
- supply the investigator with SmPC, the one best suited to ensure participant safety, and any potential updated version during the study:
  - for the test drug if marketed, to be appended to Investigator's brochure (section 4. Guidance for the investigator
- obtain any authorisation to perform the study and/or import licence for the IMP administered that may be required by the local authorities before the beginning of the study,
- provide the investigator or coordinator(s) annually, or with another frequency defined by the local regulations, with a document describing study progress which is to be sent to the IEC(s).

17.3. Concerning the investigator

17.3.1. Confidentiality - Use of information

All documents and information given to the investigator by the sponsor with respect to S95008 and study CL3-95008-002 are strictly confidential.
The investigator expressly agrees that data on his/her professional and clinical experience is collected by the sponsor on paper and computer, and stored for its sole use relating to its activities as the sponsor of clinical trials, in accordance with GCP.
He/she has a right to access, modify, and delete his/her own personal data by applying to the sponsor.

The investigator agrees that he/she and the members of his/her team will use the information only in the framework of this study, for carrying out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the sponsor. The clinical study protocol given to the investigator may be used by him/her or his/her colleagues to obtain the informed consent of study participants. The clinical study protocol as well as any information extracted from it must not be disclosed to other parties without the written authorisation of the sponsor.

The investigator must not disclose any information without the prior written consent from I.R.I.S., except to the representatives of the Competent Authorities, and only at their request. In the latter case, the investigator commits himself/herself to informing I.R.I.S. prior to disclosure of information to these authorities.

A participant screening log and a full identification and enrolment list of each participant will be completed and kept in a safe place by the investigator who should agree to provide access on site to the auditor and/or the representatives of the Competent Authorities. The information will be treated in compliance with professional secrecy.

The participant screening log must be completed from the moment the investigator checks that a participant could potentially take part in the study (by assessment of participant medical history during a visit or by examination of the medical file).

17.3.2. Organisation of the centre

Every person to whom the investigator delegates under his/her responsibility a part of the follow-up of the study (independent rater, co-investigator, nurse…) and any other person involved in the study for this centre (cardiologist, nephrologist, pharmacist,…) must figure in the "Organisation of centre" document.

This document should be filled in at the beginning of the study and updated at any change of a person involved in the study in the centre.

17.3.3. Documentation supplied to the sponsor

The investigator undertakes before the study begins:

- to provide his/her dated and signed English Curriculum Vitae (CV) (maximum 2 pages) or to complete in English the CV form provided by the sponsor and to send it to the sponsor, together with that of his/her co-investigator(s),
- to provide a detailed description of the methods, techniques, and investigational equipment, and the reference values for the parameters measured,
- to provide any other document required by local regulation (e.g. Food & Drug Administration 1572 form),
- to send, a copy of the IEC's opinion with details of its composition and the qualifications of its constituent members.

The CVs of other members of the team involved in the study (if possible in English) will be collected during the course of the study (at least, members involved in the participants' medical follow-up/study-related decision process and persons involved in the measurement of main assessment criteria).

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