2. SYNOPSIS

Name of Sponsors: **I.R.I.S., 50 rue Carnot - 92284 Suresnes Cedex - France** **Laboratorios Servier, S.L., Avenida de los Madroños, 33 - 28043 Madrid - Spain** **Servier Research & Development Ltd, Sefton House, Sefton Park, Bells Hill, Stoke Poges - SL2 4JS - United Kingdom**		*(For National Authority Use only)*
Test drug **Name of Finished Product:** Not applicable **Name of Active Ingredient:** Bumetanide - S95008		
Individual Study Table Referring to Part of the Dossier	**Volume:**	**Page:**

Title of study:

Efficacy and safety of bumetanide oral liquid formulation in children and adolescents aged from 7 to less than 18 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 0.5 mg twice a day followed by an open-label active 6-month treatment period with bumetanide (0.5 mg twice a day) and a 6-week discontinuation period after treatment stop.

Protocol No.: CL3-95008-001
EudraCT No.: 2017-004419-38

The description of the study protocol given hereafter includes the modifications of the 4 substantial amendments to the protocol applicable in all countries.

Main investigator

No international coordinator was appointed for this study.
Nine national coordinators supervised this study (no national coordinator was identified for Portugal).

Study centres:

A total of 211 patients were randomised in 10 countries (40 centres) as follows: Spain (41 patients, 7 centres), United Kingdom (29 patients, 3 centres), Brazil (27 patients, 5 centres), Poland (26 patients, 5 centres), Italy (25 patients, 6 centres), France (23 patients, 5 centres), Hungary (20 patients, 4 centres), Portugal (14 patients, 1 centre), Germany (5 patients, 3 centres) and Netherlands (1 patient, 1 centre).
Moreover, Australia, one centre in France and one centre in Italy screened patients but did not include any for this study.

Publication (reference):

Not applicable

Studied period:	**Phase of development of the study:**
Initiation date: 21 September 2018 Completion date: 13 September 2021	Phase III

Objectives:

Primary objective

The primary objective was to demonstrate the superiority of bumetanide [0.5 mg twice a day (b.i.d.)] oral liquid formulation compared to placebo in the improvement of Autism Spectrum Disorders (ASD) core symptoms, as evaluated on Childhood Autism Rating Scale second edition (CARS2), after 6 months of treatment in ASD children and adolescents aged from 7 to less than 18 years old.

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 patient's quality of life.
- To improve existing pharmacokinetic (PK) model of bumetanide in this population.

Exploratory objectives

To describe the bumetanide effect on utility index scores.

Methodology:
This study was a 6-month, randomised, double-blind, placebo-controlled, parallel groups, international, multicentre phase III study followed by a 6-month open-label active treatment period and 6-week follow-up period after treatment discontinuation.
In addition, to give the possibility to the patients to pursue the treatment with bumetanide at the end of the main study periods, it was proposed a Named Patient Basis (NPB) in European Union or a Post-Access Study Program in Brazil. In the 3 countries where it was not possible to provide the treatment via a NPB (France, Poland and Germany), an optional 6-month extension period in open-label was proposed.

The randomisation was balanced (ratio 1:1), non-adaptative and stratified on country and gender.

The study was conducted in children and adolescents from 7 to less than 18 years old presenting with ASD.

Considering the targeted population and the study duration, a Data Monitoring Committee was set up and was responsible for periodic review of patient's safety data throughout the study.

This study was performed in strict accordance with Good Clinical Practice.

The superiority of bumetanide compared to placebo in ASD was not demonstrated in this phase III study. Based on these results and a consequent negative Benefit/Risk assessment, the Sponsor decided to prematurely discontinue the S95008 development. Subsequently, the present clinical study report is an abbreviated report.

Number of patients:
Planned: 200 patients included (100 patients in each group).
Included: 211 patients (107 in the S95008 group and 104 in the placebo group).

Diagnosis and main criteria for inclusion:

- Male and female patients from 7 to less than 18 years of age.
- Primary diagnosis of ASD as per Diagnostic and Statistical Manual of Mental Disorders, 5th edition, Text Revision (DSM-5) criteria, confirmed by Autism Diagnostic Observation Schedule-Generic (ADOS-2) and Autism Diagnosis Interview Revised.
- Clinical Global Impression (CGI) – Severity (CGI-S) rating Score ≥ 4.
- CARS2 (Standard Tool or High Functioning version) total raw score ≥ 34.
- Social responsiveness Scale second edition (SRS-2) total score ≥ 66 T-Score.
- Absence of known monogenic syndrome (as Fragile X or Rett Syndrome, list not exhaustive).
- Absence of any clinically significant abnormality likely to interfere with the conduct of the study according to the judgment of the investigator.

Test drug:
Bumetanide, oral solution dosed at 0.5 mg/mL, was 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 < 25 kg: 0.02 mg/kg corresponding to 0.04 mL/kg oral liquid formulation.
- For patients with a weight ≥ 25 kg: 0.5 mg corresponding to 1 mL oral liquid formulation.

Comparator:
Placebo, oral solution, was 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 < 25 kg: 0.04 mL/kg oral liquid formulation.
- For patients with a weight ≥ 25kg: 1 mL oral liquid formulation.

Duration of treatment:

- **Run-in period:** up to 4 weeks [without Investigational Medicinal Product (IMP)].
- **Treatment period:** 52 weeks (± 28 days).
  - Double-blind treatment period: 26 weeks.
  - Open-label active treatment period: 26 weeks.
- **Follow-up period:** 6 weeks after discontinuation of IMP.
- **Optional extension treatment period**: 6 months.

As none of the efficacy endpoints were reached after 6 months of treatment in children and adolescents aged from 7 to less than 18 years and due to the identified risk of hypokalaemia and associated effects linked to the drug's diuretic activity, the Benefit/Risk of the study treatment in ASD was considered negative. Consequently, the sponsor decided to stop the S95008 development and prematurely discontinue the extension period. This decision was not related to unexpected safety concerns.

Criteria for evaluation:

Efficacy measurements:

Primary efficacy endpoint:

CARS2 total raw score: expressed mainly in terms of change from baseline to Week (W) 026.

Secondary expressions were:

- Change from baseline to W012.
- Responders at W012 and W026 visits, defined as an improvement of at least 4.5 points in the CARS2 total raw score as compared to baseline.

Secondary and exploratory efficacy endpoints:

- SRS-2 total raw score.
- CGI: CGI-Global Improvement (CGI-I) score, CGI-S score and CGI responders.
- Vineland Adaptative Behaviour Scales, Second Edition composite score and domains scores.
- Paediatric Quality of Life Inventory.
- World Health Organization Quality of Life - BREF questionnaire.
- EuroQoL-5-Dimension-3-Level questionnaire (exploratory endpoint).

Safety measurements:

- Adverse events (AEs).
- Paediatric Adverse Event Rating Scale.
- Laboratory parameters: biochemistry including electrolytes monitoring, haematology, calciuria, creatininuria, proteinuria.
- Physical examination: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), body weight, height, body mass index (BMI), Tanner Stage.
- 12-lead electrocardiogram (ECG).
- Columbia Suicide Severity Rating Scale Children's version (C-SSRS-C).
- Renal ultrasound.

Pharmacokinetic measurements:

PK samples were collected at W012 and W026. The concentrations of bumetanide were determined in plasma.

Other measurements:

- At selection visit: drug screening (urine), Beta Human Chorionic Gonadotropin for post pubertal female (blood test) and screening for hepatitis A, B and C serologic markers.
- Acceptability and palatability questionnaire.

Statistical methods:
Main Analysis Sets:

- The *Randomised Set (RS)* is constituted of included and randomised (*i.e.* for whom a therapeutic unit was randomly assigned using Interactive Web Response System) patients.
- The *Safety Set (SS)* is constituted of all patients having taken at least one dose of IMP.
- The *Safety Set Open (SSO)* is constituted of patients of the SS having taken at least one dose of bumetanide during the open-label period.
- The *Safety Set Combined (SSC)* is constituted of patients of the SS having taken at least one dose of bumetanide during the open-label period and with a delay between end of double-blind period and start of open-label period less than 30 days.

One subgroup was planned for this study for the main efficacy analysis: Age [Children (7-11) / Adolescents (12-17)].

Efficacy analysis:
All efficacy analyses were performed in the RS. P-value was only provided for the primary analysis. For the other inferential analyses, only the appropriate estimate and associated 95% Confidence Interval (CI) were displayed.

Primary endpoint: CARS2 total score
Primary analysis:
The primary estimand was defined according to the primary objective of the trial, which was to evaluate treatment effect:

- Taking into account the unfavourable outcome when patients were unable to continue taking the study drug due to an adverse event or for lack of efficacy.
- Independently of treatment discontinuations for non-medical reasons because those patients would have theoretically continued to be treated as planned in clinical practice.

The attributes of the primary estimand were defined as following:

- Population: children and adolescents aged from 7 to less than 18 years old with ASD.
- Variable: change in CARS2 total score from baseline to W026.
- Summary measure: difference in means.
- Intercurrent events:
  - Treatment discontinuation due to lack of efficacy or AE (hypothetical strategy):
    - For S95008 arm, the quantification of the treatment effect cannot ignore the situation where a patient could no longer tolerate or benefit from the treatment, from whom a continuation of the treatment was not conceivable. Bumetanide is a treatment with a rapid onset and short duration of action. So the assumption that the treatment benefit in patients who discontinued the active arm disappeared immediately upon discontinuation is clinically meaningful. CARS2 values after this intercurrent event was considered as missing and imputed using a reference based multiple imputation ("jump-to-reference" approach).
    - For placebo arm, it was considered as if patients were stayed under placebo, CARS2 values after the intercurrent event were considered as missing and imputed using a multiple imputation [Missing At Random (MAR) approach].
  - Treatment discontinuation for other reason (hypothetical strategy): a reasonable question is what difference is attributable to treatment if no such events occurred namely if patients were stayed under their randomised treatment. CARS2 values after the intercurrent events were considered as missing and imputed in both arms using multiple imputations (MAR approach).

Bumetanide was compared to placebo on the change from baseline to W026 of the CARS2 total score, using a General Linear Model including the fixed, categorical effect of treatment, gender and country as well as the continuous fixed covariate of baseline value.

Missing data as well as data considered as missing for the primary analysis due to the strategy used to handle intercurrent event were imputed in the same way, depending on the reason for study premature withdrawal:

- For all study premature withdrawals due to other reasons as well as for study premature withdrawals due to lack of efficacy or AE in placebo arm, missing data were imputed in both arms using a multiple imputation based on similar patients in the same treatment arm (missing at random assumption).

- For study premature withdrawals due to lack of efficacy or AE in S95008 arm, missing data were imputed using a reference based multiple imputation with a jump-to-reference approach (missing not at random assumption).

Sensitivity analysis: same model as for the primary analysis without adjusting on covariates country and gender (*i.e.* unadjusted analysis).

Supplementary analyses:

- Treatment policy estimand: the primary analysis was repeated using all CARS2 values reported regardless of occurrence of treatment discontinuation.
- Hypothetical estimand based on Mixed-effects Model for Repeated Measures (MMRM): patients were considered as if they had continued their randomised treatment, using longitudinal data at each planned post-baseline visit of the double-blind period. In this analysis, all data occurring after all intercurrent events were considered as missing. The MMRM model included the fixed, categorical effects of country, gender, treatment, visit, treatment by visit interaction, country by visit interaction and gender by visit interaction as well as the continuous, fixed covariates of baseline and visit-by-baseline interaction.
- W012 analysis: the primary analysis was repeated for the CARS2 total score expressed in terms of change from baseline to W012.
- CARS2 responders analysis at W012 and W026, using a logistic regression model.
- Descriptive analysis of CARS2 total score and item scores (*i.e.* subscores) at baseline, each planned post-baseline visit as well as change from baseline to each planned post-baseline visit. The proportions of CARS2 responders was also summarised at each planned post-baseline visit using descriptive statistics.

Age subgroup [Children (7-11) / Adolescents (12-17)] analysis: same model as for the primary analysis with addition of subgroups and treatment by subgroups interaction as fixed effect.

Secondary endpoints: As this is an abbreviated clinical study report, none of the planned analyses related to secondary and exploratory endpoints are described in the present report.

Study patients: disposition, baseline characteristics and extent of exposure. Descriptive statistics were provided.

Safety analysis: Descriptive statistics were provided.

Pharmacokinetic analysis: Due to the Sponsor's decision to stop the development of S95008, only descriptive statistics were provided.

SUMMARY - CONCLUSIONS

DISPOSITION OF PATIENTS AND ANALYSIS SETS

As required in the protocol, a total of 211 patients were included in the double-blind period of the CL3-95008-001 study and randomly assigned to one of the 2 groups with a balanced ratio: 107 patients in the S95008 group and 104 in the placebo group.

The table below gives the disposition of patients by group as well as the analysis sets.

During the double-blind period, 29 patients (13.7%) were withdrawn, with a higher frequency in the S95008 group (16.8%, 18 patients) than in the placebo group (10.6%, 11 patients). The most frequent reasons for premature withdrawals were non-medical reasons with a slightly higher frequency in the S95008 group (9.3% of patients) as compared to placebo group (6.7%).

At W026, 86 patients in the S95008 group and 90 in the placebo group entered in the open-label period during which all patients received S95008. Overall, 26 patients (14.8%) were withdrawn from the open-label period with a lower rate in the group of patients previously on S95008 (S95008/S95008 group) than in those previously on placebo (placebo/S95008 group) (10 patients, 11.6% *versus* 16 patients, 17.8%). The most frequent reasons for premature open-label period withdrawals were adverse events, with a lower frequency in the S95008/S95008 group (4.7% of patients) as compared to placebo/S95008 group (14.4%).
During the whole study, withdrawal was reported as related to Coronavirus disease 2019 pandemic in 4 patients, all in the S95008 group.

Disposition of patients by group

		S95008	Placebo	All
Included in the double-blind period	**n[1]**	**107**	**104**	**211**
Withdrawn in the double-blind period due to	**n (%[1])**	**18 (16.8)**	**11 (10.6)**	**29 (13.7)**
Adverse event	n (%[1])	7 (6.5)	3 (2.9)	10 (4.7)
Withdrawal non-medical reason	n (%[1])	10 (9.3)	7 (6.7)	17 (8.1)
Protocol violation	n (%[1])	1 (0.9)	1 (1.0)	2 (0.9)
Completed the double-blind period	**n (%[1])**	**89 (83.2)**	**93 (89.4)**	**182 (86.3)**
Included in the open-label period	**n[2]**	**86**	**90**	**176***
Withdrawn in the open-label period due to	**n (%[2])**	**10 (11.6)**	**16 (17.8)**	**26 (14.8)**
Adverse event	n (%[2])	4 (4.7)	13 (14.4)	17 (9.7)
Lack of efficacy	n (%[2])	-	1 (1.1)	1 (0.6)
Withdrawal non-medical reason	n (%[2])	4 (4.7)	2 (2.2)	6 (3.4)
Protocol violation	n (%[2])	2 (2.3)	-	2 (1.1)
Completed the open-label period	**n (%[2])**	**76 (88.4)**	**74 (82.2)**	**150 (85.2)**
Analysis Sets				
Randomised Set	n	107	104	211
Safety Set	n	107	104	211
Randomised Set Open	n (%[a])	86 (80.4)	90 (86.5)	176 (83.4)
Safety Set Open	n (%[b])	86 (80.4)	90 (86.5)	176 (83.4)
Randomised Set Combined	n (%[a])	83 (77.6)	84 (80.8)	167 (79.1)
Safety Set Combined	n (%[b])	83 (77.6)	84 (80.8)	167 (79.1)

%[1] Percentages are based on n[1].
%[2] Percentages are based on n[2].
[a] Percentages are based on the number of patients in the Randomised Set.
[b] Percentages are based on the number of patients in the Safety Set.
[*] 6 patients (3 patients in each treatment group) stopped the IMP during the double-blind period but continued and completed the double-blind period and did not enter in the open-label period.

BASELINE CHARACTERISTICS

Demographic data and other baseline characteristics fulfilled with the selection/inclusion criteria defined in the protocol.

The patients of the RS were from 7 to 17 years old with a mean ± Standard Deviation (SD) age of 10.4 ± 3.0 years without relevant difference between groups. They were 141 children (7-11 years) (66.8%) with a lower rate in the S95008 group (63.6%) as compared to the placebo group (70.2%) and 70 adolescents (12-17 years) (33.2%) with a higher rate in the S95008 group (36.4%) than in the placebo group (29.8%).
In both groups, most patients were male (82.5% overall), reflecting the known male over-prevalence in ASD.

All patients of the RS met criteria for ASD on ADOS-2 questionnaire. According to DSM-5 questionnaire, ASD was accompanied by intellectual impairment in 65.4% of patients or by language impairment in 68.7%, without relevant difference between groups.

The mean age (± SD) of patients at the time of the ASD diagnostic was 4.6 ± 2.5 years and the mean time since diagnosis was 6.4 ± 3.5 years, without relevant difference between groups.
Among the 154 patients with an Intellectual Quotient (IQ) test score assessable, 51.9% had a score below 70. The mean of IQ test score was 71.3 ± 26.3, without relevant difference between groups.

According to Columbia-Suicide Severity Rating Scale for Children at baseline, 10 patients with C-SSRS-C evaluable (4.9%) have had suicidal ideation or behaviour in their lifetime without relevant difference between groups and 26 patients (12.8%) have had self-injurious behaviour without suicidal intent in their lifetime with a higher rate in the S95008 group (14.9%) than in the placebo group (10.8%).

Only 9.5% of patients in the RS had received at least one previous treatment predefined as related to ASD (treatments for attention-deficit hyperactivity disorder, antipsychotic treatments and antiepileptic treatments) and 1.4% one previous therapy for ASD, within the 6 months before the selection (*i.e.* ended before inclusion). Overall, 33.6% of the patients received at least one concomitant treatment related to ASD at inclusion.

Most of the patients (81.5%) reported at least one medical history besides ASD, without relevant difference between groups.

At baseline, the mean CARS2-total raw score was 40.1 ± 4.9 without relevant difference between groups. According to CARS2 total score, the ASD was rated as severe in most patients (83.9%) with a slightly lower rate in the S95008 group (80.4%) than in the placebo group (87.5%).

No relevant difference between groups was observed for vital signs, at baseline, in the RS. The mean values in sitting position were 107.1 ± 12.7 mmHg for SBP, 68.6 ± 10.9 mmHg for DBP and 88.1 ± 15.7 bpm for HR. The patients weighed from 17.2 to 129.2 kg and were 113 to 188 cm tall. Regarding BMI, 2.4% of patients were considered underweight, 27.1% overweight and 18.1% obese.
As regards ECG parameters, one patient in the placebo group had at least one clinically significant ECG abnormality at baseline.

During the double-blind period in the SS, during the open-label period in the SSO in patients initially treated with placebo and during the combined period in the SSC in patients initially treated with S95008, around one third of patients received at least one concomitant treatment related to ASD. A total of 20.4% of patients, 8.9% and 32.5%, respectively during these 3 periods and sets, had at least one clinically relevant modification in their current therapy of ASD. Few patients (5.7%, 3.3% and 13.3%, respectively) had new added concomitant therapy during these periods.

EXTENT OF EXPOSURE
In the SS, the mean treatment duration during the double-blind period was 5.5 ± 1.5 months, with a large majority of patients (91.4%) treated more than 3 months, and the compliance was on average 95.1 ± 14.1%. No relevant difference between groups was noted for these parameters.
In the SSO in patients initially treated with placebo, the treatment duration during the open-label period was on average 5.6 ± 1.5 months with also a large majority of patients (91.0%) treated more than 3 months and an average compliance of 75.7 ± 14.9%.
In the SSC in patients initially treated with S95008, the treatment duration during the combined period was on average 11.7 ± 1.7 months with a large majority of patients (91.6%) treated more than 38 weeks and an average compliance of 88.2 ± 3.9%.

EFFICACY RESULTS
As this is an abbreviated clinical study report, only the results on the primary endpoint defined as the CARS2 total score are described in the present report.

The *primary analysis* which consisted in the estimation of the difference between S95008 and placebo in change from baseline to W026, did not show the superiority of S95008 compared to placebo after 6 months of treatment in ASD children and adolescents. The difference between treatment groups calculated as S95008 *minus* placebo showed an estimated adjusted difference (Standard Error [SE]) of -0.45 (0.61) without statistical significance (95% CI [-1.64;0.74], p-value = 0.455).

The *sensitivity analysis* and two *supplementary analyses* based on treatment policy estimand for one and MMRM model for the other as well as a *third supplementary analysis on the change from baseline to W012,* showed similar results.

The proportion of *CARSR2 responders* at W012 was 35.4% in the S95008 group *versus* 27.7% in the placebo group, with an adjusted Odds Ratio (SE) between S95008 and placebo groups of 0.70 (0.33) with a 95% CI [0.36;1.35]. The percentage of CARS2 responders at W026 was 40.0% *versus* 38.7%, respectively, with an adjusted OR of 0.94 (0.32) with a 95% CI [0.51;1.76].

The *age subgroup [Children (7-11) / Adolescents (12-17)] analysis* did not reveal age effect on the primary endpoint expressed in terms of change from baseline to W026.

Altogether, these data did not evidence a positive effect of bumetanide compared to placebo in the treatment of paediatric ASD.

SAFETY RESULTS
During the double-blind period in the Safety Set

- **Treatment emergent adverse events**

The following table summarises the main results of treatment emergent adverse events (TEAEs) during the double-blind period in the Safety Set [*i.e.* TEAEs which occurred between first intake in double-blind and minimum (last intake in double-blind + 2 days, first intake in open-label), or which started strictly before this period but which worsened (in terms of intensity) or became serious according to the investigator opinion during this period].

Overall summary for treatment emergent adverse events during the double-blind period in the Safety Set

		S95008 (N = 107)	Placebo (N = 104)
Patients having reported at least one:			
TEAE	n (%)	97 (90.7)	85 (81.7)
IMP-related TEAE	n (%)	77 (72.0)	50 (48.1)
Serious TEAE*	n (%)	11 (10.3)	5 (4.8)
IMP-related serious TEAE	n (%)	3 (2.8)	2 (1.9)
TEAE leading to IMP withdrawal	n (%)	10 (9.3)	3 (2.9)
Serious TEAE leading to IMP withdrawal	n (%)	2 (1.9)	2 (1.9)
IMP-related TEAE leading to treatment withdrawal	n (%)	8 (7.5)	2 (1.9)
IMP-related serious TEAE leading to treatment withdrawal	n (%)	1 (0.9)	2 (1.9)
Patients who died**	n (%)	-	-

** All Serious EAEs (SEAEs) during the double-blind period were emergent on treatment during the double-blind period.*
*** On treatment or not.*

In the SS, the percentage of patients with at least one TEAE during the double-blind period was higher in the S95008 group (90.7%) than in the placebo group (81.7%). A total of 630 TEAEs were reported in the S95008 group and 464 TEAEs in the placebo group.

Among the most frequently affected System Organ Classes on S95008 (*i.e.* in at least 10.0% of patients) during the double-blind period, those more frequently reported in the S95008 group than in the placebo group were General disorders and administration site conditions (54.2% of patients *versus* 43.3%), Gastrointestinal disorders (48.6% *versus* 31.7%), Metabolism and nutrition disorders (43.9% *versus* 25.0%), Renal and urinary disorders (39.3% *versus* 16.3%), Psychiatric disorders (32.7% *versus* 26.9%) and Investigations (31.8% *versus* 19.2%).

Among the most frequent **TEAEs** reported on S95008 (*i.e.* in at least 10.0% of patients)**,** those more frequently reported in the S95008 group than in the placebo group were: thirst (46.7% of patients *versus* 29.8%), polyuria (27.1% *versus* 11.5%), dry mouth (22.4% *versus* 13.5%), decreased appetite (19.6% *versus* 7.7%), hypokalaemia (19.6% *versus* 3.8%), increased appetite (15.9% *versus* 8.7%), nasopharyngitis (12.1% *versus* 4.8%) and pollakiuria (11.2% *versus* 1.9%).
Most of these emergent adverse events (EAEs) are related to bumetanide diuretic effect.

Hypokalaemia on S95008 occurred throughout the double-blind period. All hypokalaemia cases were considered as related to IMP and 2 in 2 patients (1.9%) on S95008 were reported as serious. No hypokalaemia was rated as severe or led to IMP withdrawal.

The percentage of patients reporting at least one **severe TEAE** during the double-blind period in the SS was higher in the S95008 group (17.8%) than in the placebo group (10.6%). The most frequent severe TEAE was thirst reported in 4 patients (3.7%) in the S95008 group *versus* 2 patients (1.9%) in the placebo group.

The percentage of patients with at least one **TEAE considered to be related to IMP** was higher in the S95008 group (72.0%) than in the placebo group (48.1%).
The most common IMP-related TEAEs more frequently reported on S95008 than on placebo were: thirst (41.1% of patients *versus* 26.9%), polyuria (26.2% *versus* 10.6%), hypokalaemia (19.6% *versus* 3.8%), dry mouth (15.9% *versus* 10.6%), decreased appetite (11.2% *versus* 4.8%) and pollakiuria (10.3% *versus* 1.9%). These EAEs are related to bumetanide diuretic effect.

All **SEAEs** occurring during the double-blind period were emergent on treatment with a higher rate of patients in the S95008 group (10.3%) than in the placebo group (4.8%).
Serious TEAEs were reported by no more than one patient, except petit mal epilepsy and hypokalaemia both reported by 2 patients (1.9%) in the S95008 group *versus* none in the placebo group. Overall, 3 patients (2.8%) in the S95008 group and 2 patients (1.9%) in the placebo group had at least one serious TEAE considered as IMP-related (2 hypokalaemia and 1 tonic convulsion in the S95008 group and blood potassium increased, dehydration and generalised tonic-clonic seizure (one case each) in the placebo group).
Serious TEAEs led to IMP withdrawal in 2 patients (1.9%) in both groups: tonic convulsion and autism spectrum disorder in the S95008 group and generalised tonic-clonic seizure and dehydration in the placebo group.

The percentage of patients with at least one **TEAE leading to IMP withdrawal** during the double-blind period was higher in the S95008 group (9.3%) than in the placebo group (2.9%).

- **Laboratory tests**

During the double-blind period in the SS, emergent Potentially Clinically Significant Abnormal (PCSA) biochemical values on S95008 treatment were sparse except high bicarbonate reported with a higher frequency than in the placebo group (6.5% of patients *versus* 4.2%). It should be also noted that 40.9% of patients on S95008 had at least one high emergent abnormal (no PCSA) value of urate *versus* 5.2% on placebo. Hypokalaemia (*i.e.* defined as potassium [K^+] value < 3.5 mEq/L) (21 patients on S95008 and 4 patients on placebo) were all considered as mild according to the predefined study criteria (*i.e.* [3 ; 3.5[mEq/L). Only 2 patients, both in the S95008 group (2.2%), reported at least one emergent PCSA haematological value on treatment (high eosinophils). No limits for PCSA values were considered for urinary parameters analyses; however, it should be noted that 18.3% of patients on S95008 had at least one low abnormal value of creatininuria with a higher frequency than on placebo (3.1% of patients).

- **C-SSRS-C**

No patient in the S95008 group presented suicidal ideations on treatment during the double-blind period.

- **Vital signs and clinical examination**

There were no clinically relevant mean changes in **SBP, DBP and HR** between baseline and each post-baseline value on treatment in any group, nor relevant difference between the treatment groups, during the double-blind period.

As expected in a paediatric population, the mean **weight** gradually increased during the double-blind period within each S95008 and placebo group with a mean change from baseline of 1.07 ± 3.12 kg *versus* 2.35 ± 2.66 kg at W026, respectively, without increase of mean **BMI**.

Assessment of pubertal status by **Tanner stage** showed that several patients experienced an increase of stage over the double-blind period, with roughly similar data in both groups.

There were no clinically relevant mean changes in the **ECG parameters** from baseline nor relevant difference between groups, during the double-blind period. One patient in the S95008 group, had a QT interval Fridericia's Correction (QTcF) value in the range]450-480] msec at W008. Few patients (no more than 4 depending on the visit) had QTcF changes from baseline between 30 and 60 msec without relevant difference between groups, except at W008: 4 patients on S95008 *versus* none on placebo. In addition, one patient in the S95008 group had QTcB change from baseline between 30 and 60 msec at 3 visits. Clinically significant abnormal ECGs (according to the investigator) were reported in 2 patients in the S95008 group (sinus tachycardia, T wave inversion, T wave biphasic and ST depression); one patient in the placebo group presented with an abnormality already present at selection.

<u>During the open-label period in the Safety Set Open in patients initially treated with placebo</u>

- **Treatment emergent adverse events**

The following table summarises the main results of TEAEs during the open-label period in the Safety Set Open in patients initially treated with placebo [*i.e.* TEAEs which occurred between first intake in open-label (W026 not included) and last intake in open-label + 2 days (included), or which started strictly before this period but which worsened (in terms of intensity) or became serious according to the investigator opinion during this period].

Overall summary for treatment emergent adverse events during the open-label period in the Safety Set Open in patients initially treated with placebo

		Placebo/S95008 (N = 90)
Patients having reported at least one:		
TEAE	n (%)	77 (85.6)
IMP-related TEAE	n (%)	60 (66.7)
Serious TEAE*	n (%)	2 (2.2)
IMP-related serious TEAE	n (%)	-
TEAE leading to IMP withdrawal	n (%)	11 (12.2)
Serious TEAE leading to IMP withdrawal	n (%)	-
IMP-related TEAE leading to treatment withdrawal	n (%)	8 (8.9)
IMP-related serious TEAE leading to treatment withdrawal	n (%)	-
Patients who died**	n (%)	-

** All SEAEs during the open-label period were emergent on treatment during the open-label period.*
*** On treatment or not.*

In the SSO in patients initially treated with placebo, 85.6% of patients reported 381 TEAEs during the open-label period.

The **most frequent TEAEs** (*i.e.* in at least 10% of patients) were thirst (40.0% of patients), polyuria (21.1%), hypokalaemia (17.8%), dry mouth (15.6%), pyrexia (12.2%) and vomiting (11.1%).

Hypokalaemia occurred within the first 12 weeks of treatment in 68.8% of patients and after 12 weeks in 31.3%; all were considered as related to IMP and none was severe or serious or led to IMP withdrawal.

A total of 6.7% of patients initially treated with placebo reported at least one **severe TEAE** during the open-label period. None was reported in more than one patient, except dry mouth reported in 2 patients (2.2%).

Overall, 66.7% of patients reported at least one **TEAE considered to be related to IMP**. The most frequent were thirst (36.7% of patients), polyuria (21.1%), hypokalaemia (17.8%) and dry mouth (12.2%).

Two patients (2.2%) initially treated with placebo reported 2 **SEAEs** during the open-label period, both emergent on treatment: forearm fracture and hypoglycaemic unconsciousness reported in one patient each. None was considered as related to IMP or led to IMP withdrawal.

A total of 12.2% of patients experienced at least one **TEAE leading to IMP withdrawal**.

- **Laboratory tests**

During the open-label period in patients initially treated with placebo, emergent PCSA biochemical values on S95008 treatment were sparse except high bicarbonate (4.8%, 4 patients). It should be also noted that 38.6% of patients on S95008 had at least one high emergent abnormal (no PCSA) value of urate. Hypokalaemia (*i.e* defined as K+ value < 3.5 mEq/L), reported in 16 patients, were all considered as mild according to the predefined study criteria (*i.e.* [3 ; 3.5[mEq/L). Two patients reported at least one emergent PCSA haematological value: low neutrophils and high platelets (one patient each). No limits for PCSA values were considered for urinary parameters analyses; however, it should be noted that 28.6% of patients had at least one low abnormal value of creatininuria.

- **Vital signs and clinical examination**

There were no clinically relevant mean changes in **SBP and DBP** during the open-label period. **Standing heart rate** increased on average 5/6 bpm between baseline of the open-label period and each post-baseline value.
The mean **weight** remained stable during the open-label period until W034, then slightly increased up W052 (1.16 ± 2.52 kg), with a stable **BMI**.

There were no clinically relevant mean changes in the **ECG parameters** during the open-label period. No patient had QTcB/QTcF value above 450 msec or change from baseline of the open-label period > 60 msec. Clinically significant ECG abnormalities were few and sparse in 2 patients.

<u>During the combined period in the Safety Set Combined in patients initially treated with S95008</u>

- **Treatment emergent adverse events**

The following table summarises the main results of TEAEs during the combined period in the Safety Set Combined in patients initially treated with S95008 [*i.e.* TEAEs which occurred between first intake in double-blind and last intake in open-label + 2 days (included), or which started strictly before this period but which worsened (in terms of intensity) or became serious according to the investigator opinion during this period].

Overall summary for treatment emergent adverse events during the combined period in the Safety Set Combined in patients initially treated with S95008

		S95008/S95008 (N = 83)
Patients having reported at least one:		
TEAE	n (%)	82 (98.8)
IMP-related TEAE	n (%)	70 (84.3)
Serious TEAE*	n (%)	10 (12.0)
IMP-related serious TEAE	n (%)	3 (3.6)
TEAE leading to IMP withdrawal	n (%)	4 (4.8)
Serious TEAE leading to IMP withdrawal	n (%)	3 (3.6)
IMP-related TEAE leading to treatment withdrawal	n (%)	2 (2.4)
IMP-related serious TEAE leading to treatment withdrawal	n (%)	1 (1.2)
Patients who died**	n (%)	-

** All SEAEs during the combined period were emergent on treatment during the combined period.*
*** On treatment or not.*

In the SSC in patients initially treated with S95008, 98.8% of patients reported 784 TEAEs during the combined period.

The **most frequent TEAEs** (*i.e.* in at least 20% of patients) were thirst (56.6% of patients), polyuria (33.7%), hypokalaemia (27.7%), dry mouth (26.5%), decreased appetite (21.7%) and nasopharyngitis (20.5%).

Hypokalaemia occurred mainly within the first 3 weeks of treatment (39.1% of patients) and after 26 to 38 weeks (30.4%). All were considered as related to IMP and 2 in 2 patients (2.4%) were reported as serious. No hypokalaemia was rated as severe or led to IMP withdrawal.

A total of 19.3% of patients initially treated with S95008 had at least one **severe TEAE** during the combined period. Severe TEAEs were reported by no more than 2 patients except thirst (3 patients, 3.6%).

A total of 84.3% of patients reported at least one **TEAE considered to be related to IMP**. The most frequent were thirst (48.2% of patients), polyuria (33.7%), hypokalaemia (27.7%), dry mouth (19.3%) and decreased appetite (10.8%).

Overall, 12.0% of patients experienced at least one **SEAE** during the combined period, all emergent on treatment. Serious TEAEs were reported by no more than one patient, except hypokalaemia (2 patients, 2.4%). Hypokalaemia in both patients and seizure in one patient were considered as IMP-related. Serious TEAE led to IMP withdrawal in 3 patients (3.6%): partial seizures with secondary generalisation, seizure and intentional self-injury.

Overall, 4.8% of patients experienced at least one **TEAE leading to IMP withdrawal**.

- **Laboratory tests**

During the combined period in patients initially treated with S95008, emergent PCSA biochemical values on S95008 treatment were sparse except high bicarbonate (13.3% of patients) and low bicarbonate (3.6%). It should be also noted that 47.0% of patients had at least one high emergent abnormal value of urate (including PCSA value in one patient). Hypokalaemia (*i.e.* defined as K^+ value < 3.5 mEq/L) (23 patients) were all considered as mild according to the predefined study criteria (*i.e.* [3 ; 3.5[mEq/L). Emergent PCSA haematological values were reported for high eosinophils and low neutrophils in 2 patients (2.4%) each. No limits for PCSA values were considered for urinary parameters analyses; however, it should be noted that 24.1% of patients had at least one low abnormal value of creatininuria.

- **Vital signs and clinical examination**

There were no clinically relevant mean changes in **SBP and DBP** between baseline and each post-baseline value on treatment. **Heart rate** increased on average less than 6 bpm between baseline and few post-baseline values (from W012 to D199) for standing/supine HR.
The **weight** remained stable from baseline until W008, then gradually increased from W012: on average from 0.28 ± 1.89 kg at W012 to 3.53 ± 4.30 kg at W052, with a stable mean **BMI**.

No clinically relevant mean change from baseline was observed for the **ECG parameters**. QTcF values in the range]450-480] msec were observed at W008, W030 and W038, in one patient each time. No patient had QTcF/QTcB change from baseline > 60 msec. Clinically significant ECG abnormalities were few and sparse in 2 patients.

Altogether, these data confirmed the already known safety profile of bumetanide when used in a diuretic purpose.

CONCLUSION

This 6-month, randomised, double-blind, placebo-controlled, parallel groups, international, multicentre phase III study followed by a 6-month open-label active treatment period conducted in children and adolescents aged from 7 to less than 18 years old affected by Autism Spectrum Disorders failed to demonstrate the superiority of bumetanide 0.5 mg twice a day compared to placebo after 6 months of treatment in the CARS2 total score expressed in terms of change from baseline to W026.

During the double-blind period, the rate of patients with at least one TEAE was higher on bumetanide than on placebo. As expected, and in line with the known diuretic action and safety profile of the drug, thirst, polyuria and dry mouth were the most common TEAEs reported more frequently on bumetanide than on placebo.

As expected also, hypokalaemia was more frequently reported on bumetanide than on placebo; two hypokalaemia, both on bumetanide, were reported as serious. All hypokalaemia cases were of mild intensity according to the predefined study criteria (*i.e.* [3 ; 3.5[mEq/L) and none led to IMP withdrawal.

The safety profile of bumetanide during the 12 months combined period was quite similar to the one during the 6 months double-blind period. In patients treated with bumetanide only during the open label period, the rate of patients with adverse events was lower than during the double-blind period with however a roughly similar safety profile.

No unexpected safety concerns were observed during this study. However, due to the absence of clinical benefits, the Benefit/Risk assessment was considered negative and the Sponsor decided to prematurely discontinue the S95008 development.

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