RESEARCH PROTOCOL

Bumetanide for the Autism Spectrum Clinical Effectiveness Trial

Short title:

BASCET

Multicenter double-blind randomized placebo-controlled trial

EudraCT-study number: 2016-002875-81

Version August 2017

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Protocol ID	**BASCET**
Short title	**BASCET Study**
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Version	**4.0**
Date	**August 2017**
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Pharmacy	**Pharmacy UMC Utrecht**

PROTOCOL SIGNATURE SHEET

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABC	**Aberrant Behavior Checklist**
ABC-I	**Aberrant Behavior Checklist-Irritability subscale**
ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
ADOS	**Autism Diagnostic Observation Schedule**
AE	**Adverse Event**
AR	**Adverse Reaction**
ASD	**Autism Spectrum Disorder**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CE	**Childhood Epilepsy**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EEG	**Electroencephalogram**
ERP	**Event Related Potential**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IC	**Informed Consent**
MCR	**Medical Chart Review**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NKCC1	**Na-K-Cl cotransporter-1**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party**
SRS	**Social Responsiveness Scale**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TSC	**Tuberous Sclerosis Complex**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale:
Autism Spectrum Disorder (ASD) constitute a group of devastating neurodevelopmental disorders that have no cure at present [1]. ASD is clinically defined by two core symptom domains: persistent deficits in social communication and social interaction, and restricted, repetitive patterns of behavior, interests, or activities [2]. It is estimated that approximately 1 in 100 children display signs and symptoms that lead to a diagnosis of ASD [3], making it more common than childhood cancer and juvenile diabetes together. Individual differences in ASD manifestation are characterized by substantial variability in symptomatology, severity and comorbidities [4,5]. However, a very common feature is altered sensory reactivity. Over 80% of children with ASD display hyper or hyporeactivity to sensory stimuli such as sounds or touch. Indeed, sensory processing is increasingly being marked as the fundamental process underlying not only ASD but also other neurodevelopmental disorders such as epilepsy and ADHD. A common sensory etiology between these disorders is further suggested as they frequently co-occur in patients. Moreover, sensory reactivity has been added as a symptom to the second core domain of ASD in the DSM-V. A logical progression is to find treatments that target sensory reactivity as these may have broad applicability beyond the classical borders of the DSM.

A promising example in this respect is the selective chloride transporter NKCC1 antagonist bumetanide. Bumetanide has been used for decades as a diuretic drug and its safety has been confirmed. Bumetanide has recently been proposed as a rational treatment for ASD based on its capacity to reinstall the inhibitory action of the principal neurotransmitter gamma-aminobutyric acid (GABA). More specifically, failure of GABAergic inhibition has directly been linked to sensory processing defects. Furthermore, epilepsy has been associated with altered chloride homeostasis leading to depolarizing effects of GABA. A first trial has tested bumetanide in a modest sample (n = 56) of children with ASD and showed a decrease in symptom severity after three months of treatment with bumetanide [6]. In a recent pilot study, we have confirmed the potential of bumetanide to treat autism core symptomatology. In addition, we have established a positive effect on brain activity using EEG. Building on these previous experiences, we now aim to conduct a phase IIb trial to confirm the effectiveness of bumetanide in neurodevelopmental disorders and analyze which types are most responsive to bumetanide in terms of severity, intellectual functioning, seizure frequency and comorbidity.

Here, we propose to conduct a large, multicenter, placebo-controlled randomized trial testing bumetanide plus usual care treatment versus placebo plus usual care in 172 children with signs of altered sensory reactivity and a neurodevelopmental disorders diagnosis of ASD, ADHD and/or epilepsy. With this design, we expect to confirm effectiveness of bumetanide as a cheap, safe and rational treatment option for an important subset of ASD, ADHD and/or epilepsy and to facilitate rapid implementation of this treatment in Dutch clinical guidelines for ASD treatment.

Objectives
Primary aim**:** to investigate whether 13 weeks usual care and add-on treatment with bumetanide will improve daily life functioning and reduce behavioral symptoms related to

hyperexcitability in children and adolescents with ASD, ADHD and/or epilepsy in comparison to usual care
Secondary aim: to identify biomarkers, both clinical and neurophysiological characteristics, of effectiveness of bumetanide

Study design:
Multicenter double-blind randomized placebo-controlled trial

Study population:
172 children with ASD, ADHD and/or epilepsy, between 5 and 15 years of age

Intervention:
Bumetanide or placebo treatment in the form of tablets with a dosage ranging from 0.25-1.0 mg twice daily, in addition to usual care

Main study parameters/endpoints:
Primary endpoint: Aberrant Behavior Checklist-Irritability subscale (ABC-I) at Day 91.
Secondary endpoints: other behavioral and quality of life parameters; seizure frequency

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Burden:
The main burden of this study is posed by the outpatient clinic visits to monitor the safety of the diuretic effects, which requires physical examination, blood tests and a urine test, which are of negligible and known risks.

Risks:
Bumetanide has been used as a diuretic drug for decades. In patients with conditions of fluid overload, its safety and tolerability after short and prolonged treatment has been established in all ages apart from neonates. Experience and safety of bumetanide in patients with neurodevelopmental disorders is based upon recent studies, including one randomized controlled trial in a sample of 56 children with ASD [6]. These data indicate that bumetanide significantly alleviates behavioral morbidity at dosages ranging from 1 to 2 mg daily. The main adverse events are related to the diuretic activity of the molecule leading to a decrease in electrolytes, notably mild hypokalemia are frequently reported. In addition, usage of bumetanide has been proven effective and safe in patients with temporal lobe epilepsy. However, in neonates severe side effects have been reported and therefore this age group is not included in the proposed study.

Benefit and group relatedness:
At present, care as usual has no effect on autism morbidity. As ASD can be very invalidating, it is associated with high healthcare and high societal costs. Over the past 20 years, a variety of therapies have been proposed to improve the symptoms associated with ASD [1,7]. Only two antipsychotics, aripiprazole and risperidone, marketed as Abilify and Risperdal, respectively, are approved to treat symptoms that often accompany autism, such as hyperactivity and irritability[7]. Long term use of these drugs may result in severe adverse effects such as obesity, hypertension, and diabetes type II. Given the lack of drug treatments, behavioral and developmental interventions are currently the predominant treatment approach for promoting social, adaptive and behavioral function in children with ASD based on efficacy demonstrated in empirical studies. The primary goals of these treatments are to minimize the core features and associated deficits, maximize functional independence and quality of life, and alleviate family distress. Facilitating development and learning, promoting socialization, reducing maladaptive behaviors, and educating and supporting families can help accomplish these goals. However, none of these treatments targets the underlying neurobiological defects of ASD. In the last decade, there have been major investments to develop etiology driven

treatments for ASD, but all of these attempts have failed so far. Bumetanide is a rationally driven, cheap treatment, with the potential to positively change cognitive, behavioral, societal and educational problems that patients encounter. In contrast with other existing treatments, the application of bumetanide is etiologically driven and will not affect the central nervous system in neurons in which chloride homeostasis is unaffected.

1. INTRODUCTION AND RATIONALE

The prevalence of ASD diagnosis has shown an exponential rise in the last two decades. About half of this rise seems accounted for by factors such as broader diagnostic criteria, improved awareness, lower thresholds for clinical diagnosis and higher parental age, leaving roughly half of the increase unexplained [8]. Bob Wright, co-founder of Autism Speaks, said, "Autism is a global public health crisis. The costs are staggering and will continue to rise as prevalence continues to increase. The investment we make now is essential to reducing the long-term costs of autism." (https://www.autismspeaks.org/about-us/press-releases/annual-cost-of-autism-triples). The growing awareness of the burden of ASD has fueled tremendous research efforts, but no etiology-driven treatments are currently available to treat the core symptoms of ASD. Especially the more severe forms of the disorder are accompanied by emotional, sensory and cognitive distress and can put a tremendous burden on family resources. In children under 5 years of age, ASDs have been shown to be the leading cause of disability, in terms of years lived with disability (YLDs), among all mental disorders. ASDs accounted for 7.7 million disability-adjusted life-years (DALYs) in 2010. Globally, ASDs accounted for 170 DALYs per 100 000 males (95% UI 119–237) and 50 DALYs per 100 000 females (95% UI 35–68) [9].
In the UK, mean annual costs for a child with ASD was calculated at €30000 per year [10]. The largest contributors to these costs were direct nonmedical costs, such as special education (including early intervention services), and indirect costs, such as parental productivity loss. In the Netherlands, the individual costs for ASD have not been published but total annual health care cost for psychiatric disorders in underaged subjects is estimated around €670 million (www.kostenvanziekten.nl). Taken together, there is an extremely urgent need for effective treatments for ASD.

At present, care as usual has no effect on autism morbidity. As ASD can be very invalidating, it is associated with high healthcare and high societal costs. Over the past 20 years, a variety of therapies have been proposed to improve the symptoms associated with ASD [1,7]. Only two antipsychotics, aripiprazole and risperidone, marketed as Abilify and Risperdal, respectively, are approved to treat symptoms that often accompany autism, such as hyperactivity and irritability[7]. Long term use of these drugs may result in severe adverse effects such as obesity, hypertension, and diabetes type II. Given the lack of drug treatments, behavioral and developmental interventions are currently the predominant treatment approach for promoting social, adaptive and behavioral function in children with ASD based on efficacy demonstrated in empirical studies. The primary goals of these treatments are to minimize the core features and associated deficits, maximize functional independence and quality of life, and alleviate family distress. Facilitating development and learning, promoting socialization, reducing maladaptive behaviors, and educating and supporting families can help accomplish these goals. However, none of these treatments targets the underlying neurobiological defects of ASD. In the last decade, there have been major investments to develop etiology driven treatments for ASD, but all of these attempts have failed so far. Bumetanide is a rationally

driven, cheap treatment, with the potential to positively change cognitive, behavioral, societal and educational problems that patients encounter. In contrast with other existing treatments, the application of bumetanide is etiologically driven and will not affect the central nervous system in neurons in which chloride homeostasis is unaffected.

Bumetanide treatment in neurological disorder was first linked to different forms of epilepsy, a condition highly comorbid to ASD. Recently, efficacy of bumetanide to treat ASD was suggested by multiple animal model studies[11-13], where GABAergic transmission could be reinstated through systemic bumetanide treatment leading to a reduction in ASD related symptoms. One previous trial has tested bumetanide in a modest sample (n= 56) of children with ASD showed a reduction in autism symptom severity after 3 months treatment with bumetanide 0.5-1.0mg twice daily [6]. Although these preliminary results are promising, the sample size in this trial was small and the study has been criticized for using unvalidated primary outcome measures, e.g., Childhood Autism Rating Scale (CARS) and assessment of included children were based on videos of behavior. We have conducted a pilot study (n= 8) to confirm possible efficacy and to test validated endpoints. We included both low-functioning (LFA) (n = 5) and high-functioning ASD (HFA) (n=3) subjects (age 4-18) and found that bumetanide treatment lead to a marked but variable improvement in behavioral morbidity in all patients. Improvements were particularly evident in the domain of restricted and repetitive behaviors and irritability, measured by the Aberrant Behavior Checklist–irritability subscale (ABC-I) and the Repetitive Behavior Scale-revised, both well-established endpoints in ASD treatment studies [7].

Importantly, we found that a positive effect in these symptom domains was not restricted to HFA but was also evident in LFA. This is crucial as especially behavioral symptoms in LFA can be severe and are generally less responsive to supportive interventions or guidance. To confirm an effect of bumetanide on brain activity, we further tested whether bumetanide treatment lead to changes in both resting-state electroencephalography (EEG) and event-related potentials. We found that a baseline reduction in resting-state alfa-power, an anomaly previously associated with ASD[14], could be restored by bumetanide treatment. In addition, we found that certain sensory evoked EEG parameters, such as sensory gating and mismatch negativity, improved through treatment in several of these patients. Finally, we assessed change in cognitive functioning and found that bumetanide lead to improvement in memory functioning, which further supports a positive effect of bumetanide on aberrant information processing.

It is very well feasible that bumetanide may also be effective to treat behavioral symptoms in other neurodevelopmental disorders. Altered chloride homeostasis, the target of bumetanide, has been linked to different pathological states outside of ASD, including different forms of epilepsy, sensory disorders and schizophrenia [15-24]. In particular, the behavioral burden in childhood epilepsy (CE) is reflected by the high prevalence of coinciding behavioral disorders such as ASD and attention deficit and attention deficit hyperactivity disorder (ADHD) [25]. Moreover, many children have symptoms across different neurodevelopmental domains that seem characterized by excessive behavioral responses to sensory environmental cues, e.g., rigidity, hyperactivity and irritability [25-27]. These behavioral responses may relate to the excess in cortical excitability due to depolarizing GABA, as has been suggested from post-mortem studies and animal model studies. Furthermore, anti-epileptic drugs (AED) aim to control seizures, but do normally not prevent or reduce

behavioral problems. In some cases, they may even exacerbate them or cause paradoxical behavioral effects [20,28]. More generally, paradoxical responses to benzodiazepines are a common phenomenon [29-31]. It has recently been hypothesized that such an aggravation of seizures or behavioral symptoms through GABAergic AEDs can be related to excitatory instead of inhibitory activity of GABA [20,32]. Recently we have reported on the successful treatment of a first epilepsy and ASD case with a previous paradoxical response to a benzodiazepine [33]. Overall, there is ample evidence that disturbed chloride homeostasis contributes to a wide variety of neurodevelopmental disorders which may suggest broad applicability of bumetanide to ASD with comorbidities such as ADHD and epilepsy.

This study builds upon a dedicated collaboration for Autism research, called Reach-Aut. The Reach-Aut Academic Centre for Autism collects, connects and links existing and new information about crucial care and treatment issues for people with autism. Reach-Aut conducts research related to the entire lifetime, and in doing so, conforms with the recommendations of The Health Council of the Netherlands (Gezondheidsraad) as regards knowledge infrastructure. Reach-Aut aims to establish a sustainable link between scientific theory and practice, and by the dissemination of knowledge. A subsidy for this organization has been granted by ZonMw. Through the collaboration of these sites, a large pool (> 1000, new and existing patients in total) of eligible patients is available for this study. In addition, the combination of academic, non-academic centers and institutions specialized in LFA ensures that broad experience across the full scope of ASD care will be developed, which is crucial to establish clinical effectiveness of bumetanide in the treatment for this broad spectrum of neurodevelopmental disorders. It should be noted that inclusion and treatment will be conducted at two sites, UMC Utrecht and Jonx Groningen in order to reduce administrative cost.

UMC Utrecht: The Brain Center Rudolf Magnus has a strong tradition in studying and treating neurodevelopmental disorders. This department has an extensive track record in the conduct of randomized clinical trials, including those involving young subjects with developmental disorders. Our project group clearly has the necessary expertise to conduct this study. The project group is assembled to comprise all the required skills and complement each other. This study will be conducted in close collaboration with the Clinical Trial Center (CTC) which is part of the Department of Psychiatry of UMC Utrecht, and is specialized in implementation and coordination of clinical trials across Europe. Experts within this group ensure patient safety and data integrity for studies including both adolescent and adult psychiatric patients as well as children at risk for developing psychiatric disorders, in line with Good Clinical Practice and the European Clinical Trial Directive as well as any applicable local laws and regulations. A research assistant trained by the CTC and experienced with monitoring and data management in RCTs will be coordinating the trial with supervision of the main applicants and the experts of the CTC. In addition, the department of Healthcare Innovation and Evaluation of the Julius Centre in Utrecht will supervise the statistical analysis and perform the cost-effectiveness analysis.

Jonx Groningen: Jonx has two departments, one offering general child- and adolescent psychiatry, the other autism care. Part of this last department is the Autism Team Northern-Netherlands (ATN) in Groningen. This TOPGGZ-certified team offers ambulatory diagnostic and treatment services to individuals with autism regardless of age, intellectual levels, comorbidity or complexity of ASD. Other ATN-teams can be found in Drachten and

Hoogeveen. In addition, Jonx offers assisted living facilities for adolescents and young adults (≤23 years) with autism, and also has a work-home for (older) adults with autism. Jonx serves in excess of 4000 patients each year.

Dr. H. Bruining, the PI of this project, has been involved in many clinical and preclinical studies focusing on understanding and improving treatments in developmental disorders. At present, he carries out a study assessing the neurobiological basis of the neurobehavioral impairments of both ASD and childhood epilepsy (METC 15/143, “SPACE”), focusing on behavioral manifestations of hyperexcitability. Moreover, a placebo controlled, randomized trial assessing the efficacy of bumetanide in ASD has been approved (METC 15/733, “BAMBI”). The proposed study is an etiology driven sequel of these studies. Although the BAMBI study and the current proposed BASCET study have similar hypotheses, they are complementary. In the BAMBI study, we investigate efficacy in a more homogenous subset of high functioning children with ASD in a different age range (7-15). An important aim of that study is to assess which neurocognitive and neurophysiological markers may predict efficacy of bumetanide. Such measurements are not feasible in a large proportion of (low-functioning) patients with ASD and can only be conducted in specialized centers such as the UMC Utrecht. The proposed BASCET study aims to assess the effectiveness of bumetanide by including a larger group of patients with behavioral manifestations of hyperexcitability , over the full range of neurodevelopmental disorders, from both academic and non-academic centers. In contrast to the BAMBI study, the BASCET protocol proposes to allow co-medication such as AED and antipsychotics. This will allow children with epilepsy to enroll in the study as well as those children who cannot remain without their current antipsychotic or psychostimulant medication. Once both studies are finalized, we will be able to give a more complete answer whether bumetanide is an effective treatment for children with behavioral manifestations of hyperexcitability and for which subset.

Dr. I.D.C. van Balkom, second PI on this project, is an experienced clinician, and has been involved in clinical and epidemiological studies of neurodevelopmental disorders with autism phenotypes across the lifespan. She has recently completed a matched control study on executive and daily life functioning in older adults (>55 years) with ASD and is currently studying repetitive and restrictive behaviors in primary school age children with and without ASD.

Taken together, existing preclinical and clinical data support the proposed solution to repurpose the drug bumetanide based on its mechanism of action as a rational objective. A next step towards its implementation in standard care for behavioral manifestations of hyperexcitability across neurodevelopmental disorders is to confirm bumetanide efficacy in a standard design trial across different autism treatment settings and including the full range of neurodevelopmental disorder subtypes, treatments and comorbidities.

2. OBJECTIVES

Primary aim**:** to investigate whether 13 weeks usual care and add-on treatment with bumetanide will improve daily life functioning and reduce behavioral symptoms related to hyperexcitability in children and adolescents with ASD, ADHD and/or epilepsy, in comparison to usual care
Secondary aim: to identify biomarkers, both clinical and neurophysiological characteristics, of effectiveness of bumetanide

3. STUDY DESIGN

This is a multicenter (two sites: UMC Utrecht and Jonx Groningen), double-blind, randomized, placebo-controlled trial testing the effectiveness of three months bumetanide treatment in 172 children aged 5 to 15 years with ASD, ADHD and/or epilepsy. The primary endpoint is change in the ABC-I scale at Day 91. Usual care + bumetanide will be compared with usual care + placebo.

4. STUDY POPULATION

4.1 Population (base)

Children with ASD, with and without epilepsy, between 5 and 15 years of age and an IQ≥55. As this study proposes a collaboration between different centers across the Netherlands, children can be referred from multiple centers: Expertise Centrum De Hartekamp Groep (Heemskerk), Jonx (Lentis Psychiatric Institute, Groningen), UMC Utrecht (Utrecht), Stichting Epilepsie Instellingen Nederland (Zwolle) and Intermetzo (Zeist). Yearly in total 200 new eligible patients are seen across the different sites. In addition, the participating sites together govern a prevalent population of 800 children with ASD in the intended age and IQ range. Children with ASD, ADHD and/or epilepsy at the two study/inclusion sites (i.e., Utrecht and Groningen) will be included after written informed consent.

4.2 Inclusion criteria

1. Males or females aged ≥5 years to ≤15 years;
2. Above clinical cut-off scores of altered sensory reactivity on the Sensory Profile and either a clinical ASD or ADHD diagnosis based on DSM-5 (or DSM-IV) or an epilepsy diagnosis;
3. Written informed consent.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Total IQ < 55 (WISC or WPPSI-III NL if 5 years of age) and/or inability to comply with the protocol-specified procedures for the duration of the study, including treatment, blood sampling to control diuretic effects;
2. Presence of a severe medical or genetic disorder other than related to ASD or epilepsy;
3. Serious, unstable illnesses including, gastroenterological, respiratory, cardiovascular (arrhythmias, QT interval lengthening), endocrinologic, immunologic, hematologic disease, dehydration or hypotension, electrolyte disturbances (Na <133 mmol/L, K <3.5 mmol/L or Ca <2.17 mmol/L (<13y) or <2.2 mmol/L (>13y);
4. Renal insufficiency (CKD st2-5; estimated glomerular filtration rate < 90 ml/min/1.73m2), congenital or acquired renal disease with decreased concentration capacity (tubulopathy, diabetes insipidus) and liver insufficiency interfering with excretion or metabolism of bumetanide;
5. Start of behavioral treatment during study;
6. Treatment with psychoactive medications, including antipsychotics and AEDs, **except** methylphenidate, is allowed albeit on a stable regime in terms of types and dosage from 2 months prior to the study to the end of the study;
7. Treatment with NSAIDS, aminoglycosides, digitalis, antihypertensive agents, indomethacin, probenecid, acetazolamide, Lithium, other diuretics (e.g., furosemide, hydrochlorothiazide), drugs known to have a nephrotoxic potential;
8. Documented history of hypersensitivity reaction to sulfonamide derivatives;
9. Body weight <17 kg.

4.4 Sample size calculation

The sample size calculation based upon ABC irritability subscale (the primary outcome

measure) was based on previous studies [34] comparing challenging behaviors in community populations using the ABC-irritability subscale. A sample size of 172 will provide 90% power to differentiate between placebo and bumetanide when the true difference in mean change from baseline in ABC irritability subscale score is 4.6. This assumed an SD of 9.3 [34] (i.e. an effect size of 0.5) and a 2-sided test at the 0.05 level of significance, computed with Gpower 3.1. This sample size will also be sufficient to detect an increase of 10% in in R^2 the exploratory regression analysis described below, using maximal 10 predictors. We plan to assign 190 patients, allowing for a 10% attrition rate.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The IP will consist of bumetanide 0.5 mg tablets or placebo, which will be provided as an add-on treatment, supplementary to the regular use of AEDs or other (allowed) co-medications. Patients will be given a dose between 0.5 mg and 1.0 mg twice a day (or 0.25 mg and 0.5 mg if body weight ≤ 33 kg) (before breakfast and at the end of the afternoon, at least 2.5 hours before bedtime) for 91 days. The IP will be administered in the formulation a 0.5 mg Bumetanide tablet and taken orally. Starting dosage for children > 33 kg will be 0.5 mg twice a day, then the dose will be increased to 1.0 mg twice a day (starting dosage for children between 17-33 kg will be 0.25 mg twice a day which increases to 0.5 mg twice a day) if blood electrolytes are normal and no signs of dehydration are present in the clinic visit at day 7. Dose reductions to manage side effects will be allowed at any time. The treatment period will be followed by a wash-out period to evaluate return of symptomatology and reversibility of treatment effect. The duration of the wash-out will be 28 days in concordance with the study by Lemonnier and colleagues [6].

Placebo product will be administered as comparator of the Bumetanide in exact similar tablets. The qualitative and quantitative composition in excipients of the Placebo product is comparable to that of Bumetanide 0.5 mg tablets.

5.2 Use of co-intervention

Due to the expected chance of frequent mild to moderate hypokalemia in the first phase of bumetanide application, all subjects will receive standard potassium supplementation during the 91 days of treatment. This supplementation will be administered in the form of either potassium chloride 0.5 mmol/ml or potassium chloride 600mga tablets, at an approximate dosage of 0.5 mmol/kg body weight divided over 2 gifts. Children below 30kg receive 0.5mmol/kg KCl solution, whereas children ≥30kg will receive KCl tablets MGA 600mg (equivalent to 8mmol) (see the table below). The safety checking routine is developed in collaboration with Dr. M.G. Keijzer-Veen, a child nephrologist, who will be available for consultation and triage of hypokalemia events < 3.0 in Utrecht. In Groningen, Dr. H.T. Swelheim, a pediatrician, will be available for monitoring similar hypokalemia events. Supplementation of potassium chloride to subjects without hypokalemia due to bumetanide is safe and without any adverse effect in patients with normal kidney function.

Weight	<30kg	>30kg
Dose	2dd 0.25mmol/kg KCl solution	2dd 600mga tablet

KCl suppletion protocol at start of study

5.3 Escape medication

n/a

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

The IP consists of Bumetanide 0.5 mg tablets, which will be administered orally (see IMPD). Bumetanide is a potent loop diuretic and used for several decades in the treatment of edema, including that associated with congestive heart failure or hepatic or renal disease, and hypertension. The drug belongs to the same family as furosemide, albeit showing markedly higher potency: 1 mg bumetanide has a diuretic potency equivalent to ~40 mg furosemide, which is reflected in the proposed dosage of 0.5-1.0 mg bumetanide. The primary site that bumetanide targets is the ascending limb of the loop of Henle, where the drug inhibits electrolyte reabsorption which causes the diuretic and natriuretic actions observed. Besides its actions as a diuretic, bumetanide is a selective NKCC1 antagonist, capable of decreasing internal chloride concentrations in neurons. This concentration change makes the action of GABA more hyperpolarizing, which may be useful for the treatment of ASD.

Placebo product will be administered as comparator of the bumetanide 0.5 mg tablets. The qualitative and quantitative composition in excipients of the placebo product is comparable to that of bumetanide 0.5 mg tablets.

<u>Bumetanide 0.5 mg and Placebo:</u>

Appearance
White round tablet 9mm with smooth sides with the notation “Bumetanide 0,5”
Uniformity of mass
Average weight 0.250 mg
Disintegration time
NMT 15 minutes
Shelf life
Blister: 24 months
Storage conditions
Ambient temperature
API purchased by
TioFarma, Oud-Beijerland
IMPD prepared by
TioFarma, Oud-Beijerland
Distributed
Pharmacy UMC Utrecht

6.2 Summary of findings from non-clinical studies

Main pharmacodynamics studies supporting the role of Bumetanide in ASD and related disorders are summarized in Table 1.

Table 1. Main pharmacodynamic studies supporting the use of Bumetanide in neurodevelopmental disorders relevant to ASD

Study ID	Experiment	Sample characteristics	Dose	Main findings
Dzhala et al., 2005[35]	*In vivo* and *in vitro*	Hippocampal slices from NKCC1-knockout ($Slc12a2^{-/-}$) mice (P7-P9), controls ($Slc12a2^{+/-}$) and a kainate rat model (P9-P12)	Bumetanide 0.1-0.2 mg/kg *in vivo* and 10µM *in vitro*	Bumetanide shifted the depolarized Cl^- equilibrium potential, it suppressed epileptiform activity in hippocampal slices (P7-P9) *in vitro* and attenuated kainate-induced seizure activity *in vivo*. Bumetanide had no effect in brain slices from NKCC1-knockout mice
Tyzio et al., 2006[36]	*In vitro*	Fetal and neonatal rat hippocampal slices (E18-P5)	Bumetanide 10µM	At E18, GABA is depolarized and Bumetanide hyperpolarizes it. During delivery the GABA action is transiently inhibitory and Bumetanide does not affect it
Rheims et al., 2008a[37]	*In vitro*	Brain slices from Swiss mice of both sexes (P1-P15)	Bumetanide 10µM	Bumetanide reduces intracellular chloride ($[Cl^-]_i$), GABA depolarization and network oscillations
Rheims et al., 2008b[38]	*In vitro*	Neocortical slices from Swiss mice of both sexes (P6-P17)	Bumetanide 10µM	Bumetanide blocks epileptic seizures in the neocortex where GABA is excitatory
Nardou et al., 2009[32]	*In vitro*	Hippocampal slices and interconnected intact hippocampal formations from neonatal Wistar rats (P7-P8)	Bumetanide 10µM	Bumetanide reduces $[Cl^-]_i$ and blocks spontaneous network activities. In epileptic tissue, Bumetanide reduces $[Cl^-]_i$ and the excitatory action of GABA
Valeeva et al., 2010[39]	*In vitro*	Hippocampal slices from Wistar rat of both sexes (P2-P6)	Bumetanide 0.3-10µM	At 0.3-1 µM, Bumetanide reducing $[Cl^-]_i$ modifies action potential properties that desynchronized neuronal network. Bumetanide completely blocked network activities at 5-10 µM
Mazzuca et al., 2011[40]	*In vivo* and *in vitro*	*In vivo*: Wistar rats (P0-P2); *in vitro*: primary cultures of trigeminal neurons dissociated from Wistar rats (P0)	Bumetanide 5 µmol/kg	Bumetanide has an analgesic action in newborn pups *in vivo*. It reduced $[Cl^-]_i$, reducing the depolarizing action of GABA *in vitro*
Tyzio et al., 2011[41]	*In vitro*	Neocortical and hippocampal slices of Wistar rats of both sexes (P5-P7)	Bumetanide 10µM	Bumetanide fully blocked the generation of spikes evoked by a depolarizing GABA
Nardou et al., 2011[20]	*In vitro*	Hippocampal intact formations and slices from neonatal Wistar rats (P7-P8), C57BL/6 wild-type and $NKCC1^{-/-}$ mice (P6–P7)	Bumetanide 10µM	Bumetanide has no effect in NKCC1 knock-out mice. Bumetanide ameliorated the interictal-like events that were aggravated by phenobarbital (especially when applied before phenobarbital)
Talos et al., 2012[24]	*In vitro*	24 cortical tissue samples from TSC and FCD epilepsy patients (mean age: 10.3; F/M: 16/8) and 10 controls (mean age 21.3; F/M: 6/4)	Bumetanide 10µM	Bumetanide significantly attenuated the excitatory $GABA_AR$ responses found in dysplastic neurons in acute slices from TSC tubers
Koyama et al., 2012[42]	*In vivo* and *in vitro*	Wild-type (SLC) or transgenic Sprague-Dawley male rats expressing GFP with experimental febrile seizures induced at P11 (i.e., model of complex febrile seizures)	Bumetanide 0.1 mg/kg *in vivo* and 20µM *in vitro*	Bumetanide prevented muscimol-induced migration deficits *in vitro*. In addition to preventing granule cell ectopia and limbic seizure susceptibility, Bumetanide also rescued the development of epilepsy in the *in vivo* febrile seizure model
Reid et al., 2013[43]	*In vitro*	Hippocampal slices from Long Evan male rats receiving either saline, LPS/KA (modeling inflammation) or LPS/KA with behavioral convulsions (modeling inflammation with febrile seizures) at P14	Bumetanide 10µM	Bumetanide significantly lowered burst frequencies in the inflammation and inflammation/febrile seizures groups and had no effect in the saline group

Koyama et al., 2013[44]	*In vivo* and *in vitro*	Hippocampal slices from Sprague–Dawley rats (at P4, P7 and P28)	Bumetanide 10 µmol/kg *in vivo* and 20µM *in vitro*	Bumetanide inhibited the midazolam-induced increase in $[Ca]^{2+}_i$ and cREB expression in the neonatal hippocampus *in vitro*. *In vivo*, Bumetanide enhanced the sedative effects of midazolam in P4 and P7 rats
Cleary et al., 2013[45]	*Ex vivo*	Hippocampal slices of immature Long Evan male rats following hypoxia-induced seizures (at P10)	Phenobarbital 15 mg/kg and/or Bumetanide 0.15 mg/kg or 0.3 mg/kg	Bumetanide in combination with Phenobarbital is significantly more effective than Phenobarbital alone. Serum and brain concentrations of Bumetanide showed a dose-dependent increase after administration. The combination therapy did not increase neuronal apoptosis in the second postnatal week
Tyzio et al., 2014[13]	*In vivo*	VPA rats (in utero exposed to valproate) and FRX mice (carrying fragile-X mutation)	Maternal pre-treatment 1 day before delivery with 2-2.5 mg/kg Bumetanide	Maternal Bumetanide before delivery rescued GABA developmental sequence (i.e., switched the action of GABA from excitatory to inhibitory) and autistic phenotype in offspring (at P15) of both models of autism. Maternal pre-treatment also restored gamma oscillations and other physiological values in the VPA rat offspring
Hamidi & Avoli, 2015[46]	*In vitro*	Olfactory (PC) and limbic (EC) cortical networks of male adult Sprague-Dawley rats	Bumetanide 10µM or 50µM	Low doses of Bumetanide (10 µM) did not modulate the duration or rate of ictal discharges, whereas high doses (50 µM) completely abolished ictal discharges in the PC and EC. High doses of Bumetanide increased the occurrence rate of interictal discharges albeit decreasing their duration in both cortical networks
Holmes et al., 2015[47]	*In vivo*	Male rat pups enduring recurrent flurothyl-induced seizures (from P5-P14) and controls (all tested from P18-P25)	Bumetanide 0.5 mg/kg twice daily (once before first seizure induction and once following last)	Bumetanide treatment (at the time of the seizures) prevented abnormalities in coherence and voltage correlation. Bumetanide also reversed the sociability deficits that were induced by the early-life seizures, as well as normalizing seizure threshold
Marguet et al., 2015[48]	*In vivo*	Kv7 current-deficient mice (i.e., genetic epilepsy model) and controls (P0-P14)	Bumetanide 0.2 mg/kg twice per day from P0-P14	Bumetanide has a significant normalizing effect on cortical network hyperactivity and altered hippocampal firing patterns in the genetic epilepsy model. It prevents the development of long-term hippocampal structural, physiological and behavioural changes. Bumetanide does not alter principle network activity in controls, nor does it impair neurocognitive development
Sivakumaran & Maguire, 2015[49]	*In vivo* and *in vitro*	Adult male C57BL/6 mice with KA-induced status epilepticus (*in vivo*) and 0-Mg^{2+}-induced seizure-like events (*in vitro*)	Bumetanide 0.2 or 2.0 mg/kg	Bumetanide significantly reduced ictal activity (*in vivo*) and seizure-like events (*in vitro*). In both models, Bumetanide restored the seizure-suppressing effect of diazepam
Deidda et al., 2015[50]	*In vivo* and *in vitro*	Adult Ts65Dn mice (i.e., Down syndrome model) and controls	Bumetanide 10 µM and 0.2 mg/kg daily for 1 or 4 weeks	Bumetanide restored $GABA_AR$-driven Cl^- currents, synaptic plasticity and hippocampus-dependent memory in adult Down syndrome mice
Tao et al., 2016[51]	*In vivo*	Adult mice from the reporter line Thy1-mGFP with febrile seizure (induced by HT on P11 or P14) and controls	Bumetanide 0.1 mg/kg	Bumetanide suppressed cell ectopia and restored the increase in freezing in a contextual fear conditioning paradigm (i.e., indicating memory enhancement) for P11-HT mice

Note. E: Embryonic day; *FCD*: Focal cortical dysplasia; *$GABA_AR$:* γ-aminobutyric $acid_A$ receptors*; HT:* Hyperthermia; *KA:* Kainic acid; *LPS:* Lipopolysaccharide; *NMDA:* N-methyl-D-aspartate; *P:* Postnatal day; *pCREB:* phosphorylated cyclic adenosine monophosphate-response element-binding protein; *TSC:* Tuberous sclerosis complex.

6.3 Summary of findings from clinical studies

See Table 2 and Table 3 for an overview of clinical open-label studies and trials reporting safety and efficacy of treatment with bumetanide in patients with, respectively, ASD and epilepsy. Table 4 summarizes clinical studies in newborns and children treated with bumetanide for indications other than ASD and epilepsy.

Table 2. Clinical experience with Bumetanide in patients with ASD

Study ID	Study design	Sample characteristics	Dose/ treatment	Endpoints	Main findings	Adverse events
Lemonnier & Ben-Ari, 2010[52]	Pilot study	5 patients (age range 3-11 years; F/M: 1/4) with autistic disorder	0.5 mg Bumetanide twice a day for 3 months	CARS, ABC[a], CGI, RDEG and RRB	Improvements in total scores of CARS, ABC[a], RDEG and RRB for all children	No serious adverse event
Lemonnier et al., 2012[6]	Double-blind randomised controlled study	60 patients (age range 3-11 years; F/M: 16/44) with autism or Asperger syndrome	0.5 mg Bumetanide twice a day for 3 months	CARS, CGI and ADOS-G	Bumetanide significantly reduced the CARS and CGI. ADOS-G values significantly reduced when the most severe cases were removed	Occasional mild hypokalaemia, which was treated with supplemental potassium
Lemonnier et al., 2013[53]	Single case report	1 patient (age 10 years; male) with Fragile-X syndrome	0.5 mg Bumetanide twice a day for 3 months	CARS, ADOS, ABC[a], RDEG and RRB	Improvements on all measurements	K^+ reduced to inferior limit (3.5 mmol/L)
Hadjikhani et al., 2013[54]	Open label trial pilot study	7 patients (age range 14.8-28.5 years; all males) with high-functioning ASD	1.0 mg Bumetanide a day for 10 months	Performance in emotion recognition and activation of brain areas involved in emotion processing (fMRI)	Improved emotion recognition and enhanced activation of brain regions involved in social and emotional perception during perception of emotional faces	Frequent: increased urinary output and 1 patient showed hypokalaemia after 1 month of treatment
Grandgeorge et al., 2014[55]	Single case report	1 patient (age 10 years; female) with Asperger syndrome	2.0 mg Bumetanide divided by three daily doses for 18 months	SP	Improvements on a large range of sensory behaviours i.e., auditory, vestibular, touch, multisensory and oral sensory processing, as well as sensory processing related to endurance/tone, modulation related to body position and movement, modulation of sensory input and visual input affecting emotional responses and activity level	NR
Du et al., 2015[56]	Open pilot study	60 patients (age range 2.5-6.5 years; F/M: 9/51) with ASD	One group (*n*=28) received ABA and one group (*n*=32) received both ABA and Bumetanide (0.5 mg, twice a day) for 3 months	ABC[b], CARS and CGI	The combined ABA Bumetanide group had significantly better treatment scores on the ABC and CGI, and a nonsignificant better treatment outcome on the CARS, as compared to the single treatment group	No serious adverse event

Note. ABA: Applied Behaviour Analysis; *ABC*[a]: Aberrant Behaviour Checklist; *ABC*[b]: Autism Behavior Checklist; *ADOS-G*: Autism Diagnostic Observation Schedule-Generic; *ASD*: Autism Spectrum Disorder; *CARS*: Childhood Autism Rating Scale; *CGI*: Clinical Global Impressions; *RDEG*: Regulation Disorder Evaluation Grid; *RRB*: Repetitive and Restricted Behaviour; *SP*: Sensory Profile; *NR*: Not reported.

Table 3. Clinical experience with Bumetanide in patients with epilepsy

Study ID	Study design	Sample characteristics	Dose/ treatment	Endpoints	Main findings	Adverse events
Kahle et al., 2009[57]	Single case report	1 patient (age 6 weeks; female) with multifocal seizures	A single 0.1 mg/kg dose of Bumetanide	Number, duration and frequency of seizures	Significant reductions in mean seizure duration, mean seizure frequency and intraseizure spike frequency when comparing the 2 hours prior to Bumetanide to the 2 hours following administration	No acute clinical or metabolic side effects
Eftekhari et al., 2013[58]	Pilot study	3 patients (age range 31-37 years; all males) with drug-resistant temporal lobe epilepsy	2 mg Bumetanide daily (as add-on to pre-existing AEDs) for 4 months	Seizure frequency	The patients showed seizure-day frequency reductions of 68%, 84% and 75%	NR
Bruining et al., 2015[59]	Single case report	1 patient (age 10 years; female) with epilepsy, ASD, cortical dysplasia and a 15q11.2 duplication	0.5 mg Bumetanide twice a day for 6 months	SP, RBS-R, BRIEF, several cognitive tests and EEG measurements	SP and RBS-R declined from clinical to normal levels. Significant improvements in memory and flexibility according to the BRIEF. Auditory learning and memory skills improved. Normalization of the α frequency power. Seizure frequency or severity did not change	No adverse events or discomfort due to treatment
Pressler et al., 2015[60]	Open-label, dose finding and feasibility trial	14 new-born babies (F/M: 4/10) with refractory neonatal seizures due to hypoxic ischaemic encephalopathy	Bumetanide solution for injection in 4 dose levels (0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg and 0.3 mg/kg) 4 times intravenously via slow infusion at 12 h intervals (maximum of 1.2 mg/kg) after a loading dose of phenobarbital	Adverse events, pharmacokinetics and seizure burden during 48 h continuous EEG monitoring	5 of 14 infants met the pre-specified EEG efficacy criteria; 2 of 14 infants met rescue criteria, but none both efficacy criteria (i.e., >80% seizure reduction and no need for rescue AED). Rescue drug was given to 12 infants which confounded the subsequent assessment of seizure burden	Low blood pressure reported in 7 infants: managed with adjustment of fluids. No major electrolyte disturbance reported. Hearing loss in 3 out of 11 survivors; the trial was therefore ended prematurely

Note. AEDs: Antiepileptic Drugs; *ASD*: Autism Spectrum Disorder; *BRIEF*: Behaviour Rating Inventory of Executive Function; *EEG*: Electroencephalography; *NR*: Not reported; *RBS-R*: Repetitive Behaviour Scale–Revised; *SP*: Sensory Profile.

Table 4. Clinical experience with Bumetanide in newborns and children

Study ID	Study design	Sample characteristics	Dose/ treatment	Endpoints	Main findings	Adverse events

Ward & Lam, 1977[61]	Dose and efficacy study	12 patients (age range 2 weeks-7 months; all males) in the acute group and 13 patients (age range 1 week-4 months; all males) in the long-term group with congenital heart disease presenting with cardiac failure	Acute group: One 0.015 mg/kg dose of Bumetanide; long-term group: dose ranged from 0.015 mg/kg on alternate days to 0.10 mg/kg daily (treatment length ranged from 2-40 weeks)	Huematological and biochemical tests	Bumetanide caused significant natriuresis and no significant increase in potassium excretion. In the acute cases, no fall in plasma sodium, potassium and chloride was observed. Treatment was well-tolerated	No side effects in either group
Wells et al., 1992[62]	Pharmacokinetics and pharmacodynamics study	11 term neonates (gestational age range: 37-41 weeks) with fluid retention and potentially reversible cardiac or pulmonary failure treated with ECMO	0.1 mg/kg Bumetanide intravenously for 2 minutes into the postmembrane side of the ECMO circuit	Pharmacokinetic and pharmacodynamic parameters	The diuretic, natriuretic, and kaliuretic responses of Bumetanide were linear. Significant diuresis, natriuresis and kaliuresis, although the duration of these effects was less than expected given the prolonged renal elimination of Bumetanide	NR
Marshall et al., 1998[63]	Prospective open-label study	9 patients (age range: 0.3-25 years) with oedema related to bacterial sepsis, viral pneumonia or respiratory failure	0.1 mg/kg Bumetanide every 12 hours of the 24-hour study period	Pharmacokinetic and pharmacodynamic parameters	Bumetanide caused significant increases in the urine excretion rate and electrolytes. Creatinine clearance increased after each dose. Bumetanide was an efficacious diuretic in these population	Hypokalaemia, hypochloremia and metabolic alkalosis were observed after the second dose

Note. ECMO: Extracorporeal membrane oxygenation; *NR*: Not reported.

6.4 **Summary of known and potential risks and benefits**

Refer to section 4.8 of the SPC and Table 2, 3 and 4. With regards to the specific interaction with other medications commonly used by epilepsy patients, no reports exist in Dutch pharmacovigilance registries on negative or adverse interactions between antiepileptic drugs, antipsychotic drugs or other psychoactive medications. Bumetanide and carbamazepine or oxcarbazepine is a possible interaction for which caution is warranted in Dutch practice because both drugs are associated with increased risk for hyponatremia. However, the mechanisms by which these AED or bumetanide cause hyponatremia are different and in principal unrelated. Bumetanide can stimulate renal excretion while carbamezine and oxcarbazepine can cause volume through increased sensitivity for tubular ADH (antidiuretic hormone). These effects should cancel each other out but there are casuistic reports on possible synergistic effects [64].

For this study we acquired expertise advise of our clinical pharmacist. The risk of the occurrence of hyponatremia is small in these instances of concurrent use of these AEDs and bumetanide. The planned safety checks of electrolytes are in their opinion sufficient to monitor possible effects of hyponatriemia. The relatively highest risk for hyponatremia is expected after start of treatment, which is covered by the frequent blood withdrawals in the first weeks of the treatment phase. It should also be noted that in this study dosages of AEDs will be maintained stable from one month prior to the study to the end of the washout phase

and that we will detect hyponatremia through AEDs in the screening phase through the electrolyte checking and established hyponatreamie is an exclusion criterion (4.3).

6.5 Description and justification of route of administration and dosage

It is reported that oral or intravenous bumetanide (0.5–2 mg) produces a dose-related increase in sodium, chloride and water excretion, leading to a loss of extracellular volume. This in turn activates the renin angiotensin aldosterone system and secondary potassium loss in the collecting ducts of the distal nephron [65]. On a mg/kg basis, the recommended daily dosages of bumetanide for adults according to the SPC (see Appendix 1) is: every morning 0.5 mg or 1.0 mg. Depending on the response the dose can be repeated every 6-8 hours. In special cases, the dose can be elevated. The doctor rarely prescribes more than 4.0 mg per day. In case of edema in nephrotic syndrome (fluid retention due to renal dysfunction); when the usual lower dose is inadequate, it can be elevated to 2.0-5.0 mg and when necessary repeated every 6-8 hours.

The Dutch pediatric formulary of medications ("Nederlands Kenniscentrum voor Farmacotherapie bij Kinderen" [NKFK]) recommends a starting dose between 0.01-0.1 mg/kg/dose 1-4 times daily, with a maximum dosage of 0.2 mg/kg/dose and not exceeding 10 mg/day. The proposed dosage for children and adolescents with ASD is therefore at the low end of the standard range of bumetanide.

Available clinical data with bumetanide in patients with a neurodevelopmental disorder were obtained with daily oral doses ranging from 1 to 2 mg bumetanide in several studies: an exploratory study in 5 children diagnosed with ASD [52], an RCT study in 56 children with ASD by Lemonnier et al. [6], an open-label trial using fMRI with 7 individuals with high-functioning ASD [54], an open pilot study assessing a combined treatment with 60 children with ASD [56] and our previous case study [59]. In the Lemonnier RCT study the average body weight of the ASD children was 24.1 ± 6.1 kg corresponding to 0.04 mg/kg of bumetanide. The duration of treatment of 91 days has been chosen as a significant improvement of the cognitive function was seen after a 90 day treatment period in the Lemonnier study and our own experience [6,59].

With regards to the potassium suppletion, the normal average potassium requirement is approximately 1-2 mmol/kg/d which amounts to a couple of glasses of apple or orange juice (200 ml apple juice contains approximately 4 mmol K and 200 ml orange juice nearly 8 mmol). The proposed dosage of potassium co-medication (i.e. 0,5mmol/kg) during 91 days of treatment is lower than generally supplied in addition to diuretics (1-2 mmol/kg) and is not expected to cause any complications in either the bumetanide or placebo group. Potassium suppletion is only harmful when supplied via rapid intravenous infusion and/or with persons with a renal insufficiency who cannot properly excrete potassium. Therefore, such patients are excluded through the exclusion criteria of the study.

6.6 Dosages, dosage modifications and method of administration

Dosage:

For patients with a body weight > 33 kg: The IP will be given at a dosage of 0.5 mg or 1.0 mg twice a day (before breakfast and at the end of the afternoon, at least 2.5 hours before bedtime) for 91 days. The IP will be administered orally in the formulation of a tablet 0.5 mg

containing serum or placebo. Starting dosage will be 0.5 mg twice a day, then the dose will be increased to 1.0 mg twice a day, if blood electrolytes are normal and no signs of dehydration are present after the outpatient clinic visit at D7.

For patients with a body weight between 17-33 kg: The IP or placebo dose is calculated on a body weight basis, as the provided starting dose is 0.03 mg/kg/dose and the maximum dose is 0.06 mg/kg/dose. As the tablets can be divided into two 0.25 mg tablets, starting dose for children ≥ 17 kg will be 0.5 mg/day divided over 2 dosages (i.e., 0.25 mg twice daily) and the dose will be increased to 1.0 mg/day divided over 2 dosages (i.e., 0.5 mg twice daily) if blood electrolytes are normal and no signs of dehydration are present after the clinic visit at D7.

Dosage modifications:
Patients will receive IP for a 91 days treatment period. At D7, the dose will be evaluated based upon the assessment of safety and tolerability. If the subject demonstrates no safety or tolerability concerns at D7 with the starting dosage of either 0.25 or 0.5 mg (depending on body weight) twice daily, then the dose will be increased to 0.5 or 1.0 mg (depending on body weight) twice daily (see also section 5.1). Dose modifications between 0.25-1.0 mg twice daily to manage side effects are allowed at any time in this study (see section 8.3.3).

Method of administration:
The method of administration of IP tablets will be described and provided to the patients on the package leaflet (see Appendix 2, section 3 for an example). IP will be administered twice daily; in the morning and at the end of afternoon. A minimum of 6 hours is required between doses and the second dosage should not be administered after 5 pm to avoid disruption of sleep through increased frequency of diuresis. The IP tablets must not be mixed with food or beverages. It is, however, allowed to drink a beverage (water) following the administration of the IP dose. The patients will receive a diary and are requested to write down details concerning dose administration (e.g., sickness, time of dose in case it was forgotten, etc.), for drug accountability (see Appendix 3).

6.7 Preparation and labelling of Investigational Medicinal Product

See IMPD (attached to the research file) by Tiofarma, Oud-Beijerland.

6.8 Drug accountability

Upon receipt of the IP, the pharmacist in the UMCU will (1) verify accurate delivery, (2) acknowledge receipt by signing/initialing and dating the IP release documents provided by TioFarma. A copy of these documents will be retained for the Investigator file. The IP should be stored in a secure location at room temperature by the ward.

The cartridges containing IP tablets should not be opened upon receipt at the site. The dispensing of the blinded IP to each patient will be recorded on appropriate drug accountability forms registered by the investigator. Accurate drug accountability records will be available for verification by the study and pharmacy following database lock installed by the Julius center.
IP accountability records of the pharmacy will include:

- Confirmation of delivery of the IP to the trial site;
- Dispensation of the product as a batch to the ward at UMCU and Jonx Groningen;

These pharmacy records should include dates, quantities, batch numbers and expiration dates.

IP accountability records of the investigator will include:

- Inventory at the site;
- Dispensation of the product to the patient;A calendar for tracking details of bumetanide administration (See appendix 3)
- Return to the Sponsor or alternative disposition of unused product(s) (following database lock).

Records should include dates, quantities, batch numbers, expiration dates and any randomization numbers assigned to the patients. Investigators will maintain records, which document adequately that:

- All patients will be provided with the correct IP box as per the assigned randomization number
- All IP provided by the Sponsor will be fully reconciled.

Unused IP must not be discarded or used for any purpose other than the present study. Patients are instructed to return both used and unused IPs, in their original packaging. IP that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

During the study, drug accountability will document the date the IP was dispensed to the patient. At the end of treatment, the site will open the blinded IP boxes containing the returned IP and update the accountability as required. Following database lock, unused tablets will be destructed by the study site by the investigator. The used cartridges will be checked and destructed by the study site at the end of each individual treatment by the investigator.

The investigator will perform a final review of the drug accountability forms and check all IP returns (both unused and used cartridges) prior to authorizing their destruction by the study site.

7. NON-INVESTIGATIONAL PRODUCT

n/a

8. METHODS

8.1 Study parameters/endpoints

All endpoint measurements will be taken:

- At baseline: between D -45 and D0;
- End of treatment: D91 (+/- 4 days);
- End of the wash-out period: D119 (+/- 4 days).

8.1.1 Main study parameter/endpoint

To investigate whether behavioral deficits associated with abnormal sensory reactivity reduce over time in patients taking bumetanide, we will use the Aberrant Behavior Checklist (ABC) irritability subscale. This subscale is chosen as 1) it is the most commonly used outcome scale in (neuro)behavioral trials [7], 2) a clear effect on this scale was noticed in our pilot study and 3) the irritability subscale measures behavior related to hyperexcitability. Importantly, a recent meta-analysis has supported the convergent and divergent validity of the ABC as a measure of behavior problems in neurodevelopmental disorders, such as ASD [34]. The ABC is a parent interview including five subscales: (1) Irritability, Agitation, Crying; (2) Lethargy, Social Withdrawal; (3) Stereotypic Behavior; (4) Hyperactivity, Noncompliance; and (5) Inappropriate Speech [34]. Factor analysis indicates that these subscales are statistically separate [34,66] and therefore the appropriate approach is to select a relevant subscale. Based upon previous trials and our pilot study, the ABC-irritability subscale was chosen as the primary endpoint measure. The ABC has been validated for the Dutch pediatric population. Age, sex, and IQ are largely unrelated to ABC subscale scores [34].

8.1.2 Secondary study parameters/endpoints

Quality of life and societal improvement

- The PedsQL Measurement Model is a modular approach to measure health-related quality of life (HRQOL) in healthy children and adolescents and those with acute and chronic health conditions. It is the most appropriate quality-of-life measure for use in children and youth with neurodevelopmental disorders [67];
- Quality of life (QoL) (World Health Organization QoL [WHOQOL - BREF]) and EQ5D-5L to assess the parent's quality of life, assessing the individual's perceptions in the context of their culture and value systems, and their personal goals, standards and concerns*;*
- EQ-5D-5L and the EQ5D-Youth version to assess health related quality of life in parents and children. This questionnaire enables the calculation of quality adjusted life years for the economic evaluation, either to be completed by parents (younger patients) or children (older patients);
- BRIEF to evaluate performance, behavior and integration of the child in his/her educational settings.

Measures for economic evaluation

- Productivity Cost Questionnaire (iPCQ)
- Trimbos/iMTA questionnaire for Costs associated with Psychiatric Illness (TiC-P), child version

Behavioral psychometric measures

- ABC subscales other than irritability (hyperactivity, stereotypy, inappropriate speech, and lethargy/social withdrawal). Repetitive Behavior Scale (RBS-R) to measure repetitive and restricted behaviors [68]; Social Responsiveness Scale (SRS) [69] to measure sociability in a continuous scale and the Highly Sensitive Child/Parent Scale, Sensory Profile questionnaire (SP-NL) [70] and school companion (SP-SC) to measure sensory behaviors (all parent administered).

Genetic analysis

- Genetic analysis will be conducted to test for pharmacogenetic responses

Epilepsy related variables (if applicable)

- Seizure frequency over the 28-day period preceding D0 until D119 (assessed with seizure diaries, see Appendix 4);
- Number of occasions rescue medication is necessary.

This proposal further includes a multi-method, multivariable set of outcomes including parent report along a number of different lines (including the ABC dimensions and general autism symptomatology). Overall, the expected effect of bumetanide on reducing aberrant brain activity is expected to improve behaviors on a broad scale and enhance the quality of life of caregivers and facilitate integration of the treated children at school. Therefore, the findings of the study will be easily comparable with RCTs of other therapeutic agents. Secondary outcome measures add useful information of other outcomes to children, parents and health professionals. The treatment duration is adequate to detect treatment effects based on pilot research and the results of a previously published study. The dosing schedule is also based on previously published research and was tested in our pilot study. In addition, we have included assessments to evaluate quality of life concerns and economic impact. In terms of follow-up of the treatment effects, we will evaluate return of symptomatology and reversibility of treatment effect. The duration of the wash-out will be 30 days in concordance with the study by Lemonnier et al [6]. It should be noted that all subjects will be offered bumetanide following the completion of the study in the form of compassionate use.

8.1.3 Other study parameters

Baseline data (in case of comorbid epilepsy)

- Detailed seizure and medical history with a special focus on behavioral and cognitive problems: i.e., date of seizure onset, semiology, triggers and frequency and duration of seizures; seizures will be classified according to the ILAE classification. Seizure types will be defined by caregivers prior to start of study;
- Epilepsy-related history (trauma, infections, febrile seizures, previous seizure semiology);
- Detailed history of psychomotor and neurobehavioral development;
- Neurological examination;
- Anti-epileptic and psychoactive drug history (previous and current, including response to previous treatments);
- Family history of epilepsy or other neurodevelopmental disorders.

8.2 **Randomization, blinding and treatment allocation**

A randomization list will be set up using permuted blocks by the responsible person at the Julius Center. We intend to use the secure online randomization tool hosted by the department of data management of the Julius Center for Health Sciences of the UMC Utrecht. This ensures randomization that is both easy accessible from multiple locations and tamper-proof. We will be using minimization on epilepsy (yes/no), center (UMCU/Jonx) and intelligence (TIQ 55-75, 76-110, >110). The full randomization list will contain 190 patient study numbers, 95 for bumetanide treatment and 95 for placebo treatment. At enrolment, the responsible person at the Julius Center will send requested information (patient study number and initials, date of consent) and the randomization number with allocated treatment code to the pharmacy at UMC Utrecht . The delegated person at these pharmacies will dispense the IP kit allocated to this code.

EMERGENCY UNBLINDING

There should always be a way to obtain an individual subject's treatment assignment during an emergency in blinded trials. The trial coordinator (Hilgo Bruining) is responsible for emergency unblinding: he makes the decision to unblind and is responsible for following the procedures. If the principal investigator delegates this responsibility to another member of the research team, this should be documented in the Authorisation Log.

Emergency unblinding is indicated in the following situations ONLY:

1. unblinding is necessary for the subject's emergency treatment at the investigators discretion
2. unblinding is required by local laws or regulations (in case of SUSAR)
3. the Data Monitoring Safety Board decides that unblinding is necessary for proper study management of the subjects and the overall safety of the other subjects in the study

The BASCET study will use a secured PDF document for emergency unblinding situations containing the name of the treatment in each specific kit. This list will be secured with a password and will be accessible only by the unblinded pharmacist at the UMCU. Outside normal working hours, an on-call pharmacist always has access to this document.
If the person who is required to perform the unblinding is not the investigator or a delegated study team member, he/she needs to notify the investigator or study team member that unblinding is required. Each patient and his/her parents will receive emergency instructions via a letter and a study identification card stating their name and study identification number and a telephone number to request information in case of emergency. In addition, this card will state the name and acronym of the study and the product (bumetanide or placebo) under investigation.

The date and reason that the blind was broken must be recorded in the eCRF of the unblinded patient. The trial coordinator of the study must be notified before breaking the blind, unless identification of the study drug is required for emergency therapeutic measures. The investigator needs to inform the trial coordinators or project manager of the study on the following information:

- Name and title of the person who requested the unblinding
- Patient number
- Reason for unblinding
- Date and time of unblinding

- Name of person authorising the procedure
- Signature of person performing the unblinding

8.3 Study procedures

Inclusion and treatment will occur at two sites: UMC Utrecht and Jonx Groningen. Several affiliated centers are involved in referring eligible patients to one of these sites.

8.3.1 Study phase and procedures

Following completion of the pre-study evaluations, included subjects will undergo the following phases:

- Phase I: Pre-treatment and screening (between D-45 to Day0)
    - Screening for eligibility
    - Baseline measurements
    - Randomization

- Phase II: Treatment (between D0 and D91)
    - Blood analysis at D4, D7, D14, D28, D56 (+/- 2 days)
    - End of treatment outcome measurements D91 (+/- 4 days)

- Phase III: Washout (between D91 and D119)
    - End of washout outcome measurements D119 (+/- 4 days)

An essential element of the study procedures is that the research team involved in outcome measurements (team I) cannot be involved in the check-ups for adverse effects during the treatment. This separation of tasks is crucial to avoid suspicion of treatment allocation: symptoms related to diuretic treatment such as increased diuresis or hypokalemia will raise suspicion of bumetanide treatment arm allocation and conversely, the lack of these symptoms may be interpreted as an indication of placebo allocation. Therefore, the trial procedure enforces a strict separation of tasks related to either the measurement of endpoints, or the check-ups for adverse effects during treatment. This separation is embodied in two teams both at the UMC Utrecht and at the Groningen site:

- Team 1: Responsible for screening and outcome measurements (Phase I, II and III). These tasks will be performed by the research team members of the department of Psychiatry and Child Neurology in Utrecht (including HB and DvA) and the department of the Autisme Team Noord-Nederland (ATN) in Groningen (including IB, SP and AE), as well as in the screening procedures.
- Team 2: This team consists of pediatricians and pediatric nurses of the department of pediatrics in Utrecht and Groningen who will be responsible to oversee the management of the patient's condition, including safety and blood analysis (screening is also allowed), intercurrent illnesses, tolerability and prescription of medication and follow up on the other protocol-related procedures, including the adjustment of dosage as described under section 6.6. Accordingly, these check-ups will take place not in sight of team 1 (to avoid unbinding due to observation of adverse effects), at the department of pediatric nephrology at the Wilhelmina Children’s Hospital of the UMC Utrecht and at the department of general child- and adolescent psychiatry of

Jonx Groningen. These research team members will also be responsible for the dispensation of the potassium chloride co-medication and the increase in dosage at D7 (if allowed). During the treatment phase, patients will report to this team with possible AEs including signs of dehydrations and/or electrolyte disturbance expected to have arisen from diuretic effects.

An overview of the study, with all visits and procedures, is depicted in the trial flowchart (see Table 5).

Table 5: Trial flowchart of the proposed study

Phase	Screening	Treatment						Post-treatment
Visit	Screening visit	Start of treatment					End of treatment	End of study visit
Day	**Day -45 to -1**	**Day 0**	**Day 4**	**Day 7**	**Day 14**	**Day 28**	**Day 56**	**Day 91**	**Day 119**
Allowed time windows	None	None	+/- 2 days	+/- 2 days	+/- 2 days	+/- 2 days	+/- 2 days	+/- 4 days	+/- 4 days
Informed consent	**X**								
ASD, ADHD or epilepsy diagnosis	**X**								
Demographics	**X**								
Medical (and epilepsy) history	**X**								
IQ (WISC or WPPSI)[a]	**X**								
Study eligibility confirmed	**X**								
Physical examination	**X**							**X**	**X**
Weight	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Vital signs	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Blood analysis	**X**		**X**	**X**	**X**	**X**	**X**		
Epilepsy diary check		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Urine analysis	**X**								
Concomitant therapy checking	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Primary and secondary endpoints	**X**							**X**	**X**

Note. [a] Previous IQ measurement by WISC <2 years old is also acceptable.

Phase I: evaluations and procedures for screening (D-45 to -1):

Team I will perform the following tasks:

- Date of signed informed consent;
- Confirmation of study eligibility (inclusion and exclusion criteria);
- ASD or ADHD diagnosis based on the DSM-IV or DSM-V criteria or epilepsy diagnosis and SP-NL;
- Demographics and medical history (including family history);
- IQ (short)WISC or WPPSI if 5 years old, if available < 2 years to start of study, it is not necessary to repeat it;
- Baseline measurements;
- Physical examination; vital signs; concomitant therapy;
- Blood analysis;

- Urine analysis;
- eGFR analysis (modified Schwartz formula (36.5*length (cm)/serum creatinine (umol/L));
- Serum or urine pregnancy test for females of child-bearing potential;
- Cardiac risk screening;
- Seizure diary, daily report of seizures. Frequency assessed over the total study period.

Completion and outcome of all the Phase I procedures must be reviewed by the Principal Investigator prior to patient enrolment in Phase II.

Phase II: Treatment Phase
Treatment will commence once the principal investigator confirms that the patient is eligible for inclusion and when Phase I screenings and baseline outcome measurements have been completed within the correct timeframe.

Day 0: Start of Treatment Visit at UMC Utrecht / Groningen
Team I or II will carry out the following tasks:

- Concomitant therapy check;
- Dispensing of IP;
- Dispensing of potassium chloride suppletion for 91 days (see section 5.2).

Days 4, 7, 14, 28, 56: Safety checks at UMC Utrecht / Groningen (+/- 2 days)
Team II will carry out the following tasks:

- Weight, vital signs, concomitant therapy;
- Dosage adjustment (if Potassium levels are < 3.5 mEq/L);
- Blood analysis;
- Seizure diary;
- AEs.

Day 91: End of treatment visit (+/- 4 days):
Team I will carry out the following task:

- Outcome measurements (must be measured before end of treatment);

Team II will carry out the following tasks:

- Physical examination, vital signs, concomitant therapy;
- Seizure diary;
- AEs.

Phase III: Washout phase
Day 119: End of study visit (+/- 4 days):
Team I will carry out the following tasks:

- Outcome measurements;

Team II will carry out the following task:

- Physical examination, vital signs, concomitant therapy;
- Seizure diary;
- AEs.

8.3.2 Study investigations

Behavioral parent questionnaires

Parents will be asked to fill in multiple questionnaires about the behavioral and psychological functioning of their child at Day -45/0, Day 91 and Day 119. Filling out all questionnaires can take up to 60 minutes per episode, which can be done at home. The questionnaires include:

ABC

Refer to section 8.1.1

Quality of life measurements

Refer to section 8.1.2

Measures for economic evaluation

Refer to section 8.1.2

SP-NL and SP-SC

Sensory behavioral symptoms may constitute important parameters of bumetanide treatment as they have been directly linked to GABAergic dysfunction and the imbalance between neuronal excitation and inhibition. The Sensory Profile (SP), developed by Dunn and colleagues [70], is a caregiver questionnaire that measures a child's sensory processing abilities and their impact on daily functioning. It consists of a 125-item assessment on which parents report the frequency their child responds to items in eight categories: Auditory, Visual, Taste/Smell, Movement, Body Position, Touch, Activity Level, and Emotional/Social. The SP has been translated in Dutch and has been validated with norm-scores for normal developing Dutch children of different age ranges [6,73]. A version for children between 3-12 and a version for children aged 11 and older exist. Filling out the 125 items results in 14 sensory processing category scores and a total score. The SP takes about 28 min to complete and a computer-based scoring program is available.

HSP

The highly sensitive child or parent scale (HSP) is a self-administered 12 item child or caregiver questionnaire that determines sensory sensitivity. The questions are scored on a 7 point Likert-scale.

SRS

The Social Responsiveness Scale (SRS) [69] is a caregiver questionnaire that distinguishes autism spectrum conditions from other child psychiatric conditions by identifying presence and extent of autistic social impairment. This 65-item rating scale measures the severity of autism spectrum symptoms as they occur in natural social settings. The SRS provides a clear picture of a child's social impairments, assessing social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits.

RBS-R

The Repetitive Behavior Scale-Revised (RBS-R) [68] is a caregiver questionnaire that addresses several domains of repetitive and restricted behaviors. The questionnaire consists of 43-items that are rated on a four-point Likert scale ranging from (0) “behavior does not occur”, to (3) “behavior occurs in a severe problem”. Caregivers are asked to refer to the

behavior observed in the previous month when completing the questionnaire. The RBS-R provides information on six subscales: Stereotyped Behavior, Self-injurious Behavior, Compulsive Behavior, Routine Behavior, Sameness Behavior and Restricted Behavior.

Genetic analysis
Participants and parents are asked to consent to obtain blood samples for genome analysis. The blood will be collected during the blood withdrawals required for safety analysis. Blood will be collected and stored at the laboratory of the UMC Utrecht and Certe Groningen. After blood of all consenting participants is collected genomic analysis will be performed over the entire batch. Rest material will be discarded. The genetic analysis could be performed outside the UMC Utrecht. To this end it may be necessary to send encoded whole blood samples to a research center other than the UMC Utrecht.

8.3.3 Safety investigations

Assessments of safety will be performed by the research team under supervision of a pediatric nephrologist in Utrecht (MK-V) and a pediatrician in Groningen (HS), as described under section 8.3.1. The research team will be responsible for the management of the patient's condition, including safety assessments, intercurrent illnesses, tolerability and prescription of medication and follow up of the other protocol-related AE procedures.

The following safety parameters will be assessed:

- AEs;
- Physical examination and vital signs;
- Blood laboratory tests: blood electrolytes (ionogram) and extended blood tests including liver functions and full blood count;
- Urinalysis;
- eGFR;

Adverse Events (AEs)
All AEs which occur from the date of written informed consent to the last day of IP administration or at the final study visit (whichever is the longer), will be recorded in the electronic case record form (eCRF). AEs will be documented with respect to severity, duration, relationship to the IP, management and outcome. Patients (and their parent[s]) should be specifically questioned about dehydration, orthostatic hypotension, hypersensitivity reactions, cramps, asthenia, diarrhea, myalgia, arthralgia, dizziness and nausea. Investigators should also evaluate the patient for hypokalemia, health problems including cardiovascular and renal functions and intercurrent illnesses.
It should be noted that seizure control is often poor in these patients. Seizures will be accounted for in the seizure diary and monitored by the treating neurologist (FJ). Seizures in patients with incomplete seizure control before the study will therefore not be regarded as a (S)AE.

Physical Examination and Vital Signs
The investigator will observe the patient's appearance, general health and behavior, along with measuring height (at the screening visit only) and weight. With the patient in the seated position, the following systems will be assessed:

- Skin: the exposed areas of the skin are observed and the size and shape of any lesions are noted;
- Mouth and pharynx: the lips, gums, teeth, roof of the mouth, tongue, and pharynx are inspected.

Normal results of a physical examination correspond to the healthy appearance and normal functioning of the body. Abnormal results of a physical examination include any findings that indicated the presence of a disorder, disease, or underlying condition. Body weight will be assessed in kg, with patients dressed as lightly as possible. Height will be measured in cm. Vital signs will include sitting blood pressure (mmHg) (systolic/diastolic), pulse rate (beats/min) (supine after 5 min rest) and body temperature. Additional vital sign measurements should be recorded as clinically indicated.

Blood analysis (D4, D7, D14, D28, D56)

- Blood analysis will be performed by the UMC Utrecht clinical laboratory and Certe Laboratory in Groningen. Blood parameters to be analyzed at all planned time points are sodium, potassium, chloride, uric acid, urea, creatinine, glucose, estimated glomerular filtration rate (eGFR), haematocrit, hemoglobin, erythrocytes, leukocytes, thrombocytes and total protein. Additional blood analysis measurements will be gathered at the screening (see section 8.3.3. *Extended Blood Tests [Screening]*). Results will be sent to Team 2 (see section 8.3.1) only.

Hypokalaemia is the predominant AE expected to occur. Management of hypokalaemia will depend on the current study medication dose the participant receives:

Twice daily 0.5mg

1. <u>Hypokalaemia between 3.0-3.5 mmol/ml:</u>
   - Increase of KCl suppletion to 1 mmol/kg/day
   - Repeat the blood analysis in 3-4 days. When K+ concentration is stable or increased, raise the study medication to twice daily 1mg and repeat blood analysis in 3-4 days. Maintain KCl suppletion at 1 mmol/kg/day

2. <u>Hypokalaemia between 2.5-3.0 mmol/ml:</u>
   - Stop study medication
   - Increase KCl with 0.5 mmol/kg/day
   - Blood analysis in 3-4 days. When K+ recovers start study medication at twice daily 0.5mg and repeat blood analysis in 3-4 days. Maintain KCl dosage

3. <u>Hypokalaemia < 2.5 mmol/ml:</u> Magnesium (Mg) analysis in blood, ECG analysis, discontinuation of study medication.
   a. If no abnormalities on ECG, Mg normal (>0.70 mmol/l) and no clinical symptoms:
      - No hospital admittance required
      - Increase potassium suppletion to 2 mmol/kg/day
      - Revision of blood analysis and clinical symptoms after 2 days at the outpatient department
      - Withdrawal from study
   b. Abnormalities present on ECG or symptomatic hypokalemia, and/or Mg < 0.70 mmol/L:
      - Immediate hospital admission with ECG monitor surveillance
      - Immediate extra oral dosage of potassium chloride (1 mmol/kg)

- Checking of Mg levels in addition to blood analysis
- Intravenous potassium chloride suppletion according to the protocol of the Wilhelmina Children's hospital (WKZ)
- Withdrawal from study

Twice daily 1mg

1. Hypokalemia between 3.0-3.5 mmol/ml:
   - Increase KCl supplementation with 0.5mg/kg/day
   - Repeat blood analysis in 3-4 days. When K+ is stable or increased maintain KCl supplementation at current dose

2. Hypokalemia between 2.5-3.0 mmol/ml:
   - Stop study medication
   - Increase KCl supplementation with 0.5mg/kg/day
   - Blood analysis in 3-4 days
      a. When K+ increases >3.0 mmol/ml:
         - Start study medication at twice daily 0.5mg. After two consecutive K+ levels of >3.5 increase study medication to 1.0mg twice daily. KCl dosage remains at increased dosage
      b. When K+ remains <3.0 mmol/ml withdrawal from study.

3. Hypokalemia < 2.5 mmol/ml: Mg analysis in blood, ECG analysis, discontinuation of study medication.
   a. If no abnormalities on ECG, Mg normal (>0.70 mmol/l) and no clinical symptoms:
      - No hospital admittance required
      - Increase potassium suppletion to 2 mmol/kg/day
      - Revision of blood analysis and clinical symptoms after 2 days at the outpatient department. When potassium is recovered, restart study medication at 2dd0.5mg
   b. Abnormalities present on ECG or symptomatic hypokalemia, and/or Mg < 0.70 mmol/L:
      - Immediate hospital admission with ECG monitor surveillance
      - Immediate extra oral dosage of potassium chloride (1 mmol/kg)
      - Intravenous potassium chloride suppletion according to the protocol of the WKZ

In case a patient is withdrawn due to hypokalemia, he/she will not be replaced.
Behavioral adverse events are at first evaluated by team two. If further psychiatric evaluation of behavioral adverse events is warranted, then participants are referred to the independent expert. The independent expert will consult parents and decide on further treatment, un-blinding and study termination.

Extended Blood Tests (Screening)
Laboratory tests will be performed by the local laboratory and will include the following assessments:

- Alanine Transaminase (ALAT);
- Aspartate Transaminase (ASAT),
- Gamma-glutamyltransferase (γ-GT);
- Alkaline phosphatase;
- Sodium;
- Potassium;
- Chloride;
- Calcium;
- Uric acid;
- Urea;
- Creatinine;
- Glucose;
- Haematocrit;
- Hemoglobin
- Erythrocytes
- Leukocytes
- Thrombocytes
- Total protein;

* In children, the estimated eGFR is calculated by the modified Schwartz formula (Schwartz, 2009), which uses serum creatinine (µmol/L), the child's height (cm) and a constant to estimate the glomerular filtration rate (36.5): eGFR=36.5 *(height [cm]/serum creatinine [µmol])

Urinalysis (Screening)
Urinalysis will include the following assessments:

- Sodium;
- Potassium;
- Chloride;
- Calcium;
- Protein;
- Creatinine;
- Uric acid;
- Micro-albumineria;

Patients will be provided with bottles in which to collect a urine sample before the center visit.

Cardiac Risk Screening (Screening)
During the medical check at the screening visit, a cardiac risk screening will be performed, in which family history and cardiac auscultations are carefully examined. Evidence of cardiac complaints, a positive family history suspect for arrhythmia's, or abnormal auscultative findings will lead to a referral to the pediatric cardiologist in the WKZ. This is agreed in a meeting with the cardiologists of the WKZ and a pharmacist of the hospital, who all ascertained that there is no available evidence that bumetanide increases QT-interval or causes arrhythmias. The main effects of bumetanide on the heart are mediated by electrolyte disturbances, which are controlled for by the laboratory check-ups (correspondence between the study team and the cardiologist and pharmacist in this regard can be found in Appendix

5).

Checking for concomitant therapy/medical management of adverse events
Any medications, except those listed below, which are considered necessary for the patient's welfare, and which will not interfere with the study medication, may be given at the discretion of the Investigator. Concomitant therapy should be recorded from the date of written informed consent to the last day of IP administration or at the final study visit (whichever is the longer). Two types of medications should be used with caution or excluded from the clinical studies with bumetanide: drugs whose therapeutic activity may affect potassium levels and drugs associated with ototoxicity. Administration of all concomitant drugs must be reported in the appropriate section of the eCRF along with dosage information, dates of administration and reasons for use. Additionally, any unplanned diagnostic, therapeutic or surgical procedure performed during the study period must be recorded in the appropriate section of the eCRF. Special care should be taken in questioning of the patients (or patient's parent[s]) on any self-medication taken by the patient.

Behavioral and neurological surveillance
Behavioral and seizure control is generally variable in patients with epilepsy and difficult to manage. Although no adverse effects of bumetanide on these aspects of the disease are expected, changes in behavioral and seizure morbidity may occur during the trial and may cause concern whether these are attributable to the IP. To address these possible concerns it will be possible to consult a neurologist or child psychiatrist of the UMC Utrecht or Jonx by phone. In addition, patients (with active epilepsy) and their parents will be instructed to keep a diary of seizure occurrence and frequency (see Appendix 4, seizure diary). In any case, if the seizure frequency is increased by more than 50 %, IP treatment will be stopped.

Medications that may NOT be administered
The patient must not take any of the following medications during the course of this study (during the treatment period and prior to the start of treatment, as specified below and also in the exclusion criteria under section 4.3). Should any of these medications be required for the treatment of the patient, the patient must be withdrawn from the study:

- Aminoglycosides. Despite potentiation, ototoxicity has not been tested for bumetanide. The ototoxic effects of aminoglycosides may be increased by the concomitant administration of diuretics such as bumetanide. Aminoglycosides are a group bactericidal drugs that are used to combat infections due to aerobic, Gram-negative bacteria. Neomycin, kanamycin, gentamycin and amikacin are the most likely to cause problems with hearing. They produce cochleotoxicity through a poorly understood mechanism. Aminoglycosides also cause nephrotoxicity by inhibiting protein synthesis in renal cells, particularly in repeated dose treatments. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure;

- Non-steroidal anti-inflammatory drugs (NSAIDs) are prohibited. NSAID inhibit the effect of bumetanide. Diuretics may enhance the nephrotoxicity of NSAIDs. NSAIDs constitute a heterogeneous group of compounds that share similar therapeutic effects as well as side effects. Since these drugs can be obtained without a prescription, they

are potentially available for long-term use. The dominant ototoxic effect of salicylates appears to be the production of tinnitus as well a reversible mild to moderate symmetric sensorineural hearing loss. Patients who may require NSAIDs or Aminoglycosides will be withdrawn from the study and replaced;

- Other drugs to be avoided include digoxin, antihypertensive agents, indomethacin, probenecid, acetazolamide, lithium and drugs known to have a nephrotoxic potential;

- Patients may not start concurrent psychotherapy, social skills training, or behavioral interventions (e.g., applied behavior analysis) during the study. If the patient had been receiving these services prior to the study, they will have the option of continuing such services during the study;
- The use of any herbal/natural products or other "folk remedies" should be discouraged, but use of those products, as well as the use of vitamins, nutritional supplements, and all other concomitant medications must be recorded in the eCRF;
- Use of other diuretics (e.g., furosemide, hydrochlorothiazide).

Guidance on medications to be administered/patient management for expected AEs
Based on the inclusion/exclusion criteria, it is anticipated that the need for concomitant medications will be limited. Melatonin could be administered at the Investigator's discretion for sleep aid. Administration of Vitamin D is also accepted. Long-term or regular use of salicylates or paracetamol should be avoided.

8.4 **Withdrawal of individual subjects**

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Patients (and their parent[s]) will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and are not obliged to state their reasons. Any withdrawals must be fully documented in the eCRF and should be followed up by the Investigator. Additionally, the Investigators may withdraw a patient at any time if they consider this to be in the patient's best interest.

Patients **MUST** be discontinued for any of the following reasons:

- Hypokalaemia <2.5mmol/L;
- Prescription of antimicrobial aminoglycosides;
- Prescription of non-steroidal anti-inflammatory drugs (NSAIDs);
- Serious intercurrent illness or significant worsening of intercurrent illness;
- Withdrawal of patient consent;
- If in the Investigator's opinion continuation in the study would be detrimental to the well-being of the patient;
- At the specific request of the Sponsor.

Patients **MAY** be discontinued for any of the following reasons:

- Protocol violations, including non-compliance with study procedures, patient lost to follow-up and patient refusal;

- Intolerable or persistent AEs.

If a patient fails to return for a scheduled visit/follow up, attempts should be made to contact the patient (the parent[s]) to ensure that the reason for not returning is not an AE. Likewise, if a patient (and/or the patient's parent[s]) declares his/her wish to discontinue from the study e.g., for personal reasons, an attempt should be made to establish that the true reason is not an AE (bearing in mind the patient is not obliged to state his/her reasons). If the IP therapy is prematurely discontinued, the primary reason for discontinuation must be recorded in the appropriate section of the eCRF and all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation following the patient's withdrawal should be made in the eCRF and any AEs followed up until resolution or a period of 30 days from the last dose of IP has elapsed, whichever is the longer.

8.4.1 Specific criteria for withdrawal

n/a

8.5 Replacement of individual subjects after withdrawal

All patients leaving the study early, regardless of the reason, will be requested to return to the site for an Early Termination visit to finalize participation. If the patient is not willing to complete all measures, priority will be given to the ABC-I, the primary outcome measure. There are no consequences if a patient also refuses this.

Recruitment will continue until at least 172 patients are evaluable. An evaluable patient is defined as a patient who has:

- Met all of the inclusion and none of the exclusion criteria specified in Sections 4.1 and 4.2 within the specified time-frame;
- Received the allotted course of treatment and is assessable for the primary efficacy endpoint;
- Had their eCRF completed.

Patients experiencing hypokalemia <3 mEq/L will be not replaced. A patient should be replaced if he regularly spits out or regurgitates the IP.

8.6 Follow-up of subjects withdrawn from treatment

In case a subject discontinues before the end of the study, no replacement is needed due to the methods that were selected for our statistical plan.

8.7 Premature termination of the study

If the study is terminated prematurely, for whatever reason, the accredited METC will be notified immediately.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by investigators or staff will be recorded. It should be noted that seizure control is often poor in these patients. Seizures will be accounted for in the seizure diary and monitored by the treating neurologist (FJ in Utrecht and BD in Groningen). Seizures in patients with incomplete seizure control before the study will therefore not be regarded as a (S)AE.

Severity of the adverse event is rated according to the NCI Common Terminology for Adverse Events (CTCAE) rating scale. The scale consists of 5 grades:

<u>Adverse Events:</u>

Grade 1 Mild:	Asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention indicated
Grade 2 Moderate:	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL

<u>Serious Adverse Events:</u>

Grade 3:	Severe or medically significant but not immediately life- threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated.
Grade 5:	Death related to AE.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse event.
SAEs that result in death or are life threatening should be reported expeditedly. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse event. This is for a preliminary report with another 8 days for completion of the report.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorized medicinal product;
   - Investigator's Brochure for an unauthorized medicinal product.

The sponsor will expeditedly report the following SUSARs through the web portal *ToetsingOnline* to the METC :

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

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.
The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

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

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

An independent DSMB will be established to perform ongoing safety surveillance and to perform interim analyses on the safety data, this committee should be an independent committee. The composition, roles and responsibilities of this committee are described in the charter of the DSM charter, which is attached to the research file (K5. Charter Data Safety Monitoring Board).
The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

10. STATISTICAL ANALYSIS

Comparisons at baseline and after treatment within patients will be analyzed. Descriptive statistics of continuous outcomes will be presented by group and include sample size, mean, median, standard deviation, minimum and maximum.

10.1 Primary study parameter(s)

Summary statistics including mean and standard deviation for continuous variables and frequencies for categorical variables will be compared. Effect analysis will be done on an intention-to-treat basis. Linear mixed effects models will be used to assess differences in changes in clinical outcomes over time. Missing data will not be imputed: linear mixed effects models give unbiased estimates [74] under the assumption that data will be missing at random. We intend do subgroup analysis on IQ, severity, comorbidity (previous seizures, hyperactivity) and age. T-tests or Chi2 will be used to test for differences in baseline demographics between the treatment and placebo groups. To explore the relationship between treatment outcome and patient characteristics, we will do both linear and ordinal regression. For the ordinal regression, the treatment outcome will be categorized into improved, unchanged and deteriorated, using the value of 4.6 points from the power analysis as a cut-off point. A significance level of [alpha] = 0.05 will be used for all analyses. P values will be adjusted for multiple comparisons using the Bonferroni correction. SPSS version 22 (or newer) will be used for most analyses except the mixed effects modelling, which will be done with R version 3.1.2 (or newer).

10.2 Secondary study parameter(s)

See 10.1

10.3 Other study parameters

n/a

10.4 Interim analysis

n/a

10.5 Economic evaluation

Cost-effectiveness will be studied by relating cost-differences between bumetanide and placebo treatment group to differences in ABC irritability scores (primary outcome) between both strategies. Furthermore, costutility will be assessed by studying cost differences between groups in relation to quality of life changes, both in children and in their parents. Data on health care use will be derived from Electronic Medical Record. Patient costs and productivity losses of parents will be elicited through parent administered questionnaires (Productivity Cost Questionnaire and TiC-P). Data will be presented both from a health care and a societal perspective. Bootstrap methods will be used to present uncertainty around cost-effectiveness estimates. The economic evaluation will have a similar timeframe as the clinical study.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki, version of Fortaleza, 2013, and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

Recruitment

Children with ASD, ADHD and/or epilepsy, at the participating sites will be included in the study after written informed consent. Yearly in total 200 new eligible patients are seen across the network of ReachAUT and the participating sites. In addition, these institutions together govern a prevalent population of 800 children with ASD in the intended age and IQ range and without psychoactive medication. As the treatment is well-tolerated and patients and their parents have no alternative treatment options, we expect that parents and children will be eager to participate. Even with a very conservative estimate of 25% consent and taking an additional drop out of 10% into account, the amount of eligible patients ensures enough participation within the time frame. Each potentially eligible patient (and their parents) will be informed of the study's objectives and overall requirements. Prior to conducting any of the pre-entry tests not performed routinely in the treatment of the patient, the investigator will explain the study fully to the patient and his/her parent(s) using the prior informed consent document (PICD).

Consent

Each potentially eligible patient (and their parents[s]) will be informed by the Investigator (HB in Utrecht and IB in Groningen) of the study's objectives and overall requirements. Prior to conducting any of the pre-entry tests not performed routinely in the treatment of the patient, the Investigator will explain the study fully to the patient and his/her parent(s) using the Pediatric Inform Consent Document. Following this information, the patients and his/her parents/legal guardian are given at least 2 weeks to read the information and decide whether to participate or not. If a parent is willing for the patient to participate in the study, they will be requested to give written informed consent for children under 12 years of age. In case of patients 12 years or older, patients themselves also need to sign consent. The informed consent will be signed and personally dated by a single or both parent(s) according to local laws and regulations and by the Investigator. A copy of the signed form(s) will be provided to the parent(s) and the original(s) retained with the source documents

11.3 Objection by minors or incapacitated subjects

We will work according to the ‘Verzet’ act of the ‘Nederlandse Vereniging van Kindergeneeskunde’. For each individual participant, a record will be kept including information about the investigation sessions and any details of interest.

11.4 Benefits and risks assessment, group relatedness

The proposed study could establish a novel safe and groundbreaking treatment for children with neurodevelopmental disorders, for which there is no existing pharmacological treatment. As we emphasized in this protocol, there are so far no clear targets for sensory processing disorder treatments, but this should not imply that novel treatment developments will be delayed and that these devastating disorders remain outcasts. Consistent with this notion, the intended research could lead to elucidation of a new therapeutic concept as well as an

opportunity for further, advanced investigation of the neurophysiological mechanism of sensory processing deficits in a variety of neurodevelopmental disorders.

This study will be performed among children with ASD, ADHD and epilepsy as this disorder manifests from young age and if untreated will devastate the lives of these children. In addition, it is well recognized that intervention at the intended ages (and far less at later or adult ages) may enhance plasticity of the brain and restore important developmental capabilities for learning or behavioral adaptation.

Bumetanide has been used for over 20 years in the treatment of treatment of fluid retention (edema) in congestive heart failure, liver disease (cirrhosis) and kidney disease including nephrotic syndrome. Its safety and efficacy is well-established. The current study could demonstrate an added value of bumetanide addition regarding symptom improvement. This could prevent or shorten hospitalizations, with the associated high costs for society as well as the negative impact on the patients' lives.

In the face of the limited additional burden for the patient when participating in the current trial as compared to routine treatment, and the possible positive outcome for future treatment, offering participation to selected patients appears to be justified

Routine care consists of less extensive monitoring of symptomatology and (neuro)cognitive functioning compared to the tests proposed in this study. Therefore, enrolled patients may benefit from extended clinical examinations during study participation.

The data obtained from genetic analyses will serve primarily the research purposes of this study. All data will be saved under anonymous codes to prevent potential linking of psychiatric or biologic information to individuals. At the informed consent form parents can indicate to wish to be informed on incidental findings with clinical relevance.

11.5 **Compensation for injury**

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

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

11.6 **Incentives**

n/a

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Privacy laws and regulations will be adhered to during the complete study. The collection and processing of participants' personal information will be limited to what is necessary to ensure the study's scientific practicability, the evaluation of efficacy, adherence, side effects and the investigational product's safety. Information collected about participants during this clinical investigation will be treated confidentially. The investigator or her co-workers will collect data and transfer it without recording the patient's name or date of birth. Instead, data will be coded with a participant identification number.

The file with the key to the code will be managed by one person. The source documents will be kept in a locked file cabinet in the office of the study coordinator with limited access of the research personnel. In accordance with national laws and guidelines and the specifications of the ICH-GCP guidelines, the investigators are obligated to archive all documents pertaining to the study for the legally required time period. The anamnestic and medical history interview at Screening will be directly entered into the eCRF (i.e., Research Online), which will therefore serve as source document.

The acquired data will be entered into an eCRF that is accessible via the internet, called Research Online. The eCRF will be build and supported by the department of data management of the Julius Center (supervisor Diane van der Doest). Their specialized data management tool will be used, called Research Online 2.0 (RO2). RO2 is an advanced web based electronic data capture system for collecting, managing and reporting high quality clinical research data according to ICH-GCP standards. It allows for creating databases for a variety of study designs including clinical trials. Investigators will receive personal user names and passwords for this purpose, and data will be encrypted for transfer. More detail on the handling and storage of collected data can be found in the separate SOP on Data Management. For more information about the eCRF software Research Online and the department of data management of the Julius Center, see http://www.juliussupport.nl/Fields-of-Expertise/Data-management.

In addition, we will use SLIM (Study Logistics and Information Manager) as a software system that enables the GCP-compliant registration and access to personal data of study subjects for digital and paper communication purposes. It also stores informed consent details as well as in- and exclusion data. Finally, it records the dropout or opt out status of a subject. SLIM for this study will be developed and supported by the same data management team of the Julius Center (supervisor Diane van der Doest), who will support Research Online (see above). Parents will be given the choice to be informed about their appointments for this study by email or by regular paper mail.

12.2 Monitoring and Quality Assurance

Associated investigators will be carefully selected and comprehensively informed and trained regarding Good Clinical Practice (GCP), all study procedures and the required examinations and documentation.

All monitoring activities will be in line with national laws and guidelines and the specifications of the ICH-GCP guidelines. Study monitors will visit the study site at regular intervals to monitor the execution of the study. Monitors will have access to all documents that are needed to perform their task according to the above mentioned guidelines. Monitors will check whether requirements to conduct the study are met and study procedures are followed correctly, and will check the study site's documentation, the participants' source data, eCRF entries, and the correct maintenance of the Investigator Site File. Investigators will permit trial-related monitoring, audits, ERB reviews and regulatory inspections, providing direct access to source data and study documents.

Monitoring will be conducted by Julius Clinical, an academic research organization (ARO) that combines strong scientific leadership and operational excellence to conduct innovative national and global clinical trials. Julius Clinical originates from the Clinical Trial Services Unit based at the University Medical Center Utrecht (UMC Utrecht), and was transferred into a separate legal entity, with head office in Zeist, The Netherlands. Julius Clinical remains closely linked to her parent organization, the UMCU Utrecht. Monitoring activities that will be performed for this study are:

- Presence and completeness of Trial Master File (TMF) and/or Investigator File (IF); Investigators are responsible for completing the TMF/IF and for assembling the essential documents
- Monitoring of the rate of inclusion and the percentage of drop-outs
- 25% check on Informed Consent Forms (ICFs)(completeness & availability)
- 25% verification of existence and identity of the participant
- 100% check on Serious Adverse Event (SAE) procedure for all reported SAEs
- 100% check on in- and exclusion criteria for the first 10 enrolled subjects per site and 25% of the remaining enrolled subjects per site (25% of 76 = 19 patients per site); a random selection will be made upfront.
- 25% verification of endpoints and 25% of the secondary endpoints.
- 100% SDV of the first 3 patients per site; thereafter complete SDV of 25% of the remaining patients, including check on missed SAEs. For questionnaires completed by the investigator only scores will be checked and questionnaires completed by parents will be checked on completeness.
- Check the presence of instructions for the execution of study procedures and if study personnel has been properly trained on these procedures; check equipment and facilities
- Check on GLP/GMP certification of laboratory/ Pharmacy. Check on the prescription and the return and storage of IMP.

The monitor will write a monitoring visit report, using Julius Clinical SOPs and report templates, and send a copy by e-mail to the coordinating investigator and the quality manager of the appropriate division after every visit (within 15 working days).

12.3 **Amendments**

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 **Annual progress report**

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 **Temporary halt and (prematurely) end of study report**

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 **Public disclosure and publication policy**

The results of the study will be submitted for publication in an international peer-reviewed journal adhering to applicable privacy laws and regulations. Publication strategy will be determined by the principal investigator.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Bumetanide is an inhibitor of the Na-K-Cl co-transporters called NKCC1 and NKCC2. NKCC1 is widely distributed throughout the body, as well as in many regions of the brain. NKCC2 is specifically found in cells of the thick ascending limb of the loop of Henlé in nephrons, the basic functional units of the kidney. Bumetanide has been used since several decades as a loop diuretic used in the treatment of edema, including that associated with congestive heart failure or hepatic or renal disease, and hypertension. It belongs to the same family as Furosemide. Furosemide also antagonizes the chloride exporter KCC2 and would therefore exert a chloride lowering effect [75] [76] and has therefore no purpose in this context. Pharmacological and clinical studies have shown that 1 mg bumetanide has a diuretic potency equivalent to approximately 40 mg furosemide. The site of bumetanide action is the ascending limb of the loop of Henlé. Recent studies have shown that bumetanide also blocks the NKCC1 co-transporter in the brain, and decreases internal chloride concentration in neurons. This concentration change makes the action of GABA more hyperpolarizing, which may be useful for treatment of the neurobehavioral problems associated with ASD.

Due to overexpression of neuronal NKCC1 and low expression of KCC2 (K+ Cl− co-transporter isoform 2), *immature* neurons have a high intracellular Cl− concentration, rendering GABAa receptor-mediated Cl− currents depolarizing (excitatory state) instead of hyperpolarizing (inhibitory state) as in *mature* neurons [35,37,77]. This may at least partially explain the high incidence of seizures and poor response to conventional AEDs in the new-born infants. The switch from excitatory to inhibitory function is assumed to happen around birth but varies between species and between different brain regions. Indeed, the levels of intracellular chloride have been shown to be elevated in immature neurons and are progressively reduced in a brain structure and neuronal age dependent developmental sequence [36,78].

Recurrent seizures and other traumatic insults can lead to down-regulation of KCC2 and to a re-establishment of NKCC1-dependent depolarizing GABAergic signaling [79]. Several *in vitro* and *in vivo* studies suggest that bumetanide can switch the GABA equilibrium potential of immature neurons or abnormal mature neurons from depolarizing to hyperpolarizing, resulting in a reduced neuronal firing [35,37,77,79] (see Table 1). In addition, chloride levels are increased in epilepsies and a variety of brain disorders and lesions, including cerebrovascular infarcts and spinal cord lesions [20,35,37,79-81]. Furthermore, bumetanide can augment phenobarbital anticonvulsive action in different rodent models including a hypoxic rat model [82,83]. These observations suggest that drugs that reduce intracellular chloride levels may be helpful in reinstating normal/low levels of chloride and thereby a powerful GABAergic inhibition.

b. Previous exposure of human beings with the test product and/or products with a similar biological mechanism

Bumetanide was introduced in the seventees as a diuretic agent and was widely used for the treatment of fluid retention. In the early 1980s, several articles and reviews summarized the safety and the efficacy of bumetanide in patients with conditions related to fluid overload. Following IV administration, forced diuresis commences within a few minutes and within 30–60 minutes following oral administration. Peak diuretic activity generally occurs within 1–2 hours following oral and within 15–30 minutes after IV administration. Diuresis is dose-dependent and generally complete within 4–6 hours following oral administration.

Efficacy of bumetanide in newborns and children has shown to be efficacious and generally well tolerated. Available studies indicate that acute or repeated administration of bumetanide in toddlers and children result in a significant diuretic activity is associated with loss of electrolytes. For details on the clinical experience with bumetanide in different age groups we refer to Table 4 and the SPC (Appendix 1).

In addition to its use for conditions associated with fluid overload, there is recent experience with the application of bumetanide in ASD and seizure conditions.

Bumetanide application in patients with epilepsy
A case study has reported that a daily dose of 2.0 mg bumetanide for 4 months led to a reduction of seizure frequency and EEG discharges in three patients with temporal lobe epilepsy [58]. The route of administration and the dose distribution over the day was not specified in this report. With regard to infants, a single case study of a 6-week old infant with multifocal seizures due to meningitis demonstrated that a single dose of 0.1 mg/kg IV bumetanide was associated with a significant reduction of electrographic seizures by more than 40% (36 ± 7 seizures per hour in a 2-h window before treatment compared to 21 ± 7 seizures in the 2-h window following the administration of bumetanide) [57]. Clinical studies assessing the efficacy of bumetanide in epilepsy are summarized in Table 3.

Bumetanide application in patients with ASD
Three open-label pilot studies [52,54,56], two single case reports [55,59] and a double-blind randomized controlled study [6] report on the safety and efficacy of bumetanide in children diagnosed with ASD. Another single case report assessed the efficacy of bumetanide to treat Fragile-X syndrome [53]. A summary of clinical studies assessing the efficacy of bumetanide in ASD is provided in Table 2.

Lemonnier and colleagues investigated the efficacy and safety of bumetanide in 5 children (3.5 to 11.5 years of age) with ASD who received 0.5 mg Burinex® (half a 1 mg tablet per dosing) twice a day (morning and evening) for 90 days [52]. Several behavioral scales demonstrated an improvement in total scores for all children at the end of the 90 days treatment. Bumetanide provided better cognitive regulation in keeping with the improved presence reported by the parents. Frequent monitoring (first weekly, then monthly) of known adverse events such as dehydration, orthostatic hypotension, hyper-sensitivity, cramps, asthenia, diarrhea, myalgia, arthralgia, nausea and dizziness was performed. Potassium and sodium levels remained stable and no serious adverse effects related to the treatment were reported.

Another study assessed the effect of bumetanide on emotional face recognition capacity using fMRI [54]. This study involved 7 male patients (14.8 to 28.5 years of age) with high-functioning ASD on 1.0 mg bumetanide twice daily for 10 months. Improved emotion recognition corresponded to the observed enhanced activation of brain regions involved in social and emotional perception. Besides mild hypokalemia, no other adverse events were reported.

Due and colleagues included 60 children (2.5 to 6.5 years of age) with ASD who received either solely Applied Behavior Analysis (ABA) or both ABA and 0.5 mg bumetanide twice a day for 3 months [56]. The combined group (ABA + bumetanide) demonstrated significant better treatment scores on the ABC and CGI, in addition to non-significant improvements on the CARS, as compared to the ABA-only group. No serious adverse events were observed in the group receiving bumetanide.

A randomized double-blind placebo-controlled study was performed by Lemonnier et al. including 56 children with ASD [6]. ASD diagnoses had been made by a clinical child psychiatrist using International Classification Disease (ICD-10) and confirmed by the Autism Diagnostic Interview-Revised. Patients eligible for enrolment were 3 to 11 years old. Exclusion criteria included history of seizures or other neurological disorders and treatment with other treatments (notably psychotropic agents, including risperidone) with the exception of melatonin to improve sleep disorders. The primary endpoint was the CARS measured before and after 90 days of treatment. The secondary endpoints were CGI and ABC scores, measured before and after 90 days of treatment. Patients received either 0.5 mg bumetanide or placebo (lactose) twice daily (morning and evening). Bumetanide treatment resulted in a decrease in CARS total score, with an average improvement of 1.8 units for the placebo group and 5.6 units for the bumetanide group (p=0.004). Bumetanide treatment also resulted in an improvement in CGI disease severity index score compared to the placebo group (2.04 versus 1.56, p=0.017) suggesting a clinical amelioration. Bumetanide treatment was generally well tolerated. The most frequent reported in the study article was a decrease in plasma potassium level: 22 out of 59 children (37%) and 6 out of 19 adolescents (32%) experienced decreased kalmia, which were treated with oral potassium supplements.

A single case report demonstrated that 2.0 mg bumetanide for 18 months, led to significant improvements on a large range of sensory behaviors in a 10-year old girl with Asperger syndrome [55].

We have published a case of a 10-year-old girl with ASD, epilepsy, cortical dysplasia, and a 15q11.2 duplication who had exhibited marked behavioral arousal after previous treatment with clobazam, a benzodiazepine, was prescribed [59]. We hypothesized that this response indicated the presence of depolarizing excitatory GABA and started bumetanide treatment with monitoring of behavior, cognition and EEG. The bumetanide treatment resulted in markedly clinical improvements in sensory behaviors, rigidity, and memory performance, which was substantiated by questionnaires and cognitive assessments. At baseline, the patient's EEG showed a depression in absolute α power, an electrographic sign previously related to ASD, which was normalized with bumetanide. The treatment did not cause adverse effects, hypokalemia or discomfort through diuretic effects.

These studies demonstrate the safety and efficacy of bumetanide in children and adolescents diagnosed with ASD and/or epilepsy.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

Diuretics like bumetanide or furosemide are organic anions that reversibly inhibit NKCC1 transporters in a variety of preparations by a concentration-dependent mechanism [20,23,32,84-86]. The conclusion is that sensitivity of NKCC1 to bumetanide varies depending on level of activation the co-transporter and the experimental conditions [87].

NKCC1 is also expressed in the brain where it is responsible for chloride homeostasis that influences the polarity of GABA neurotransmission from excitatory to inhibitory, which was suggested to be important for early neuronal development [85]. Bumetanide is a selective NKCC1 antagonist, which can lead to a decrease of intracellular neuronal chloride concentration [Cl^-] if NKCC1 is still active. A decrease in chloride concentrations makes the action of GABA more hyperpolarizing; this is the initial triggering event for explaining the efficacy of bumetanide in neurodevelopmental disorders [78].

In vitro and in vivo pharmacological studies have been carried out to assess the activity of bumetanide on NKCC1 and the GABAergic system. A summary of these studies, which support the use of bumetanide in neurodevelopmental disorders such as ASD, is provided in Table 1.

The activity of bumetanide on the GABAergic system is often studied with brain slice preparations. Bumetanide was found to convert the depolarization to a hyperpolarization and a powerful inhibition [35,36,40]. Bumetanide also reduced the duration required for a neuron to recuperate the chloride levels after a strong stimulation by GABA and a large chloride influx. These effects were absent in mice lacking the NKCC1 gene indicating that NKCC1 is directly responsible for these effects [20,35].

With regard to epilepsy, many pharmacodynamics studies support the inhibitory effect of bumetanide on epileptiform activity in brain slices. Bumetanide was found to suppress epileptiform activity in hippocampal slices [35,43] and to block epileptic seizures in the neocortex [37,38]. In addition, bumetanide ameliorated the interictal-like events that were aggravated by phenobarbital [20] and reduced ictal activity in a status epilepticus model [49]. In an *in vivo* febrile seizure model, bumetanide rescued the development of epilepsy and prevented granule cell ectopia and limbic seizure susceptibility [42]. Recent studies demonstrate that bumetanide treatment *in vivo* prevents abnormalities in coherence and voltage correlation and it normalizes seizure threshold, which were induced by early-life seizures [47]. The drug also significantly normalized cortical network hyperactivity in a genetic epilepsy model [48]. Lastly, recent studies demonstrated that bumetanide restored synaptic plasticity and memory in a Down syndrome model [50] and enhanced memory in a febrile seizure model [51].

With regard to ASD, the actions of bumetanide were studied ex vivo on two models of autism [13]. In both models, immature hippocampal neurons recorded during the first weeks postnatally have indeed a higher concentration of chloride and depolarizing actions of GABA than match-aged control animals. These studies provide substantial confirmation that the

regulation of intracellular chloride is mal operative in autism in the hippocampus. Moreover, Tyzio and colleagues recently found significant reduction of intracellular chloride levels in cortical neurons of two animal models in slices Fragile X and in utero valproate [13].
They also demonstrated that maternal pre-treatment with bumetanide restored gamma oscillations and other physiological correlates in the VPA rat offspring.

Bumetanide appears to be an efficient drug to enhance GABAergic inhibition in a variety of pathological conditions. Taken together, these studies show that 30 years after the registration of bumetanide as a diuretic, this molecule also belongs to the class of psychotropic agents.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Bumetanide toxicity has been extensively assessed in several animal species: [88] in rats, rabbits, dogs and monkeys. Doses of 10-140 mg/kg/day in rats, 0.1-1.2 mg/kg in dogs and 0.1-5 mg/kg/day in monkeys were tested. Long term toxicity studies are available in 3 animal species: rat (13-26-78 weeks), dog (4-26-52 weeks) and baboons (26 weeks). These studies indicate that bumetanide is safe and that toxicity is mostly related to the diuretic activity of the compound and is similar to that reported for other loop diuretics such as furosemide. No severe toxicity or deterioration of clinical condition was apparent. In most repeated dose studies decreased body weight was recorded. Serum and urine chemistry revealed decreases in electrolytes, which were noted in all tested species. No effects on total blood counts examinations were observed, eg neutropenia, low platelets nor anemia.

Long-term effects of bumetanide were studied in the rat and the dog, while larger dosages of up to 140 mg/kg per day have been used in rat studies as compared to lower doses in dog studies. This discrepancy is explained by a rapid metabolism in rats [89]. In contrast, bumetanide is excreted largely unchanged in the dog and in man [90]. The rat and dog are the two species on which most of the preclinical toxicological evaluation of bumetanide was performed due their similar excretion and metabolism mechanism of this drug in comparison to humans.

The kidneys are the most likely organ for toxicity. Distribution studies in dogs have shown a high kidney exposure to bumetanide. Kidney changes were seen at the microscopic examination in all treatment groups in one 13-week study in male rats. So far, no toxic effects on the CNS have been noticed in short-term and long-term tests. No teratogenic potential was shown in various rodent species, apart from a slight embryo lethal effect was reported in rats and rabbits. There was no bumetanide related effect on fertility and peri-post-natal development in rats. The genotoxic potential of bumetanide has been re-evaluated according to current ICH standards. No evidence of genotoxicity was found. The predictability of the toxicity studies with bumetanide is good: the adverse effects experienced by patients with fluid retention are also mostly related to its diuretic activity.

e. Analysis of potential effect

Several studies have assessed the safety and efficacy of bumetanide in adults with congestive heart failure or renal disease; Table 6 summarizes the safety data. These studies

show that bumetanide is generally well-tolerated and that AEs were related to the diuretic activity of the drug. A review based on 58 clinical studies, including a total of 493 patients, studied the adverse reactions to bumetanide [91]. Results were compared with adverse reactions of 220 patients treated with furosemide. Both agents were administered to patients with edema secondary to congestive heart failure, and renal and hepatic disease. The adverse reactions were listed according to the organ system involved. Those found to have occurred with the greatest frequency involved the special sense organs (i.e., impaired hearing, vertigo) and the skin (i.e., rash, pruritus, hives, sweating) and musculoskeletal (i.e., muscle weakness, cramps, arthritic pain) systems.

Table 6. Long term safety of Bumetanide in adults with fluid retention

Study ID	Sample characteristics	Treatment/dose	Main adverse effects
Handler et al., 1981[92]	34 Patients (age range: 48-81; *M*=63; *SD*=3.5) with oedema due to congestive heart failure	Bumetanide orally once or twice daily. Total daily dosage *M*=2.3 mg; range: 1-6 mg/day; for 6 months	Moderately severe recurrent urticaria (*n*=1), abdominal cramp (*n*=1), weight loss, uric acid increase and electrolyte imbalance
Konecke, 1981[93]	31 Patients (age range: 50-85; *M*=62.5) with oedema due to congestive heart failure	Bumetanide range: 1-12 mg/day; for 1-24 months	Variable minor changes in serum sodium, potassium, chloride and uric acid (generally remained within normal limits); no major side effects
Dixon et al., 1981[94]	21 Patients (age *M*=60; *SD*=1.8) with peripheral oedema due to congestive heart failure	Bumetanide 1.0 mg tablets, maximum dosage of 10 mg/day; for 1 week-18 months	Variable minor changes in serum potassium, chloride and uric acid
Whelton, 1981[95]	18 Patients (age range: 22-72; *M*=48) with oedema due to renal disease	Bumetanide range: 1-18 mg/day; *M*=4.2 mg/day; for 1 week-30 weeks	Increase in uric acid in all patients, significant potassium decrease from baseline after 1 week (non-significant thereafter), weight loss, no abnormalities on physical examination

Precautions and safety alerts

The SPC (see Appendix 1) reports potential side effects that can occur, which can be traced back to the diuretic effect of bumetanide:

Hypovolemia: Especially in elderly, hypovolemia can occur, which can cause headaches, dry mouth, dehydration, impaired vision, dizziness, orthostatic hypotension, syncope and a tendency for thrombosis.

Sodium deficiency:

In a strict salt-free diet, individuals should take into account a possible resulting NaCl-deficiency, which can lead to a reduced effect of diuretics and an increase in potassium excretion. Such a salt deficiency can manifest itself in calf cramps, loss of appetite, weakness, dizziness, drowsiness, vomiting or confusion.

Potassium deficiency:

Potassium deficiency can manifest as neuromuscular symptoms (muscle weakness, paresis), intestinal symptoms (vomiting, constipation, meteorism), renal symptoms (polyuria) or cardiac symptoms. Severe potassium loss may result in confusion or paralytic ileus, which may result in coma.

Magnesium deficiency:

Magnesium deficiency which rarely results in tetany and cardiac arrhythmias.

Calcium deficiency:
Bumetanide can reduce calcium levels in the blood.

Asymptomatic hyperuricemia has been observed in some patients. When patients with severe chronic renal failure are intensively treated, painful convulsions and an increase in amylase serum levels may occur. Skin rash (exanthema, erythema multiforme) and a change in the blood count, particularly thrombocytopenia, probably caused by bumetanide, has occurred in a few cases. Abdominal complaints have been reported occasionally. Hearing loss has been reported but could not be confirmed with certainty by audiometry

See section 4.4 of the SPC (Appendix 1) for a description of special warnings and precautions for use.

With regards to the specific interaction with other medications commonly used by epilepsy patients, no reports exist in Dutch pharmacovigilance registries on negative or adverse interactions between antiepileptic drugs, antipsychotic drugs or other psychoactive medications. Bumetanide and carbamazepine or oxcarbazepine is a possible interaction for which caution is warranted in Dutch practice because both drugs are associated with increased risk for hyponatremia. The mechanisms by which these AED or bumetanide cause hyponatremia are different and in principal unrelated. Bumetanide can stimulate renal excretion while carbamezine and oxcarbazepine can cause volume through increased sensitivity for tubular ADH (antidiuretic hormone). These effects should cancel each other out but there are casuistic reports on possible synergistic effects [64].

For this study we acquired expertise advise of our clinical pharmacist. The risk of the occurrence of hyponatremia is small in these instances of concurrent use of these AEDS and bumetanide. The planned safety checks of electrolytes are in their opinion sufficient to monitor possible effects of hyponatriemia. The relatively highest risk for hyponatremia is expected after start of treatment, which is covered by the frequent blood withdrawals in the first weeks of the treatment phase. It should also be noted that in this study dosages of AEDs will be maintained stable from one month prior to the study to the end of the washout phase and that we will detect hyponatremia through AEDs in the screening phase through the electrolyte checking and established hyponatreamie is an exclusion criterion (4.3).

Tolerability and safety in new-borns and children with edema or seizure
Bumetanide has been used in preterm and full term new-borns to treat fluid volume overload due to cardiac or pulmonary diseases. Despite limited publications, there is substantial evidence to show that pediatric populations respond to diuretics such as bumetanide in a manner comparable to adults. The main differences are related to pharmacokinetic parameters in new-borns where NKCC1 co-transporter activity is still emerging in the kidneys.

Reports on the use of bumetanide in children or adolescents are scarce. Most of prescriptions are off-label in this age group. Table 3 (and part of Table 4 [57,60]) summarizes the main published studies [61-63]. Available studies indicate that acute or repeated

administrations (i.e., several weeks) of bumetanide at dosages between 0.06 and 7.2 mg in new-borns, toddlers and children result in a significant diuretic activity associated with loss of electrolytes. Despite the lack of direct comparisons between the age groups, the effect of bumetanide is anticipated to be similar in children above 2 years of age and adults.

Recently, a trial tested the use of bumetanide for treatment of neonatal seizures ("NEMO" trial) [60]. In this study, the dose and feasibility of intravenous bumetanide was tested as an add-on to phenobarbital for treatment of neonatal seizures. In this open-label, dose finding, and feasibility phase 1/2 trial, full-term infants were recruited younger than 48 h who had hypoxic ischemic encephalopathy and electrographic seizures, and who were not responding to a loading-dose of phenobarbital. New-born babies were allocated to receive an additional dose of phenobarbital and one of four bumetanide dose levels, by using a bivariate Bayesian sequential dose-escalation design to assess safety and efficacy. No short-term dose-limiting toxic effects were observed, although three out of 11 surviving infants had hearing impairment, confirmed on auditory testing between 17 and 108 days of age. The most common non-serious adverse reactions were moderate dehydration in one, mild hypotension in seven, and mild to moderate electrolyte disturbances in 12 infants. The trial was stopped early because of possible increased risk for hearing loss. A causal link between bumetanide and hearing loss was refuted on the basis of the occurrence in 3 of 11 children and the possibility of interaction with aminoglycosides, which had been given to 2 of the 3 children in which hearing loss was established. Following this study, researchers of the same consortium conducted a Pilot evaluation of the population pharmacokinetics of bumetanide in term new-born infants with seizures [96]. In this study, 14 infants were included, of which 13 were being cooled (a common intervention in this disorder). No relationship was found between bumetanide exposure and its efficacy (reduction in seizure burden) or its toxicity (hearing loss).

Tolerability and safety in children with ASD

Three open-label pilot studies [52,54,56], three single case reports [53,55,59] and a double-blind randomized controlled study [6] have assessed the safety and efficacy of bumetanide in children diagnosed with neurodevelopmental disorders (i.e., ASD and Fragile-X syndrome). Main findings and reported adverse events of these studies are described in Table 2.

The most important data for safety and tolerability of bumetanide in ASD, comes from the RCT described in 13.1b [6]. Out of the 60 children initially included in the study, six patients (*n*=3 in each group) withdrew shortly after the start: two patients due to enuresis (*n*=1 in each group); two patients due to agitation-related behavior as a consequence of risperidone/methylphenidate discontinuation (*n*=1 in each group); one patient (bumetanide group) due to hypokalemia and one patient (placebo group) due to eczema. All other patients (*n*=54 in each group) completed 90 days of treatment. During the trial, six bumetanide-treated children developed a mild hypokalemia, which was treated with potassium-gluconate syrup. Bumetanide treatment seemed well-tolerated. Furthermore, bodyweight and sodium were not altered and no dehydration was observed.

f. Pharmacokinetic considerations

Absorption

Bumetanide is well absorbed after oral administration with a bioavailability reaching between 80 and 95% (see section 5.2 of the SPC in Appendix 1). The elimination half-life ranges from between 0.75 to 2.5 hours. No active metabolites are known. Bumetanide is poorly distributed due to high protein binding. Renal excretion accounts for approximately half the clearance with hepatic excretion responsible for the other half. Several studies report lower clearance of bumetanide in new-borns compared to adults due to incomplete kidney maturation, which increases proportionally with postnatal age [75,97-102]. There is an expected increase in half-life and a reduced plasma clearance in the presence of renal or hepatic disease. Peak plasma concentrations in healthy individuals following oral doses of 0.5 to 2.0 mg are dose related and occur after 0.5-2 hours [103].

Metabolism
Bumetanide is partially metabolized by liver and kidney [101]. Major urinary metabolite is the 3′-alcohol derivative. The major hepatic metabolite is the 2′-alcohol derivative. Minor metabolites include the 4′-alcohol, N-desbutyl, and 3′-acid derivatives. Metabolites in urine and bile are present as conjugates, principally glucuronide conjugates. Conjugates of bumetanide and its metabolites do not appear in faeces.

Elimination
Bumetanide is eliminated with half-lives ranging from 0.4 to 2.6 h depending on the modus of administration. Bumetanide and its metabolites are for about 70-80% excreted in urine and 50% unchanged; about 10-20% in feces, almost completely as metabolites apparently via biliary elimination [104-106].

Pharmacokinetic studies with furosemide or bumetanide have shown that new-borns do not eliminate loop diuretics efficiently due to decreased renal and metabolic clearance. As stated within the CHMP "Concept paper on the impact of renal immaturity when investigating medicinal products intended for pediatric use" (CPMP/PEG/35132/03), the kidney reaches maturity until between one and two years of age. The paper highlights that adult levels of glomerular filtration rate (GFR) (120 ml/min/1.73 m2) are reached between one and two years of age. This suggests that the pharmacokinetics of bumetanide in children (2-11 years) is close to that observed in adult. Due to the targeted age range (i.e., 6-15 years old), we do not expect major changes in the pharmacokinetic parameters of bumetanide 0.5 mg/tablet compared with adults receiving 1 mg of bumetanide.

g. Study population

Children with ASD, ADHD and/or epilepsy, between 5 and 15 years of age and an IQ> 55. Children at the participating sites (i.e., Utrecht and Groningen) will be included in the study after written informed consent. Yearly in total 200 new eligible patients are seen across the four sites. In addition, the participating sites together govern a prevalent population of 800 children with ASD in the intended age and IQ range.

h. Interaction with other products

Drugs with ototoxic potential: the use of bumetanide in patients that receive aminoglycoside antibiotics like amikacin (Amikin), streptomycin, neomycin, gentamicin (Garamycin),

erythromycin (E-Mycin, Eryc), kanamycin (Kantrex), tobramycin (Nebcin), netilmycin (Netromycin), vancomycin (Vancocin) should be avoided. In life-threatening conditions, bumetanide treatment will be stopped and the patients withdrawn from the study.

The use of non-steroidal anti-inflammatory drugs should also be avoided.

Other drugs can be avoided including digitals, antihypertensive agents, indomethacin, probenecid, acetazolamide, Lithium and drugs known to have a nephrotoxic potential. These medicines cannot be prescribed during the study due to the inclusion and exclusion criteria (see the study protocol for more details).

Cytochromes P450 involved in bumetanide metabolism have not been identified.

i. Predictability of effect

It has been shown that GABA excites immature neurons instead of inhibiting them because of elevated intracellular chloride that reverse the polarity of GABA actions and an abrupt shift leads to an excitatory/inhibitory shift of GABA actions [36]. It was further shown that the shift is abolished in a variety of brain disorders, including 2 animal models of ASD [13]. This effect has been related to reduction of intracellular chloride thereby reinstalling physiological actions of GABA. In these animal models, bumetanide also reduced aberrant brain oscillations and “autistic” behavior. Consequently, it was shown that bumetanide produces an improvement of hyperexcitable behaviors measured by CARS and CGI in children with ASD (3-11 years old) in a double blind randomized study, with little or no side effects [6]. A favorable effect on behavioral and cognitive functioning was further confirmed in our case study [59].

The ABC-I is chosen as the primary endpoint of this study as this questionnaire is an extensively validated measure of behavioral problems in neurodevelopmental disorders [34] and it measures behavior related to hyperexcitability. Besides that, it is the most commonly used outcome scale in (neuro)behavioral trials [7].

j. Can effects be managed?

There is no specific antidote or antagonist for bumetanide.

13.2 **Synthesis**

Here, we propose to conduct a large, multicenter, placebo-controlled randomized trial testing bumetanide plus usual care treatment versus placebo plus usual care in 172 children with ASD,ADHD and/or epilepsy. With this design, we expect to confirm effectiveness of bumetanide as cheap, safe and rational treatment option for an important subset of neurodevelopmental disorders and to facilitate rapid implementation of this treatment in Dutch clinical guidelines for ASD treatment.

At present, care as usual has no effect on autism morbidity. As ASD can be very invalidating, it is associated with high healthcare and high societal costs. Over the past 20 years, a variety of therapies have been proposed to improve the symptoms associated with ASD [1,7]. Only two

antipsychotics, aripiprazole and risperidone, marketed as Abilify and Risperdal, respectively, are approved to treat symptoms that often accompany autism, such as hyperactivity and irritability[7]. These drugs also have severe side effects, including obesity, hypertension, and diabetes type II. Given the lack of drug treatments, behavioral and developmental interventions are currently the predominant treatment approach for promoting social, adaptive and behavioral function in children with ASD based on efficacy demonstrated in empirical studies. The primary goals of these treatments are to minimize the core features and associated deficits, maximize functional independence and quality of life, and alleviate family distress. Facilitating development and learning, promoting socialization, reducing maladaptive behaviors, and educating and supporting families can help accomplish these goals. However, none of these treatments targets the underlying neurobiological defects of ASD. In the last decade, there have been major investments to develop etiology driven treatments for ASD, but all of these attempts have failed so far. Bumetanide may become the first potential rational drug treatment to reduce ASD morbidity. Bumetanide is a very cheap treatment, with the potential to positively change cognitive, behavioral, societal and educational problems that patients encounter. In contrast with other existing treatments, the application of bumetanide is etiologically driven and will not affect the central nervous system in neurons in which chloride homeostasis is unaffected.

Hypotheses:
1. 91 days of (add-on) treatment with bumetanide will effectively reduce sensory hyperreactivity-related behavioral symptoms and improve day-to-day functioning in comparison to baseline measurements.
2. Efficacy of bumetanide is associated with clinical and comorbidity parameters related to hyperexcitable networks in patients with ASD, ADHD and/or epilepsy.

We propose that available toxicology reports are sufficient to allow conclusion on the relationship of the toxic effects and treatment with bumetanide in the new target population: patients with ASD, ADHD and/or epilepsy. Bumetanide is well tolerated, independent of the age group. The main adverse events are related to the diuretic activity of the molecule leading to a decrease in electrolytes. To reduce this risk, the study excludes patients with electrolyte disturbances. In addition, extensive blood laboratory tests will be conducted throughout the trial to assess blood electrolyte levels. Hypokalemia is frequently reported. In close collaboration with Dr. M.G. Keijzer-Veen, a pediatric nephrologist, a blood analysis protocol is developed to reduce the risk of hypokalemia events. These blood analyses will be carried out at six time points throughout the study (i.e., at Screening, D4, D7, D14, D28 and D56) and will be supervised by the pediatric nephrologist.

It is reported that oral or intravenous bumetanide (0.5-2 mg) produces a dose related increase in diuresis, sodium, potassium and chloride excretion [65]. The comparison with the use of bumetanide for conditions of fluid overload [61-63] [92-95] shows that the proposed dosage for children and adolescents with neurodevelopmental disorders is at the low end of the standard range of bumetanide.

In summary, the rationale for the application and dosage of bumetanide is based upon the following elements. The dose range proposed for the treatment of children and adolescent with ASD is similar to the treatment of fluid retention. Pharmacokinetics of bumetanide in children and adolescents with mature glomerular filtration should not be different from the

adults. The mechanism of action of bumetanide is well-defined and its successful application for ASD and ASD with comorbid epilepsy could mean the first rational treatment for behavioral problems in these devastating disorders. The Dutch paediatric formulary of medications recommends a starting dose between 0.01-0.1/kg/dose and 0.2 mg/kg/dose with a maximum dosage of 10 mg/day. Our proposed dosage is well within this range. The risk of common adverse events is reduced by our strict exclusion criteria and the extensive blood monitoring throughout the entire trial.

We expect that bumetanide as add-on treatment enhances the therapeutic effect of AED and reduces the behavioral effects caused by hyperexcitable brain circuitry. Therefore, patients can continue their regular AED treatment whilst enrolling in this study. This possibility lowers the risk of seizure or behavioural aggravation due to discontinuation of these drugs.

In conclusion, based on the preclinical and clinical data, we consider that the risk-benefit relationship of bumetanide allows the initiation of phase IIb studies in ASD, ADHD and/or epilepsy children and adolescents within the dose range. The use of bumetanide as add-on treatment for these patients is a rationale and feasible option, as bumetanide is a safe and well tolerable drug for children with limited side effects.

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APPENDIX 1: Summary of Product Characteristics (in Dutch)

Samenvatting van de productkenmerken

1. NAAM VAN HET GENEESMIDDEL
Bumetanide CF 1 mg, tabletten
Bumetanide CF 2 mg, tabletten
Bumetanide CF 5 mg, tabletten

2. KWALITATIEVE EN KWANTITATIEVE SAMENSTELLING
Bumetanide CF 1, 2 en 5 mg, tabletten bevatten respectievelijk 1, 2 en 5 mg bumetanide per tablet.
Voor de volledige lijst van hulpstoffen, zie rubriek 6.1.

3. FARMACEUTISCHE VORM

Tabletten

Bumetanide CF 1 mg, tabletten
Witte tot nagenoeg witte tablet. De tablet is vlak aan de ene zijde en heeft een breukstreep aan de
andere kant met een opdruk “1” boven en “BMT” onder de breukstreep.

Bumetanide CF 2 mg, tabletten
Witte tot nagenoeg witte tablet. De tablet is vlak aan de ene zijde en heeft een breukstreep aan de
andere kant met een opdruk “2” boven en “BMT” onder de breukstreep.

Bumetanide CF 5 mg, tabletten
Witte tot nagenoeg witte tablet. De tablet is vlak aan de ene zijde en heeft een breukstreep aan de
andere kant met een opdruk “5” boven en “BMT” onder de breukstreep.

De tablet kan verdeeld worden in gelijke doses.

4. KLINISCHE GEGEVENS

4.1 Therapeutische indicaties
Acuut longoedeem en oedeem ten gevolge van decompensatio cordis, levercirrose, nefrotisch syndroom en geneesmiddelen. Diuretische therapie bij chronische nierinsufficiëntie. Geneesmiddelintoxicaties waarbij geforceerde diurese is gewenst.

4.2 Dosering en wijze van toediening
Doorgaans kan iedere ochtend 0,5 tot 1 mg gegeven worden. Afhankelijk van de reactie van de patiënt kan de dosering om de 6-8 uur worden herhaald. In hardnekkige gevallen kan de dosering worden verhoogd tot een bevredigend resultaat wordt verkregen. De onderhoudsdosering dient, wanneer mogelijk, te worden teruggebracht tot minder dan de aanvangsdosering.
Het zal zelden nodig zijn om meer dan 4 mg per dag toe te dienen.

Oedeem bij nefrotisch syndroom:
Indien met de gebruikelijke lage dosering onvoldoende effect wordt bereikt kan de dosering worden verhoogd tot 2-5 mg, indien nodig iedere 6-8 uur te herhalen.

4.3 Contra-indicaties

Precomateuze leverfunctieafwijkingen.
Ernstige elektrolytendepletie, met name kaliumdeficiëntie.
Acute nefritis en acute nierinsufficiëntie, gepaard met anurie.

4.4 Bijzondere waarschuwingen en voorzorgen bij gebruik

Bij patiënten, die gedurende langere tijd of met hoge doses worden behandeld, is het aan te bevelen regelmatig het elektrolytengehalte (met name natrium, magnesium en kalium) te bepalen, vooral van patiënten die lijden aan andere ziekten (bijvoorbeeld levercirrose) of bij gelijktijdige behandeling met andere medicijnen (waaronder protonpompremmers) die geassocieerd worden met het ontstaan van hyponatriëmie, hypomagnesiëmie of hypokaliëmie (zie rubriek 4.5). Bij patiënten met een zoutarm dieet kan een NaCl-tekort ontstaan.
Bij gelijktijdige behandeling met corticosteroïden, bij eenzijdige voeding en bij misbruik met laxeermiddelen dient rekening gehouden te worden met het ontstaan van hypokaliëmie. Het verdient aanbeveling om altijd, maar zeker bij hogere doseringen en bij patiënten met een nierfunctiestoornis de kaliumconcentratie van het plasma regelmatig te controleren en zo nodig een aanvullende kaliumtherapie te geven. De eerste keus in de genoemde gevallen is de toediening van een kaliumsparend diureticum. Dit is in bijzonder van belang bij een gelijktijdige behandeling met digitalisglycosiden, omdat een kaliumtekort de verschijnselen van digitalisintoxicatie kan provoceren of verergeren. De symptomen van kaliumtekort zijn zwakte, duizeligheid, lethargie, beenkrampen, gebrek aan eetlust, braken of mentale verwardheid.
Bij patiënten met ernstige decompensatio cordis dient de behandeling onder strenge controle en met de nodige terughoudendheid te geschieden.
Evenals dat het geval is bij andere diuretica, kan een ongunstige beïnvloeding van de koolhydraatstofwisseling optreden waardoor een bestaande koolhydraatintolerantie of diabetes mellitus kunnen verergeren. Bij diabetici en patiënten met een mogelijke latente diabetes dient regelmatig het glucosegehalte in urine en bloed te worden gecontroleerd.
Een toename van het urinezuur gehalte in het serum is mogelijk, men dient daarom voorzichtig te zijn met patiënten die ooit hyperurikemie hebben gehad.
Een therapie met diuretica kan reeds bestaande symptomen van urinewegobstructie verergeren (bijv. bij patiënten met prostaathypertrofie).
Met name bij bejaarden dient men voorzichtig te beginnen en in het begin van de behandeling laag te doseren, omdat abrupte diurese kan leiden tot hypovolemie en dientengevolge tot symptomen van circulatoire insufficiëntie.
Sterke diurese bij een gestoorde nierfunctie kan een reversibele nierfunctievermindering veroorzaken.
Een adequate vochttoevoer is bij dergelijke patiënten noodzakelijk. Indien het ureumgehalte in het bloed aanzienlijk toeneemt of oligurie ontstaat is staken van de behandeling gewenst.
Bumetanide dient - tot er meer ervaring in de pediatrie mee is verkregen - niet aan kinderen te worden toegediend.
Bumetanide bevat lactose. Patiënten met zeldzame erfelijke aandoeningen als galactose-intolerantie, Lapp lactasedeficiëntie of glucose-galactose malabsorptie, dienen dit geneesmiddel niet te gebruiken.

4.5 Interacties met andere geneesmiddelen en andere vormen van interactie

Bumetanide kan het effect van antihypertensiva versterken. Een verlaging van de dosering hiervan kan gewenst zijn indien Bumetanide wordt gebruikt voor de behandeling van oedeem bij hypertensie.

Gelijktijdige behandeling met corticosteroïden of misbruik van laxeermiddelen kan hypokaliëmie doen ontstaan (zie rubriek 4.4). Bij gelijktijdig gebruik van digitalisglycosiden kan hypokaliëmie digitalisintoxicatie provoceren of verergeren (zie rubriek 4.4).
Bumetanide kan de uitscheiding van lithiumionen afremmen, zodat bij gelijktijdige behandeling met lithiumzouten de kans bestaat op een lithiumintoxicatie.
Regelmatige controle van de lithiumconcentratie in het plasma is dan noodzakelijk.
De bloeddrukverlagende werking van bumetanide wordt tegengegaan door acetylsalicylzuur, indometacine en vermoedelijk ook de meeste andere antiphlogistica.
De nefrotoxische en ototoxische werking van aminoglycoside-antibiotica kan bij gelijktijdig gebruik van bumetanide worden versterkt. De optredende gehoorstoornissen kunnen irreversibel zijn.Gelijktijdige toediening dient derhalve beperkt te blijven tot vitale indicaties.
Bij gelijktijdig gebruik met cefaloridine kan nierbeschadiging optreden, vooral bij patiënten met nierinsufficiëntie.
Gelijktijdig gebruik van bumetanide (parenteraal) en cisplatinum kan leiden tot beschadigingen aan het gehoor die soms irreversibel zijn.

4.6 Vruchtbaarheid, zwangerschap en borstvoeding

Over het gebruik van bumetanide bij zwangerschap bij de mens bestaan onvoldoende gegevens om de mogelijke schadelijkheid te kunnen beoordelen. In dierexperimenteel onderzoek werden geen teratogene effecten gezien. Diuretica mogen slechts op zeer strikte indicatie worden toegepast in de zwangerschap. Zij kunnen de placentaire doorbloeding verminderen: bij toepassing in de zwangerschap dienen hematocriet, elektrolyten en intra-uteriene groei gecontroleerd te worden. Ook kunnen diuretica na gebruik in de zwangerschap bij de neonaat de elektrolytenbalans verstoren. Het is niet bekend of bumetanide overgaat in de moedermelk. Gebruik van bumetanide tijdens lactatie wordt daarom afgeraden.

4.7 Beïnvloeding van de rijvaardigheid en het vermogen om machines te bedienen

Aangezien in incidentele gevallen hypovolemie kan optreden, aanleiding gevend tot onder andere duizeligheid en verminderd gezichtsvermogen, kan vooral bij het begin van de behandeling het vermogen om deel te nemen aan het verkeer of gebruik te maken van machines nadelig beïnvloed worden.

4.8 Bijwerkingen

In de loop van de behandeling kunnen complicaties optreden, welke terug te voeren zijn op de diuretische werking.

Hypovolemie:
Vooral bij bejaarden kan hypovolemie optreden, die de oorzaak kan zijn van hoofdpijn, droge mond, uitdroging, verminderd gezichtsvermogen, duizeligheid, orthostatische hypotensie, syncope en neiging tot trombose.

Natrium-deficiëntie:
Bij een streng zoutloos dieet dient men rekening te houden met een eventueel daaruit resulterende NaCl-deficiëntie, hetgeen kan leiden tot verminderde werking van diuretica en toename van de kaliumuitscheiding. Een dergelijk zoutgebrek kan zich uiten in kuitkrampen, gebrek aan eetlust, gevoel van zwakte, duizeligheid, slaperigheid, braken of verwardheid.

Kaliumdeficiëntie:
Kaliumdeficiëntie kan zich manifesteren als neuromusculaire symptomen (spierzwakte, paralyse), intestinale symptomen (braken, constipatie, meteorisme), renale symptomen

(polyurie) of cardiale symptomen. Ernstig kaliumverlies kan resulteren in paralytische ileus of verwardheid, welke kan resulteren in coma.

Magnesium-deficiëntie:
Magnesium-deficiëntie welke zeer zelden resulteert in tetanie en hartritmestoornissen.

Calcium-deficiëntie:
Bumetanide kan de calciumspiegel in het bloed verlagen.

Asymptomatische hyperurikemie is bij sommige patiënten waargenomen. Bij een intensieve behandeling van patiënten met ernstige chronische nierinsufficiëntie kunnen pijnlijke spierkrampen en stijging van het amylasegehalte van het serum ontstaan. Huiduitslag (exantheem, erythema exsudativum multiforme) en een verandering in het bloedbeeld, met name trombocytopenie, waarschijnlijk veroorzaakt door bumetanide, is in enkele gevallen voorgekomen. Buikklachten zijn een enkele keer gemeld. Gehoorverlies is gemeld maar kon niet met zekerheid door audiometrie worden bevestigd.

Melding van vermoedelijke bijwerkingen
Het is belangrijk om na toelating van het geneesmiddel vermoedelijke bijwerkingen te melden. Op deze wijze kan de verhouding tussen voordelen en risico's van het geneesmiddel voortdurend worden gevolgd. Beroepsbeoefenaren in de gezondheidszorg wordt verzocht alle vermoedelijke bijwerkingen te melden via het Nederlands Bijwerkingen Centrum Lareb, website: www.lareb.nl.

4.9 Overdosering

De symptomen bij overdosering zijn gerelateerd aan het farmacologisch effect van bumetanide, namelijk versterkte natriurese en diurese met als gevolg acute hypotensie, dehydratie, hyponatriëmie en hypokaliëmie met de daarbij behorende symptomen. Er bestaan geen specifieke maatregelen. Na ingestie van een grote hoeveelheid tabletten kan men overwegen de patiënt te laten braken of de maag te spoelen. Ondersteunende maatregelen bestaan uit het herstellen van de vocht- en elektrolytenbalans.

5. FARMACOLOGISCHE EIGENSCHAPPEN

5.1 Farmacodynamische eigenschappen

Bumetanide is een krachtig lisdiureticum met een snelle en korte werking. Farmacologische en klinische proeven hebben aangetoond, dat de diuretische werking van 1 mg bumetanide overeenkomt met ongeveer 40 mg furosemide. Er moet echter wel rekening gehouden worden met verschil in individuele reacties. Het diuretisch effect van bumetanide is dosisafhankelijk. Patiënten die niet reageren op een lage aanvangsdosering reageren gewoonlijk als de dosis wordt verhoogd. Bumetanide blijkt het grootste effect uit te oefenen op het proximale deel van de lis van Henle, maar kan tevens een additionele werking hebben op de proximale tubulus.

De diurese begint 30 minuten na orale toediening, terwijl maximaal effect na 1 tot 2 uur wordt bereikt. Het diuretisch effect is praktisch voltooid binnen 4-6 uur. Na intraveneuze toediening begint de diurese binnen enkele minuten en houdt doorgaans na ongeveer 2 uur op.

5.2 Farmacokinetische eigenschappen

Bumetanide wordt goed geabsorbeerd na orale toediening met een biologische beschikbaarheid tussen de 80 en 95%. Bumetanide wordt voor ongeveer 50% door de nier en voor ongeveer 50% door de lever geklaard. Bij lever- of nierziekte neemt de

eliminatiehalfwaardetijd toe. De halfwaardetijd varieert tussen de 40-150 minuten. Er zijn geen actieve metabolieten bekend.

5.3 Gegevens uit preklinisch veiligheidsonderzoek

Geen bijzonderheden.

6. FARMACEUTISCHE GEGEVENS

6.1 Lijst van hulpstoffen

Microkristallijne cellulose (E460), lactose monohydraat, magnesiumstearaat (E470b), maïszetmeel, natriumlaurylsulfaat.

6.2 Gevallen van onverenigbaarheid

Niet van toepassing.

6.3 Houdbaarheid

Bumetanide CF 1 mg: blister: 48 maanden – flacon: 60 maanden
Bumetanide CF 2 mg: blister: 36 maanden – flacon: 60 maanden
Bumetanide CF 5 mg: blister: 48 maanden – flacon: 60 maanden

6.4 Speciale voorzorgsmaatregelen bij bewaren

Voor dit geneesmiddel zijn er geen speciale bewaarcondities.

6.5 Aard en inhoud van de verpakking

- Al/Al of Al/PVC/PVDC blisterverpakking: 20, 28, 30, 50, 56, 60, 84 en 120 tabletten.
- Polypropyleen pot met polyethyleen deksel: 200 tabletten.

Niet alle genoemde verpakkingsgrootten worden in de handel gebracht.

6.6 Speciale voorzorgsmaatregelen voor het verwijderen

Geen bijzondere vereisten.

7. HOUDER VAN DE VERGUNNING VOOR HET IN DE HANDEL BRENGEN

Centrafarm B.V.
Nieuwe Donk 3
4879AC Etten-Leur
Nederland

8. NUMMERS VAN DE VERGUNNING VOOR HET IN DE HANDEL BRENGEN

RVG 23140 Bumetanide CF 1 mg, tabletten
RVG 24313 Bumetanide CF 2 mg, tabletten
RVG 23141 Bumetanide CF 5 mg, tabletten

9. DATUM VAN EERSTE VERLENING VAN DE VERGUNNING/HERNIEUWING VAN DE

VERGUNNING

Bumetanide CF 1 mg, tabletten: 22 maart 1999
Bumetanide CF 2 mg, tabletten: 7 september 1999
Bumetanide CF 5 mg, tabletten: 22 maart 1999

10. DATUM VAN HERZIENING VAN DE TEKST

Laatste gedeeltelijke wijziging betreft rubriek 4.4: 2 februari 2016

APPENDIX 2: Package leaflet (in Dutch)

BIJSLUITER: INFORMATIE VOOR DE GEBRUIKER

Bumetanide CF 1 mg, tabletten
Bumetanide CF 2 mg, tabletten
Bumetanide CF 5 mg, tabletten

Bumetanide

Lees goed de hele bijsluiter voordat u dit geneesmiddel gaat gebruiken want er staat belangrijke informatie in voor u.
- Bewaar deze bijsluiter. Misschien heeft u hem later weer nodig.
- Heeft u nog vragen? Neem dan contact op met uw arts of apotheker.
- Geef dit geneesmiddel niet door aan anderen, want het is alleen aan u voorgeschreven. Het kan schadelijk zijn voor anderen, ook al hebben zij dezelfde klachten als u.
- Krijgt u last van een van de bijwerkingen die in rubriek 4 staan? Of krijgt u een bijwerking die niet in deze bijsluiter staat? Neem dan contact op met uw arts of apotheker.

Inhoud van deze bijsluiter

1. WAT IS BUMETANIDE CF EN WAARVOOR WORDT DIT MIDDEL GEBRUIKT?

Bumetanide behoort tot de groep geneesmiddelen die lisdiuretica heten. Het is een vochtafdrijvend geneesmiddel (plastablet) met een snelle en korte werking.

Bumetanide tabletten worden toegepast bij de behandeling van acuut longoedeem (het plotseling vasthouden van vocht in de longen) en van oedemen (het vasthouden van lichaamsvocht), veroorzaakt door hart, lever- of nierziekte of door geneesmiddelen. Zij kunnen ook gebruikt worden om diurese (een verhoging van de urineproduktie) op te roepen in geval van overdosering van een ander geneesmiddel. Bumetanide kan ook worden voorgeschreven als vochtafdrijvende therapie bij langdurig onvoldoende functioneren van de nieren.

2. WANNEER MAG U DIT MIDDEL NIET GEBRUIKEN OF MOET U ER EXTRA VOORZICHTIG MEE ZIJN?

Wanneer mag u dit middel niet gebruiken?
- U bent allergisch voor één van de stoffen in dit geneesmiddel. Deze stoffen kunt u vinden in rubriek 6.
- Bij leveraandoeningen die zo ernstig zijn dat de patiënt bijna bewusteloos is.
- Bij ernstig elektrolyten gebrek, vooral een gebrek aan kalium in het bloed.
- Bij acute ontsteking van de nieren en bij het plotseling onvoldoende functioneren van de nier, waarbij de nieren geen urine produceren.

Wanneer moet u extra voorzichtig zijn met dit middel?

- Indien u gedurende langere tijd of met hoge doses wordt behandeld met bumetanide dient het gehalte aan zouten (met name natrium, magnesium en kalium) in uw bloed regelmatig gecontroleerd te worden. Dit geldt vooral voor patiënten die lijden aan andere ziekten (bijvoorbeeld levercirrose) of voor patiënten die gelijktijdig behandeld worden met andere medicijnen (waaronder bepaalde maagzuurremmers) die ook kunnen leiden tot een verstoring van de zoutbalans.
- Voorzichtigheid is geboden bij patiënten met een onvoldoende functionerend hart, een gestoorde nierfunctie, een vergrote prostaat, in het verleden opgetreden hyperurikemie (verhoogde urinezuurspiegel in het bloed bij jicht), of een zoutarm dieet.
- Wanneer u gelijktijdig behandeld wordt met corticosteroïden (zie ook "Gebruikt u nog andere geneesmiddelen?"), wanneer u een nierfunctiestoornis heeft, bij eenzijdige voeding of bij misbruik van laxeermiddelen kan er een kaliumtekort ontstaan. Dit is vooral van belang bij een gelijktijdige behandeling met digitalisglycosiden, omdat een kaliumtekort de verschijnselen van digitalisintoxicatie (=vergiftiging) kan opwekken of verergeren (zie ook "Gebruikt u nog andere geneesmiddelen?"). Verschijnselen van kaliumtekort zijn zwakte, duizeligheid, slaapzucht, beenkrampen, gebrek aan eetlust, braken of geestelijke verwardheid. Indien bij u één van deze verschijnselen optreedt, waarschuw dan uw arts.
- Bumetanide kan, net als andere vochtafdrijvende middelen, de suikerstofwisseling veranderen. Bij patiënten met suikerziekte dient dan ook door de arts regelmatig het suikergehalte in bloed en urine te worden gecontroleerd.
- Bij ouderen dient de behandeling met lage doseringen te worden gestart.
- Bumetanide is vooralsnog niet geschikt voor gebruik bij kinderen.

Gebruikt u nog andere geneesmiddelen?
Gebruikt u naast Bumetanide CF nog andere geneesmiddelen, of heeft u dat kort geleden gedaan of bestaat de mogelijkheid dat u in de nabije toekomst andere geneesmiddelen gaat gebruiken? Vertel dat dan uw arts of apotheker.
Een wisselwerking wil zeggen dat geneesmiddelen elkaars werking(en) en/of bijwerking(en) kunnen beïnvloeden. Een wisselwerking kan optreden bij gelijktijdig gebruik van bumetanide met:

- bloeddrukverlagende middelen
- corticosteroïden (geneesmiddelen met onder andere een ontstekingsremmende werking)
- misbruik van laxeermiddelen
- digitalisglycosiden (b.v. digoxine, wordt toegepast bij hartritmestoornissen en onvoldoende functioneren van het hart)
- lithium (middel gebruikt voor de behandeling van manieën, manisch depressiviteit en depressie)
- acetylsalicylzuur en indometacine en mogelijk ook andere middelen uit de groep stoffen waar deze middelen toe behoren (NSAID's) (pijnstillers met ontstekingsremmende werking)
- aminoglycosiden (bepaalde antibiotica)
- cefaloridine (antibioticum uit de cefalosporinegroep)
- cisplatine (middel toegepast bij de behandeling van bepaalde vormen van kanker)

Wanneer u zulke geneesmiddelen gebruikt moet u hiermee rekening houden en advies vragen aan uw arts of apotheker.

Let op, bovenstaande geneesmiddelen kunnen bij u bekend zijn onder een andere naam, vaak de merknaam. In deze rubriek wordt alleen de werkzame stof of therapeutische groep van het geneesmiddel genoemd en niet de merknaam. Kijk daarom altijd goed op de verpakking en in de bijsluiter van de geneesmiddelen die u al gebruikt, wat de werkzame stof of therapeutische groep is van het middel.

Zwangerschap en borstvoeding
Over het gebruik van bumetanide tijdens de zwangerschap en de periode van borstvoeding bestaan onvoldoende gegevens om de mogelijke schadelijkheid voor het kind te beoordelen. Bumetanide dient daarom alleen na overleg met de arts te worden gebruikt tijdens de zwangerschap. Het geven van borstvoeding tijdens gebruik van bumetanide wordt afgeraden.

Rijvaardigheid en het gebruik van machines
Vooral in het begin van de behandeling kan het soms voorkomen dat teveel vocht wordt afgedreven. Dit kan aanleiding geven tot onder andere duizeligheid en een verminderd gezichtsvermogen. Deze verschijnselen kunnen de rijvaardigheid en het reactievermogen tijdelijk nadelig beïnvloeden.

Bumetanide CF bevat lactose.
Indien uw arts u heeft meegedeeld dat u bepaalde suikers niet verdraagt, neem dan contact op met uw arts voordat u dit geneesmiddel inneemt.

3. HOE GEBRUIKT U DIT MIDDEL?

Gebruik dit geneesmiddel altijd precies zoals uw arts of apotheker u dat heeft verteld. Twijfelt u over het juiste gebruik? Neem dan contact op met uw arts of apotheker.

Dosering en wijze van gebruik
De dosering dient door de arts te worden vastgesteld. Alle aanbevolen doseringen zijn slechts een richtlijn. Soms kan uw arts een lagere dosis voorschrijven, in het bijzonder wanneer u voor de eerste keer bumetanide tabletten gebruikt. Een gebruikelijke dosering is:

Bij volwassenen
Doorgaans dient u iedere ochtend 0,5 mg (1 halve tablet van 1 mg) of 1 mg (1 hele tablet van 1 mg of een halve tablet van 2 mg) in te nemen.
Afhankelijk van uw reactie op dit geneesmiddel kan de dosering om de 6-8 uur worden herhaald.
In bijzondere gevallen kan de dosis verder verhoogd worden. Uw arts zal u zelden meer dan 4 mg per dag voorschrijven.

Bij oedemen bij nefrotisch syndroom (vochtophopingen door nierfunctiestoornissen)
Bij onvoldoende effect met de gebruikelijke lagere doseringen kan de dosis worden verhoogd tot 2-5 mg, zo nodig kan deze dosering elke 6-8 uur herhaald worden.

De tabletten kunnen het beste zonder kauwen en met een ruime hoeveelheid water worden ingenomen.

Heeft u te veel van dit middel ingenomen?
Indien u een overdosering vermoedt of bemerkt dient u onmiddellijk een arts te waarschuwen.

Bent u vergeten dit middel te gebruiken?
Indien u Bumetanide CF vergeten bent in te nemen moet u de dosis alsnog innemen. Als u dit ontdekt kort voor of op het moment dat u aan de volgende dosering toe bent moet u de vergeten dosering niet meer innemen, maar gewoon het doseringsschema volgen alsof er niets gebeurd is. Neem geen dubbele dosis om een vergeten dosis in te halen. In geval van twijfel, raadpleeg uw arts.

Als u stopt met het gebruik van dit middel
Blijf deze tabletten gebruiken totdat uw arts u aangeeft te stoppen.

Heeft u nog andere vragen over het gebruik van dit geneesmiddel? Neem dan contact op met uw arts of apotheker.

4. MOGELIJKE BIJWERKINGEN

Zoals elk geneesmiddel kan ook dit geneesmiddel bijwerkingen hebben, al krijgt niet iedereen daarmee te maken.

De volgende bijwerkingen kunnen voorkomen: hoofdpijn, een droge mond, uitdroging, verminderd gezichtsvermogen, duizeligheid, duizeligheid bij plotseling opstaan, plotseling optredende bewusteloosheid, neiging tot trombose (ontstaan van bloedstolsels), kuitkrampen, verminderde eetlust, gevoel van zwakte, slaperigheid, een afwijkend bloedbeeld en huiduitslag. Een enkele keer komt gehoorverlies voor.

Verder kunnen braken, verwardheid, spierzwakte, verlamming, verstopping, ophoping van gassen of lucht in de maag of darmen, abnormaal veel en vaak plassen, verlamming van de darmspier, hartritmestoornissen en spierkramp voorkomen.

Het melden van bijwerkingen
Krijgt u last van bijwerkingen, neem dan contact op met uw arts of apotheker. Dit geldt ook voor mogelijke bijwerkingen die niet in deze bijsluiter staan. U kunt bijwerkingen ook rechtstreeks melden via het Nederlands Bijwerkingen Centrum Lareb, website: www.lareb.nl. Door bijwerkingen te melden, kunt u ons helpen meer informatie te verkrijgen over de veiligheid van dit geneesmiddel.

5. HOE BEWAART U DIT MIDDEL?

Buiten het zicht en bereik van kinderen houden.

Voor dit geneesmiddel zijn er geen speciale bewaarcondities.
Bewaren in de oorspronkelijke verpakking.

Gebruik dit geneesmiddel niet meer na de uiterste houdbaarheidsdatum. Die is te vinden op de doos of de blisterverpakking na “Niet te gebruiken na” of "EXP". Daar staat een maand en een jaar. De laatste dag van die maand is de uiterste houdbaarheidsdatum.

Spoel geneesmiddelen niet door de gootsteen of de WC en gooi ze niet in de vuilnisbak. Vraag uw apotheker wat u met geneesmiddelen moet doen die u niet meer gebruikt. Ze worden dan op een verantwoorde manier vernietigd en komen niet in het milieu terecht.

6. INHOUD VAN DE VERPAKKING EN OVERIGE INFORMATIE

Welke stoffen zitten er in dit middel?
De werkzame stof in dit middel is bumetanide. Eén tablet Bumetanide CF 1 mg, 2 mg of 5 mg bevat respectievelijk 1 mg, 2 mg of 5 mg bumetanide.

De andere stoffen in dit middel zijn microkristallijne cellulose (E460), lactose monohydraat, magnesiumstearaat (E470b), maiszetmeel en natriumlaurylsulfaat.

Hoe zien Bumetanide CF tabletten eruit en hoeveel zit er in een verpakking?
Bumetanide CF 1 mg, tabletten: witte tot nagenoeg witte tablet. De tablet is vlak aan de ene zijde en heeft een breukstreep aan de andere kant met een opdruk “1” boven en “BMT” onder de breukstreep.

Bumetanide CF 2 mg, tabletten: witte tot nagenoeg witte tablet. De tablet is vlak aan de ene zijde en heeft een breukstreep aan de andere kant met een opdruk “2” boven en “BMT” onder de breukstreep.

Bumetanide CF 5 mg, tabletten: witte tot nagenoeg witte tablet. De tablet is vlak aan de ene zijde en heeft een breukstreep aan de andere kant met een opdruk “5” boven en “BMT” onder de breukstreep.

De tablet kan verdeeld worden in gelijke doses.

Bumetanide CF 1 mg, 2 mg of 5 mg, tabletten is verkrijgbaar in de volgende verpakkingen:
- Kartonnen buitenverpakking met doordrukstrips: 20, 28, 30, 50, 56, 60, 84 en 120 tabletten per verpakking
- Polypropyleen pot met polyethyleen deksel: 200 tabletten per verpakking

Niet alle genoemde verpakkingsgrootten worden in de handel gebracht.

Houder van de vergunning voor het in de handel brengen en fabrikant
Vergunninghouder
Centrafarm B.V.
Nieuwe Donk 3
4879 AC Etten-Leur
Nederland

Fabrikant
Centrafarm Services B.V.
Nieuwe Donk 9
4879 AC Etten-Leur
Nederland

Niche Generics Ltd.
151 Baldoyle Industrial Estate
Dublin
Ierland

In het register ingeschreven onder:
RVG 23140 Bumetanide CF 1 mg, tabletten
RVG 24313 Bumetanide CF 2 mg, tabletten
RVG 23141 Bumetanide CF 5 mg, tabletten

Deze bijsluiter is voor het laatst goedgekeurd in februari 2016.

Gedetailleerde informatie over dit geneesmiddel is beschikbaar op de website van het CBG (www.cbgmeb.nl).

APPENDIX 3: Drug accountability calendar for participants (example)

Bumetanide kalender

Deze kalender is van ……

Week 1

Dag	Datum	Dosis 1	Dosis 2	Bijzonderheden
1				
2				
3				
4				
5				
6				
7				

APPENDIX 4: Aanvalsdagboek

Epilepsiedagboek

Patiëntnummer: .....
Visite datum: .....

Maand/jaar:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag
Code	*e.g., C*															
Aantal	*e.g., 2*															
Noodmedicatie	*e.g., Nee*															
	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht
Code																
Aantal																
Noodmedicatie																

Maand/jaar:

	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag	Overdag
Code															
Aantal															
Noodmedicatie															
	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht	Nacht
Code															
Aantal															
Noodmedicatie															

Aanval classificatie:

Code:	Aanvallen	
A	Partieel	Bij bewustzijn
B		Veranderd/verminderd bewustzijn
C		Partieel overgaand in gegeneraliseerd
D	Gegeneraliseerd (motor)	Tonisch-clonisch
E		Tonisch
F		Atonisch
G		Myoclonus
H		Myoclonus-atoon
I		Clonisch
J		Clonisch-tonisch-clonisch
K		Epileptische spasmes
L	Gegeneraliseerd (absences)	Typisch
M		Atypisch
N		Myoclone absence
O		Ooglid myoclonus
P	Onbekend	Niet geclassificeerd
Q	Geen aanvallen	

APPENDIX 5: Minutes on ECG evaluation in the BAMBI and BASCET study

Attending:

- Dr. Strengers (pediatric cardiologist)
- Dr. Krings (pediatric cardiologist)
- M. Korpershoek (resident pediatric cardiology)
- D. van Andel (PhD student Psychiatry)
- J. Sprengers (PhD student Psychiatry)

The meeting was scheduled on initiative of the Department Pediatric Cardiology to discuss the value of ECG measurement in the BAMBI and BASCET protocols. Two questions were discussed:

1. *What is de value of ECG screening to evaluate the primary effects of bumetanide on the heart?*

The most common cardiac pathologies in children are congenital abnormalities. When clinically relevant these abnormalities are usually picked up before the age of seven. The most common abnormalities expected in the BAMBI and BASCET participants are arrhythmias. ECG does not adequately detect these. More relevant is careful cardiac history taking. When cardiac history is suspect for arrhythmias children should be referred to the pediatric cardiologist, who will perform ultrasound cardiac evaluation. ECG is not important to screen for cardiac disorders that exclude children from participation.

To the knowledge of the cardiologists there is no cardiac interaction of bumetanide. Therefore ECG recording is not required. In addition, consultation with one of the residents hospital pharmacy (I. Favie 21-11-2016) revealed that screening on Pubmed or CredibleMeds revealed no record of Bumetanide increasing QTc risk.

Taken together there is no evidence that bumetanide interacts directly with cardiac function. This warrants omission of ECG measurements to assess primary effects of bumetanide on the heart.

2. *What is the value of ECG screening to evaluate the secondary effects of bumetanide on the heart?*

The main effect of bumetanide on the heart is mediated via electrolyte disturbances (most importantly hypokalemia). Electrolyte disturbances are detected via laboratory screening as is secured via the study protocol. Following serious electrolyte disturbances an ECG recording is acquired. Nevertheless, treatment exists of correcting the electrolyte disturbances. Base level ECG recordings are not required to guide clinical evaluation following cardiac arrhythmias following electrolyte disturbances. Therefore, base level and post treatment ECG recording has no additional value in the current protocol and can be omitted.