Ben-Ari. *Rare Dis Orphan Drugs J* 2024;3:2
DOI: 10.20517/rdodj.2023.22

Rare Disease and Orphan Drugs Journal

Original Article

Open Access

Bumetanide to treat autism spectrum disorders: are complex administrative regulations fit to treat heterogeneous disorders?

Yehezkel Ben-Ari

Neurochlore, Scientific Campus of Iuminy, Marseille 13288, France.

Correspondence to: Prof. Yehezkel Ben-Ari, Neurochlore, Scientific Campus of Iuminy, 163 Route de Luminy, Marseille 13288, France. E-mail: Ben-ari@neurochlore.fr

How to cite this article: Ben-Ari Y. Bumetanide to treat autism spectrum disorders: are complex administrative regulations fit to treat heterogeneous disorders? *Rare Dis Orphan Drugs J* 2024;3:2. https://dx.doi.org/10.20517/rdodj.2023.22

Received: 25 Jul 2023 **First Decision:** 7 Oct 2023 **Revised:** 14 Nov 2023 **Accepted:** 8 Dec 2023 **Published:** 3 Jan 2024

Academic Editors: Daniel Scherman, Antonio Persico **Copy Editor:** Dan Zhang **Production Editor:** Dan Zhang

Abstract

Introduction: Extensive experimental observations suggest that the regulation of ion fluxes and, notably, chloride are impacted in autism spectrum disorders (ASD) and other neurodevelopmental disorders. The specific NKCC1 cotransporter inhibitor Bumetanide has been shown to attenuate electrophysiological and behavioral features of ASD in experimental models. Both pilot and phase 2 double-blind randomized independent trials have validated these effects with thousands of children treated successfully. Both brain imaging and eye tracking observations also validate these observations. However, final large phase 3 trials failed, with no significant differences between placebo and treated children.

Methods: Here, I discuss the possible reasons for these failures and discuss the exclusive reliance on complex patent cooperation Treaty (PCT) regulations. Indeed, available data suggest that bumetanide responders could be identified by relying notably on EEG measures, suggesting that biological sub-populations of patients might benefit from the treatment.

Results: These observations raise important debates on whether treating only a % of children with ASD is acceptable.

Discussion: It is likely that in many disorders, the heterogeneity of the pathological event precludes a single general treatment for all, suggesting that trials centered on selective populations of responders might be essential for large

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

clinical trials to succeed.

Keywords: Autism spectrum disorders, brain imaging, eye tracking

INTRODUCTION

The purpose of this study is to discuss the possible reasons underlying the systematic failures of large phase 3 clinical trials to succeed in spite of successful phase 2 and a large number of pilot trials. The current study discusses these issues relating to ASD and the NKCC1 chloride cotransporter inhibitor Bumetanide.

THE GABA DEVELOPMENTAL SHIFT

In 1989, we reported that immature hippocampal neurons have high $(Cl^-)_I$ levels, GABA excitatory actions, and a network-driven pattern that we called Giant Depolarizing Potentials (GDPs)[1]. The commonality between these features is their time course, disappearing around the 2nd week post-natal, at least in the hippocampus. These three features had been observed with different properties in various systems[2-10]. This developmental reduction of $(Cl^-)_I$ level has been observed in a long list of animal species and brain structures extending from insects to birds, frogs, and mammals[4,11]. This list is, in fact, growing continuously, rendering it difficult to quote all papers - it is safe to say that exceptions to this sequence are rare. Excitatory actions of GABA underlie the roles of GABA as a trophic factor, mediated by a depolarization that is sufficient to remove the voltage-dependent Mg++ block of NMDA channels and activate voltage-gated calcium currents leading to an increase of intracellular Ca^{++} levels[12-18]. This sequence has received considerable support from studies showing that NKCC1 /KCC2-two cotransporters that control00- are developmentally regulated, underlying the early depolarizing actions of GABA[19]. In fact, early in utero, the blockade of NKCC1 exerts deleterious actions, confirming the importance of the trophic actions of GABA via the calcium influx it induces[20,21]. Therefore, this developmental shift corresponds to the different roles exerted during development and subsequently by the same transmitter. This is not unprecedented; other ionic currents seem to shift developmentally, exerting different actions during development and adults[22-25] (i.e., Ach subunit compositions or NMDA voltage-dependent blockade regulation).

THE GABA SHIFT IN DISEASE

Curiously, a long list of disorders is associated with a sort of return to an immature state. In a review published 15 years ago, I called this phenomenon -the neuro-archeology concept, suggesting that early (in utero notably) insults deviate developmental sequences, leading to aberrant networks endowed with immature features[26]. In infantile epilepsies caused, for instance, by genetic mutations that impact migration, removing the dysplasia by surgical interventions can alleviate the disorder or at least drastically reduce seizure occurrence. The neuro-archeology concept posits that misplaced neurons are the direct cause of the disorder and the one treatable, whereas the inaugurating insult is not as it occurs well before the diagnosis has been determined and is thus not accessible to treatments. Therefore, using drugs that selectively block these immature ensembles might open therapeutic avenues by imposing a selective pharmaceutical blockade of the perturbing networks[27].

Indeed, the NKCC1/KCC2 ratio and activity are hampered severely in many brain disorders, leading to high $(Cl^-)_I$ levels and excitatory actions of GABA. Pathological conditions often lead to overactivity of NKCC1 via a long chain of now well-identified steps and an internalization of KCC2 and, hence, a strong reduction of the capacity of neurons to reduce their $(Cl^-)_I$ levels. This has been shown in ASD, Brain trauma, cerebral infarcts, heart failure and ischemic episodes, spinal cord lesions, chronic pain, various types of

epilepsies, Parkinson's and Alzheimer's disease, and even various types of cancers and tumors including Glioblastoma, pancreatic, lung, liver and other types of cancer[28-35]. In many animal models, restoring low $(Cl^-)_I$ levels in NKCC1 Kos or with the specific NKCC1 inhibitor Bumetanide attenuates the pathological features of the disorder[28]. Collectively, these observations suggest that drugs capable of reducing high$(Cl^-)_I$ levels might effectively attenuate many disorders. We, therefore, embarked on clinical trials by prioritizing ASD as the initial focus, as epilepsies are hampered by the recurrent seizures that enhance $(Cl^-)_I$ level and NKCC1 activity and reduce KCC2 efficacy.

CLINICAL TRIALS: SUCCESS AND FAILURE

The typical process required to reach an agreement for the use of a treatment involves a sequential series of steps, usually three or four. This includes conducting pilot trials initially, followed by phase 2 trials, which continue until the treatment is considered pivotal by regulatory authorities, and then at least a large confirmatory phase 3 trial, involving hundreds of patients across multiple centers/countries. The preclinical experimental data on animal models were available and indispensable toxicity tests were not needed. Indeed, the NKCC1 inhibitor Bumetanide has been used for 4 decades by hundreds of thousands of patients suffering from hypertension or brain edema without severe sequels and no long-lasting neurological or general biological deficiencies[36,37]. Classically, Bumetanide induces a diuresis associated with occasional dehydration and headaches. In contrast to criticisms repeatedly raised in the literature, Bumetanide did not affect hearing-except in cases involving high dosages administrated with heavy levels of antibiotics in two-day-old children with severe encephalopathy; however, this observation does not apply to children or adolescents with ASD[38,39]. The ubiquity distribution of NKCC1 in the body and poor brain penetrability have also been evoked to challenge its usefulness[40]. However, the precise levels of NKCC1 in the brain during disorders in humans remain unknown, and the wide distribution of NKCC1 might be an advantage when considering the overall clinical manifestations of ASD and others[41].

Results from our initial pilot trials showed a clear-cut attenuation of the severity of social interactions and agitation[42,43]. We then conducted two randomized double-blind studies, one in a single center (Brest) and the other in many centers (Limoges, Paris, Rouen, Marseille, Lyon, Nice) including 87 children /adolescents -2-18 years old. This large sample was needed as the EMA authorities required a treatment for the entire pediatric population. Again, the results were in keeping with our preliminary observations and validated by similar trials in other countries[44]. Notably, several independent trials using exactly the same approach in terms of dosages and examinations revealed similar attenuations[45-48] [Table 1]. A recent Egyptian trial also observed attenuation of ASD (80 children, 3-12 years old) with a significant amelioration of CARS in the treated group versus placebo[49]. A Dutch group also reported partial attenuation of some symptoms[50]. They found that bumetanide attenuates the autistic features of ASD but not the recurrent seizures[51]. Other studies showed adecrease in amygdala activation through constrained visual interactions[52,53]. These studies collectively showed that after treatment, participants with autism exhibit faster and more accurate recognition of emotions in a behavioral task, and that there was an increase in social brain activation in response to faces in an unconstrained stimulus presentation of dynamic faces[54].

These results suggested that it is worth pursuing this to a Phase 3 development aimed at obtaining market authorization. To align with the requirements of the European Medicine Agency (EMA), this included the entire pediatric population in two separate trials (2-7 and 7-18 years old)[55]. Participants received a bumetanide syrup with a taste agreeable to children /adolescents. In this trial, approximately 210 patients were recruited in each trial and underwent a double-blind randomized treatment. They received the lowest effective doses of Bumetanide, specifically 0.5 mg twice daily, adjusted according to body weight. Improvement from childhood autism rating scale (CARS) baseline score was used as the first criterion and

Table 1. List of clinical trials using Bumetanide to treat ASD

Country	*n*	Age (year)	Rating scale	Dose	Duration	End points	Side effects	Ref.
China	119	3-6	CARS, ADOS, CGI, SRS	0.5 mg twice/day	3 months	Improvement in CARS score	Mild (polyuria, hypokalemia)	Dai *et al.*[46]
Sweden	6	3-14	CARS	0.5 mg twice/day	4-12 weeks	Improvement in CARS score	Mild (polyuria)	Fernell *et al.*[47]
Netherland	92	7-15	CARS, ADOS, SRS	0.5 mg twice/day	3 months	Improvement in CARS and SRS score	Mild (hypokalemia)	Sprengers *et al.*[50]
China	83	3-6	CARS, ADOS, CGI	0.5 mg twice/day	3 months	Reduction in CARS score, CGI-I	Mild (polyuria)	Li *et al.*[45]
Netherland	15	8-21	ABC-I (TSC)	0.5 mg twice/day	3 months	Improvement in ABC-I score EEG	Mild (hypokalemia)	Van Andel *et al.*[51]
Tunisia	29	Average 7.9	ADI-R, CARS, CGI	0.1 mg/day	12 months	Improvement in CARS score	Mild (hypokalemia)	Hajri M, Ben Amor A, Abbes Z, et al. Bumetanide in the management of autism. Tunisian experience in Razi Hospital. *Tunis Med* 2019;97(8-9):971-7. [PMID:32173844]
France	9	Average 21.4	Eye tracking	1 mg/day	10 months	fMRI	None	Hadjikhani *et al.*[54]
France	88	2-18	CARS, SRS, CGI	0.5-2 mg twice/day	3 months	Improvement in CARS, CGI, SRS score	Mild (hypokalemia)	Lemonnier *et al.*[44]
China	60	Average 4.5	ABC, CARS, CGI	0.5 mg twice/day	3 months	Improvement in ABC, CARS, CGI score	None	Du *et al.*[48]
France	7	Average 19.3	ADOS, fMRI emotion recognition	1 mg/day	10 months	Improvement performance for emotion recognition	Mild (polyuria)	Hadjikhani *et al.*[52]
France	60	3-11	CARS, SRS, ADOS	1 mg/day	3 months	Improvement in CARS, ADOS score	Mild (hypokalemia)	Lemonnier *et al.*[42]
France	5	3-11	CARS, ABC, CGI RDEG, RRB	1 mg/day	3 months	Improvement in CARS, CGI	None	Lemonnier *et al.*[43]

ASD: autism spectrum disorders; CARS: childhood autism rating scale; SRS: social responsive scale; CGI: scaleclinical global impression.

other measures of ASD severity as the second criterion, including social responsive scale (SRS) and the clinical global impression (CGI) Scale. The results were negative[56]. There was no significant difference between treatment and placebo, mainly because of high placebo improvements alleviating the difference with treatment. This situation is not unprecedented; clinical trials on Fragile X were successful initially but failed subsequently in large trials[57]. In fact, most trials on these neurodevelopmental disorders succeeded in early phase 2 but failed subsequently [Figure 1]. What are the possible explanations for this failure?

DISCUSSION AND CONCLUSIONS

What underlies the failures? Searching for explanations

The simplest explanation, of course, is that the treatment does not deserve to be used as it failed to meet a statistical difference. However, there are other explanations that might underlie the failures of large phases after initial success.

Firstly, there is an extreme heterogeneity of ASD. This spectrum of disorders is indeed very heterogeneous, with some children having primarily a given sequel and others very different ones.

Figure 1. Allegorical scheme of the failure of large clinical trials notably in the last phase.

Secondly, it is possible that the evaluation by so many different centers (40 in > 15 different countries) is a problem -although our analysis did not reveal a predominance of successes or failures in a given center.

Thirdly, it is possible that, as for other phase 3 trials, inclusion criteria were less restrictive than for phase 2. In phase 3, the included patients had to have a CARS total score of 34 points and a weight ≥ 11 kg. Serious, unstable illnesses including gastroenterological, respiratory, cardiovascular (QT interval lengthening), endocrinology, immunologic or hematologic disease, and renal or hepatic dysfunction and neurological disorders such as seizures and microcephaly were not allowed. Admittedly, this is always the case considering the large recruitment needed.

Perhaps most importantly, considering the heterogeneity of ASD, it is possible that a single treatment for such a variety of clinical manifestations is unlikely to be efficient. We therefore reasoned that if indeed there are subpopulations including responders, it might be possible to identify them using Machine Learning or other similar tools. The group of Bruining had successful results in adolescents with ASD treated with the same syrup but identified upon their EEG[58]. The analysis of the EEG helps predict which patient will respond to bumetanide treatment and which will not. The authors showed that patients treated with bumetanide had an increase in the absolute power of *alpha* frequency and of a measure they developed of excitatory/inhibitory ratio[58]. This important observation is also in line with the observations from similar events that led to the general conclusion that large trials are doomed to fail unless they are concentrated on specific populations of patients identified early on as being efficient. This is one of the reasons that trials on ASD features are centered on genetic forms of ASD or associated with ASD features like Fragile X or Tuberous Sclerosis. However, whether these genetic forms of ASD are sufficiently homogeneous to lead to successful large trials is presently unknown; there are indications that they are not as homogeneous as thought with regard to the semiology of neurological and psychiatric sequels.

Bearing this in mind, we have recently conducted a reanalysis of the data of phase 3 using ML approaches. Here, without any a priori, we searched for subpopulations that respond to the treatment with a significant improvement in CARS but without relying on specific a priori parameters. Our preliminary results are promising, with an identification of a significant % of patients responding positively to the treatment (in preparation).

However, this raises other important issues. Why did the trial show improvement in the population receiving the placebo? This situation is not unprecedented, especially among children. It is not easy to take this into account and reduce the placebo factor[59]. Furthermore, from a broader viewpoint, is it acceptable to develop treatment valid for some but not for other children with ASD? On one hand, it seems difficult to eliminate the possibility of treating selected populations, and on the other hand, excluding others also raises complex ethical issues. I posit that unless inclusion criteria are modified to reduce the number of patients recruited, we might fail to have significant results leading to market approval. It is likely that machine learning (ML) and other approaches might facilitate the identification of subpopulations, thus reducing ASD heterogeneity and enhancing the likelihood of success and eventual market approval. The bumetanide responders belong to a subpopulation with some unique features, perhaps corresponding to unique in utero insults. Indeed, we have succeeded in identifying at birth a subpopulation of babies who will have a diagnosis of ASD later, relying on an analysis of maternity data with machine learning. Analysis of the parameters impacting the decision-making process of the ML program to identify babies who may later receive a diagnosis of ASD suggests some common early insults. It might help better understand the etiology of ASD and its underlying heterogeneity; the developmental stage during which the initial insults occur is at the core of the symptoms observed later. From a therapeutic perspective, it might improve the results of large trials and, most importantly, help treat at least a subpopulation of children. It is perhaps time to review the methodologies employed in large-scale trials to bolster the likelihood of achieving successful outcomes.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

The author declared that there is no conflicts of interest.

Ethical approval and consent to participate

I also declare my role as a CEO and shareholder of Neurochlore, a startup dedicated to developing treatments for ASD and Fragile X. This manuscript does not involve issues of data reporting. With the exception of funds received from the French Public Investment Bank (BPI), funding for my research and development is entirely provided by the private financial resources of Neurochlore.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2024.

REFERENCES

1. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol* 1989;416:303-25. DOI PubMed PMC

2. Ben-ari Y, Tseeb V, Raggozzino D, Khazipov R, Gaiarsa J. γ-Aminobutyric acid (GABA): a fast excitatory transmitter which may regulate the development of hippocampal neurones in early postnatal life. *Prog Brain Res* 1994;102:261-73. DOI PubMed
3. Ben-Ari Y. Excitatory actions of gaba during development: the nature of the nurture. *Nat Rev Neurosci* 2002;3:728-39. DOI PubMed
4. Ben-Ari Y. The GABA excitatory/inhibitory developmental sequence: a personal journey. *Neurosci* 2014;279:187-219. DOI PubMed
5. Yamada J, Okabe A, Toyoda H, Kilb W, Luhmann HJ, Fukuda A. Cl- uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. *J Physiol* 2004;557:829-41. DOI PubMed PMC
6. Watanabe M, Fukuda A. Development and regulation of chloride homeostasis in the central nervous system. *Front Cell Neurosci* 2015;9:371. DOI PubMed PMC
7. Luhmann HJ, Kirischuk S, Sinning A, Kilb W. Early GABAergic circuitry in the cerebral cortex. *Curr Opin Neurobiol* 2014;26:72-8. DOI PubMed
8. Chabrol FP, Eglen SJ, Sernagor E. GABAergic control of retinal ganglion cell dendritic development. *Neurosci* 2012;227:30-43. DOI PubMed
9. Zhang RW, Wei HP, Xia YM, Du JL. Development of light response and GABAergic excitation-to-inhibition switch in zebrafish retinal ganglion cells. *J Physiol* 2010;588:2557-69. DOI PubMed PMC
10. Ben-Ari Y, Krnjević K, Reiffenstein RJ, Reinhardt W. Inhibitory conductance changes and action of gamma-aminobutyrate in rat hippocampus. *Neurosci* 1981;6:2445-63. DOI PubMed
11. Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 2007;87:1215-84. DOI
12. Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R. Ca^{2+} oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus. *Neuron* 1997;18:243-55. DOI PubMed
13. Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL. GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. *Trends Neurosci* 1997;20:523-9. DOI
14. Heck N, Kilb W, Reiprich P, et al. GABA-A receptors regulate neocortical neuronal migration in vitro and in vivo. *Cereb Cortex* 2007;17:138-48. DOI
15. Inada H, Watanabe M, Uchida T, et al. GABA regulates the multidirectional tangential migration of GABAergic interneurons in living neonatal mice. *PLoS One* 2011;6:e27048. DOI PubMed PMC
16. Kirischuk S, Sinning A, Blanquie O, Yang JW, Luhmann HJ, Kilb W. Modulation of neocortical development by early neuronal activity: physiology and pathophysiology. *Front Cell Neurosci* 2017;11:379. DOI PubMed PMC
17. Griguoli M, Cherubini E. Early correlated network activity in the hippocampus: its putative role in shaping neuronal circuits. *Front Cell Neurosci* 2017;11:255. DOI PubMed PMC
18. Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E. GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. *Proc Natl Acad Sci U S A* 2004;101:3967-72. DOI PubMed PMC
19. Rivera C, Voipio J, Payne JA, et al. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 1999;397:251-5. DOI PubMed
20. Khalilov I, Chazal G, Chudotvorova I, et al. Enhanced synaptic activity and epileptiform events in the embryonic KCC2 deficient hippocampus. *Front Cell Neurosci* 2011;5:23. DOI PubMed PMC
21. Wang DD, Kriegstein AR. Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits. *Cereb Cortex* 2011;21:574-87. DOI PubMed PMC
22. Zheng JJ, Lee S, Zhou ZJ. A developmental switch in the excitability and function of the starburst network in the mammalian retina. *Neuron* 2004;44:851-64. DOI PubMed
23. Spitzer NC. Neurotransmitter switching in the developing and adult brain. *Annu Rev Neurosci* 2017;40:1-19. DOI PubMed
24. Moody WJ, Bosma MM. Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. *Physiol Rev* 2005;85:883-941. DOI PubMed
25. Dehorter N, Vinay L, Hammond C, Ben-Ari Y. Timing of developmental sequences in different brain structures: physiological and pathological implications. *Eur J Neurosci* 2012;35:1846-56. DOI PubMed
26. Ben-Ari Y. Neuro-archaeology: pre-symptomatic architecture and signature of neurological disorders. *Trends Neurosci* 2008;31:626-36. DOI PubMed
27. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science* 2002;298:1418-21. DOI PubMed
28. Ben-Ari Y. NKCC1 chloride importer antagonists attenuate many neurological and psychiatric disorders. *Trends Neurosci* 2017;40:536-54. DOI PubMed
29. Lozovaya N, Eftekhari S, Cloarec R, et al. GABAergic inhibition in dual-transmission cholinergic and GABAergic striatal interneurons is abolished in Parkinson disease. *Nat Commun* 2018;9:1422. DOI PubMed PMC
30. Thompson EG, Sontheimer H. A role for ion channels in perivascular glioma invasion. *Eur Biophys J* 2016;45:635-48. DOI PubMed PMC
31. Garzon-Muvdi T, Schiapparelli P, ap Rhys C, et al. Regulation of brain tumor dispersal by NKCC1 through a novel role in focal adhesion regulation. *PLoS Biol* 2012;10:e1001320. DOI PubMed PMC
32. Damier P, Hammond C, Ben-Ari Y. Bumetanide to treat parkinson disease: a report of 4 cases. *Clin Neuropharmacol* 2016;39:57-9. DOI PubMed

33. Gagnon M, Bergeron MJ, Lavertu G, et al. Chloride extrusion enhancers as novel therapeutics for neurological diseases. *Nat Med* 2013;19:1524-8. DOI PubMed PMC
34. Savardi A, Borgogno M, De Vivo M, Cancedda L. Pharmacological tools to target NKCC1 in brain disorders. *Trends Pharmacol Sci* 2021;42:1009-34. DOI PubMed
35. Capsoni S, Arisi I, Malerba F, D'Onofrio M, Cattaneo A, Cherubini E. Targeting the cation-chloride co-transporter NKCC1 to re-establish GABAergic inhibition and an appropriate excitatory/inhibitory balance in selective neuronal circuits: a novel approach for the treatment of Alzheimer's disease. *Brain Sci* 2022;12:783. DOI PubMed PMC
36. Oberbauer R, Krivanek P, Turnheim K. Pharmacokinetics and pharmacodynamics of the diuretic bumetanide in the elderly. *Clin Pharmacol Ther* 1995;57:42-51. DOI PubMed
37. Flamenbaum W, Friedman R. Pharmacology, therapeutic efficacy, and adverse effects of bumetanide, a new "loop" diuretic. *Pharmacotherapy* 1982;2:213-22. DOI PubMed
38. Pressler RM, Boylan GB, Marlow N, et al; NEonatal seizure treatment with Medication Off-patent (NEMO) consortium. Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): an open-label, dose finding, and feasibility phase 1/2 trial. *Lancet Neurol* 2015;14:469-77. DOI
39. Ben-Ari Y, Delpire E. Phenobarbital, midazolam, bumetanide, and neonatal seizures: The devil is in the details. *Epilepsia* 2021;62:935-40. DOI PubMed PMC
40. Löscher W, Kaila K. Reply to the commentary by Ben-Ari and delpire: bumetanide and neonatal seizures: fiction versus reality. *Epilepsia* 2021;62:941-6. DOI PubMed
41. Delpire E, Ben-Ari Y. A wholistic view of how bumetanide attenuates autism spectrum disorders. *Cells* 2022;11:2419. DOI PubMed PMC
42. Lemonnier E, Degrez C, Phelep M, et al. A randomised controlled trial of bumetanide in the treatment of autism in children. *Transl Psychiatry* 2012;2:e202. DOI PubMed PMC
43. Lemonnier E, Ben-Ari Y. The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects. *Acta Paediatr* 2010;99:1885-8. DOI PubMed
44. Lemonnier E, Villeneuve N, Sonie S, et al. Effects of bumetanide on neurobehavioral function in children and adolescents with autism spectrum disorders. *Transl sych* 2017;7:e1056. DOI PubMed PMC
45. Li Q, Zhang L, Shan H, et al. The immuno-behavioural covariation associated with the treatment response to bumetanide in young children with autism spectrum disorder. *Transl sych* 2022;12:228. DOI PubMed PMC
46. Dai Y, Zhang L, Yu J, et al. Improved symptoms following bumetanide treatment in children aged 3-6 years with autism spectrum disorder: a randomized, double-blind, placebo-controlled trial. *Sci Bull* 2021;66:1591-8. DOI PubMed
47. Fernell E, Gustafsson P, Gillberg C. Bumetanide for autism: open-label trial in six children. *Acta Paediatr* 2021;110:1548-53. DOI PubMed PMC
48. Du L, Shan L, Wang B, et al. A pilot study on the combination of applied behavior analysis and bumetanide treatment for children with autism. *J Child Adolesc Psychopharmacol* 2015;25:585-8. DOI
49. Shaker E, Agami OE, Salamah A. Bumetanide, a diuretic that can help children with autism spectrum disorder. *CNS Neurol Disord Drug Targets* 2023;23:536-42. DOI PubMed
50. Sprengers JJ, van Andel DM, Zuithoff NPA, et al. Bumetanide for core symptoms of autism spectrum disorder (BAMBI): a single center, double-blinded, participant-randomized, placebo-controlled, phase-2 superiority trial. *J Am Acad Child Adolesc Psychiatry* 2021;60:865-76. DOI
51. Van Andel DM, Sprengers JJ, Oranje B, Scheepers FE, Jansen FE, Bruining H. Effects of bumetanide on neurodevelopmental impairments in patients with tuberous sclerosis complex: an open-label pilot study. *Mol Autism* 2020;11:30. DOI PubMed PMC
52. Hadjikhani N, Zürcher NR, Rogier O, et al. Improving emotional face perception in autism with diuretic bumetanide: a proof-of-concept behavioral and functional brain imaging pilot study. *Autism* 2015;19:149-57. DOI
53. Hadjikhani N, Åsberg Johnels J, Zürcher NR, et al. Look me in the eyes: constraining gaze in the eye-region provokes abnormally high subcortical activation in autism. *Sci Rep* 2017;7:3163. DOI PubMed PMC
54. Hadjikhani N, Åsberg Johnels J, Lassalle A, et al. Bumetanide for autism: more eye contact, less amygdala activation. *Sci Rep* 2018;8:3602. DOI PubMed PMC
55. Crutel V, Lambert E, Penelaud PF, et al. Bumetanide oral liquid formulation for the treatment of children and adolescents with autism spectrum disorder: design of two phase III studies (SIGN Trials). *J Autism Dev Disord* 2021;51:2959-72. DOI PubMed PMC
56. Fuentes J, Parellada M, Georgoula C, et al. Bumetanide oral solution for the treatment of children and adolescents with autism spectrum disorder: results from two randomized phase III studies. *Autism Res* 2023;16:2021-34. DOI PubMed
57. Tobe R, Zhu Y, Gleissl T, et al. Predictors of placebo response in three large clinical trials of the V1a receptor antagonist balovaptan in autism spectrum disorder. *Neuropsychopharmacol* 2023;48:1201-16. DOI PubMed PMC
58. Juarez-Martinez EL, Sprengers JJ, Cristian G, et al. Prediction of behavioral improvement through resting-state electroencephalography and clinical severity in a randomized controlled trial testing bumetanide in autism spectrum disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging* 2023;8:251-61. DOI
59. Masi A, Lampit A, Glozier N, Hickie IB, Guastella AJ. Predictors of placebo response in pharmacological and dietary supplement treatment trials in pediatric autism spectrum disorder: a meta-analysis. *Transl sych* 2015;5:e640. DOI PubMed PMC