AUTISM SPECTRUM DISORDER



# A phase 2 clinical trial of a vasopressin V1a receptor antagonist shows improved adaptive behaviors in men with autism spectrum disorder

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**There are no approved pharmacological therapies to address the core symptoms of autism spectrum disorder (ASD), namely, persistent deficits in social communication and social interaction and the presence of restricted, repetitive patterns of behaviors, interests, or activities. The neuropeptide vasopressin has been implicated in the regulation of social behaviors, and its modulation has emerged as a therapeutic target for ASD. The phase 2 VANILLA clinical trial reported here evaluated balovaptan, an orally administered selective vasopressin V1a receptor antagonist, in 223 men with ASD and intelligence quotient ≥70. The drug was administered daily for 12 weeks and was compared with placebo. Participants were randomized to placebo ($n = 75$) or one of three balovaptan dose arms (1.5 mg, $n = 32$; 4 mg, $n = 77$; 10 mg, $n = 39$). Balovaptan treatment was not associated with a change from baseline compared with placebo at 12 weeks in the primary efficacy endpoint (Social Responsiveness Scale, 2nd Edition). However, dose-dependent and clinically meaningful improvements on the Vineland-II Adaptive Behavior Scales composite score were observed for participants treated with balovaptan 4 or 10 mg compared with placebo. This was driven principally by improvements in the Vineland-II socialization and communication scores. Balovaptan was well tolerated across all doses, and no drug-related safety concerns were identified. These results support further study of balovaptan as a potential treatment for the socialization and communication deficits in ASD.**

## INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by core symptoms of persistent deficits in social communication and social interaction, and the presence of restricted, repetitive patterns of behaviors, interests, or activities (*1*). The prevalence of ASD is estimated to range from 1.68 to 2.24% (*2*, *3*) among school-aged children and to be 1.1% in adults (*4*), although data for adult populations are scarce. ASD is diagnosed about four times more frequently in boys compared with girls (*3*).

There are no approved medicines to treat the core symptoms of ASD. At present, treatment for core symptoms of ASD focuses on the pediatric population and is primarily limited to cognitive and behavioral interventions, for example, speech and language therapy and Applied Behavior Analysis (*5*, *6*). However, clinical trials assessing these strategies are often underpowered, and evidence for their effectiveness in adults, in particular, is limited (*6*). Currently, approved pharmacological treatments in the United States (aripiprazole and risperidone) are limited to use in irritability associated with ASD. Although these drugs and other psychotropic medications are associated with side effects (e.g., tardive dyskinesia, weight gain, and sedation), the use of psychotropic drugs to treat associated psychiatric comorbidities is common (*7*).

Although diagnosis is made based on clinical observations, tests for diagnosing ASD are well established, most notably, the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R). In contrast, there is no generally accepted assessment for evaluating improvement of the core symptoms of ASD in clinical practice or clinical trials, particularly in adults with ASD (*8*, *9*). Changes in symptoms are often assessed using scales designed to evaluate ASD severity (*8*), such as the caregiver-rated Social Responsiveness Scale, 2nd Edition (SRS-2). Recently, a working group evaluated all available tests to measure changes in social communication and social interactions and recommended a number of clinician- and caregiver-rated scales including the Vineland-II Adaptive Behavior Scales and suggested deprioritization of the SRS-2 (*9*).

Although the etiology of ASD is complex and multifactorial, involving genetic and environmental factors, several neural pathways and circuits underlying social behavior have been identified. The neuropeptide vasopressin has been implicated in modulation of brain circuits regulating social behaviors (*10*, *11*). Vasopressin is produced in the paraventricular and supraoptic nuclei of the hypothalamus, which project to key brain regions including the olfactory system, prefrontal cortex, entorhinal cortex, amygdala, and hippocampus (*12*). In the brain, vasopressin acts on the G protein–coupled vasopressin receptors V1a and V1b. The V1a receptor is the dominant receptor subtype in the brain, whereas the expression of V1b receptor is restricted, predominantly to the hippocampus (*13*, *14*).

Alteration of vasopressin expression or expression of its receptors affects social behaviors, such as social anxiety, pair bonding, and aggression in a species-dependent manner (*10*). In humans, studies have suggested that acute administration of intranasal vasopressin modulates cooperativity (*15*, *16*) and memory and perception of facial expressions (*17*–*19*), potentially in a sex-dependent manner (*16*, *18*). Several studies have also reported a potential linkage of microsatellite DNA in *AVPR1A*, the gene encoding the V1a receptor, with social behaviors (*20*, *21*).

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Animal models of ASD have been used to explore the effect of V1a receptor inhibition on social behaviors. Fetal exposure to the antiepileptic drug valproate increases the risk of ASD in children (*22*) and causes social deficits in the corresponding rat model of ASD (*23*, *24*). V1a receptor antagonist infusion has been shown to have prosocial effects in this rat valproate model of ASD (*24*), suggesting that V1a receptor inhibition could improve social behaviors in humans.

Given the connection between vasopressin signaling and social behavior, and the observed effects in the rat valproate model, we considered the V1a receptor as a potential therapeutic target for the treatment of ASD. A small ($n$ = 19) crossover, placebo-controlled, proof-of-mechanism study (ClinicalTrials.gov identifier: NCT01474278) evaluated an intravenously administered highly selective V1a receptor inhibitor (RG7713/RO5028442) in adult men with ASD with an intelligence quotient (IQ) ≥70 using exploratory biomarkers including eye tracking and behavioral assessments (*25*). Administration of a single dose of RG7713 was associated with a statistically significant increase in orienting preference to biological motion and a trend for improvement in the composite score of the eye-tracking assessments (*25*). RG7713 was also associated with a reduced ability to detect lust and a trend for reduced ability to detect fear (*25*). Although RG7713 could not be developed further for chronic use in humans owing to its properties (*26*), this study provided preliminary evidence that V1a receptor inhibition may improve social and communication behaviors in ASD (*25*) and encouraged further research and the development of an orally available compound more suitable for use outside a research setting.

Balovaptan (RG7314/RO5285119) is an orally administered selective V1a receptor competitive antagonist, with similar in vitro potency and selectivity as RG7713. Phase 1 clinical studies of balovaptan did not identify any safety or tolerability concerns with either single doses up to 76 mg or repeated doses up to 52 mg daily for 2 weeks, respectively, in healthy male volunteers (ClinicalTrials.gov identifier: NCT01418963).

Here, we report the results of the phase 2 Vasopressin ANtagonist to Improve SociaL Communication in Autism (VANILLA) clinical trial, which evaluated three doses of balovaptan (1.5, 4, and 10 mg), compared with a placebo in adult men with moderate to severe ASD and with an IQ ≥70. Balovaptan was administered daily for 12 weeks. The objective of the study was to assess the safety, tolerability, and effect of balovaptan on social communication and social interaction deficits.

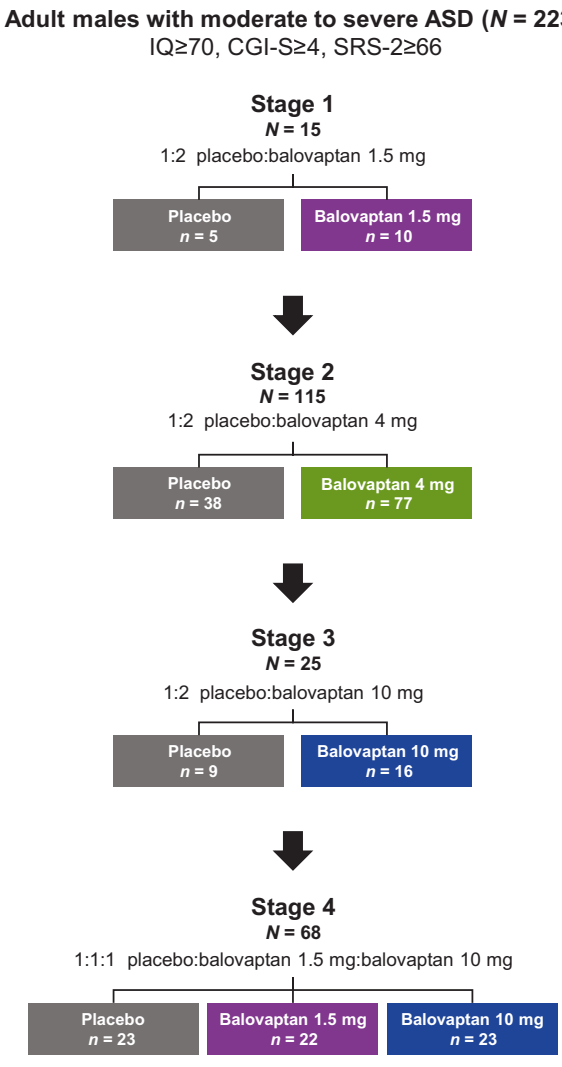


**Fig. 1. VANILLA trial study design.** The VANILLA trial was a staggered parallel-group, multicenter, randomized, double-blind, placebo-controlled phase 2 trial to evaluate the efficacy and safety of orally administered balovaptan at a 1.5-, 4-, or 10-mg dose daily for 12 weeks in adult males with ASD. Participants were randomized in stage 1 at 2:1 to balovaptan 1.5 mg daily or placebo. Participants were randomized in stage 2 at 2:1 to balovaptan 4 mg daily or placebo and in stage 3 at 2:1 to balovaptan 10 mg daily or placebo. Participants were randomized in stage 4 at 1:1:1 to balovaptan 1.5 mg daily, balovaptan 10 mg daily, or placebo. Transitions between stages and escalation to the next dose were supported by safety data review performed by an internal monitoring committee (IMC) and an independent scientific oversight committee (SOC). CGI-S, Clinical Global Impression–Severity.

## RESULTS

### Study design and balovaptan dose selection in the VANILLA trial

The VANILLA clinical trial (ClinicalTrials.gov identifier: NCT01793441) was a sequential cohort, parallel-group, multicenter, randomized, double-blind, placebo-controlled phase 2 trial. The study proceeded through four sequentially recruited stages (Fig. 1), with stages 1 to 3 designed to assess escalating doses of balovaptan and stage 4 added to increase the sample size of the balovaptan 1.5- and 10-mg cohorts.

The lower dose of balovaptan (1.5 mg) was selected to provide some initial safety information at a dose expected to produce limited pharmacodynamic effect. The 4-mg dose of balovaptan referred to estimates using modeling calculations of a V1a receptor occupancy based on a peripheral ex vivo platelet aggregation assay and pharmacokinetic/pharmacodynamic modeling. This predicted a V1a receptor occupancy of >80% in the brain over 24 hours. The 10-mg dose was predicted to provide a receptor occupancy of >90% in most of the dosed participants. Available safety data were reviewed before initiating the balovaptan 4- and 10-mg dose arms.

### Participants in the VANILLA trial

The VANILLA trial enrolled 223 participants meeting criteria for ASD as defined in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) and International Classification of Diseases, 10th Revision (ICD-10). A total of 398 individuals were screened for eligibility across 26 sites in the United States between

January 2014 and May 2016 (Fig. 2). Participants were randomized to receive placebo ($n$ = 75), balovaptan 1.5 mg ($n$ = 32), balovaptan 4 mg ($n$ = 77), and balovaptan 10 mg ($n$ = 39) (Fig. 2).

Participant demographics and baseline characteristics are presented in Table 1, with the median age ranging from 22 to 26 years and median IQ ranging from 95.5 to 98.5 across treatment groups. Baseline severity of ASD (Table 1) was also similar across groups as measured by the ADOS communication/social interaction total score (median ranging from 12.0 to 13.0) and CGI-S scales (median 4.0 in all groups). Although the ADOS, 2nd Edition (ADOS-2) scale was not used as an entry criterion, of the 223 participants in the VANILLA trial, 215 exceeded the threshold on ADOS-2 for an autism diagnosis, 5 met criteria for autism spectrum, and only 3 did not meet criteria (nonspectrum). Baseline SRS-2 $T$ scores were 77.7 ± 7.4 (mean ± SD) in the placebo group and 78.0 ± 8.0, 77.9 ± 7.3, and 75.4 ± 6.5 in the balovaptan 1.5-, 4-, and 10-mg treatment groups, respectively. Visual inspections showed no major departures from a normal distribution among the study population (fig. S1). There was no clear relationship between baseline SRS-2 total proxy $T$ scores and Vineland-II composite or domain scores (table S1).

Caregivers responsible for completing key assessments were in the following relationships with the study participants: 75% mothers, 12% fathers, 5% other custodial adults, 4% teachers, and 4% other. There was little change in caregivers and, therefore, little change in raters for caregiver-completed assessments during the trial.

Before enrollment and throughout the study, 81 to 86% of participants across treatment groups were on concomitant pharmacological treatments, with the most common being selective serotonin reuptake inhibitors (28 to 35% across groups). Antipsychotic agents were taken by 15 to 28% of participants across treatment groups, and central nervous system stimulants were taken by 13 to 26% of participants. There were no imbalances in the percentage of participants taking psychotropic drugs across the treatment arms.

The 12-week treatment period was completed by 192 (86.1%) randomized participants, with the safety follow-up 6 weeks after the last dose (week 18) completed by 186 (83.4%) randomized participants. The most common causes for discontinuation were withdrawal by subject ($n$ = 16) and withdrawal due to adverse events ($n$ = 8) (table S2). More than 90% of study medication was taken by all except six participants as recorded in their treatment diaries (four participants missed >15%, one participant missed between 10 and 15%, and one participant did not document dosing and was withdrawn from study treatment).

Plasma concentrations of balovaptan were monitored throughout the study using a sparse sampling design that enabled characterization, using population pharmacokinetic modeling, of maximum plasma concentration ($C_{max}$) and area under the curve (AUC) at steady state, at both individual and population levels. The AUC and $C_{max}$ values were as predicted. Visual inspection of individual pharmacokinetic time courses and the treatment diary records and pharmacokinetic analysis measured over 12 weeks of treatment allowed the assessment of compliance, which was determined to be good for most of the participants.

## Primary efficacy outcome measured by the SRS-2

The primary efficacy outcome was the change from baseline at week 12 in ASD symptoms as measured by the caregiver-rated SRS-2 total $T$ score. The $T$ score was analyzed separately on three datasets obtained by pooling participant data from each active drug dose

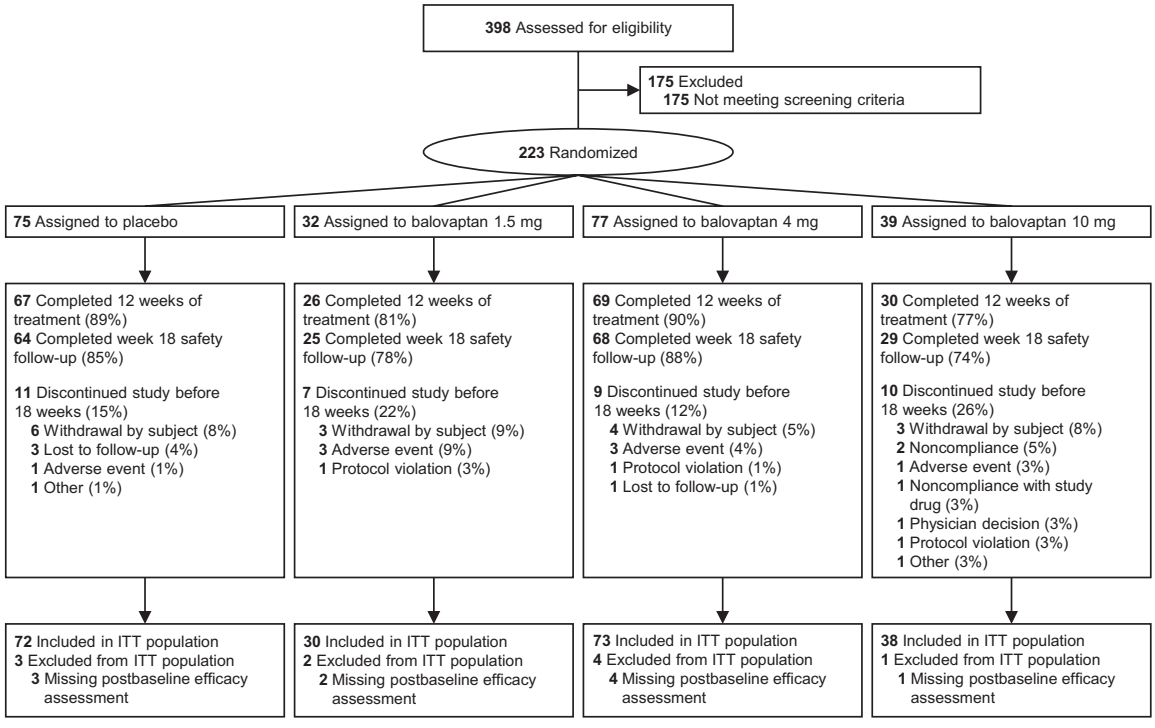


**Fig. 2. CONSORT flow diagram for the VANILLA phase 2 trial.** A total of 398 individuals were screened for eligibility across 26 sites in the United States between January 2014 and May 2016. The VANILLA trial enrolled 223 participants meeting predefined criteria for ASD. Participants were randomized to receive placebo ($n$ = 75), balovaptan 1.5 mg ($n$ = 32), balovaptan 4 mg ($n$ = 77), and balovaptan 10 mg ($n$ = 39). CONSORT, Consolidated Standards of Reporting Trials; ITT, intent-to-treat.

**Table 1. Participant demographics and baseline characteristics.**

| | Placebo | Balovaptan | | |
|---|---|---|---|---|
| | | 1.5 mg | 4 mg | 10 mg |
| **Participant demographics, safety population** | | | | |
| *N* | 75 | 32 | 77 | 39 |
| Age, years | | | | |
| Mean (SD) | 24.7 (6.3) | 28.2 (7.8) | 24.5 (6.6) | 23.9 (5.0) |
| Median | 23.0 | 26.0 | 22.0 | 22.0 |
| Minimum to maximum | 18–45 | 18–45 | 18–42 | 18–38 |
| Race, *n* (%) | | | | |
| Asian | 2 (2.7) | 0 | 1 (1.3) | 0 |
| Black or African American | 7 (9.3) | 3 (9.4) | 4 (5.2) | 4 (10.3) |
| White | 63 (84.0) | 27 (84.4) | 69 (89.6) | 34 (87.2) |
| Other | 3 (4.0) | 2 (6.3) | 3 (3.9) | 1 (2.6) |
| Ethnicity, *n* (%) | | | | |
| Hispanic or Latino | 4 (5.3) | 3 (9.4) | 3 (3.9) | 5 (12.8) |
| Not Hispanic or Latino | 70 (93.3) | 28 (87.5) | 72 (93.5) | 33 (84.6) |
| Not reported | 0 | 0 | 2 (2.6) | 1 (2.6) |
| Unknown | 1 (1.3) | 1 (3.1) | 0 | 0 |
| **Baseline characteristics, ITT population** | | | | |
| *N* | 72 | 30 | 73 | 38 |
| **ADOS-2** | | | | |
| Communication total | | | | |
| Mean (SD) | 4.4 (1.8) | 4.4 (1.7) | 4.4 (1.7) | 4.7 (1.7) |
| Median | 5.0 | 4.0 | 4.0 | 4.0 |
| Minimum to maximum | 1–8 | 2–8 | 1–8 | 2–8 |
| Communication/social interaction total | | | | |
| Mean (SD) | 13.3 (3.9) | 13.0 (3.7) | 13.1 (4.3) | 13.7 (4.0) |
| Median | 13.0 | 12.0 | 13.0 | 13.0 |
| Minimum to maximum | 5–21 | 7–22 | 2–22 | 7–21 |
| Imagination/creativity | | | | |
| Mean (SD) | 0.9 (0.7) | 0.8 (0.8) | 1.1 (0.7) | 0.9 (0.8) |
| Median | 1.0 | 1.0 | 1.0 | 1.0 |
| Minimum to maximum | 0–2 | 0–2 | 0–3 | 0–3 |
| Social interaction total | | | | |
| Mean (SD) | 8.9 (2.6) | 8.6 (2.5) | 8.7 (3.0) | 9.1 (2.7) |
| Median | 9.0 | 8.0 | 9.0 | 9.0 |
| Minimum to maximum | 4–14 | 4–14 | 1–14 | 5–14 |
| Stereotyped/restricted interest total | | | | |
| Mean (SD) | 1.8 (1.7) | 1.6 (1.2) | 2.0 (1.6) | 1.4 (1.7) |
| Median | 2.0 | 1.0 | 2.0 | 1.0 |
| Minimum to maximum | 0–7 | 0–4 | 0–7 | 0–7 |
| **CGI-S** | | | | |
| Mean (SD) | 4.4 (0.6) | 4.4 (0.6) | 4.4 (0.5) | 4.4 (0.6) |
| Median | 4.0 | 4.0 | 4.0 | 4.0 |
| Minimum to maximum | 4–6 | 4–6 | 4–6 | 4–6 |

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| | Placebo | Balovaptan | | |
|---|---|---|---|---|
| | | 1.5 mg | 4 mg | 10 mg |
| **SRS-2 total *T* score (caregiver)** | | | | |
| Mean (SD) | 77.7 (7.4) | 78.0 (8.0) | 77.9 (7.3) | 75.4 (6.5) |
| Median | 77.5 | 77.0 | 77.0 | 75.5 |
| Minimum to maximum | 59–95 | 66–97 | 63–94 | 66–91 |
| **Vineland-II Adaptive Behavior Scales, composite** | | | | |
| Mean (SD) | 59.5 (13.1) | 56.4 (14.2) | 62.6 (12.8) | 59.8 (11.2) |
| Median | 62.0 | 60.0 | 63.0 | 62.0 |
| Minimum to maximum | 25–85 | 27–79 | 28–89 | 37–82 |
| **Wechsler Abbreviated Scale of Intelligence, full-scale IQ score** | | | | |
| Mean (SD) | 96.6 (15.1) | 100.1 (17.5) | 99.5 (17.2) | 97.3 (17.8) |
| Median | 98.5 | 97.0 | 96.0 | 95.5 |
| Minimum to maximum | 71–142 | 71–143 | 70–141 | 72–138 |

with data from the concurrently randomized placebo participants in the corresponding stages 1 to 4: dataset 1: 1.5-mg dose ($n$ = 32) versus placebo ($n$ = 28) using data from stages 1 and 4; dataset 2: 4-mg dose ($n$ = 77) versus placebo ($n$ = 38) using stage 2 data; and dataset 3: 10-mg dose ($n$ = 39) versus placebo ($n$ = 32) using stage 3 and 4 data.

A mean improvement in symptoms on the SRS-2 was reported across all groups, including placebo at week 12 from baseline (SRS-2 total $T$ score least-squares mean, dataset 1: placebo, −13.09; balovaptan 1.5-mg dose, −12.93; dataset 2: placebo, −11.39; balovaptan 4-mg dose, −10.83; dataset 3: placebo, −8.33; balovaptan 10-mg dose, −11.26) (Table 2 and table S3). No statistically significant differences in change from baseline in SRS-2 $T$ scores were observed at the 12-week primary endpoint for balovaptan treatment compared with placebo for all evaluated doses: estimated treatment difference (Δ) and effect size (ES) versus placebo, 1.5-mg dose Δ = 0.15, ES = 0.02, $P$ = 0.96; 4-mg dose Δ = 0.56, ES = 0.07, $P$ = 0.74; 10-mg dose Δ = −2.93, ES = −0.31, $P$ = 0.25 (Fig. 3 and Table 2). Similar results were obtained using SRS-2 raw scores (ES, active against placebo: balovaptan 1.5-mg dose, −0.01; balovaptan 4-mg dose, 0.07; balovaptan 10-mg dose, −0.35). These results were not statistically significant ($P$ value versus placebo: balovaptan 1.5-mg dose, 0.98; balovaptan 4-mg dose, 0.72; and balovaptan 10-mg dose, 0.22).

### Secondary efficacy outcomes measured by the Vineland-II Adaptive Behavior Scales

A key secondary outcome was the change from baseline on the Vineland-II scale, which assesses adaptive behaviors and skills in three defined domains: communication, socialization, and daily living skills. Visual inspection of baseline scores on the Vineland-II composite and domain scale suggested no major departures from normality among the VANILLA study participants (fig. S1). Improvements were observed on the Vineland-II composite score across balovaptan treatment groups compared with placebo, with the magnitude of the treatment effect increasing with dose. Estimated treatment difference (Δ) and ES versus placebo with nominal $P$ values were as follows: 1.5-mg dose Δ = 1.96, ES = 0.24, $P$ = 0.41; 4-mg dose Δ = 3.95, ES = 0.59, $P$ = 0.005; 10-mg dose Δ = 4.87, ES = 0.49, $P$ = 0.074 (Fig. 4 and Table 2). In contrast to the SRS-2, observed changes from baseline were, on average, minimal in the placebo group on the Vineland-II composite and domain scores and were small to moderate on other scales (table S3). Similar results were obtained from the sensitivity analyses, which evaluated this outcome on the overall pooled population, according to the baseline observed carried forward method, and on the per-protocol population (table S4). In addition, results were similar after removing composite scores in cases where the difference between the highest and lowest domain standard score was greater than 35 (maximum to minimum domain standard score ≥35 removed; table S4), because, according to the Vineland-II manual, such composite scores should not be considered meaningful representations of the individual's overall level of functioning (*27*).

On individual Vineland-II domain scores (Table 2), improvement with balovaptan treatment compared with placebo was observed in the socialization and communication domain standard scores. On the daily living skills domain score, an improvement compared with placebo was observed for the balovaptan 4-mg dose but not the 1.5- or 10-mg doses.

To assess whether the treatment effects observed with balovaptan were clinically meaningful, we evaluated our results against the minimally clinically important difference (MCID) for the Vineland-II composite score. This was defined in a recent report using pooled data from multiple consortia and registries, from which it was estimated that the MCID for the Vineland-II composite score in adults with ASD and IQ ≥70 was 3.4 or 3.8 points, depending on the method applied (*28*). In the VANILLA trial, a higher proportion of participants in the balovaptan 4- and 10-mg treatment groups showed an improvement of ≥4 points on the Vineland-II composite score from baseline compared with placebo. The proportion of responders increased in a dose-dependent fashion (placebo, 37.9%; balovaptan 1.5-mg dose, 40.0%; balovaptan 4-mg dose, 45.6%; balovaptan 10-mg dose, 55.2%) (fig. S2).

To explore the relationship of changes in the Vineland-II scores with balovaptan concentration, individual values of Vineland-II

**Table 2. Primary and secondary efficacy outcomes: Change at week 12 from baseline.** SRS-2: decreasing score = improvement; Vineland-II: increasing score = improvement; ABC: decreasing score = improvement; RBS-R: decreasing score = improvement; ADAMS: decreasing score = improvement; STAI: decreasing score = improvement; CGI-I: decreasing score = improvement.

| Dose (mg) | Change from baseline, least-squares mean | | Estimated treatment difference | 90% CI | ES | *P** |
|---|---|---|---|---|---|---|
| | Balovaptan | Placebo | | | | |
| **SRS-2 (caregiver, total *T* score)** | | | | | | |
| 1.5 | −12.93 | −13.09 | 0.15 | −4.62, 4.92 | 0.02 | 0.957 |
| 4 | −10.83 | −11.39 | 0.56 | −2.24, 3.36 | 0.07 | 0.740 |
| 10 | −11.26 | −8.33 | −2.93 | −7.19, 1.32 | −0.31 | 0.254 |
| **Vineland-II Adaptive Behavior Scales** | | | | | | |
| Vineland-II Adaptive Behavior composite standard score | | | | | | |
| 1.5 | 4.47 | 2.51 | 1.96 | −2.00, 5.92 | 0.24 | 0.410 |
| 4 | 4.25 | 0.30 | 3.95 | 1.66, 6.24 | 0.59 | 0.005 |
| 10 | 7.91 | 3.04 | 4.87 | 0.40, 9.33 | 0.49 | 0.074 |
| Vineland-II Adaptive Behavior socialization standard score | | | | | | |
| 1.5 | 5.99 | 5.52 | 0.48 | −5.69, 6.64 | 0.04 | 0.897 |
| 4 | 4.14 | 1.44 | 2.70 | −0.27, 5.67 | 0.31 | 0.135 |
| 10 | 9.00 | 3.03 | 5.97 | 0.14, 11.80 | 0.46 | 0.093 |
| Vineland-II Adaptive Behavior communication standard score | | | | | | |
| 1.5 | 7.58 | 1.93 | 5.65 | −2.57, 13.87 | 0.33 | 0.254 |
| 4 | 5.43 | −0.68 | 6.10 | 1.71, 10.49 | 0.47 | 0.023 |
| 10 | 9.97 | 2.50 | 7.46 | 0.13, 14.79 | 0.46 | 0.094 |
| Vineland-II Adaptive Behavior daily living skills standard score | | | | | | |
| 1.5 | 0.20 | 1.28 | −1.08 | −4.36, 2.19 | −0.16 | 0.582 |
| 4 | 3.76 | 0.80 | 2.96 | 0.91, 5.01 | 0.49 | 0.018 |
| 10 | 5.59 | 4.16 | 1.44 | −2.49, 5.37 | 0.17 | 0.543 |
| **ABC** | | | | | | |
| ABC total I (irritability) | | | | | | |
| 1.5 | −1.99 | −2.85 | 0.86 | −1.67, 3.39 | 0.16 | 0.571 |
| 4 | −1.64 | −1.07 | −0.57 | −2.16, 1.02 | −0.12 | 0.553 |
| 10 | −3.42 | −2.99 | −0.43 | −2.55, 1.69 | −0.09 | 0.734 |
| ABC total II (lethargy) | | | | | | |
| 1.5 | −5.28 | −4.40 | −0.88 | −4.37, 2.60 | −0.12 | 0.672 |
| 4 | −3.86 | −2.89 | −0.97 | −3.18, 1.24 | −0.15 | 0.469 |
| 10 | −6.98 | −5.94 | −1.03 | −3.55, 1.48 | −0.19 | 0.494 |
| ABC total III (stereotype) | | | | | | |
| 1.5 | −0.03 | −1.06 | 1.03 | −0.51, 2.56 | 0.32 | 0.267 |
| 4 | −1.22 | −1.08 | −0.14 | −1.03, 0.76 | −0.05 | 0.803 |
| 10 | −1.80 | −1.34 | −0.46 | −1.53, 0.60 | −0.20 | 0.469 |
| ABC total IV (hyperactivity) | | | | | | |
| 1.5 | −4.14 | −2.59 | −1.55 | −4.49, 1.39 | −0.25 | 0.381 |
| 4 | −2.22 | −0.36 | −1.86 | −3.50, −0.22 | −0.38 | 0.063 |
| 10 | −3.90 | −3.65 | −0.25 | −2.97, 2.47 | −0.04 | 0.878 |

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| Dose (mg) | Change from baseline, least-squares mean | | Estimated treatment difference | 90% CI | ES | *P** |
|---|---|---|---|---|---|---|
| | Balovaptan | Placebo | | | | |
| ABC total V (inappropriate speech) | | | | | | |
| 1.5 | −1.26 | −0.44 | −0.81 | −2.00, 0.37 | −0.33 | 0.254 |
| 4 | −0.87 | −0.74 | −0.13 | −0.81, 0.55 | −0.07 | 0.747 |
| 10 | −1.27 | −1.50 | 0.22 | −0.58, 1.03 | 0.13 | 0.645 |
| **RBS-R (overall score)** | | | | | | |
| 1.5 | −5.48 | −5.58 | 0.11 | −7.23, 7.45 | 0.01 | 0.980 |
| 4 | −5.95 | −5.42 | −0.53 | −4.08, 3.01 | −0.05 | 0.803 |
| 10 | −11.72 | −9.19 | −2.54 | −7.51, 2.44 | −0.23 | 0.397 |
| **ADAMS** | | | | | | |
| ADAMS depressed mood total | | | | | | |
| 1.5 | −0.93 | −1.12 | 0.20 | −1.44, 1.84 | 0.06 | 0.840 |
| 4 | −1.76 | −0.73 | −1.03 | −2.11, 0.05 | −0.32 | 0.118 |
| 10 | −1.32 | −1.98 | 0.66 | −0.93, 2.25 | 0.19 | 0.488 |
| ADAMS general anxiety total | | | | | | |
| 1.5 | −1.81 | −2.62 | 0.81 | −0.95, 2.56 | 0.22 | 0.445 |
| 4 | −1.85 | −0.82 | −1.03 | −2.12, 0.06 | −0.32 | 0.120 |
| 10 | −1.83 | −1.41 | −0.42 | −1.92, 1.09 | −0.13 | 0.644 |
| ADAMS manic/hyperactive behavior total | | | | | | |
| 1.5 | −1.91 | −1.63 | −0.28 | −1.61, 1.06 | −0.10 | 0.731 |
| 4 | −1.81 | −0.67 | −1.14 | −1.94, −0.34 | −0.48 | 0.020 |
| 10 | −1.28 | −1.51 | 0.22 | −1.23, 1.68 | 0.07 | 0.797 |
| ADAMS obsessive/compulsive behavior total | | | | | | |
| 1.5 | −1.00 | −1.13 | 0.13 | −0.73, 0.99 | 0.07 | 0.803 |
| 4 | −0.89 | −0.66 | −0.23 | −0.79, 0.33 | −0.14 | 0.496 |
| 10 | −1.03 | −1.02 | −0.01 | −0.59, 0.56 | −0.01 | 0.974 |
| ADAMS social avoidance total | | | | | | |
| 1.5 | −2.82 | −2.49 | −0.33 | −2.72, 2.06 | −0.07 | 0.818 |
| 4 | −2.61 | −1.66 | −0.95 | −2.13, 0.23 | −0.27 | 0.186 |
| 10 | −2.99 | −2.89 | −0.11 | −2.07, 1.86 | −0.03 | 0.927 |
| **STAI** | | | | | | |
| 1.5 | 1.02 | 0.44 | 0.59 | −5.05, 6.23 | 0.05 | 0.862 |
| 4 | −2.60 | −1.33 | −1.27 | −4.72, 2.19 | −0.14 | 0.543 |
| 10 | −2.82 | −2.55 | −0.27 | −3.73, 3.20 | −0.03 | 0.898 |
| **CGI-I** | | | | | | |
| 1.5 | 3.34 | 3.35 | −0.00 | −0.50, 0.49 | −0.00 | 0.989 |
| 4 | 3.29 | 3.44 | −0.14 | −0.43, 0.15 | −0.17 | 0.414 |
| 10 | 3.28 | 3.10 | 0.18 | −0.20, 0.56 | 0.22 | 0.424 |

*Nominal *P* value applies to secondary endpoints.

composite (fig. S3A) and domain scores (fig. S3, B and C) that changed from baseline were plotted against balovaptan steady-state AUC values. The proportion of participants with a positive change from baseline in the Vineland-II composite score increased with increasing AUC (fig. S3A).

### Additional secondary efficacy outcomes

Additional secondary outcome measures evaluated the effect of balovaptan on general symptom severity and other ASD symptoms, such as restricted and repetitive behaviors, irritability, mood, and anxiety, using the Aberrant Behavior Checklist (ABC), Clinical Global

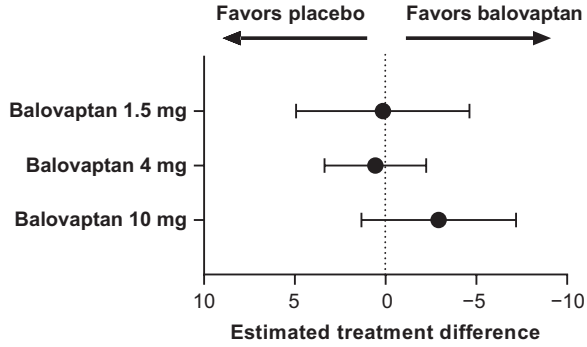


**Fig. 3. Estimated treatment difference at week 12 between balovaptan and placebo on the caregiver-rated SRS-2 *T* score.** Analysis was performed separately on three datasets obtained by pooling participant data from each active dose group with those from the concurrently randomized placebo group administered in the corresponding stage of the study: dataset 1: 1.5-mg dose ($n = 32$) versus placebo ($n = 28$), using stage 1 and 4 data; dataset 2: 4 mg ($n = 77$) versus placebo ($n = 38$), using stage 2 data; and dataset 3: 10 mg ($n = 39$) versus placebo ($n = 32$), using stage 3 and 4 data. Estimated treatment difference (Δ) and ES versus placebo: 1.5-mg dose Δ = 0.15, ES = 0.02, $P = 0.96$; 4-mg dose Δ = 0.56, ES = 0.07, $P = 0.74$; 10-mg dose Δ = −2.93, ES = −0.31, $P = 0.25$. Estimated treatment difference is the difference between the least-squares means of the three balovaptan dose groups and placebo, calculated separately by dataset. Error bars indicate 90% confidence intervals (CIs).

Impression–Improvement (CGI-I), Repetitive Behavior Scale–Revised (RBS-R), State-Trait Anxiety Inventory (STAI), and Anxiety, Depression and Mood Scale (ADAMS).

No consistent treatment effect was observed on the ABC, RBS-R, ADAMS, STAI, and CGI-I scales (Table 2). However, on the CGI-I scale, we saw a numerically higher percentage of responders (defined as scores of 1 or 2) with all three balovaptan doses compared with placebo at week 12 (% responders: dataset 1: placebo, 8.7%; balovaptan 1.5-mg dose, 30.8%; dataset 2: placebo, 13.5%; balovaptan 4-mg dose, 23.1%; dataset 3: placebo, 15.4%, balovaptan 10-mg dose, 23.3%) (fig. S4).

## Exploratory outcomes measured by the Pediatric Quality of Life Scale

Among the exploratory outcomes, health-related quality of life was assessed using the Pediatric Quality of Life Inventory (PedsQL) Generic Core Scale, Cognitive Functioning Scale, and Family Impact Module. Improvements from baseline at week 12 were observed in the balovaptan 10-mg dose group compared with placebo on each of the following scales: Generic Core Scale (mean ± SD; placebo, 3.9 ± 13.0; balovaptan 1.5-mg dose, 2.0 ± 13.7; balovaptan 4-mg dose, 6.2 ± 10.8; balovaptan 10-mg dose, 9.8 ± 10.1), Cognitive Functioning Scale (mean ± SD; placebo, 5.7 ± 20.4; balovaptan 1.5-mg dose, 3.0 ± 21.3; balovaptan 4-mg dose, 9.3 ± 19.4; balovaptan 10-mg dose, 9.7 ± 15.5), and Family Impact Module (mean ± SD; placebo, 5.2 ± 15.1; balovaptan 1.5-mg dose, 4.1 ± 13.9; balovaptan 4-mg dose, 4.3 ± 14.3; balovaptan 10-mg dose, 8.3 ± 18.7) (tables S5 and S6).

## Safety of balovaptan treatment in the VANILLA trial

The proportion of participants reporting any adverse events was similar across study cohorts. Adverse events were reported in 64.0% of the placebo group and 78.1, 66.2, and 66.7% of the balovaptan 1.5 mg–, 4 mg–, and 10 mg–treated participants, respectively (Table 3 and

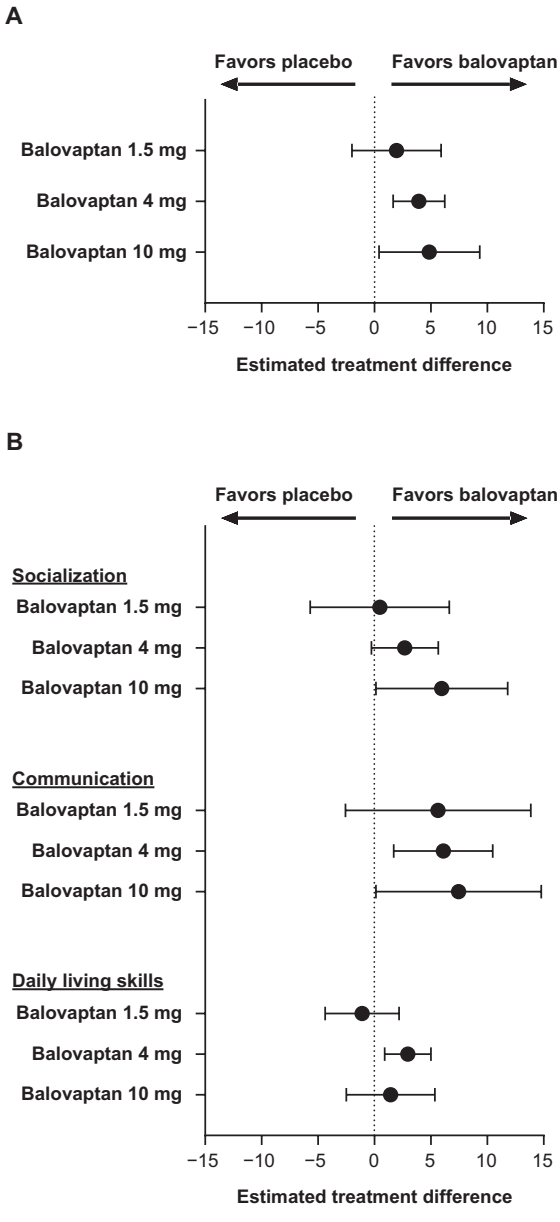


**Fig. 4. Estimated difference at week 12 between balovaptan treatment and placebo on the Vineland-II scale.** (**A**) Composite standard score and (**B**) individual standard scores for the socialization, communication, and daily living skills domains of the Vineland-II Adaptive Behavior Scale. Estimated treatment difference (Δ) on the Vineland-II composite score and ES versus placebo with nominal *P* values were as follows: 1.5-mg dose Δ = 1.96, ES = 0.24, $P = 0.41$; 4-mg dose Δ = 3.95, ES = 0.59, $P = 0.005$; 10-mg dose Δ = 4.87, ES = 0.49, $P = 0.07$. Estimated treatment difference and ES on the Vineland-II socialization domain score with nominal *P* values were as follows: 1.5-mg dose Δ = 0.48, ES = 0.04, $P = 0.90$; 4-mg dose Δ = 2.70, ES = 0.31, $P = 0.14$; 10-mg dose Δ = 5.97, ES = 0.46, $P = 0.09$. Estimated treatment difference and ES on the Vineland-II communication domain score with nominal *P* values were as follows: 1.5-mg dose Δ = 5.65, ES = 0.33, $P = 0.25$; 4-mg dose Δ = 6.10, ES = 0.47, $P = 0.02$; 10-mg dose Δ = 7.46, ES = 0.46, $P = 0.09$. Estimated treatment difference and ES on the Vineland-II daily living skills domain score with nominal *P* values were as follows: 1.5-mg dose Δ = −1.08, ES = −0.16, $P = 0.58$; 4-mg dose Δ = 2.96, ES = 0.49, $P = 0.02$; 10-mg dose Δ = 1.44, ES = 0.17, $P = 0.54$. Estimated treatment difference is the difference between the least-squares means of the three balovaptan dose groups and placebo, calculated separately by dataset. Error bars indicate 90% CIs.

table S7). The most common adverse events were headache, which was reported more often in participants receiving placebo (21.3%) compared with balovaptan treatment (1.5-mg dose, 12.5%; 4-mg dose, 13.0%; 10-mg dose, 12.8%). On the basis of comparison of frequency and intensity across study cohorts, no particular adverse event term was considered as a safety liability because of the administration of balovaptan.

Within the 12-week treatment period, there were seven serious adverse events in four participants, with one serious adverse event in a participant (suicidal ideation) treated with placebo, five serious adverse events in two participants treated with balovaptan 1.5 mg (participant 1: sinus node dysfunction; participant 2: rhabdomyolysis 2 events, acute psychosis, and agitation), and one serious adverse event in one participant (syncope) treated with balovaptan 4 mg (table S2). With the exception of the serious adverse event of rhabdomyolysis in a participant treated with balovaptan 1.5 mg, none of the other serious adverse events were considered related to study treatment by the responsible investigator. Treatment was withdrawn in eight participants (placebo, one; balovaptan 1.5-mg dose, three; balovaptan 4-mg dose, three; balovaptan 10-mg dose, one) because of adverse events (table S2).

Monitoring of heart rate, blood pressure including orthostatic challenge testing, and electrocardiograms (ECGs) did not identify safety signals considered related to the treatment with balovaptan (table S8). Orthostatic challenge testing was included given the hypothetical liability of an impairment in blood pressure regulation due to inhibition of peripheral vascular V1a receptors. No apparent imbalance in the percentage of participants meeting the definition of orthostatic hypotension (decrease in systolic and/or diastolic blood pressure by at least 20 and 10 mmHg, respectively) emerged across treatment cohorts (table S8). No balovaptan-related abnormalities in any measurements of clinical chemistry, hematology, and urinalysis were identified. Creatine phosphokinase values were variable, but overall, no balovaptan-associated safety signals in terms of toxicity in skeletal muscle were identified. Also, no safety signals from physical or neurological examinations were identified.

### Pharmacokinetics of balovaptan

Balovaptan was rapidly absorbed with a median time to maximum concentration of 3 hours and eliminated with a half-life of 47 hours. Food was previously shown to have no relevant effect on the pharmacokinetics of balovaptan. Steady-state pharmacokinetic parameters were proportional to balovaptan dose, with moderate intersubject variability. Steady-state concentrations of balovaptan were reached by week 2 of dosing (table S9).

## DISCUSSION

In the VANILLA phase 2 clinical trial, daily administration of the selective V1a receptor antagonist balovaptan was not associated with a statistically significant change from baseline in the SRS-2 score compared with placebo at 12 weeks in men with an IQ ≥70. However, a clinically meaningful and dose-dependent improvement on the Vineland-II composite score was observed for the 4- and 10-mg doses of balovaptan compared with placebo, and a higher proportion of balovaptan-treated participants had improvements exceeding the Vineland-II MCID (*28*). These treatment differences increased with dose and were driven by improvements on the Vineland-II scores for the socialization and communication domains. No significant improvements over placebo were seen in other secondary endpoints, including the RBS-R, ABC, ADAMS, STAI, and CGI-I, although benefits were observed on the PedsQL exploratory endpoint assessing health-related quality of life. Balovaptan was well tolerated across all dose arms with no safety concerns identified, and pharmacokinetic parameters were as predicted.

The Vineland-II scale is designed for use in ages from 0 to 90 years and evaluates adaptive behaviors defined as the performance of daily activities required for personal and social sufficiency (*27*). Because there are age-appropriate norms, the scale can be used to study adaptive behavior development. The skills measured by the Vineland-II scale are grouped in three defined domains: communication, socialization, and daily living skills (*9*, *27*). The Vineland-II scale is widely used in research and clinical practice and has been recommended by experts (*9*) and by the European Medicines Agency (*29*) for use in clinical trials. Other features of the Vineland-II scale include its excellent psychometric properties and inter-rater reliability (*9*). However, whereas the VANILLA trial had a 12-week outcome, the conventional time frame for assessing improvements on the Vineland-II scale is at least 6 months (*9*). There is some precedence for observing improvements in less than 6 months. For example, in an exploratory

**Table 3. Overview of adverse events in the safety study population up to week 18.**

| | Number of participants (%) | | | |
|---|---|---|---|---|
| | **Placebo** ***n*** **= 75** | **Balovaptan 1.5 mg** ***n*** **= 32** | **Balovaptan 4 mg** ***n*** **= 77** | **Balovaptan 10 mg** ***n*** **= 39** |
| Adverse event type | | | | |
| Any | 48 (64.0) | 25 (78.1) | 51 (66.2) | 26 (66.7) |
| Severe | 4 (5.3) | 6 (18.8) | 5 (6.5) | 1 (2.6) |
| Serious | 1 (1.3) | 2 (6.3) | 1 (1.3) | 0 |
| Deaths | 0 | 0 | 0* | 0 |
| Withdrawals due to adverse events | 1 (1.3) | 3 (9.4) | 3 (3.9) | 1 (2.6) |

*One death due to suspected heart failure occurred outside the reporting period, ~16 weeks after last dosing.

post hoc analysis of the 12-week arbaclofen phase 2 trial in children with ASD, greater improvement in arbaclofen-treated participants over placebo in the socialization domain was observed when restricting the analysis to participants with a consistent rater across the trial (*30*).

Using Vineland-II scores of >9000 individuals with ASD collated from several consortia and registries, a recent study estimated the MCID for the composite score and domains using distribution and anchor-based methods. The MCID for the composite standard score was estimated to be between 3.4 and 3.8, depending on the method applied in adults with ASD and IQ ≥70 (*28*). In the VANILLA trial, the balovaptan 4- and 10-mg dose treatment groups exceeded this threshold, with mean improvements from baseline of 4.25 and 7.91, respectively; mean scores for the placebo group did not reach the MCID. In addition, in contrast to the SRS-2, no clinically significant changes were observed in the placebo group on the Vineland-II scale. The apparent insensitivity of the Vineland-II scale to large placebo effects could be due to its well-recognized resistance to change in a short time frame and because the items are based on objective, observable skills. Also, given that the rater does not ask items directly during the interview, the informant is not aware of the concepts being explored.

In the current study, consistent improvement on the SRS-2 was observed for all groups, with no difference between treatment groups. The degree of improvement in the placebo group suggests the potential for a strong susceptibility to placebo effects when using this scale in multicenter drug trials. Similar results were observed in multicenter phase 2 trials of the metabotropic glutamate receptor 5 (mGluR5) antagonists mavoglurant (*31*) and basimglurant (*32*) for adolescents and adults with fragile X syndrome and the *N*-methyl-D-aspartate receptor antagonist memantine for children with ASD (*33*). In these trials, the placebo groups also experienced 6- to ~10-point improvements on the SRS-2 at 12 weeks across trials. In addition, a single-site trial of oxytocin for ASD reported 12-point mean improvements in both the oxytocin and placebo groups, and an exploratory analysis suggested that the caregiver's belief of treatment assignment had an effect on the SRS-2 score (*34*). By contrast, several small single-site studies have not reported notable improvements with placebo on the SRS-2 (*35*–*37*), which may suggest that the degree of placebo effect and performance of the SRS-2 may depend on study design and conduct. Because the SRS-2 is a caregiver-reported test, expectation bias may have influenced SRS-2 scores across groups in the VANILLA trial, particularly given that the vast majority of the caregivers who completed the test were the parents of the trial participants (75% mothers and 12% fathers). Also, the use of a threshold on the SRS-2 score as an entry criterion in the VANILLA trial may have led to baseline inflation. While the VANILLA trial was ongoing, a working group on ASD trial endpoints deprioritized the SRS-2 score as an outcome measure of change in symptoms in clinical trials owing to lack of validation and the potential influence of other factors such as age and expressive language (*9*).

Additional secondary outcome measures evaluated the effect of balovaptan on other ASD symptoms, such as stereotyped/restricted and repetitive behaviors, irritability, mood, and anxiety (ABC, RBS-R, STAI, and ADAMS scales). No clear differences were observed between balovaptan and placebo on these scales. However, in part reflecting that the VANILLA trial restricted enrollment to adults with IQ ≥70, baseline scores were very low on some of these scales, such as ABC and RBS-R, and therefore participants had little room for improvement on these scales. In addition, as previously reported, notable placebo effects were observed. Differences between placebo and the balovaptan 4-mg daily dose were observed on the ADAMS manic/hyperactivity behavior and ABC hyperactivity scores. However, this observation is likely due to chance given the variability in the placebo groups and lack of any similar trend in the balovaptan 10-mg daily dose groups. These factors suggest that a number of these scales may not be appropriate for assessing change in the population enrolled in the VANILLA trial and preclude determinations of whether balovaptan treatment affects these behaviors/symptoms.

Among the exploratory outcomes, it is encouraging that trends for improvements in the PedsQL Generic Core Scale, Cognitive Functioning Scale, and Family Impact Module were observed with 10-mg daily dose of balovaptan compared with placebo, although these results need to be replicated in an independent study.

Like vasopressin, oxytocin plays an important role in regulating social behaviors and has been investigated as a potential treatment for ASD (*38*, *39*). However, evidence from phase 2 efficacy clinical trials of oxytocin have yielded mixed results, with some studies showing improvements with oxytocin administration on some outcome measures (*35*–*37*, *40*, *41*) and others showing no improvement with oxytocin in other outcome measures, including for the core symptoms of ASD (*34*, *42*, *43*). Although the role of oxytocin in social behaviors has been demonstrated, clinical studies to date have not shown its utility in treating the core symptoms of ASD. With the exception of a recent trial to study the effect of intranasal oxytocin on the core social symptoms of ASD that enrolled 106 participants with ASD (*43*), most of these trials recruited small numbers of participants (8 to 60 participants). The disparate findings have been attributed to variations in dose, route of administration, study duration, age, and heterogeneity of the oxytocin system (*38*, *39*).

No balovaptan-associated safety signals emerged from the VANILLA trial, supporting the further clinical development of this molecule in larger confirmatory phase 3 trials in adult participants with ASD and in phase 2 trials for pediatric populations. Given the early stage of clinical development, further studies will need to continue diligent safety surveillance of balovaptan.

It is important to note that the VANILLA trial was restricted to adult males with IQ ≥70, SRS-2 score ≥66, and CGI-S score ≥4 (moderately ill) and had a relatively short treatment duration of 12 weeks. Additional studies are needed to confirm the VANILLA trial results and to generalize them to broader populations, including female individuals, children and adolescents, and individuals with an IQ below 70, to determine the long-term effects of balovaptan treatment, and to expand on the safety and tolerability profile of balovaptan. In addition, although improvements were observed on the Vineland-II and PedsQL scales with balovaptan over placebo, other assessments used in the VANILLA trial did not corroborate these findings. The staggered cohort study design and sequential enrollment allowed for the assessment of ascending doses of balovaptan but was associated with interim safety and efficacy analyses for decisions to proceed to the next dose and adjustments for sample size.

The vasopressin system has also been targeted by Parker *et al.* (*44*) in a recent clinical trial of 30 children with ASD. Daily intranasal administration of vasopressin for 4 weeks resulted in a beneficial impact on core symptoms of autism in these children (*44*). Although using different therapeutic strategies, both the Parker *et al.* study and our VANILLA trial point to a central role of vasopressin

in modulating social behavior in ASD. The phase 2 aV1ation trial (ClinicalTrials.gov identifier: NCT02901431) currently under way is evaluating balovaptan safety and efficacy in both male and female children and adolescents (ages 5 to 17 years) with ASD and an IQ ≥70 over a double-blind treatment period of 6 months. Future larger and well-controlled studies in adults and children with ASD will shed further light on the potential usefulness of targeting the vasopressin pathway to address socialization and communication challenges and potentially pave the way for the development of a new pharmacological treatment for these core symptoms of ASD. In conclusion, the phase 2 VANILLA trial did not meet its primary efficacy endpoint of improvement on the SRS-2 with balovaptan treatment compared with placebo in adult males with ASD and IQ ≥70 at 12 weeks. However, an efficacy signal for balovaptan treatment was observed on the Vineland-II Adaptive Behavior Scales composite score. This suggests that V1a receptor inhibition improves social behaviors and communication in adult participants with ASD and adds evidence for a role of vasopressin in human social behaviors. The results also suggest that the signals observed in the single-dose proof-of-mechanism trial with RG7713 (*25*) represented real effects on measures of social cognition. No balovaptan-associated safety issues emerged from the VANILLA trial, and pharmacokinetic parameters were in line with expectations based on phase 1 clinical studies. Further studies are needed to evaluate the potential for balovaptan to improve social communication and social interaction deficits within broader ASD patient populations, including children.

## MATERIALS AND METHODS

### Study design

The VANILLA trial (ClinicalTrials.gov identifier: NCT01793441) was a sequential dose cohort, parallel-group, multicenter, randomized, double-blind, placebo-controlled phase 2 trial to evaluate the efficacy and safety of orally administered balovaptan at 1.5-, 4-, or 10-mg daily doses for 12 weeks in adult males with ASD and an IQ ≥70. The VANILLA trial was conducted at 26 clinical sites in the United States.

Institutional review board/ethics committee approval was obtained before study initiation at each site. The study was conducted in accordance with the principles of the Declarations of Helsinki, International Conference on Harmonisation E6 guidelines for Good Clinical Practice, and applicable U.S. Food and Drug Administration requirements. All participants and their caregivers provided written informed consent and provided U.S. Health Insurance Portability and Accountability Act of 1996 authorization before participation in the study. Participant recruitment efforts were managed by the study sites and included consulting patient registries, working with advocacy groups, and identifying patients through the clinics. Site efforts were supplemented with advertising campaigns via multiple platforms.

#### *Participant eligibility*

Eligible participants included men (18 to 45 years of age) meeting the criteria for ASD based on both the *DSM-5* and ICD-10. Key inclusion criteria included CGI-S score ≥4, SRS-2 *T* score ≥66, and IQ ≥70, with language, hearing, and vision capabilities compatible with completion of study measurements, as judged by the investigator. Participants must either have lived with or have had substantial periods of contact with a caregiver willing and able to attend on-site assessments, oversee participant compliance, and report on participant status via completion of study assessments. Full eligibility requirements are listed in table S10.

#### *Design of the VANILLA trial*

The VANILLA trial was an exploratory study designed to assess both the appropriate dosing of balovaptan as well as preliminary efficacy and safety. The study began with an evaluation of the balovaptan 1.5-mg dose to provide initial safety data at a dose expected to produce limited pharmacodynamic effect. Overall, the study proceeded through four sequentially recruited stages (Fig. 1), with stages 1 to 3 designed to assess escalating doses of balovaptan and stage 4 added to increase sample size of the balovaptan 1.5- and 10-mg dose cohorts. Transitions between stages and escalation to the next dose were supported by safety data review performed by an IMC and an independent SOC. Differences in the final cohort sizes for the different dose groups were accounted for in the statistical analysis plan (see "Statistical methods" section), which specified that the primary efficacy analysis was to be performed separately on three datasets obtained by comparing data from the active dose group with the concurrently randomized placebo group only.

#### *Randomization and masking*

Eligible participants were randomized within a 5-week screening window, in order of enrollment via an independent interactive voice or web response system provider. Participants were randomized in stage 1 as 2:1 to balovaptan 1.5 mg daily or placebo; stage 2 as 2:1 to balovaptan 4 mg daily or placebo; stage 3 as 2:1 to balovaptan 10 mg daily or placebo; and stage 4 as 1:1:1 to balovaptan 1.5 mg daily, balovaptan 10 mg daily, or placebo.

Participants, site personnel, and the sponsor were blinded to treatment assignments, with the exception of the individual responsible for pharmacokinetic data analysis and the IMC/SOC for specified data reviews. The IMC/SOC reviews occurred between stages 1 and 2; for a preplanned interim efficacy analysis after ~50 participants completed 12 weeks of treatment in stage 2; and for the decision to increase sample size in stage 4.

### Balovaptan treatment

Balovaptan or placebo was administered orally daily for 12 weeks, with the first dose on day 1 after predose and baseline assessments, with a 6- to 7-week follow-up period after the last dose. Study medication was provided as balovaptan 0.5-, 4-, and 5-mg hard capsules or matching placebo capsules, with the same number of capsules with identical appearance and weight administered for all treatment arms within each stage. To monitor compliance, participants were asked to keep a diary of all doses taken or missed and to return all unused study drug at each study visit and at treatment end.

### Efficacy outcomes and assessments

Efficacy outcomes were assessed at screening/baseline and prespecified visits (table S11), with the first postdose assessments at day 7 after 1 week of treatment. The primary outcomes were safety and tolerability of 12-week treatments of balovaptan in participants with ASD and efficacy in treating social communication deficits as measured by change from baseline to week 12 on the SRS-2 Caregiver Report Form. Secondary efficacy outcomes included changes from baseline at week 12 in behavior as measured by ABC, RBS-R, STAI, ADAMS, and CGI-I scales; adaptive functioning and skills as measured by Vineland-II Adaptive Behavior Scales; and pharmacokinetics and exposure-response relationships of balovaptan. Additional exploratory outcomes are listed in Supplementary Materials and Methods; results of the exploratory outcomes

will be reported separately. Efficacy assessments were administered on site at the clinic. The SRS-2, ABC, RBS-R, ADAMS, and Vineland-II questionnaires were completed by the designated caregivers of the participants.

### Safety outcomes

Safety outcome measures included spontaneously reported adverse events, and clinical and laboratory assessments including orthostatic challenge testing, ECGs, physical and neurological examination, suicidality monitoring using the Columbia–Suicide Severity Rating Scale, blood chemistry, and hematological monitoring.

### Pharmacokinetic assessments

Blood samples for pharmacokinetic determination of plasma concentrations of balovaptan and its metabolite M3 were taken at day 1, week 2 (day 14), and week 12, predose and postdose at 2, 4, and 6 hours (±30 minutes). An additional predose sample was taken at week 6. Pharmacokinetic outcomes included concentration of balovaptan per time point, and $AUC_{0-24h,ss}$, $C_{max}$, and $C_{min}$ of balovaptan.

### Sample size calculations

In line with the hypothesis-generating nature of this study, the planned sample size was adapted through the course of the study to collect sufficient data on the effects of safe and potentially effective doses in participants with ASD, as well as to minimize the number of participants exposed to a nonefficacious dose. Following interim safety and pharmacokinetic reviews, the planned final sample size at the end of stage 4 was placebo, $n = 67$; balovaptan 1.5-mg dose, $n = 30$; balovaptan 4-mg dose, $n = 70$; and balovaptan 10-mg dose, $n = 35$. This sample size would provide 80% power to detect an ES of 0.55 with a balovaptan 1.5-mg dose, 0.43 with a 4-mg dose, and 0.52 with a 10-mg dose at a one-sided 5% (two-sided 10%) significance level.

### Statistical methods

The primary efficacy analysis was performed separately on three datasets obtained by pooling participant data from each active dose with those from the concurrently randomized placebo administered in the corresponding stage of the study: dataset 1: 1.5 mg versus placebo, using stage 1 and 4 data; dataset 2: 4 mg versus placebo, using stage 2 data; and dataset 3: 10 mg versus placebo, using stage 3 and 4 data. This statistical analysis plan, defined and documented before the database was locked, was designed to address the addition of the various study stages as a result of protocol amendments and to consider the unbalanced exposure of participants over time to a potentially more active dose. This approach also respects the randomization principle by comparing the dose of interest to the placebo simultaneously randomized.

The analysis of primary and secondary continuous efficacy endpoints was performed on the observed case dataset from the ITT population, defined as all participants who provided informed consent, were randomized, received at least one study dose, and completed baseline assessments and at least one postdose assessment. Primary and secondary continuous efficacy endpoints, expressed as changes from baseline, were analyzed using mixed-model repeated measures (MMRM), including main effects for baseline score (where available), treatment, stage (where a dose was administered in different stages) and visit (where repeat visits occurred), and the interactions baseline*visit, treatment*stage, treatment*visit, stage*visit, and stage*visit*treatment. Visit was fitted as a repeated effect with an unstructured correlation structure across visits within each participant. All main effects and interactions were retained in the final model regardless of their statistical significance.

Results of the analysis are presented as point estimates with 90% CIs and associated $P$ values for the adjusted mean (least-squares mean) differences between balovaptan doses and placebo. The extent of the treatment comparison is also reported in terms of Cohen's $d$ ES, derived by dividing the estimate of the mean treatment difference by the pooled SD for the corresponding endpoint obtained by MMRM.

For the Vineland-II composite score, additional sensitivity analyses were performed to assess the robustness of the results. These included an analysis on the overall pooled population; an analysis after discharging composite standard scores of patients showing a difference between the highest domain standard score and the lowest score greater than 35, as the composite would not be considered a meaningful representation of the individual overall level of functioning (maximum to minimum domain standard score, ≥35 removed) (*27*); an analysis with baseline observed carried forward in case of missing postbaseline data; and an analysis on the per-protocol population, defined as the ITT population without major protocol deviations.

All statistical analyses were performed without adjustment for multiple comparisons due to the exploratory nature of the phase 2 study. Exploratory analyses were performed on the overall population participating in the study, obtained by pooling data across all the stages, and are summarized by means of descriptive statistics. The safety study population included all participants who received at least one study dose. Statistical analyses were performed using SAS version 9.2 (SAS Institute).

## SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Distribution and q-q plots of baseline scores on the SRS-2 and Vineland-II composite and domain scores.

Fig. S2. Percentage of participants exceeding the MCID for the Vineland-II composite score at week 12.

Fig. S3. Individual drug exposure versus change from baseline at week 12 on Vineland-II composite and domain scores.

Fig. S4. Frequency of responders on the CGI-I scale at week 12.

Table S1. Correlations at baseline between SRS-2 total proxy $T$ score and Vineland-II Adaptive Behavior Scales composite and domain scores.

Table S2. Serious adverse events and withdrawals due to adverse events.

Table S3. Change from baseline and ES at week 12 on outcome assessments in the placebo group.

Table S4. Sensitivity analyses of the Vineland-II Adaptive Behavior composite standard score.

Table S5. Change from baseline at week 12 in the PedsQL Generic Core Scales, Cognitive Functioning Scale, and Family Impact Module in the ITT population.

Table S6. Analyses of the changes from baseline at week 12 in the PedsQL Generic Core Scales, Cognitive Functioning Scale, and Family Impact Module in the ITT population.

Table S7. Adverse events reported in >5% of participants in any treatment group by Medical Dictionary for Regulatory Activities Preferred Term.

Table S8. Treatment-emergent orthostatic hypotension.

Table S9. Balovaptan steady-state pharmacokinetic parameters.

Table S10. Full eligibility criteria.

Table S11. Efficacy assessment schedule.

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Science Translational Medicine

# A phase 2 clinical trial of a vasopressin V1a receptor antagonist shows improved adaptive behaviors in men with autism spectrum disorder

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**Modulating vasopressin in ASD**

The neuropeptide vasopressin has been implicated in the regulation of social behaviors in animals and humans. The VANILLA clinical trial evaluated balovaptan, an oral selective vasopressin V1a receptor antagonist, in 223 men with autism spectrum disorder (ASD). This 12-week phase 2 trial showed no improvement on the primary endpoint (SRS-2 score) but did show improvement on the secondary outcome measure of adaptive behaviors assessed by the Vineland-II scale (Bolognani *et al*.). In a related clinical study involving a 4-week intranasal administration of vasopressin to children with ASD, improvements were observed on the SRS-2 primary outcome measure (Parker *et al*.). Both drugs were well tolerated and had an acceptable safety profile, suggesting that modulating the vasopressin pathway may be a useful therapeutic strategy for ASD.