ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

RESEARCH ARTICLE

Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial

Robert K. Naviaux[1,2,3,4], Brooke Curtis[5], Kefeng Li[1,2], Jane C. Naviaux[1,6], A. Taylor Bright[1,2], Gail E. Reiner[1,6], Marissa Westerfield[7], Suzanne Goh[8], William A. Alaynick[1,2], Lin Wang[1,2], Edmund V. Capparelli[13], Cynthia Adams[9], Ji Sun[9], Sonia Jain[10], Feng He[10], Deyna A. Arellano[9], Lisa E. Mash[7,11], Leanne Chukoskie[7,12], Alan Lincoln[5] & Jeanne Townsend[6,7]

[1]The Mitochondrial and Metabolic Disease Center, University of California, San Diego School of Medicine, 214 Dickinson St., Bldg CTF, Rm C102, San Diego, 92103-8467, California
[2]Department of Medicine, University of California, San Diego School of Medicine, 214 Dickinson St., Bldg CTF, Rm C102, San Diego, 92103-8467, California
[3]Department of Pediatrics, University of California, San Diego School of Medicine, 214 Dickinson St., Bldg CTF, Rm C102, San Diego, 92103-8467, California
[4]Department of Pathology, University of California, San Diego School of Medicine, 214 Dickinson St., Bldg CTF, Rm C102, San Diego, 92103-8467, California
[5]Alliant International University, 10455 Pomerado Road, San Diego, California, 92131
[6]Department of Neurosciences, University of California, San Diego School of Medicine, 9500 Gilman Drive., La Jolla, CA, 92093-0662
[7]The Research in Autism and Development Laboratory (RAD Lab), University of California, 9500 Gilman Drive, La Jolla, CA, 92093-0959
[8]Pediatric Neurology Therapeutics, 7090 Miratech Dr, San Diego, CA, 92121
[9]Clinical and Translational Research Institute (CTRI), University of California, San Diego, La Jolla, CA, 92037
[10]Department of Family Medicine and Public Health, University of California, San Diego, La Jolla, CA, 92093
[11]Department of Psychology, San Diego State University, 5500 Campanile Drive, San Diego, CA, 92182
[12]Institute for Neural Computation, University of California, 9500 Gilman Drive, La Jolla, 92093-0523
[13]Department of Pediatrics, and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego School of Medicine, 9500 Gilman Drive, La Jolla, CA, 92093-0657

Correspondence
Robert K. Naviaux, The Mitochondrial and Metabolic Disease Center, University of California, San Diego School of Medicine, 214 Dickinson St., Bldg CTF, Rm C102, San Diego, CA 92103-8467. Tel: 619-543-2904; Fax: 619-543-7868;
E-mail: Naviaux@ucsd.edu

Funding Information
All funding for this study was philanthropic. This work was supported in part by gifts from the William Wright Family Foundation, the UCSD Christini Fund, the Autism Research Institute (ARI), the Lennox Foundation, the Gupta Family and Satya Fund, the Agrawal Family, Linda Clark, the N of One Autism Research Foundation, the Rodakis Family, the It Takes Guts Foundation, the UCSD Mitochondrial Disease Research Fund, Dr. Elizabeth Mumper Cooper, and the Daniel and Kelly White Family. Funding for the mass spectrometers was provided by a gift from the Jane Botsford Johnson Foundation. The funders of the study had no role in study design, data collection or analysis, decision to publish, or preparation of the manuscript.

Abstract

Objective: No drug is yet approved to treat the core symptoms of autism spectrum disorder (ASD). Low-dose suramin was effective in the maternal immune activation and Fragile X mouse models of ASD. The Suramin Autism Treatment-1 (SAT-1) trial was a double-blind, placebo-controlled, translational pilot study to examine the safety and activity of low-dose suramin in children with ASD. **Methods**: Ten male subjects with ASD, ages 5–14 years, were matched by age, IQ, and autism severity into five pairs, then randomized to receive a single, intravenous infusion of suramin (20 mg/kg) or saline. The primary outcomes were ADOS-2 comparison scores and Expressive One-Word Picture Vocabulary Test (EOWPVT). Secondary outcomes were the aberrant behavior checklist, autism treatment evaluation checklist, repetitive behavior questionnaire, and clinical global impression questionnaire. **Results**: Blood levels of suramin were 12 ± 1.5 μmol/L (mean $\pm$ SD) at 2 days and 1.5 ± 0.5 μmol/L after 6 weeks. The terminal half-life was 14.7 ± 0.7 days. A self-limited, asymptomatic rash was seen, but there were no serious adverse events. ADOS-2 comparison scores improved by -1.6 ± 0.55 points ($n = 5$; 95% CI = -2.3 to -0.9; Cohen's $d = 2.9$; $P = 0.0028$) in the suramin group and did not change in the placebo group. EOWPVT scores did not change. Secondary outcomes also showed improvements in language, social interaction, and decreased restricted or repetitive behaviors. **Interpretation**: The safety and activity of low-dose suramin showed promise as a novel approach to treatment of ASD in this small study.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Received: 25 February 2017; Revised: 18 April 2017; Accepted: 20 April 2017

doi: 10.1002/acn3.424

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT02508259

Introduction

Autism affects 1–2% of children in the United States.[1,2] Dozens of single genes and chromosomal copy number variants (CNVs)[3] increase the relative risk of autism spectrum disorder (ASD) nearly 5–50 times over the current background risk. Yet no single gene or CNV causes ASD in 100% of children who carry the mutation,[4] and no single DNA mutation accounts for more than 1–2% of all ASD.[5] Specific environmental factors have also been shown to increase the risk of ASD.[6,7] However, no single child has all of the known genetic risk factors for ASD, or is exposed to all the same environmental risks. Although the noncore symptoms of ASD are highly heterogeneous from child to child, making each child unique, the same core features used for diagnosis – abnormalities in social communication, restricted interests, repetitive behaviors, adherence to routine, and/or atypical sensory behaviors – are by definition expressed in every child. One approach to addressing the challenge of many etiologies of ASD is to define a common pathophysiology that can contribute to the core diagnostic symptoms, regardless of the initiating genetic and environmental triggers. We hypothesized that there is a conserved cellular response to metabolic perturbation or danger that is shared by all children with ASD. This is called the cell danger hypothesis.[8] Aspects of the cell danger response (CDR) are also referred to as the integrated stress response.[9–11] Preclinical studies showed that the cell danger response in mice produced a treatable metabolic syndrome that was maintained by purinergic signaling. Antipurinergic therapy with suramin corrected both the behavioral and metabolic features of these genetic and environmental mouse models of ASD.[12–14]

The formulation of the cell danger hypothesis was based on the recognition that similar metabolic pathways were coordinately regulated as an adaptive response to cellular threat regardless of whether the perturbation was caused by a virus,[15] a bacterium,[16] genetic forms of mitochondrial disease,[10] or neurodevelopmental disorders with complex gene–environment pathogenic mechanisms like autism.[17] These metabolic pathways traced to mitochondria. Mitochondria are responsible for initiating and coordinating innate immunity[18] and produce stereotyped changes in oxidative metabolism under stress[19] that lead to the regulated release of purine and pyrimidine nucleotides like ATP and UTP through cell membrane channels.[20] Inside the cell, ATP is an energy carrier. Outside the cell, extracellular ATP (eATP) is a multifunctional signaling molecule, a potent immune modulator,[21] and a damage-associated molecular pattern (DAMP) that can activate microglia, and trigger IL-1β production and inflammasome assembly.[22] Extracellular purines like ATP, ADP, and adenosine, and pyrimidines like UTP are ligands for 19 different purinergic (P2X, P2Y, and P1) receptors.[23] The intracellular concentration of ATP (iATP) in mammalian cells is typically 1–5 mmol/L,[24] but drops when ATP is released through membrane channels under stress. Typical concentrations of extracellular adenine nucleotides in the unstirred water layer at the cell surface where receptors and ligands meet are about 1–10 μmol/L, near the effective concentration for most purinergic receptors,[25] but can increase when ATP is released during cell stress. Concentrations of eATP in the blood are another 500 times lower (10–20 nmol/L).[26] Purinergic effectors like ATP are also coreleased with canonical neurotransmitters like glutamate, dopamine, and serotonin during depolarization at every synapse in which they have been studied[23] and play key roles in activity-dependent synaptic remodeling.[27] These and other features[28–30] led us to test the hypothesis that the CDR[8] was maintained by purinergic signaling.[12–14]

Suramin has many actions. One of its best-studied actions is as an inhibitor of purinergic signaling. It is the oldest member of a growing class of antipurinergic drugs (APDs) in development.[31] Suramin was first synthesized in 1916,[32] making it one of the oldest manmade drugs still in medical use. It is used to treat African sleeping sickness (trypanosomiasis), and remains on the World Health Organization list of essential medications. Concerns about the toxicity of high-dose suramin arose when the cumulative antitrypanosomal dose was increased 5 times or more over several months to treat AIDS or kill cancer cells during chemotherapy. When blood levels were maintained over 150 μmol/L for 3–6 months at a time to treat cancer, a number of dose-limiting side effects were described.[32] These included adrenal insufficiency, anemia, and peripheral neuropathy. In contrast, mouse studies suggested that high-dose suramin was not necessary to treat autism-like symptoms. These studies showed that low-dose suramin that produced blood levels

 © 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

of about 5–10 μM was effective in treating ASD-like symptoms and did not produce toxicity even when used for at least 4 months.[12,14]

Here, we report the findings of the Suramin Autism Treatment-1 (SAT-1) trial, the first direct test of suramin, the cell danger hypothesis, and the relevance of abnormal purinergic signaling in children with ASD. These data help form the foundation for future studies that will test the safety and efficacy of suramin, provide fresh directions for the development of new antipurinergic drugs, and add support to the hypothesis that a potentially treatable metabolic syndrome may contribute to the pathogenesis of autism.

Materials and Methods

Study design and participants

The SAT-1 trial was an investigator-initiated, phase I/II, double-blind, placebo-controlled, randomized clinical trial to examine the safety and activity of single-dose suramin or placebo in 10 children with autism spectrum disorders (ASD). All children met DSM-5 diagnostic criteria for autism spectrum disorder, and received confirmatory testing by Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2) examination. Inclusion criteria were male subjects, ages 4–17 years, living in the San Diego, California region, with a confirmed diagnosis of ASD. Exclusion criteria included children who weighed less than the 5th percentile for age, took prescription medications, or had laboratory evidence of liver, kidney, heart, or adrenal abnormalities. Children living more than a 90-min drive from the testing sites in La Jolla, CA were excluded to eliminate the possibility of aberrant behaviors resulting from extended car travel. Children with known syndromic forms of ASD caused by DNA mutation or chromosomal copy number variation (CNV) were excluded in this first study. Families were asked not to change their children's therapy (e.g., supplements, speech, and behavioral therapies) or diet throughout the study period. The study was conducted between 27 May 2015 (date of the first child to be enrolled) and 3 March 2016 (date of the last child to complete the study).

Regulatory approvals, registration, CONSORT, and informed consent

The research plan, clinical trial protocol, informed consents, advertising, and amendments were approved by the University of California, San Diego (UCSD) Institutional Review Board (IRB Project #150134) before implementation. The study was authorized by the U.S. Food and Drug Administration (IND#118212), and conformed to the World Medical Association Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects,[33] and the International Council for Harmonization (ICH) E6 Good Clinical Practice (GCP) guidelines. The trial was registered with clinical trials.gov (https://clinicaltrials.gov/ct2/show/NCT02508259). Reporting of the SAT-1 trial conformed to CONSORT 2010 guidelines.[34] Signed informed consent, with additional consent for video and still image photography, were obtained from the parents of all participants before enrollment and randomization. Storyboards and social stories were created to review with parents, help children visualize and prepare for the study, and create the opportunity to ask questions (Figure S1, Data S2).

Randomization and masking

Twenty male subjects with ASD were screened. Sixteen met entry criteria. Ten participants could be matched by age, nonverbal IQ, and ADOS scores into five pairs. The randomization sequence was generated electronically by the biostatistical team. Subjects within each pair were allocated to receive suramin or saline according to the prospectively determined randomization sequence. The randomization sequence was concealed from the clinical team and implemented by the UCSD investigational pharmacy, which prepared drug and placebo for infusion. The design was double blind. The mask was not broken until all subjects had completed the study and all clinical data had been collected.

Diagnostic and outcome procedures

The diagnosis of each of the enrolled participants was confirmed by ADOS-2[35] comparison scores of $\geq$7. Nonverbal IQ was tested by Leiter-3 examination.[36] The primary behavioral outcomes were ADOS scores and language assessed by standardized vocabulary testing. Expressive vocabulary was assessed by Expressive One-Word Picture Vocabulary Test (EOWPVT).[37] Primary outcomes were measured at baseline, and 2 days and 6 weeks after infusion. Secondary outcomes were the Aberrant Behavior Checklist (ABC),[38] Autism Treatment Evaluation Checklist (ATEC),[39,40] Clinical Global Impression of Improvement (CGI)[41] (Data S1), and Repetitive Behavior Questionnaire (RBQ).[42] Secondary outcomes were measured at baseline, and 7 days and 6 weeks after infusion.

Protocol deviations

The original protocol was designed to collect electroencephalography (EEG), heart rate variability (HRV), balance, gait, fine motor, and sensory motor data as secondary outcomes. However, the wide range in ages and abilities, small subject numbers, and task compliance difficulties made collection of these data incomplete and

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

insufficiently powered to draw any conclusions. In addition, we found that major language advances were in the form of new speech fluency and new interest in speech and social communication, and not in new vocabulary. Peabody Picture Vocabulary Testing (PPVT) did not capture this new interest in communication. These data were incomplete and insufficiently powered for analysis.

Drug and placebo administration

Suramin was provided as the hexasodium salt (MW 1429.2 g/mol) in 1 g lyophilized vials by Bayer Pharma AG (Leverkusen, Germany), under Dr. Naviaux's IND #118212. Lot #BXNOGW1, expiration date of 3 September 2018, was used in these studies. A 1 g vial was reconstituted in 10 mL of sterile water for infusion to prepare a 10% (100 mg/mL) solution. All infusions were conducted at the University of California, San Diego School of Medicine Clinical and Translational Research Institute (CTRI) in La Jolla, CA. Height and weight were recorded, vital signs and capillary oxygen saturation (pulse oximetry) measured, physical and neurological examinations were conducted, and urine and blood for safety monitoring, pharmacology, and metabolomics were collected before the infusion. Each child then received a 50 mg test dose (0.5 mL of a freshly reconstituted 10% solution) of suramin in 5 mL of saline, or 5 mL of saline only given by slow intravenous (IV) push over 3 min, followed by a 10-mL flush of saline. One hour after the test dose, vital signs were repeated and a single infusion of either suramin (20 mg/kg, minus the 50 mg test dose, in 50 mL, up to a maximum of 1 g) or saline (50 mL IV) was given over 30 min, followed by a 10-mL flush of saline. One hour after completion of the infusion, vital signs and the physical and neurological examinations were repeated, blood was collected for safety monitoring and pharmacology, and the family discharged to home. A typical infusion visit to the Clinical Translational Research Institute (CTRI) lasted about 4 h from start to finish.

Safety and adverse event monitoring

Blood and urine samples were collected for safety and toxicity monitoring at 5 times throughout the study: at baseline (32 ± 6 days before the infusion; mean ± SEM),

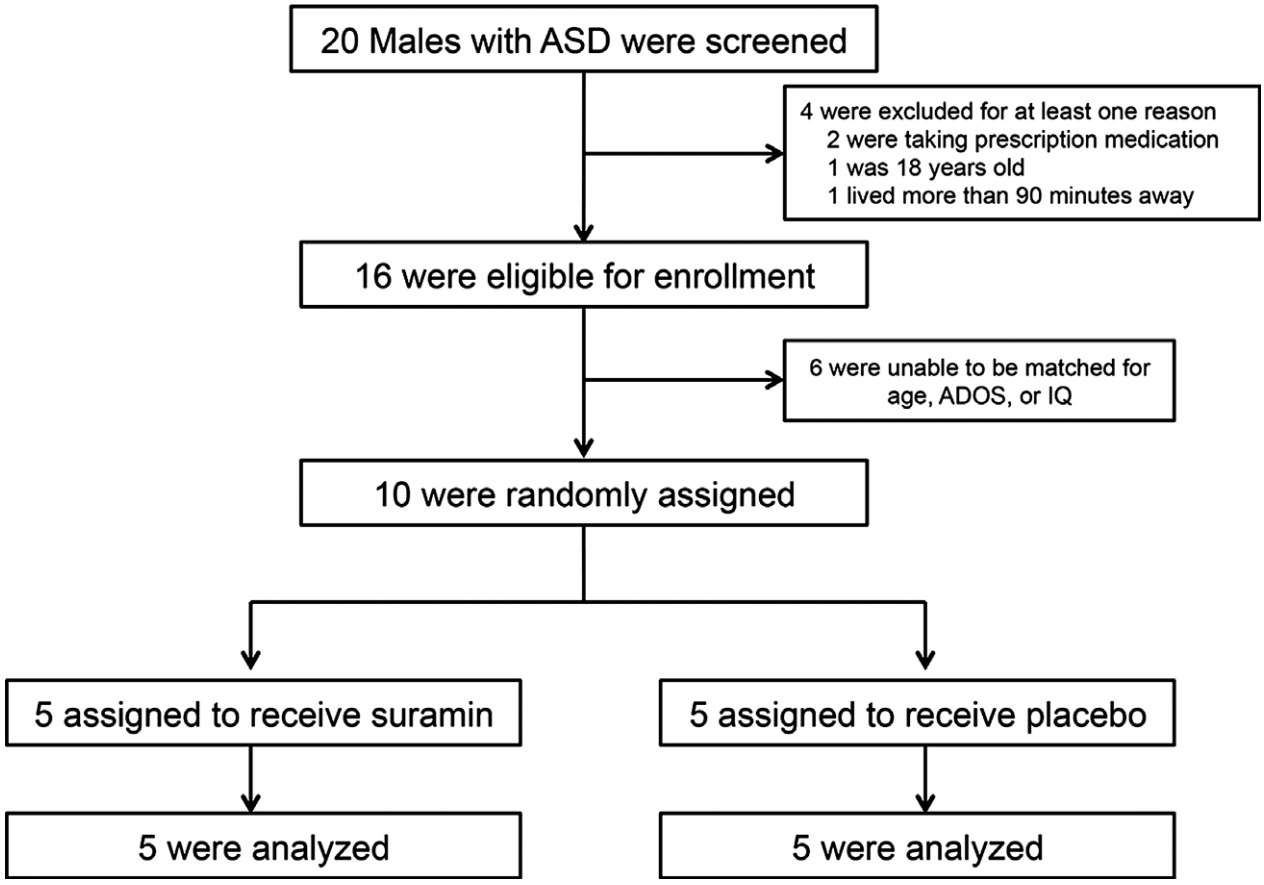


Figure 1. Trial profile.

 © 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

immediately before the infusion, 1 h after the infusion, 2 days after, and 45 days after the infusion. Unexpected and adverse events were recorded as they occurred and graded in severity according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.03 (CTCAE) scale. Additional pharmacovigilence monitoring included daily scripted phone calls in the first week, then 4 weekly calls until the exit examinations at 6 weeks. Each child received a formal neurological examination by a board-certified pediatric neurologist at baseline and at the end of the study. An independent data safety monitoring board (DSMB) reviewed the data and IRB communications for the study.

Pharmacokinetics

Plasma samples were collected for suramin pharmacokinetics (PK) before the infusion, at 1 h, 2 days, and 45 days postinfusion. Suramin concentrations were measured by high-performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) as described previously.[13] See Supplemental Methods for details. The small number of PK samples per subject prevented a standard, noncompartmental analysis in individual subjects. The suramin drug concentrations were analyzed using a population PK approach with post hoc empiric Bayesian estimate of PK parameters in individual subjects. The PK data were fit to a two-compartment model using the computer program NONMEM (ICON, Dublin, Ireland).[43] PK parameters were scaled allometrically with volume terms scaled to linear body weight ($kg^{1.0}$) and clearance terms scaled to weight ($kg^{0.75}$). Scaled adult suramin parameters of compartmental volumes of distribution and clearance were used as initial parameter estimates and between subject variability only estimated for clearance (CL) and the peripheral volume of distribution (V_d).

Pharmacometabolomics

Targeted, broad-spectrum, plasma metabolomic analysis, covering 63 biochemical pathways, was performed by LC-MS/MS as described previously[44] with minor modifications. In all, 431 of 610 targeted metabolites were measureable in plasma. See Supplemental Methods for details.

Table 1. Group characteristics.

Parameter	Suramin group Mean ± SD (range) or Number	Placebo group Mean ± SD (range) or Number	*P* value[2]
Number (male subjects)	5	5	N/A
Age (years)	8.9 ± 3.3 (5.7–13.6)	9.2 ± 3.8 (6.2–14.7)	0.88
Leiter IQ	82 ± 7.8 (75–92)	79 ± 8.8 (66–87)	0.69
ADOS Score	8.6 ± 0.9 (8–10)	9.4 ± 1.3 (7–10)	0.30
Weight (kg)	32 ± 14 (23–55)	40 ± 23 (24–80)	0.53
Weight percentile	64 ± 16 (42–84)	78 ± 30 (25–98)	0.40
Height (cm)	136 ± 23 (118–174)	137 ± 28 (113–180)	0.92
BSA[1] (m^2)	1.09 ± 0.32 (0.87–1.63)	1.21 ± 0.46 (0.87–1.99)	0.64
Body mass index (kg/m^2)	16.8 ± 1.1 (15.5–18.1)	19.9 ± 3.1 (16.2–24.7)	0.07
Head circumference (cm)	54.3 ± 2.8 (51.5–57.5)	54.5 ± 2.3 (51.5–57)	0.90
HC percentile	75 ± 30 (35–99)	75 ± 27 (42–97)	0.97
Age at ASD diagnosis (yrs)	3.2 ± 0.5 (2.5–3.75)	2.7 ± 0.3 (2.5–3.0)	0.10
Paternal age at birth (yrs)	37 ± 3.2 (35–41)	43 ± 12 (33–64)	0.62
Maternal age at birth (yrs)	35 ± 2.8 (32–38)	41 ± 6 (33–47)	0.053
Sibling with ASD	0	1	0.99
History of GI issues – current	0	1	0.99
Maintains a gluten-free diet	0	1	0.99
IVF conception	1	0	0.99
C-section delivery	1	1	0.99
History of premature birth	0	1	0.99
History of epilepsy[3] – current	0	0	0.99
History of developmental regression(s)	3	2	0.99
History of asthma – current	0	0	0.99
ASD symptom improvement with fever	2	1	0.99

BSA, body surface area; HC, head circumference; GI, gastrointestinal; IVF, in vitro fertilization; ASD, autism spectrum disorder.
[1]Mosteller method.
[2]Student's *t*-test for continuous data; Fisher's exact test for categorical data.
[3]Patients taking prescription drugs were excluded from the study. This included anticonvulsant medications.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

Sample size calculation and statistical analysis

This was a pilot study designed to obtain activity data and effect size estimates upon which future sample size calculations could be based. No data on suramin in autism were available for sample size calculations prior to this study. Each child was used as his own control to examine before and after treatment effects in a paired *t*-test design for the analysis of the ADOS, EOWPVT, ABC, ATEC, RBQ, and blood and urine safety data. Paired, nonparametric analysis was done by Wilcoxon signed-rank sum test. Categorical data, such as the presence or absence of adverse events or historical symptoms, was analyzed by Fisher's exact test. Two-way ANOVA (treatment × time), with Sidak post hoc correction, was used to analyze the 6-week summaries captured by the ADOS, CGI, and blood and urine safety analysis. Cohen's *d* – calculated as the mean difference of the paired, within-subject scores before and after treatment, divided by the standard deviation of the differences – was used as an estimate of effect size. Metabolomic data were log-transformed, scaled by control standard deviations, and analyzed by multivariate partial least squares discriminant analysis (PLSDA), with pairwise comparisons and post hoc correction for multiple hypothesis testing using Fisher's least significant difference method in MetaboAnalyst,[45] or the false discovery rate (FDR) method of Benjamini and Hochberg. Metabolites with variable importance in projection (VIP) scores determined by PLSDA that were greater than 1.5 were considered significant. Methods were implemented in Stata (Stata/SE12.1, StataCorp, College Station, TX), Prism (Prism 6, GraphPad Software, La Jolla, CA), or R. Significant metabolites were grouped into pathways and their VIP scores summed to determine the rank-ordered significance of each biochemical pathway.

Results

Participant disposition and demographics

Figure 1 illustrates the CONSORT flow diagram for patient recruitment, allocation, and analysis in the SAT-1 study. The two treatment groups were well matched (Table 1). The mean age was 9.1 years (range = 5–14). The mean nonverbal Leiter IQ was 80 (range = 66–92). The mean ADOS-2 comparison score was 9.0 (range = 7–10).

Safety monitoring and adverse events

Extensive monitoring revealed no serious toxicities (CTCAE grades 3–5). Neurologic examinations showed there was no peripheral neuropathy (Table 2). Analysis of free cortisol, hemoglobin, white blood cell count (WBC), platelets, liver transaminases, creatinine, and urine protein showed no differences in children who received suramin and placebo (Fig. 2). Five children who received suramin

Table 2. Summary of adverse or unanticipated events.

No.	Events	Suramin (*N* = 5)	CTCAE[1] grade	Placebo (*N* = 5)	CTCAE[1] grade	*P* value[2]
1	Asymptomatic rash	5	1	0	–	0.0079
2	Uncomplicated URI[3]	2	1	2	1	0.99
3	Headache	1	1	0	–	0.99
4	Emesis × 1	1[4]	1	1[5]	1	0.99
5	Hyperactivity	2[6]	1	1	1	0.99
6	Hypoglycemia[7]	1	2	1	2	0.99
7	Leukocytosis	0	–	1[8]	1	0.99
8	Enuresis	1[9]	1	0	–	0.99
9	Peripheral neuropathy	0	–	0	–	0.99
	Total:	13	–	6	–	0.12
	Nonrash AEs:	8	–	6	–	0.77

[1]CTCAE, common terminology criteria for adverse events v4.03. Mild to moderate = Grades 1–2; Serious = Grades 3–5.
[2]Fisher's exact test.
[3]URI, upper respiratory tract infection, common cold. Infusions occurred October–February.
[4]In 7-year-old after pizza and slushee consumption after playing youth league basketball.
[5]In a 6-year-old after a car ride.
[6]In a 5- and 14-year-old intermixed with periods of calm focus in first week (the 14-year-old) or first 3 weeks (the 5-year-old).
[7]Six weeks after the infusion, after several days of a URI and fasting before lunch. Hypoglycemia was asymptomatic and corrected after a normal lunch.
[8]Leukocytosis (12.2k WBC) occurred on the day of the saline infusion and preceded a URI.
[9]In a 7-year-old briefly for a few days while sick with a cold. None of the events required medical intervention. No serious adverse events (SAEs) occurred in this study.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

developed a self-limited, evanescent, asymptomatic, fine macular, patchy, morbilliform rash over 1–20% of their body (Fig. 2HI). This peaked 1 day after the infusion and disappeared spontaneously in 2–4 days. The mean number of AEs per participant was 1.9 (1.2 in the placebo group and 2.6 in the suramin group; 1.6 in the suramin group for a nonrash AE; RR = 1.3; 95% CI = 0.5–3.4; $P = 0.77$; Table 2). No serious adverse events (SAEs) occurred in this study. An independent data and safety monitoring board (DSMB) reviewed this information, as well as the clinical safety and toxicity data and IRB communications from the study, and found no safety concerns.

Pharmacokinetics

Pharmacokinetic analysis showed that at 1 h after intravenous infusion of 20 mg/kg (558 ± 41 mg/m^2; mean ± SD; Table S1), the suramin concentration was 104 ± 11.6 μmol/L (Fig. 3A). The distribution phase half-life was 7.4 ± 0.55 h. The suramin levels rapidly fell below 100 μmol/L and into the target range before day 2 in all subjects, with an average plasma level of suramin of 12.0 ± 1.5 μmol/L on day 2 (Fig. 3B, Table S1). Target concentrations of 1.5–15 μmol/L were maintained between 2 days and 6 weeks following the dose (Fig. 3). The steady-state volume of distribution was 0.83 ± 0.014 L/kg (22.7 ± 2.6 L/m^2). The clearance was 1.95 ± 0.21 mL/h/kg (0.056 ± 0.011 L/h/m^2). The terminal elimination phase half-life ($t_{1/2}$) was 14.7 ± 1.4 days (Fig. 3B,D). A two-compartment PK model showed excellent fit between measured and predicted plasma levels ($r^2 = 0.998$; Fig. 3C). These data are the first in the published literature on the pharmacokinetics of suramin in a pediatric population.

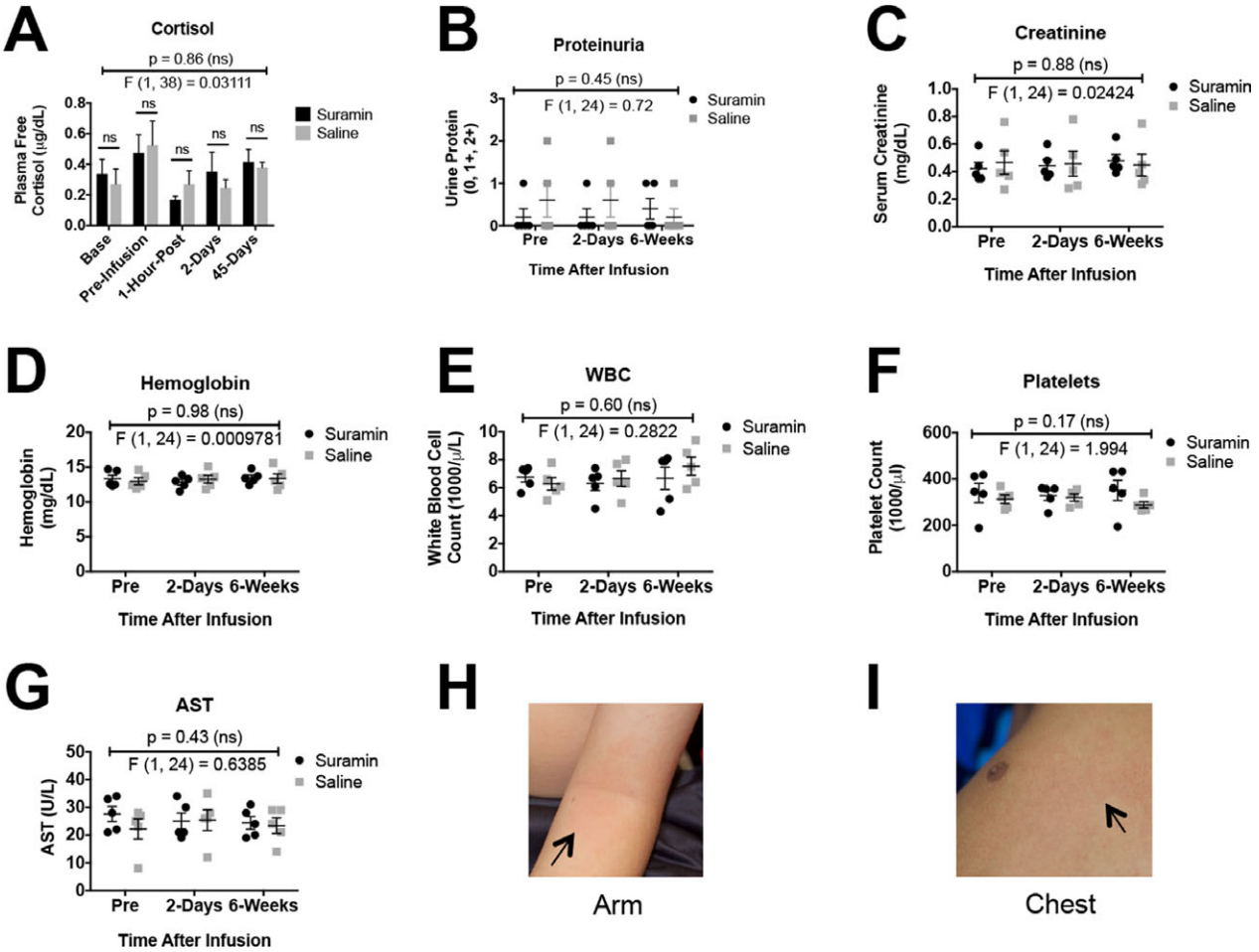
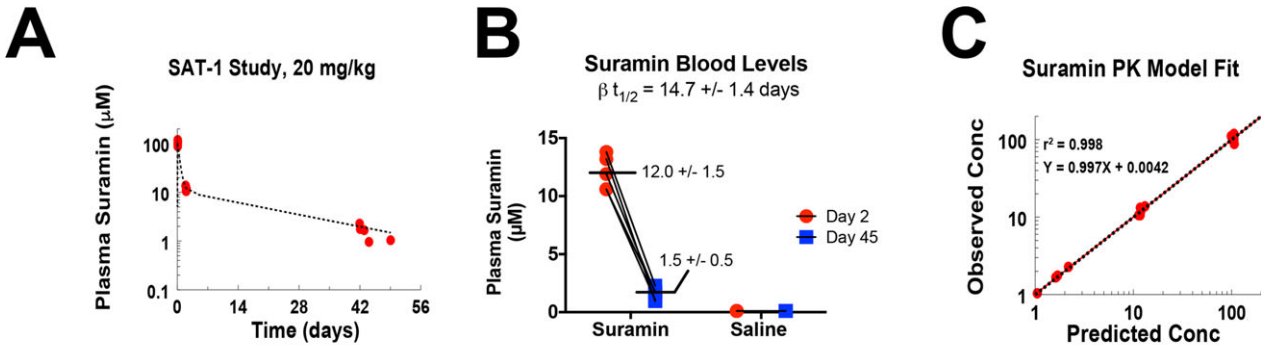


Figure 2. Safety monitoring. (A) Free cortisol, (B) proteinuria, (C) creatinine, (D) hemoglobin, (E) white blood cells (WBC), (F) platelets, (G) aspartate aminotransferase (AST), (H) rash – antecubital fossa, (I) chest. Data were analyzed by two-way ANOVA to test for treatment, time, and treatment × time interaction effects. *P* and *F* values reflect the treatment effect. Only the rash was significantly different between suramin and placebo groups.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.



D

Parameter	Symbol	Mean ± sd
Beta elimination half-life	$t_{1/2}\,\beta$	14.7 ± 1.4 days
Alpha elimination half-life	$t_{1/2}\,\alpha$	7.41 ± 0.55 hours
Clearance	CL	1.95 ± 0.21 mL/hr/kg
Steady state volume of distribution	Vd_{ss}	0.83 ± 0.014 L/kg

Figure 3. Pharmacokinetics of single-dose suramin in children with autism spectrum disorders. (A) Two-compartment model of suramin blood concentrations. The first 48 h were dominated by the distribution phase. Over 90% of the model is described by the elimination phase. (B) Plasma suramin concentrations. (C) A two-compartment model correlated well with measured values. (D) Pediatric PK parameters of suramin.

Pharmacometabolomics

Targeted plasma metabolomics was performed immediately before infusion, at 2 days, and 6 weeks after the infusion. The rank order of the top 35 of 48 significant metabolites 6 weeks after suramin treatment is illustrated in Figure 4. The rank order after 2 days is illustrated in Figure S2. Consistent with our previously published work using mouse models, the metabolic effects of suramin resulted in a decrease of the cell danger response[8] and restored more normal metabolism.[12,13] Purine metabolism was the single most changed pathway (Table 3, Table S2). Suramin increased healthy purines such as AICAR, which is an activator of the master metabolic regulator AMP-dependent protein kinase (AMPK). 1-Methyl-adenine (1-MA) was also increased. 1-MA is derived from 1-methyl-adenosine, a recently recognized marker of new protein synthesis and cell growth. Suramin decreased other purines in the plasma such as cAMP and dGDP (Fig. 4, Tables S3 and S4). Improvements in 1-carbon, folate, methionine, and cysteine metabolism were also found (Table 3, and Figure S3). Figure 5 illustrates the similarities found in the pharmacometabolomic response to suramin in MIA[13] and Fragile X mouse models[12] and in children with ASD in this study. Twenty-one of the 28 (75%) pathways changed in ASD were also changed by suramin treatment in the mouse models of ASD (Fig. 5).

Outcomes

The primary outcome measures were ADOS-2 and Expressive One-Word Picture Vocabulary (EOWPVT) scores (Table 4). Parents reported that after suramin treatment, the rate of language, social, behavioral, and developmental improvements continued to increase for 3 weeks, then gradually decreased toward baseline over the next 3 weeks. The blood levels of suramin at 3 weeks were estimated to be 4.2 ± 0.5 μmol/L using our PK model. ADOS-2 comparison scores at 6 weeks improved by an average of −1.6 ± 0.55 points (mean ± SD; $n = 5$; 95% CI = −2.3 to −0.9; Cohen's $d = 2.9$; $P = 0.0028$) in the suramin treatment group and did not change in the saline group. We calculated P values by both parametric and nonparametric methods (Table 4). The mean ADOS comparison score in the suramin-treated group was 8.6 ± 0.4 at baseline and 7.0 ± 0.3 at 6 weeks. Two-way ANOVA of ADOS scores of suramin and placebo groups measured at baseline and at 6 weeks were also significant (treatment × time

 © 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

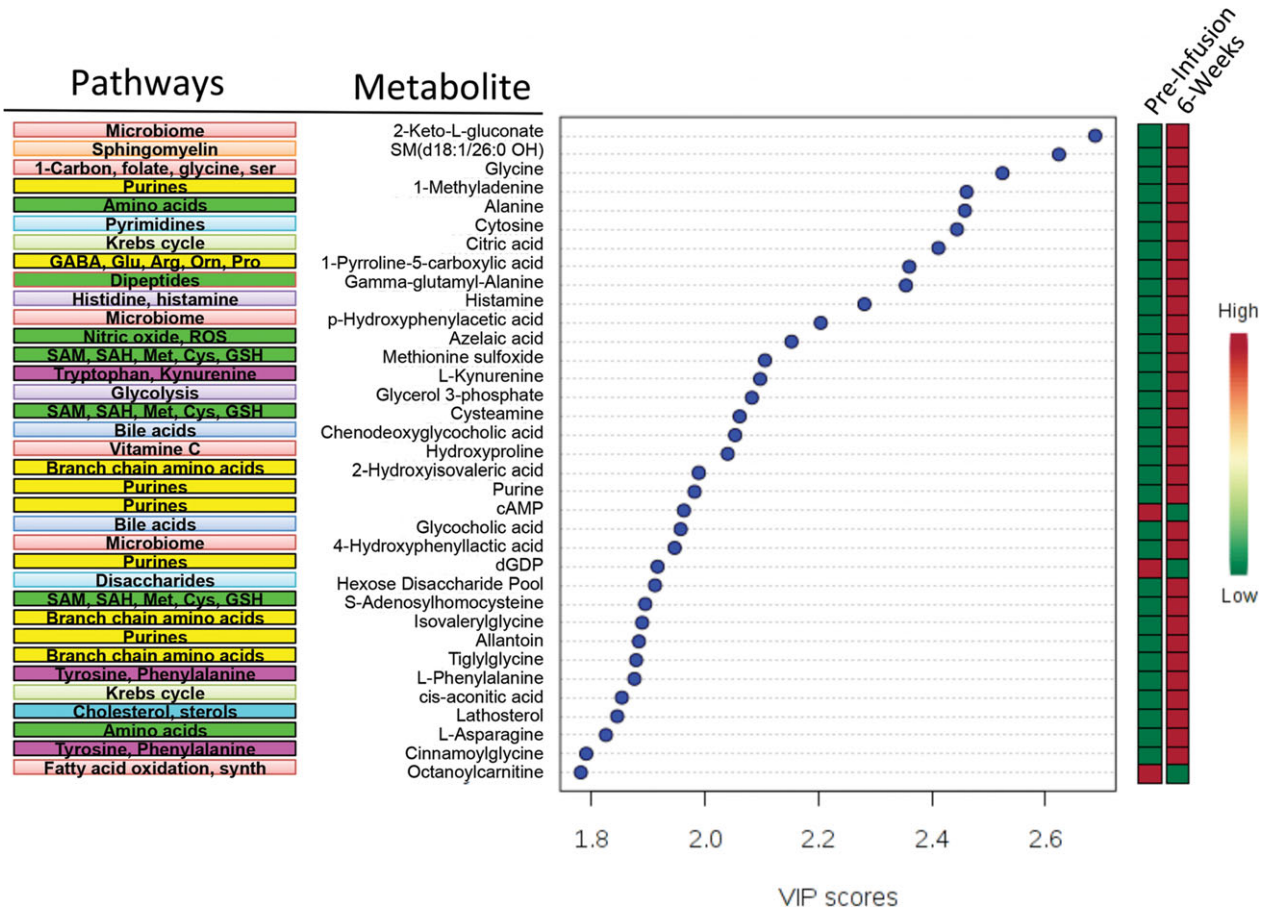


Figure 4. Suramin pharmacometabolomics. Rank order of metabolites and pathways that were changed by suramin at 6 weeks after treatment.

interaction $F(1, 8) = 12.0$; $P = 0.0085$; Figure S4A). ADOS scores were not changed in the saline-treated group (Table 4). EOWPVT scores did not change (Table 4). Several secondary outcome measures also showed improvements. These included improvements in ABC, ATEC, and CGI scores (Table 4). The Repetitive Behavior Questionnaire (RBQ) scores did not capture a change.

Discussion

The aim of the SAT-1 trial was to test the safety, pharmacokinetics, and pharmacodynamics of low-dose suramin in children with ASD. A self-limited rash was seen, but no serious adverse events occurred. Pharmacometabolomic analysis showed that the pathways changed by suramin treatment in ASD were previously known mediators of the cell danger response (CDR)[8] and that purine metabolism was changed most. Seventy-five percent of the pathways changed by suramin in children with ASD were also changed by suramin in mouse models.[12–14]

Safety

Suramin has been used safely for nearly a century to treat both children and adults with African sleeping sickness. Although side effects occurred occasionally, these could be minimized by attention to patient nutritional status, proper dose, administration procedures, and measured blood levels of suramin.[46] The low dose of suramin used in this study produced blood levels of 1.5–15 μmol/L for 6 weeks. Previous studies have never examined the side-effect profile of suramin in this low-dose range. The side-effect profile of high-dose suramin (150–270 μmol/L) is known from cancer chemotherapy studies.[32] The side-effect profile from medium-dose suramin (50–100 μmol/L) is known from African sleeping sickness studies.[46] However, the side-effect profile of low-dose suramin (5–15 μmol/L) used for antipurinergic therapy (APT) in autism is unknown. Low-dose suramin was found to be safe in five children with ASD, ages 5–14 years, in this study.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

Table 3. Suramin pharmacometabolomics: biochemical pathways changed at 6-weeks.

No.	Pathway name	Measured metabolites in the pathway (*N*)	Expected pathway proportion (*P* = N/429)	Expected hits in sample of 48 (P × 48)	Observed hits in the top 48 metabolites	Fold enrichment (obs/exp)	Impact (sum VIP score)	Fraction of impact (VIP score) explained (% of 94.6)	Increased	Decreased
1	Purine metabolism	26	0.061	2.9	5	1.7	10.2	11%	3	2
2	SAM, SAH, methionine, cysteine, glutathione	15	0.035	1.7	5	3.0	9.5	10%	5	0
3	Microbiome metabolism	18	0.042	2.0	4	2.0	8.4	9%	4	0
4	Branch chain amino acid metabolism	12	0.028	1.3	4	3.0	7.4	8%	4	0
5	Bile acid metabolism	6	0.014	0.7	3	4.5	5.7	6%	3	0
6	Fatty acid oxidation and synthesis	37	0.086	4.1	3	0.7	5.0	5%	0	3
7	Amino acid metabolism (alanine)	4	0.009	0.4	2	4.5	4.3	5%	2	0
8	Krebs cycle	9	0.021	1.0	2	2.0	4.3	5%	2	0
9	Pyrimidine metabolism	9	0.021	1.0	2	2.0	4.2	4%	2	0
10	Sphingomyelin metabolism	36	0.084	4.0	2	0.5	4.1	4%	2	0
11	1-Carbon, folate, formate, glycine, serine	5	0.012	0.6	2	3.6	4.0	4%	2	0
12	GABA, glutamate, arginine, ornithine, proline	6	0.014	0.7	2	3.0	3.9	4%	2	0
13	Tyrosine and phenylalanine metabolism	3	0.007	0.3	2	6.0	3.7	4%	2	0
14	Cholesterol, cortisol, nongonadal steroid	16	0.037	1.8	2	1.1	3.5	4%	2	0
15	Gamma-glutamyl and other dipeptides	2	0.005	0.2	1	4.5	2.4	2%	1	0
16	Histidine, histamine, carnosine metabolism	4	0.009	0.4	1	2.2	2.3	2%	1	0
17	Nitric oxide, superoxide, peroxide metabolism	2	0.005	0.2	1	4.5	2.2	2%	1	0
18	Tryptophan, kynurenine, serotonin, melatonin	6	0.014	0.7	1	1.5	2.1	2%	1	0
19	Glycolysis and gluconeogenesis metabolism	7	0.016	0.8	1	1.3	2.1	2%	1	0
20	Vitamin C (ascorbate) metabolism	2	0.005	0.2	1	4.5	2.0	2%	1	0
21	Amino-sugar, hexose metabolism	5	0.012	0.6	1	1.8	1.9	2%	1	0
22	Phospholipid metabolism	73	0.170	8.2	1	0.1	1.6	2%	0	1
								Subtotal:	42	6
								Total:	48	

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

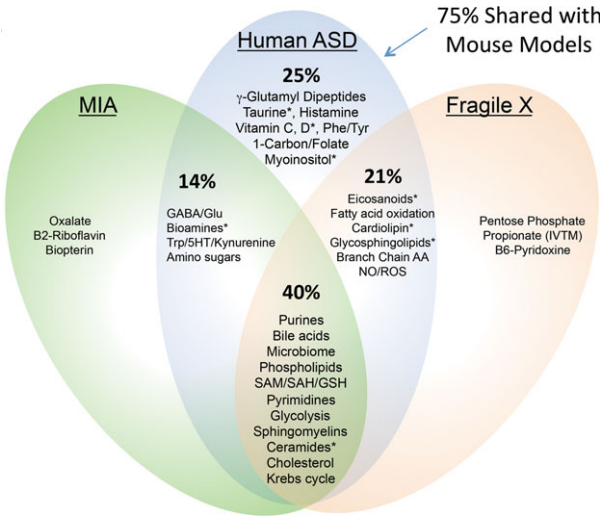


Figure 5. Shared biochemical pathways. 75% of the pathways that were altered by suramin in children with ASD were also altered in the mouse models. Asterisks (*) indicate pathways that were changed at 2 days, but not at 6 weeks after treatment.

Study limitations

Limitations of the SAT-1 study included its small size and the suboptimal timing of the outcome measurements. Parents reported that the rate of new behavioral and developmental improvements continued to increase for the first 3 weeks after the single dose of suramin, as blood levels of suramin fell from 12 to 4 μmol/L, then gradually decreased toward baseline over the next 3 weeks, as blood levels fell further from 4 to 1.5 μmol/L. This pattern of response suggested a threshold effect at about 4 μmol/L that could not have been predicted on the basis of what was known about suramin before this study, and outcomes were not measured at 3 weeks.

Another potential limitation of the trial was the self-limited rash. The rash was asymptomatic and resolved spontaneously in a few days. In theory, the rash may have biased parents in a way that caused them to either improve their scores on the ABC, ATEC, RBQ, and CGI, or to report more side-effects or adverse behaviors at both the 7-day and 6-week reports. Examiner-based ADOS scoring was more resistant to this potential bias, since the rash was not visible on exposed skin to the outcome examiners at any time. However, a design limitation of the study was that one of the two ADOS examiners was also assigned to conduct scripted phone interviews with the families, and might have been susceptible to unconscious bias even though the study remained blinded and the rash preceded any significant examiner-based outcomes by one and a half months.

Another potential weakness of this study was that ADOS scoring was not designed to be, and is not typically used as, a repeated measure of outcomes in autism treatment studies. This has occurred historically for two counterbalancing reasons: (1) because it is generally believed that ADOS scores are diagnostic and are not sensitive to change once the diagnosis is established, and (2) because training effects have the potential to produce improvements that are artifactual. With regard to the first point, under the right circumstances ADOS scores can be sensitive to change and have recently been used successfully as an outcome measure in a large autism treatment study.[47] With regard to the second point, if training effects occurred, they were asymmetric, since improvements were only observed in the suramin treatment group and were not observed in the placebo group (Table 4).

Psychopharmacology

Suramin has objective central nervous system (CNS) effects in animal models[12–14] and children with autism despite being unable to penetrate the blood–brain barrier.[48] Suramin also has a number of peripheral effects on innate immunity, metabolism, pain, gut, autonomic, inflammatory, and other pathways regulated by purinergic signaling that may contribute to the beneficial effects observed.[8,23] Previous studies have shown that suramin is taken up into the CNS at the level of the brainstem, although not appreciably into the cerebrum or cerebellum.[13] There are eight circumventricular organs (CVOs) in the brain that contain neurons that lack a blood–brain barrier.[49] The area postrema in the brainstem is one of these CVOs that monitors the chemistry of the blood and transduces this information to higher centers in the brain for neuroendocrine, affective, cognitive, and behavioral integration. Rather than being a disadvantage, the peripheral actions and indirect CNS effects of suramin may have certain advantages by minimizing the risk of CNS toxicity. While new antipurinergic drugs (APDs) may soon be developed that can pass the blood–brain barrier, this appears not to be required to produce the behavioral effects of suramin in ASD.

Conclusions

The SAT-1 trial examined the effects of low-dose suramin or placebo in 10 children with autism spectrum disorder. No safety concerns were found. A two-compartment pharmacokinetic model permitted accurate forecasting of plasma drug levels from 1 h to 6 weeks after the infusion. Metabolomic studies confirmed the importance of the cell danger response (CDR)[8] and purinergic signaling.[12–14] A single intravenous dose of suramin was associated with improved scores for language, social interaction, and

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

Table 4. Outcomes.

Outcome			Suramin						Placebo					
Instrument	Factor or behavior	Time after treatment (days)	Difference from baseline (mean ± SD)	95% CI	d^1	*N*	P^2	P^3	Difference from baseline (mean ± SD)	95% CI	d^1	*N*	P^2	P^3
Primary outcomes														
ADOS-2	Comparison	45	−1.6 ± 0.55	−2.3 to −0.9	2.9	5	0.0028	0.038	−0.4 ± 0.55	−1.1 to +0.28	0.7	5	0.18	0.16
	Raw	45	−4.6 ± 1.9	−7.0 to −2.2	2.4	5	0.0062	0.039	−0.4 ± 1.8	−2.7 to +1.9	0.22	5	0.65	0.58
	Social	45	−3.2 ± 1.9	−5.6 to −0.8	1.7	5	0.020	0.043	0.0 ± 1.7	−2.2 to +2.2	0	5	0.99	0.71
	Restr/Rep	45	−1.4 ± 0.89	−2.5 to −0.29	1.6	5	0.025	0.059	−0.4 ± 2.1	−3.0 to +2.2	0.19	5	0.69	0.58
EOWPVT	Vocabulary	45	−4.2 ± 8.3	−14.5 to +6.1	−0.51	5	0.32	0.50	+2.0 ± 4.6	−3.8 to +7.8	0.43	5	0.39	0.50
Secondary outcomes														
ABC	Stereotypy	7	−3.6 ± 2.1	−6.2 to −1.0	1.7	5	0.018	0.043	+0.4 ± 1.9	−2.0 to +2.8	−0.21	5	0.67	0.68
	Stereotypy	45	−4.0 ± 2.3	−6.9 to −1.1	1.7	5	0.019	0.042	+1.0 ± 4.3	−4.3 to +6.3	−0.23	5	0.63	0.69
ATEC	Total	7	−10 ± 7.7	−20 to −0.46	1.3	5	0.044	0.043	+7.2 ± 14	−10 to +25	−0.51	5	0.32	0.35
	Language	7	−2.2 ± 1.5	−4.0 to −0.36	1.4	5	0.021	0.059	0.0 ± 4.1	−5.0 to +5.0	0	5	0.99	0.89
	Sociability	7	−3.6 ± 2.6	−6.8 to −0.36	1.4	5	0.025	0.063	−0.8 ± 2.8	−4.3 to +2.6	0.29	5	0.55	0.58
	Language	45	−2.0 ± 1.4	−2.7 to −0.49	1.4	5	0.034	0.059	−0.2 ± 2.9	−3.8 to +3.4	0.07	5	0.88	0.79
CGI	Overall ASD	45	−1.8 ± 1.04	−3.4 to −0.15	1.7	5	0.05	n/a	0.0 ± 0.34	−0.55 to +0.55	0	5	0.99	n/a
	E. Language	45	−2.0 ± 1.04	−3.6 to −0.35	1.9	5	0.01	n/a	0.0 ± 0.34	−0.55 to +0.55	0	5	0.99	n/a
	Social Inter.	45	−2.0 ± 1.04	−3.6 to −0.35	1.9	5	0.01	n/a	0.0 ± 0.34	−0.55 to +0.55	0	5	0.99	n/a
RBQ	Total	45	−3.2 ± 5.8	−10.4 to +4.0	0.55	5	0.28	0.22	−0.8 ± 3.3	−4.9 to 3.3	0.24	5	0.62	0.47

ADOS-2, autism diagnostic observation schedule, 2nd edition; EOWPVT, Expressive One-Word Picture Vocabulary Test; ABC, aberrant behavior checklist; ATEC, autism treatment evaluation checklist; CGI, clinical global impression survey; RBQ, repetitive behavior questionnaire; Restr/Rep, restricted or repetitive behaviors; Overall ASD Sx, overall ASD symptoms; E. Language, expressive language; Social Inter., social interaction. *Analysis***.** ADOS, EOWPVT, ABC, ATEC, and RBQ scores were analyzed by paired analysis before and after treatment using each subject as their own control. CGI was analyzed by two-way ANOVA (symptom × time before and after treatment) with post hoc correction. Nonparametric *P* values were not calculated (n/a). *Interpretation***.** ADOS, ABC, ATEC, CGI, and RBQ are severity scores; negative differences from baseline reflect decreased severity, that is, improvement. EOWPVT is a performance score; negative differences reflect a decrease.

[1]A positive Cohen's *d* reflects improvement, and a negative *d* reflects a decrease by convention. Cohen's *d* is likely an overestimate of the actual treatment effect based on the large mean differences and small standard deviations found before and after treatment in this small study.

[2]*P* value from parametric paired *t*-test analysis.

[3]*P* value from nonparametric paired Wilcoxon signed-rank sum analysis.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

decreased restricted or repetitive behaviors measured by ADOS, ABC, ATEC, and CGI scores. None of these improvements occurred in the five children who received placebo. The generalizability of these findings is unknown. Future studies will be needed to confirm these findings in larger numbers of children with ASD, and to evaluate whether a few doses of suramin given over a few months are safe and might facilitate continued improvements.

Special note from the authors

Suramin is not approved for the treatment of autism. Like many intravenous drugs, when administered improperly by untrained personnel, at the wrong dose and schedule, without careful measurement of drug levels and monitoring for toxicity, suramin can cause harm. Careful clinical trials will be needed over several years at several sites to learn how to use low-dose suramin safely in autism, and to identify drug–drug interactions and rare side effects that cannot currently be predicted. We strongly caution against the unauthorized use of suramin.

Acknowledgments

RKN thanks the patients and families who gave their time and effort in helping to make this study possible. We thank Dr. Richard Haas, Dr. Doris Trauner, and Dr. Stephen Edelson for their advice in planning the study. We thank Dr. Judy S. Reilly for critical reading of the manuscript and suggestions for improvements. RKN also thanks Jonathan Monk for assistance with the Cytoscape visualizations, Marlene Samano and Nicole Suarez, and Maeve Taaffe, Lee Vowinkel, Dennis Perpetua, Jessica Nasca, Peewee Buquing, and Patricia Moraes for their expert clinical assistance at the UCSD Clinical Translational Research Institute, and Thaine Ross and Melinda Stafford for their expert assistance in the Investigational Pharmacy. RKN extends a special thanks to graphic artists Suzanne Parlett and Qamdyn Hale for help in creating the storyboards used in the study.

Author Contributions

Dr. Robert Naviaux raised the funding, obtained the regulatory approvals, conceived, designed, and directed the trial, analyzed the data, prepared the figures, and wrote the manuscript. Dr. Curtis, Dr. Westerfield, and Ms. Mash performed the neurodevelopmental testing, provided clinical coordination, and edited the manuscript. Dr. Reiner helped design the study, coordinated patient infusions and clinical care, and edited the manuscript. Dr. Li, Dr. Jane Naviaux, and Dr. Wang performed the metabolomic and pharmacokinetic analysis, analyzed the data, prepared the figures, and wrote parts of the manuscript. Dr. Jain and Ms. He helped design the study, prepared the randomization key, performed biostatistical analyses, and edited the manuscript. Dr. Bright directed the data compilation, integrity, and completeness analysis, provided independent biostatistical analysis, and edited the manuscript. Dr. Goh helped design the study, performed neurologic examinations, and edited the manuscript. Dr. Alaynick helped design the study and edited the manuscript. Dr. Capparelli analyzed the pharmacokinetic data, prepared the figures, and wrote parts of the manuscript. Dr. Sun and Ms. Adams provided investigational pharmacy support, implemented the clinical mask, and edited the manuscript. Ms. Arellano provided clinical coordination and edited the manuscript. Dr. Chukoskie helped design the study, analyzed the data, critically reviewed and edited the manuscript. Dr. Lincoln and Dr. Townsend helped design the study, directed the neurodevelopmental studies, wrote and edited the manuscript.

Conflict of Interest

RKN has filed a provisional patent application related to antipurinergic therapy of autism and related disorders and is a scientific advisory board member for the Autism Research Institute and the Open Medicine Foundation. EVC is a DSMB member for Cempra Pharmaceuticals and The Medicines Company, and a consultant for Alexion. SG is co-owner of MitoMedical. The other authors declare no conflicts of interest.

References

1. Zablotsky B, Black LI, Maenner MJ, et al. Estimated prevalence of autism and other developmental disabilities following questionnaire changes in the 2014 National Health interview survey. Natl Health Stat Report 2015;13:1–20.
2. Christensen DL, Baio J, Van Naarden Braun K, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years–Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. MMWR Surveill Summ 2016;65:1–23.
3. Pinto D, Pagnamenta AT, Klei L, et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 2010;466:368–372.
4. Richards C, Jones C, Groves L, et al. Prevalence of autism spectrum disorder phenomenology in genetic disorders: a systematic review and meta-analysis. Lancet Psychiatry 2015;2:909–916.
5. Talkowski ME, Minikel EV, Gusella JF. Autism spectrum disorder genetics: diverse genes with diverse clinical outcomes. Harv Rev Psychiatry 2014;22:65–75.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

6. Kalkbrenner AE, Schmidt RJ, Penlesky AC. Environmental chemical exposures and autism spectrum disorders: a review of the epidemiological evidence. Curr Probl Pediatr Adolesc Health Care 2014;44:277–318.
7. Zerbo O, Iosif AM, Walker C, et al. Is Maternal Influenza or Fever During Pregnancy Associated with Autism or Developmental Delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) Study. J Autism Dev Disord 2013;43(1): 25–33.
8. Naviaux RK. Metabolic features of the cell danger response. Mitochondrion 2014;16:7–17.
9. Silva JM, Wong A, Carelli V, Cortopassi GA. Inhibition of mitochondrial function induces an integrated stress response in oligodendroglia. Neurobiol Dis 2009;34: 357–365.
10. Nikkanen J, Forsstrom S, Euro L, et al. Mitochondrial DNA Replication Defects Disturb Cellular dNTP Pools and Remodel One-Carbon Metabolism. Cell Metab 2016;23:635–648.
11. Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science 2011;333:1109–1112.
12. Naviaux JC, Wang L, Li K, et al. Antipurinergic therapy corrects the autism-like features in the Fragile X (Fmr1 knockout) mouse model. Mol Autism 2015;6:1.
13. Naviaux JC, Schuchbauer MA, Li K, et al. Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. Transl Psychiat 2014;4: e400.
14. Naviaux RK, Zolkipli-Cunningham Z, Nakayama T, et al. Antipurinergic Therapy Corrects the Autism-Like Features in the Poly(IC) Mouse Model. PLoS ONE 2013;8(3): e57380.
15. Wikoff WR, Kalisak E, Trauger S, et al. Response and recovery in the plasma metabolome tracks the acute LCMV-induced immune response. J Proteome Res 2009;8:3578–3587.
16. Degtyar E, Zusman T, Ehrlich M, Segal G. A Legionella effector acquired from protozoa is involved in sphingolipids metabolism and is targeted to the host cell mitochondria. Cell Microbiol 2009;11:1219–1235.
17. James SJ, Cutler P, Melnyk S, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 2004;80:1611–1617.
18. West AP, Shadel GS, Ghosh S. Mitochondria in innate immune responses. Nat Rev Immunol 2011;11: 389–402.
19. Naviaux RK. Oxidative shielding or oxidative stress? J Pharmacol Exp Ther 2012;342:608–618.
20. Lohman AW, Isakson BE. Differentiating connexin hemichannels and pannexin channels in cellular ATP release. FEBS Lett 2014;588:1379–1388.
21. Trautmann A. Extracellular ATP in the immune system: more than just a "danger signal". Sci Signal 2009;2:pe6.
22. Riteau N, Baron L, Villeret B, et al. ATP release and purinergic signaling: a common pathway for particle-mediated inflammasome activation. Cell Death Dis 2012;3:e403.
23. Burnstock G. The Paton Lecture: Purinergic signalling: from discovery to current developments. Exp Physiol 2014;99(1):16–34.
24. Imamura H, Nhat KP, Togawa H, et al. Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. Proc Natl Acad Sci USA 2009;106:15651–15656.
25. Jacobson KA, Balasubramanian R, Deflorian F, Gao ZG. G protein-coupled adenosine (P1) and P2Y receptors: ligand design and receptor interactions. Purinergic Signalling 2012;8:419–436.
26. Adams JB, Audhya T, McDonough-Means S, et al. Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. Nutr Metab 2011;8:34.
27. Jia M, Li MX, Fields RD, Nelson PG. Extracellular ATP in activity-dependent remodeling of the neuromuscular junction. Dev Neurobiol 2007;67:924–932.
28. Naviaux RK. Mitochondria and Autism. In: Buxbaum JD, Hof PR, eds. The Neuroscience of Autism Spectrum Disorders. Waltham, MA: Academic Press, Elsevier, 2012:179–193.
29. Naviaux RK. Mitochondrial control of epigenetics. Cancer Biol Ther 2008;7:1191–1193.
30. Wallace DC, Fan W. Energetics, epigenetics, mitochondrial genetics. Mitochondrion 2010;10:12–31.
31. Burnstock G. Pathophysiology and therapeutic potential of purinergic signaling. Pharmacol Rev 2006;58:58–86.
32. Stein CA. Suramin: a novel antineoplastic agent with multiple potential mechanisms of action. Can Res 1993;53 (10 Suppl):2239–2248.
33. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013;310:2191–2194.
34. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Trials 2010;11:32.
35. Gotham K, Risi S, Pickles A, Lord C. The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. J Autism Dev Disord 2007;37:613–627.
36. Grondhuis SN, Mulick JA. Comparison of the Leiter International Performance Scale-Revised and the Stanford-Binet Intelligence Scales, 5th Edition, in children with autism spectrum disorders. Am J Intellect Dev Disabil 2013;118:44–54.
37. Adams-Chapman I, Bann C, Carter SL, et al. Language outcomes among ELBW infants in early childhood. Early Hum Dev 2015;91:373–379.

 © 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

38. Kaat AJ, Lecavalier L, Aman MG. Validity of the aberrant behavior checklist in children with autism spectrum disorder. J Autism Dev Disord 2014;44:1103–1116.
39. Geier DA, Kern JK, Geier MR. A Comparison of the Autism Treatment Evaluation Checklist (ATEC) and the Childhood Autism Rating Scale (CARS) for the quantitative evaluation of autism. J Mental Health Res Intellect Disabil 2013;6:255–267.
40. Rimland B, Edelson S. Autism treatment evaluation checklist. Autism Research Institute 2000 https://www.autism.com/ind_atec [cited 2016 June 15].
41. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry (Edgmont). 2007;4:28–37.
42. Honey E, McConachie H, Turner M, Rodgers J. Validation of the repetitive behaviour questionnaire for use with children with autism spectrum disorder. Res Autism Spectr Disord 2012;6:355–364.
43. Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetic parameters. II. Biexponential model and experimental pharmacokinetic data. J Pharmacokinet Biopharm 1981;9:635–651.
44. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. Proc Natl Acad Sci USA 2016;113(37):E5472–E5480.
45. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0-making metabolomics more meaningful. Nucleic Acids Res 2015;43(W1):W251–W257.
46. Hawking F. Suramin: with special reference to onchocerciasis. Advances Pharmacol Chemother 1978;15:289–322.
47. Pickles A, Le Couteur A, Leadbitter K, et al. Parent-mediated social communication therapy for young children with autism (PACT): long-term follow-up of a randomised controlled trial. Lancet 2016;388:2501–2509.
48. Hawking F. Concentration of Bayer 205 (Germanin) in human blood and cerebrospinal fluid after treatment. Trans R Soc Trop Med Hyg 1940;34:37–52.
49. Siso S, Jeffrey M, Gonzalez L. Sensory circumventricular organs in health and disease. Acta Neuropathol 2010;120:689–705.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Single-dose suramin pharmacokinetics.
Table S2. Suramin pharmacometabolomics. Pathways changed at 2 days.
Table S3. Suramin pharmacometabolomics. Metabolites changed at 2 days.
Table S4. Suramin pharmacometabolomics. Metabolites changed at 6 weeks.

Figure S1. Storyboard illustration of each step of the infusion day visit.
Figure S2. Suramin pharmacometabolomics. Rank order of metabolites and pathways that were changed by suramin at 2 days after treatment.
Figure S3. Suramin pharmacometabolomics pathway visualization. (A) After 2 days. (B) After 6 weeks. Metabolites indicated in red were increased, and those in green were decreased compared to controls (see *z*-score scale in upper right).
Figure S4. Outcomes. (A) 6 Weeks ADOS comparison scores by two-way ANOVA. (B) 6 Weeks ADOS comparison score improvement after suramin. (C) 6 Weeks ADOS social affect score improvement after suramin. (D) 6 Weeks ADOS restricted and repetitive behavior score improvement after suramin. (E) 2 days ADOS comparison scores were not changed. (F) no change in 6 weeks ADOS scores in subjects receiving saline placebo. (G) no change in 6 weeks ADOS social affect scores in subjects receiving placebo. (H) no change in 6 weeks ADOS restricted and repetitive behavior scores in subjects receiving placebo. (I) no change in 6 weeks Expressive One-Word Picture Vocabulary scores. (J) 7-day improvement in ABC stereotypy scores after suramin. (K) 6-week Improvement in ABC stereotypy scores after suramin. (L) 7-day Improvement in ATEC total scores after suramin. (M) no change in 6 weeks EOWPVT scores after saline. (N) no change in 7 days ABC stereotypy scores after saline. (O) no change in 6 weeks ABC stereotypy scores after saline. (P) no change in 7 days ATEC total scores after saline. (Q) improved ATEC speech, language, and communication scores 7 days after suramin. (R) improved ATEC sociability scores 7 days after suramin. (S) improved ATEC speech, language, and communication scores 6 weeks after suramin. (T) improved ADOS comparison scores after dropping a subject who missed the 6-week visit ($N = 4$). (U) no change in 7 days ATEC speech, language, and communication after saline. (V) no change in 7 days ATEC sociability after saline. (W) no change in 6 weeks ATEC speech, language, and communication scores 6 weeks after saline (X) no change in EOWPVT scores after dropping subject who missed the 6-week visit ($N = 4$). (Y) no change in 2 days ADOS scores after suramin. (Z) no change in 6 weeks RBQ total scores after suramin. (aa) improved core symptoms of ASD and other behaviors by CGI at 6 weeks after suramin. *P* values: *0.05, **0.01, ***0.001. (bb) Top 3, most changed symptoms named by parents in the 6-week CGI. (cc) no change in 2 days ADOS scores after saline. (dd) no change in 6 weeks RBQ total scores after saline.
Data S1. Clinical Global Impression (CGI) questionnaire.
Data S2. Social Stories to Accompany the Storyboard Panels Describing Each Step of the Infusion Day Visit.

© 2017 The Authors. *Annals of Clinical and Translational Neurology* published by Wiley Periodicals, Inc on behalf of American Neurological Association.

Supporting Information

Naviaux RK, Curtis B, Li K, Naviaux JC, Bright AT, Reiner G, Westerfeld M, Goh S, Alaynick WA, Wang L, Capparelli EV, Adams C, Sun J, Jain S, He F, Arellano DA, Mash L, Chukoskie L, Lincoln A, Townsend J. **Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial**. 2017, *Annals of Clinical and Translational Neurology*.

Contents

1. **Supplemental Materials and Methods**
2. **Supplemental Results**
3. **Supplemental References**
4. **Supplemental Figures Legends**
5. **Supplemental Tables**
6. **Supplemental Figures**
7. **Supplemental Data**

    S1. Clinical Global Impression (CGI) questionnaire

    S2. Social stories to accompany the storyboard

Supplemental Materials and Methods

Diagnostic and Outcome Procedures

Examiner-based outcomes (ADOS and EOWPVT) were assessed at 2-days and 6-weeks after the infusion. Parent-based outcomes (ABC, ATEC, CGI, and RBQ) were assessed at 7-days and 6-weeks after the infusion. To minimize the effects of natural behavioral variability, the parents were instructed to mark a behavior as changed only if it was persistently changed for at least 1 week. Storyboards and accompanying social stories were created to illustrate each step of the study for parents to review with each child before the study (Figure S1, and Supplemental Data S2).

Safety and Adverse Event Monitoring

Blood and urine for safety and toxicity monitoring were collected immediately before the infusion, 1 hour after the infusion, 2 days after, and 45 days after the infusion. Vital signs and anthropomorphic measurements were also collected. Safety surveillance included 18 vital sign and anthropometric features, 19 complete blood count (CBC) parameters, 20 blood chemistry measures, 3 thyroid and cortisol measures, and 5 lipid measures at the 5 time points. 24 urinalysis features were measured at 4 times: baseline, pre-infusion, 2-days post-infusion, and 45-days post-infusion.

Verification of Data Completeness and Transcription Accuracy

Standardized questionnaire responses and the ADOS-2 and EOWPVT scores (5,490 cells of data) were compiled in spreadsheets from the original hard copy forms and from the electronic medical records. A total of 87 cells (1.6%) of the 5,490 outcome scores were either left blank,

asked about a symptom that did not apply, or were missing. One participant missed the 6-week ADOS and EOWPVT evaluations because of scheduling difficulties. His 2-day results were used as an estimate of his 6-week scores. ADOS scores remained significant when this subject was dropped from the analysis (Figure S4T). EOWPVT results were also unchanged (Figure S4X). The 4,210 cells of laboratory and vital sign data were also collected and reviewed. When specific cells of data were found to be missing, they were manually confirmed by inspection of the original questionnaire, laboratory results, and clinical data sheets. A random generator program was written that randomly selected 5% of the data. These randomly selected cells of data that were then manually checked for transcription accuracy by reviewing the hard copy responses and Red Cap electronic medical records.

Standardized Testing and Questionnaires

Two observational examinations were performed by a clinician at 3 time points: baseline (56 ± 8 days; mean ± SEM; before the infusion), 2-days post-infusion, and 6-weeks post-infusion. The two examiner-based metrics were the Autism Diagnostic Observation Schedule, 2nd edition (ADOS-2)[1, 2], with video and audio files recorded on 3 cameras, and the Expressive One Word Picture Vocabulary Testing (EOWPVT)[3]. Both of these observational metrics were administered by a trained and certified examiner using approved test materials. Three standardized questionnaires were completed by parents at 3 time points: baseline, 7-days post-infusion, and 6-weeks post-infusion. The three standardized questionnaires completed by parents were the 58-question Aberrant Behavior Checklist (ABC)[4], the 75-item Autism Treatment Evaluation Checklist (ATEC)[5, 6], and the 33-item repetitive behavior questionnaire (RBQ)[7]. Parents were asked to complete these three instruments with reference to how their child behaved in the

previous 7 days. At the end of the six weeks, we included a 24-question Clinical Global Impression (CGI)[8] questionnaire (Supplementary Data S1). In addition, parents were asked to list the 3 top behaviors or symptoms that they observed to be most changed over the previous 6-weeks. To minimize the misinterpretation of natural day-to-day variations in symptoms, parents were asked to mark a symptom as changed in the 6-week CGI only if it had lasted for at least 1 week.

Storyboards and Social Stories

We commissioned a graphic artist to prepare a storyboard of each step of the procedure (Figure S1). The panel contents and color schemes were reviewed, and revisions recommended, by a 16-year old artist with Asperger syndrome to optimize the informational value and minimize any sensory issues. Next, our developmental neuropsychologist created social stories to accompany each panel of the storyboard. The social stories are shown in Supplementary Data S2.

Phone Interviews, Parent Reports, and Clinical Observations

Scripted phone interviews were conducted daily for the first week, then weekly until the completion of the study for each child 6-weeks after the infusion. Parents also kept study journals throughout the six weeks to document their observations. These scripted and narrative observations were used to permit discovery of any changes in ASD, behavior, or constitutional symptoms such as sleep and appetite, or any adverse or unanticipated events. The parent reports also provided insight regarding the timing and pattern of the responses after the infusion that were not predicted prior to the study, and were not adequately captured by the scheduled observations.

Daily Calls. Parents were contacted by phone on days 1-7 after the infusion to ensure close follow-up and to provide the opportunity for parents to report any positive or negative observations. These calls followed the script below:

"Hi. This is _____ (state your name) at UCSD. This is our daily follow-up call to see how you and your son are doing as part of the autism study."

1. How have things been going since the infusion? Any changes since yesterday?
2. Have there been any improvements? What things are most improved?
3. Have there been any setbacks, or negative things you've noticed? What are these?
4. How is he eating?
5. How is he sleeping?
6. Are there any problems, suggestions, or concerns that I can pass on to the doctors or a nurse?

Weekly Calls. Parents were called weekly on days 14, 21, 28, and 35 after the infusion to ensure close follow-up and to provide the opportunity for parents to report any positive or negative observations. These calls followed the script below:

"Hi. This is _____ (state your name) at UCSD. This is our weekly follow-up call to see how you and your son are doing as part of the autism study."

1. How have things been going since the infusion? Any changes since last week?
2. Have there been any improvements? What things are most improved?
3. Have there been any setbacks, or negative things you've noticed? What are these?
4. How is he eating?
5. How is he sleeping?
6. Are there any problems, suggestions, or concerns that I can pass on to the doctors or a nurse?

Clinical Global Impression (CGI)

We developed a 24-question Clinical Global Impression (CGI) instrument designed to assess the core symptoms of autism spectrum disorders and some of the most common comorbid features (Supplementary Material A1). The CGI instrument scoring system was the traditional 7-point,

CGI-Improvement scale[8]. In this scale, the historian gives a score of 0 if the symptom "was never a problem", a 1 for "very much improved", a 4 for "no change", and a 7 for "very much worse". In addition to the 24 structured questions, we asked the parents to write in the top 3 symptoms or behaviors that were most changed over the 6 weeks since the suramin infusion (Supplementary Material A1). This hybrid design of structured and open-ended responses permitted us to capture a large number of clinical outcomes associated with single-dose suramin treatment.

Metabolomics

Targeted, broad-spectrum, plasma metabolomic analysis of 610 metabolites from 63 biochemical pathways was performed by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) as described[9] with minor modifications. 431 metabolites were above the lower limit of quantitation (LLOQ) in this study. Venous blood was collected between the hours of 8 am and 5 pm, at least 3 hours after the last meal, into lithium-heparin vacutainer tubes (BD #367884). Plasma was separated by centrifugation at 900g x 10 minutes at room temperature within one hour of collection. The resulting fresh lithium-heparin plasma was transferred to labeled 1.2 ml or 2.0 ml externally threaded, cryotubes with a minimum headspace air gap for storage at -80˚C for analysis. Samples were analyzed on an AB SCIEX QTRAP 5500 triple quadrupole mass spectrometer equipped with a Turbo V electrospray ionization (ESI) source, Shimadzu LC-20A UHPLC system, and a PAL CTC autosampler. Typically, 90 µl of plasma was thawed on ice and transferred to a 1.7 ml Eppendorf tube. Five (5.0) µl of a cocktail containing 25-35 commercial stable isotope internal standards, and 5.0 µl of 57 stable isotope internal standards that were custom-synthesized in *E. coli NCM3722, Caenorhabditis elegans N2,*

and *Komagataella phaffii* (ATCC 76273; formerly known as *Pichia pastoris*) by metabolic labeling with ^{13}C-glucose and ^{13}C-bicarbonate, were added, mixed, and incubated for 10 min at 20˚C to permit small molecules and vitamins in the internal standards to associate with plasma binding proteins. Macromolecules (protein, DNA, RNA, glycans, etc.) were precipitated by extraction with 4 volumes (400 µl) of cold (-20˚C), acetonitrile:methanol (50:50) (LCMS grade, Cat# LC015-2.5 and GC230-4, Burdick & Jackson, Honeywell), vortexed vigorously, and incubated on crushed ice for 10 min, then removed by centrifugation at 16,000g x 10 min at 4˚C. The supernatants containing the extracted metabolites and internal standards in the resulting 40:40:20 solvent mix of acetonitrile:methanol:water were transferred to labeled cryotubes and stored at -80˚C for LC-MS/MS analysis.

LC-MS/MS analysis was performed by scheduled multiple reaction monitoring (sMRM) under Analyst v1.6.2 software control in both negative and positive mode with rapid polarity switching (50 ms). Nitrogen was used for curtain gas (set to 30), collision gas (set to high), ion source gas 1 and 2 (set to 35). The source temperature was 500˚C. Spray voltage was set to -4500 V in negative mode and 5500 V in positive mode. The values for Q1 and Q3 mass-to-charge ratios (m/*z*), declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were determined and optimized for each MRM for each metabolite. Ten microliters of extract was injected by PAL CTC autosampler via a 10 µl stainless steel loop into a 250 mm × 2.0 mm, 4µm polymer based NH2 HPLC column (Asahipak NH2P-40 2E, Showa Denko America, Inc., NY) held at 25°C for chromatographic separation. The mobile phase was solvent A: 95% water with 20 mM $(NH_4)_2CO_3$ (Sigma, Fluka Cat# 74415-250G-F), 5% acetonitrile, and 38 mM NH_4OH (Sigma, Fluka Cat# 17837-100ML), final pH 9.75; solvent B:

100% acetonitrile. Separation was achieved using the following gradient: 0-3.5 min: 95%B, 3.6-8 min: 85% B, 8.1-13 min: 75% B, 13.5–35 min: 0% B, 36–46 min: 95% B, 46.1 min: end. The flow rate was 200 µl/min. Pump pressures ranged from 920-2600 psi over the course of the gradient. All the samples were kept at 4°C during analysis. The chromatographic peaks were identified using MultiQuant (v3.0, Sciex), confirmed by manual inspection, and the peak areas integrated.

Suramin Quantitation

Suramin concentrations were measured by LC-MS/MS as previously described with modifications[10]. Plasma suramin samples were collected at 1 hour, 2 days and 42 days post-infusion. Heparinized plasma, 90 µl was used. Ten (10) µl of 50 µM stock of trypan blue was added to achieve an internal standard concentration of 5 µM. This was incubated at room temperature for 10 min to permit metabolite interaction with binding proteins, then extracted with 4 volumes (400 µl) of pre-chilled methanol-acetonitrile (50:50) to produce a final concentration of 40:40:20 (methanol:acetonitrile:H_2O), and precipitated on ice for 10 minutes. The samples were deproteinated and macromolecules removed by precipitation on crushed ice for 10 min. The mixture was centrifuged at 16,000g for 10 min at 4°C and the supernatant was transferred to a new tube and kept at -80°C for further LC-MS/MS analysis.

Suramin was analyzed on an AB SCIEX QTRAP 5500 triple quadrupole mass spectrometer equipped with a Turbo V electrospray ionization (ESI) source, Shimadzu LC-20A UHPLC system, and a PAL CTC autosampler. Ten microliters of extract were injected onto a Kinetix F5 column (100 × 2.1 mm, 2.6 µm; Phenomenex, CA) held at 30°C for chromatographic separation.

The mobile phase A was water with 20 mM ammonium acetate (NH_4OAC) (pH 7) and mobile phase B was methanol with 20 mM NH_4OAC (pH 7). Elution was performed using the following gradient: 0-1.5 min-0% B, 1.6-3 min-15% B, 3.1-7 min-60% B, 7.1-13 min-100% B, 14 min-0% B, 18 min-0% B, 18.1 minute-end. The flow rate was 400 µl/min. All the samples were kept at 4°C during analysis. Suramin and trypan blue were detected using MRM scanning mode with the dwell time of 180 ms. MRM transitions for the doubly-charged form of suramin were 647.0 m/z for the (Q1) precursor and 382.0 m/z for the (Q3) product. MRM transitions for trypan blue were 435.2 (Q1) and 185.0 (Q3). Absolute concentrations of suramin were determined using a standard curve prepared in plasma to account for matrix effects, and the peak area ratio of suramin to the internal standard trypan blue. The declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) were -104, -9.5, -32 and -16.9, and -144.58, -7, -57.8 and -20.94, for suramin and trypan blue, respectively. The ESI source parameters were set as follows: source temperature 500 °C; curtain gas 30; ion source gas 1, 35; ion source gas 2 35; spray voltage -4500 V. Analyst v1.6 was used for data acquisition and analysis.

Supplemental Results

Safety Monitoring and Adverse Events

The rash caused by suramin in this study was not raised and did not itch. It was not urticarial. The children did not appear to notice it. Any residual rash was covered by clothing and not visible on exposed skin at the 2-day evaluation. Parents were instructed not to discuss it with the neuropsychology team to decrease the chance of examiner bias. Video camera records of the

ADOS testing confirmed the absence of any visible rash. The rash was a known risk of suramin treatment that was described in the informed consent documents.

Pharmacokinetics

Additional pharmacokinetic results are illustrated in Table S1. Although no behavioral outcomes were significant at 2 days after infusion, we found that 28 biochemical pathways were changed by suramin 2-days after the infusion (Table S2). Twenty-two of these (79%) remained changed at the 6-week time point (see Table 3). The rank order of metabolites most changed at day 2, and their associated metabolic pathway is illustrated in Figure S2. The full list of 61 metabolites on day 2 and 48 metabolites at 6-weeks that were significantly changed by suramin appears in Tables S3-S4. A wallchart-style biochemical pathway map was created in Cytoscape to illustrate the organization of metabolites that were increased and decreased by suramin treatment (Figure S3).

Pharmacometabolomics

The small number of subjects in this trial precluded conventional treatment group analysis because of high false discovery rates associated with measuring 431 metabolites in groups with just 5 subjects. However, by using each child as their own control in a paired analysis of pre-infusion and post-infusion results, the pharmacometabolomic effects of suramin could be characterized (see Table 3 and Figures 4-5, Table S2 and Figure S2).

Treatment Outcomes

ADOS comparison scores were improved in the suramin treatment group at 6-weeks (Figure S4AB) but were unchanged in the saline group (Supplemental Figure S4AF). ADOS scores at 2-days after treatment were not changed (Figure S4E). EOWPVT scores were not changed (Figure S4I). Secondary outcomes included Aberrant Behavior Checklist (ABC), Autism Treatment Evaluation Checklist (ATEC), the Clinical Global Impression (CGI), and the Repetitive Behavior Questionnaire (RBQ). Suramin treatment was associated with improvements in the ABC, ATEC, and CGI, but not in the RBQ (Figure S4). Three of 24 symptoms covered in the CGI were significant (Figure S4aa). Parents were also asked to specify the three top, most-changed behaviors as an unstructured component of the CGI at 6-weeks after the infusion. Five symptoms were named that achieved statistically significant results. The most-changed behaviors were social communication and play, speech and language, calm and focus, stims or stereotypies, and coping skills (Figure S4bb).

Supplemental References

1. Lord C, Risi S, Lambrecht L, et al. The Autism Diagnostic Observation Schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. Journal of autism and developmental disorders. 2000;30(3):205-23.
2. Lord C, Rutter M, DiLavore P, Risi S, Gotham K, Bishop S. Autism Diagnostic Observation Schedule–2nd edition (ADOS-2). Los Angeles, CA: Western Psychological Corporation. 2012.
3. Adams-Chapman I, Bann C, Carter SL, Stoll BJ, Network NNR. Language outcomes among ELBW infants in early childhood. Early Hum Dev. 2015 Jun;91(6):373-9.
4. Kaat AJ, Lecavalier L, Aman MG. Validity of the aberrant behavior checklist in children with autism spectrum disorder. Journal of autism and developmental disorders. 2014;44(5):1103-16.
5. Geier DA, Kern JK, Geier MR. A Comparison of the Autism Treatment Evaluation Checklist (ATEC) and the Childhood Autism Rating Scale (CARS) for the Quantitative Evaluation of Autism. J Mental Health Research in Intellectual Disabilities. 2013;6:255-67.
6. Rimland B, Edelson S. Autism treatment evaluation checklist: statistical analyses. Autism Research Institute. 2000.
7. Honey E, McConachie H, Turner M, Rodgers J. Validation of the repetitive behaviour questionnaire for use with children with autism spectrum disorder. Research in Autism Spectrum Disorders. 2012;6(1):355-64.
8. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry (Edgmont). 2007 Jul;4(7):28-37.
9. Naviaux RK, Naviaux JC, Li K, et al. Metabolic features of chronic fatigue syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2016 Sep 13;113(37):E5472-80.
10. Naviaux JC, Schuchbauer MA, Li K, et al. Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. Translational psychiatry. 2014;4:e400.
11. Pickles A, Le Couteur A, Leadbitter K, et al. Parent-mediated social communication therapy for young children with autism (PACT): long-term follow-up of a randomised controlled trial. Lancet. 2016 Nov 19;388(10059):2501-9.

Supplemental Figure Legends

1. **Figure S1.** Storyboard illustration of each step of the infusion day visit.
2. **Figure S2.** Suramin pharmacometabolomics. Rank order of metabolites and pathways that were changed by suramin at 2-days after treatment.
3. **Figure S3.** Suramin pharmacometabolomics pathway visualization. (A) After 2 days. (B) After 6 weeks. Metabolites indicated in red are increased, and those in green are decreased compared to controls (see z-score scale in upper right).
4. **Figure S4.** Outcomes. (A) 6-week ADOS Comparison Scores by 2-Way ANOVA. (B) 6-Week ADOS Comparison Score Improvement after Suramin. (C) 6-Week ADOS Social Affect Score Improvement after Suramin. (D) 6-Week ADOS Restricted and Repetitive Behavior Score Improvement after Suramin. (E) 2-Day ADOS Comparison Scores were not changed. (F) No change in 6-Week ADOS Scores in subjects receiving saline placebo. (G) No change in 6-Week ADOS Social Affect Scores in subjects receiving placebo. (H) No change in 6-Week ADOS Restricted and Repetitive Behavior Scores in subjects receiving placebo. (I) No change in 6-week Expressive One Word Picture Vocabulary scores. (J) 7-Day improvement in ABC stereotypy scores after suramin. (K) 6-week Improvement in ABC stereotypy scores after suramin. (L) 7-Day Improvement in ATEC total scores after suramin. (M) No change in 6-week EOWPVT scores after saline. (N) No change in 7-day ABC stereotypy scores after saline. (O) No change in 6-week ABC stereotypy scores after saline. (P) No change in 7-day ATEC total scores after saline. (Q) Improved ATEC speech, language, and communication scores 7-days after suramin. (R) Improved ATEC sociability scores 7-days after suramin. (S) Improved ATEC speech, language, and communication scores 6-weeks after suramin. (T) Improved ADOS comparison scores after dropping a

subject who missed the 6-week visit (N = 4). (U) No change in 7-day ATEC speech, language, and communication after saline. (V) No change in 7-day ATEC sociability after saline. (W) No change in 6-week ATEC speech, language, and communication scores 6-weeks after saline (X) No change in EOWPVT scores after dropping subject who missed the 6-week visit (N = 4). (Y) No change in 2-day ADOS scores after suramin. (Z) No change in 6-week RBQ total scores after suramin. (aa) Improved core symptoms of ASD and other behaviors by CGI at 6-weeks after suramin. P values: * = 0.05; ** = 0.01; *** = 0.001. (bb) Top 3, most-changed symptoms named by parents in the 6-week CGI. (cc) No change in 2-day ADOS scores after saline. (dd) No change in 6-week RBQ total scores after saline.

Supplemental Tables

1. **Table S1.** Single-dose suramin pharmacokinetics.
2. **Table S2.** Suramin pharmacometabolomics. Pathways changed at 2-days.
3. **Table S3.** Suramin pharmacometabolomics. Metabolites changed at 2-days.
4. **Table S4.** Suramin pharmacometabolomics. Metabolites changed at 6-weeks.

Supplemental Data

1. **S1.** Clinical Global Impression (CGI) questionnaire.

2. **S2.** Social Stories to Accompany the Storyboard Panels Describing Each Step of the Infusion Day Visit.

Table S1. Single-dose suramin pharmacokinetics.

Pair Block	ID	Age (yrs)	Height (m)	Weight (kg)	BSA* (m^2)	20 mg/kg Dose (mg)	Dose (mg/m^2)	1-Hour Plasma Conc (µM)	2-Day Plasma Conc (µM)	45-Day Plasma Conc (µM)	Plasma Half-Life (days)
1	001	11	1.395	34.4	1.15	680	591	101.2	13.2	0.96	12.6
2	007	5	1.189	22.9	0.87	460	529	87.9	11.9	1.67	14.7
3	014	14	1.74	54.7	1.63	1000	613	110.9	10.6	1.04	14.9
4	012	6	1.18	23.1	0.87	460	529	118.6	13.8	2.28	16.5
5	005	7	1.271	25.1	0.95	500	526	101.8	10.6	1.76	15.0
						Mean:	558	104.1	12.0	1.54	14.7
						sd:	41	11.6	1.5	0.5	1.4

*Mosteller method. BSA: body surface area.

Table S2. Suramin pharmacometabolomics. Pathways changed at 2-days.

No.	Pathway Name	Measured Metabolites in the Pathway (N)	Expected Pathway Proportion (P = N/431)	Expected Hits in Sample of 61 (P * 61)	Observed Hits in the Top 61 Metabolites	Fold Enrichment (Obs/Exp)	Impact (Sum VIP Score)	Fraction of Impact (VIP Score) Explained (% of 119.7)	Increased	Decreased
1	Purine Metabolism	26	0.060	3.7	9	2.4	17.6	15%	4	5
2	Bile Salt Metabolism	6	0.014	0.8	4	4.7	11.9	10%	4	0
3	Microbiome Metabolism	18	0.042	2.5	4	1.6	9.3	8%	4	0
4	Branch Chain Amino Acid Metabolism	12	0.028	1.7	4	2.4	7.3	6%	4	0
5	Eicosanoid and Resolvin Metabolism	13	0.030	1.8	4	2.2	7.1	6%	0	4
6	Phospholipid Metabolism	74	0.172	10.5	3	0.3	5.7	5%	0	3
7	SAM, SAH, Methionine, Cysteine, Glutathione	15	0.035	2.1	3	1.4	5.6	5%	2	1
8	GABA, Glutamate, Arginine, Ornithine	6	0.014	0.8	3	3.5	4.7	4%	3	0
9	Pyrimidine Metabolism	9	0.021	1.3	2	1.6	4.3	4%	1	1
10	Glycolysis and Gluconeogenesis Metabolism	7	0.016	1.0	2	2.0	4.3	4%	2	0
11	Gamma-Glutamyl and other Dipeptides	2	0.005	0.3	2	7.1	3.8	3%	2	0
12	Sphingomyelin Metabolism	36	0.084	5.1	2	0.4	3.6	3%	0	2
13	Bioamines and Neurotransmitter Metabolism	9	0.021	1.3	2	1.6	3.3	3%	0	2
14	Krebs Cycle	9	0.021	1.3	2	1.6	3.3	3%	2	0
15	Vitamin D (Calciferol) Metabolism	3	0.007	0.4	1	2.4	3.1	3%	0	1
16	Cardiolipin Metabolism	7	0.016	1.0	2	2.0	3.1	3%	2	0
17	Glycosphingolipid Metabolism	12	0.028	1.7	1	0.6	2.1	2%	1	0
18	Taurine, Hypotaurine Metabolism	2	0.005	0.3	1	3.5	2.0	2%	0	1
19	Nitric Oxide, Superoxide, Peroxide	2	0.005	0.3	1	3.5	1.9	2%	0	1
20	Histidine, Histamine, Carnosine Metabolism	4	0.009	0.6	1	1.8	1.8	2%	1	0
21	Tyrosine and Phenylalanine Metabolism	3	0.007	0.4	1	2.4	1.8	2%	1	0
22	Fatty Acid Oxidation and Synthesis	37	0.086	5.2	1	0.2	1.8	2%	0	1
23	Cholesterol, Cortisol, Non-Gonadal Steroid	16	0.037	2.3	1	0.4	1.8	2%	1	0
24	Amino Acid Metabolism	4	0.009	0.6	1	1.8	1.8	1%	1	0
25	Endocannabinoid Metabolism	4	0.009	0.6	1	1.8	1.7	1%	0	1
26	Amino-Sugar, Galactose, & Non-Glucose	5	0.012	0.7	1	1.4	1.6	1%	1	0
27	Tryptophan, Kynurenine, Serotonin	6	0.014	0.8	1	1.2	1.6	1%	1	0
28	Ceramide Metabolism	34	0.079	4.8	1	0.2	1.5	1%	1	0
								Subtotals	38	23
								Totals	61	

Table S3. Suramin pharmacometabolomics. Metabolites changed at 2-days.

No.	Metabolite	Pathway Name	VIP Score	Z Score	AUC Ratio (Post/Pre)
1	Chenodeoxyglycocholic acid	Bile Salt Metabolism	3.171	1.610	2.787
2	1,25-Dihydroxyvitamin D3	Vitamin D (Calciferol) Metabolism	3.134	-1.447	0.273
3	Glycocholic acid	Bile Salt Metabolism	3.090	2.020	2.344
4	Taurodeoxycholic acid Pool	Bile Salt Metabolism	3.048	1.326	2.614
5	2-Keto-L-gluconate	Microbiome Metabolism	2.994	2.586	1.264
6	Taurocholic acid	Bile Salt Metabolism	2.615	1.102	2.183
7	2,3-Diphosphoglyceric acid	Glycolysis and Gluconeogenesis Metabolism	2.600	0.990	1.198
8	Cytosine	Pyrimidine Metabolism	2.556	2.055	1.689
9	p-Hydroxyphenylacetic acid	Microbiome Metabolism	2.546	1.464	1.192
10	11(R)-HETE	Eicosanoid and Resolvin Metabolism	2.400	-0.875	0.748
11	Hypoxanthine	Purine Metabolism	2.267	-1.000	0.745
12	Deoxyguanosine diphosphate	Purine Metabolism	2.264	-1.276	0.889
13	Glycylproline	Gamma-Glutamyl and other Dipeptides	2.205	1.212	1.773
14	Allantoin	Purine Metabolism	2.195	0.926	1.663
15	L-Isoleucine	Branch Chain Amino Acid Metabolism	2.136	0.815	1.094
16	GC(18:1/22:0)	Glycosphingolipid Metabolism	2.123	1.057	1.399
17	Cysteamine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	2.075	1.398	1.107
18	LysoPC(16:0)	Phospholipid Metabolism	2.067	-0.908	0.777
19	Taurine	Taurine, Hypotaurine Metabolism	2.042	-0.942	0.786
20	1-Methyladenine	Purine Metabolism	2.033	1.337	1.631
21	SM(d18:1/20:1)	Sphingomyelin Metabolism	2.033	-1.250	0.745
22	PA(16:0/16:1)	Phospholipid Metabolism	1.998	-0.813	0.793
23	Cyclic adenosine monophosphate	Purine Metabolism	1.949	-0.681	0.855
24	Azelaic acid	Nitric Oxide, Superoxide, Peroxide Metabolism	1.929	-2.024	0.914
25	Shikimate-3-phosphate	Microbiome Metabolism	1.886	1.033	1.047
26	Indoxyl sulfate	Microbiome Metabolism	1.858	0.702	1.280
27	1-Methylhistidine	Histidine, Histamine, Carnosine Metabolism	1.848	0.899	1.145
28	Purine	Purine Metabolism	1.847	1.137	1.203
29	L-Phenylalanine	Tyrosine and Phenylalanine Metabolism	1.839	0.957	1.164
30	Malonic acid	Fatty Acid Oxidation and Synthesis	1.833	-0.825	0.904
31	Methionine sulfoxide	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.817	1.738	1.331
32	L-Valine	Branch Chain Amino Acid Metabolism	1.808	0.749	1.165
33	24,25-Epoxycholesterol	Cholesterol, Cortisol, Non-Gonadal Steroid Metabolism	1.807	1.014	1.362
34	Orotic acid	Pyrimidine Metabolism	1.787	-0.612	0.670
35	AICAR	Purine Metabolism	1.787	1.310	1.309
36	Isovalerylglycine	Branch Chain Amino Acid Metabolism	1.783	0.852	1.951
37	Alanine	Amino Acid Metabolism (not otherwise covered)	1.776	1.066	1.193
38	Xanthosine	Purine Metabolism	1.764	-1.316	0.821
39	Anandamide	Endocannabinoid Metabolism	1.713	-0.709	0.684
40	Citramalic acid	Krebs Cycle	1.704	1.229	1.121
41	Cysteine-S-sulfate	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.682	-0.644	0.869
42	PG(16:0/16:0)	Phospholipid Metabolism	1.664	-0.667	0.549
43	Dopamine	Bioamines and Neurotransmitter Metabolism	1.653	-0.642	0.877
44	Glycerol 3-phosphate	Glycolysis and Gluconeogenesis Metabolism	1.651	1.151	1.187
45	5-HETE	Eicosanoid and Resolvin Metabolism	1.646	-0.671	0.866
46	Myoinositol	Amino-Sugar, Galactose, & Non-Glucose Metabolism	1.645	0.785	1.286
47	L-Glutamic acid	Bioamines and Neurotransmitter Metabolism	1.641	-0.619	0.797
48	Gamma-Aminobutyric acid	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.626	1.101	1.068
49	L-Kynurenine	Tryptophan, Kynurenine, Serotonin, Melatonin Metabolism	1.617	0.625	1.099
50	Citric acid	Krebs Cycle	1.590	0.759	1.142
51	SM(d18:1/20:0)	Sphingomyelin Metabolism	1.576	-0.770	0.712
52	Gamma-glutamyl-Alanine	Gamma-Glutamyl and other Dipeptides	1.575	0.896	1.294
53	Tiglylglycine	Branch Chain Amino Acid Metabolism	1.562	0.657	1.141
54	L-Proline	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.548	0.603	1.155
55	CL(18:2/18:2/18:2/18:2)	Cardiolipin Metabolism	1.538	0.506	1.181
56	CL(18:2/18:2/18:2/18:1)	Cardiolipin Metabolism	1.535	0.474	1.102
57	11,12-Epoxyeicosatrienoic acid	Eicosanoid and Resolvin Metabolism	1.535	-0.634	0.828
58	Ceramide(d18:1/18:2)	Ceramide Metabolism	1.521	0.530	1.337
59	Guanosine	Purine Metabolism	1.519	-0.766	0.702
60	Prostaglandin J2	Eicosanoid and Resolvin Metabolism	1.509	-0.601	0.649
61	N-Acetylglutamic acid	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.505	0.613	1.120

Table S4. Suramin pharmacometabolomics. Metabolites changed at 6-weeks.

No.	Metabolite	Pathway Name	VIP Score	Z Score	AUC Ratio (Post/Pre)
1	2-Keto-L-gluconate	Microbiome Metabolism	2.686	2.365	1.239
2	SM(d18:1/26:0 OH)	Sphingomyelin Metabolism	2.622	2.002	1.671
3	Glycine	1-Carbon, Folate, Formate, Glycine, Serine Metabolism	2.523	1.891	1.392
4	1-Methyladenine	Purine Metabolism	2.459	2.259	2.287
5	Alanine	Amino Acid Metabolism (not otherwise covered)	2.456	1.687	1.322
6	Cytosine	Pyrimidine Metabolism	2.442	2.582	1.932
7	Citric acid	Krebs Cycle	2.410	1.772	1.363
8	1-Pyrroline-5-carboxylic acid	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	2.358	1.922	1.299
9	Gamma-glutamyl-Alanine	Gamma-Glutamyl and other Dipeptides	2.353	1.725	1.644
10	Histamine	Histidine, Histamine, Carnosine Metabolism	2.279	1.312	1.219
11	p-Hydroxyphenylacetic acid	Microbiome Metabolism	2.203	2.226	1.306
12	Azelaic acid	Nitric Oxide, Superoxide, Peroxide Metabolism	2.151	2.558	1.120
13	Methionine sulfoxide	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	2.104	2.083	1.409
14	L-Kynurenine	Tryptophan, Kynurenine, Serotonin, Melatonin Metabolism	2.096	1.751	1.303
15	Glycerol 3-phosphate	Glycolysis and Gluconeogenesis Metabolism	2.081	1.731	1.294
16	Cysteamine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	2.060	2.007	1.157
17	Chenodeoxyglycocholic acid	Bile Salt Metabolism	2.052	1.650	2.858
18	Hydroxyproline	Vitamin C (Ascorbate) Metabolism	2.039	3.005	1.210
19	2-Hydroxyisovaleric acid	Branch Chain Amino Acid Metabolism	1.988	1.146	1.234
20	Purine	Purine Metabolism	1.980	1.650	1.307
21	Cyclic adenosine monophosphate	Purine Metabolism	1.962	-1.544	0.701
22	Glycocholic acid	Bile Salt Metabolism	1.956	1.945	2.270
23	4-Hydroxyphenyllactic acid	Microbiome Metabolism	1.945	1.172	1.294
24	Deoxyguanosine diphosphate	Purine Metabolism	1.915	-1.583	0.864
25	Hexose Disaccharide Pool	Amino-Sugar, Galactose, & Non-Glucose Metabolism	1.911	1.220	2.121
26	S-Adenosylhomocysteine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.894	0.971	1.417
27	Isovalerylglycine	Branch Chain Amino Acid Metabolism	1.888	0.901	2.027
28	Allantoin	Purine Metabolism	1.882	1.068	1.798
29	Tiglylglycine	Branch Chain Amino Acid Metabolism	1.878	1.310	1.302
30	L-Phenylalanine	Tyrosine and Phenylalanine Metabolism	1.875	1.381	1.245
31	cis-aconitic acid	Krebs Cycle	1.852	0.928	1.278
32	Lathosterol	Cholesterol, Cortisol, Non-Gonadal Steroid Metabolism	1.844	1.079	1.284
33	L-Asparagine	Amino Acid Metabolism (not otherwise covered)	1.824	1.581	1.360
34	Cinnamoylglycine	Tyrosine and Phenylalanine Metabolism	1.790	2.218	1.190
35	Octanoylcarnitine	Fatty Acid Oxidation and Synthesis	1.780	-1.451	0.703
36	L-Cystine	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.774	1.060	1.190
37	Uridine	Pyrimidine Metabolism	1.764	0.928	1.244
38	Mevalonic acid	Cholesterol, Cortisol, Non-Gonadal Steroid Metabolism	1.673	1.036	1.386
39	Chenodeoxycholic acid	Bile Salt Metabolism	1.670	1.575	2.080
40	Guanidinoacetic acid	SAM, SAH, Methionine, Cysteine, Glutathione Metabolism	1.644	1.254	1.217
41	2-Hydroxyisocaproic acid	Branch Chain Amino Acid Metabolism	1.622	0.997	1.306
42	Decanoylcarnitine	Fatty Acid Oxidation and Synthesis	1.617	-1.157	0.644
43	3-Hydroxy-cis-5-tetradecenoylcarnitine	Fatty Acid Oxidation and Synthesis	1.612	-1.056	0.734
44	Hippuric acid	Microbiome Metabolism	1.559	0.881	1.602
45	PE (18:0/18:0)	Phospholipid Metabolism	1.555	-1.331	0.644
46	L-Proline	GABA, Glutamate, Arginine, Ornithine, Proline Metabolism	1.546	0.749	1.196
47	SM(d18:1/18:2)	Sphingomyelin Metabolism	1.508	0.750	1.424
48	L-Serine	1-Carbon, Folate, Formate, Glycine, Serine Metabolism	1.505	0.954	1.152

Supplemental Data S1. Clinical Global Impression of Improvement questionnaire

Clinical Global Impression

UCSD Suramin Autism Treatment Study

Child's Name: _____

Your Name: _____ (please print)

Date: _____

INSTRUCTIONS

Please answer the following by assessing the full 6-week period after the infusion, compared to your child's behavior before the infusion. If a symptom changed over the 6 weeks, please write in the time after the infusion for maximum change in weeks (wks) or days (d). Please note "wks" for weeks and "days" or "d" for days. For example, if a symptom started to change after 1 week, but didn't reach maximum for 2 weeks, you would write in: "2 wks".
If a symptom didn't change check box "4". If it was never a problem check box "0".

24-Point Autism Symptom Assessment

No.	Over the 6 weeks, how would you assess each of the following?	0 Never a Problem	1 Very Much Improved	2 Much Improved	3 Minimally Improved	4 No Change	5 Minimally Worse	6 Much Worse	7 Very Much Worse	Time after Infusion for maximum change (wks or days) Write-In
1	Overall symptoms of autism severity or delayed development?	☐	☐	☐	☐	☐	☐	☐	☐	
2	Receptive language?	☐	☐	☐	☐	☐	☐	☐	☐	
3	Expressive language?	☐	☐	☐	☐	☐	☐	☐	☐	
4	Difficulty following verbal commands?	☐	☐	☐	☐	☐	☐	☐	☐	
5	Flapping or self-stimulation?	☐	☐	☐	☐	☐	☐	☐	☐	
6	Sensory issues like problems with touch, texture, taste, smell, sound, light, etc.?	☐	☐	☐	☐	☐	☐	☐	☐	
7	Insistence on sameness or difficulty with transitions?	☐	☐	☐	☐	☐	☐	☐	☐	
8	Anxiety or panic attacks?	☐	☐	☐	☐	☐	☐	☐	☐	
9	Tantrums or Meltdowns?	☐	☐	☐	☐	☐	☐	☐	☐	
10	Obsessive and/or compulsive behaviors?	☐	☐	☐	☐	☐	☐	☐	☐	
11	Self-Injurious behavior?	☐	☐	☐	☐	☐	☐	☐	☐	
12	Outbursts of anger or aggression?	☐	☐	☐	☐	☐	☐	☐	☐	
13	Lack of imaginative, make-believe, or age-appropriate play?	☐	☐	☐	☐	☐	☐	☐	☐	
14	Lack of desire for social interaction?	☐	☐	☐	☐	☐	☐	☐	☐	
15	Hyperactivity?	☐	☐	☐	☐	☐	☐	☐	☐	
16	Lethargy or fatigue?	☐	☐	☐	☐	☐	☐	☐	☐	
17	Inattention?	☐	☐	☐	☐	☐	☐	☐	☐	
18	Lack of eye contact or gaze avoidance?	☐	☐	☐	☐	☐	☐	☐	☐	
19	Problems sleeping?	☐	☐	☐	☐	☐	☐	☐	☐	
20	Sound sensitivity or ear covering?	☐	☐	☐	☐	☐	☐	☐	☐	
21	Feeding problems?	☐	☐	☐	☐	☐	☐	☐	☐	
22	Gross motor problems like trouble with abnormal walking or running?	☐	☐	☐	☐	☐	☐	☐	☐	
23	Fine motor problems like trouble with buttons, zippers, snaps, or tripod grasp?	☐	☐	☐	☐	☐	☐	☐	☐	
24	Problems with bowel movements?	☐	☐	☐	☐	☐	☐	☐	☐	

Comments and recommendations:

Page 1 of 2

INSTRUCTIONS

Write down the 3 symptoms that changed the most during the 6 weeks after infusion

Child's Name: _____

Date: _____

No. Over the 6 weeks, what 3 symptoms changed the most?

		1 Very Much Improved	2 Much Improved	3 Minimally Improved	4 No Change	5 Minimally Worse	6 Much Worse	7 Very Much Worse	Write-In: Time after Infusion for maximum change (wks or days)
1		☐	☐	☐	☐	☐	☐	☐	☐
2		☐	☐	☐	☐	☐	☐	☐	☐
3		☐	☐	☐	☐	☐	☐	☐	☐

Supplemental Data S2. Social Stories to Accompany the Storyboard Panels Describing Each Step of the Infusion Day Visit.

Check-in. "Hello again! You and your mom or dad are at our clinic today! We will do lots of different things, and meet different people. Everybody here is really nice. First, you will check in at the front desk, to let the doctor and nurses know that you are here. You might have to wait a few minutes before the nurse gets you. That's okay. You can sit in a chair and play with any toys that you brought with your today."

Numbing Medicine. "Then you will meet the nurse. She is really nice and friendly. You will sit in a chair or on the bed, and the nurse will put a special medicine on your arms, on the inside of your elbows (right where it bends.) The medicine will make your arms tingly and numb, and might tickle a little. That's okay, that's how we know that the medicine is working."

Height and Weight. "The nurse will take you to another room. You will stand on a scale and measure your weight, and you will stand tall to measure how tall you are. The nurse will also measure your blood pressure with a special bracelet that goes around your arm. She will take your temperature by touching your forehead with a fast thermometer."

Urine Sample. "If you didn't pee in a cup at home before you came to the clinic today, you will pee in a cup at the doctor's office in the bathroom. Mom or Dad will go with you if you need help."

Blood Sample. "After the bathroom, you will see the nurse again. Your arm will be nice and numb. The nurse will put a special needle in your arm, take some blood, then take out the needle and leave in a little plastic tube called an IV. Great job! That didn't hurt too much, and you sat so nice and still! The nurse will take some blood out of the tube, put some medicine in the tube, then wrap up your arm so you can go and play! We have lots of toys to play with. Or you can plan with the toys that you brought with you."

IV. "After some play time, you will sit down or lay down quietly, with no walking or jumping. A long tube called and IV will put medicine into the little tube in your arm. You can watch TV or play with your iPad, or even some Legos. Mom or Dad will sit with you the whole time."

Post-Infusion Free Time. "Next, the big tube gets put away, your arm gets wrapped up again, and you get to play some more! Or watch more TV. Have fun with your mom or dad."

Thank You Gift. "The nurse will then take the little tube out of your arm. Then you are done! Great job! You get to pick a present or have a treat, then go home with Mom or Dad. Thank you for being such a good helper today, and sitting so nicely and quietly. You had a good quiet mouth and gentle hands, and that makes Mom and Dad so happy. You did great!"

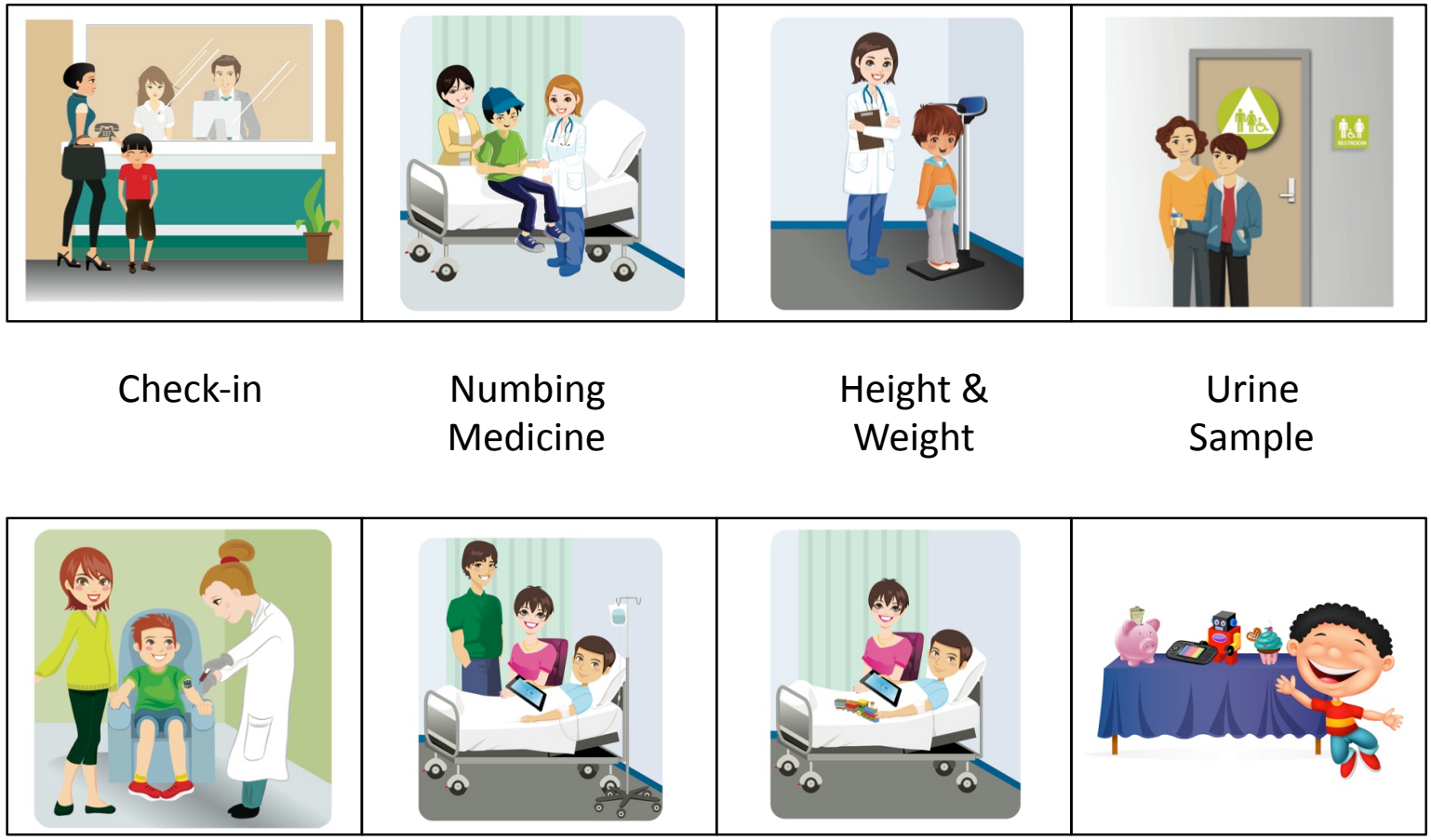


FIGURE S1
Storyboard illustration of each step of the infusion day visit

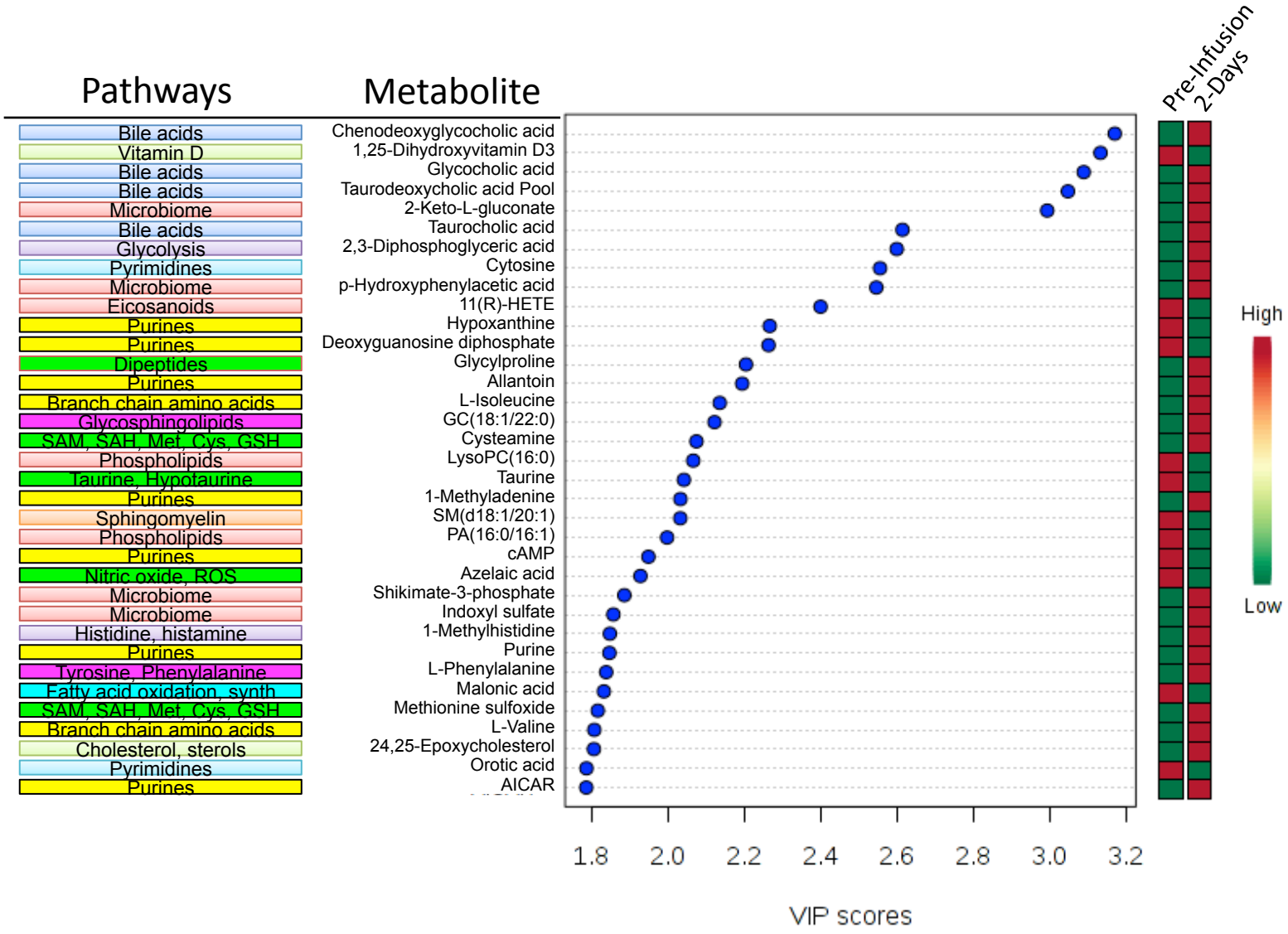
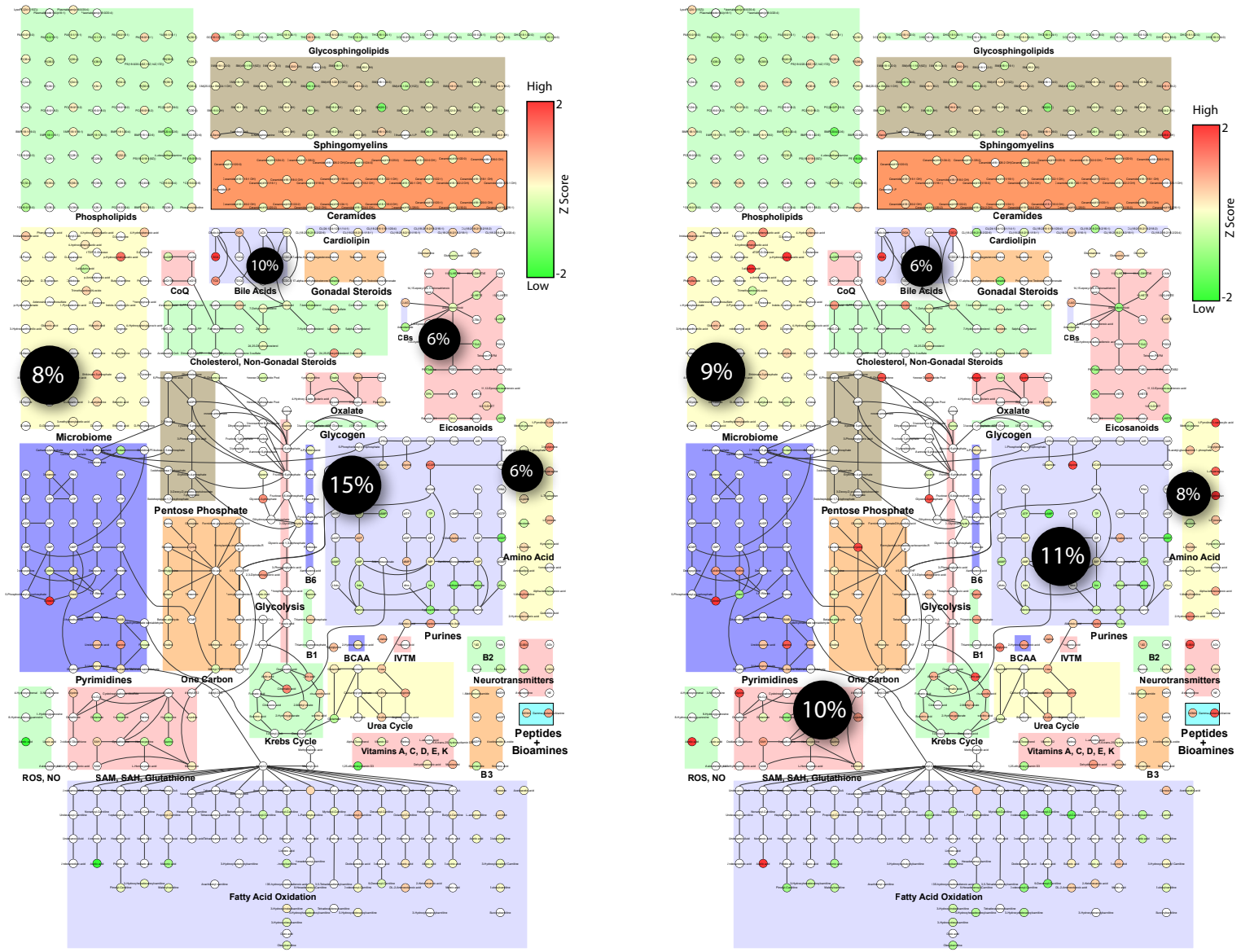


FIGURE S2

Suramin pharmacometabolomics. Metabolites and pathways changed at 2 days



2-Days 6-Weeks

FIGURE S3. Suramin pharmacometabolomics. Pathway visualization.

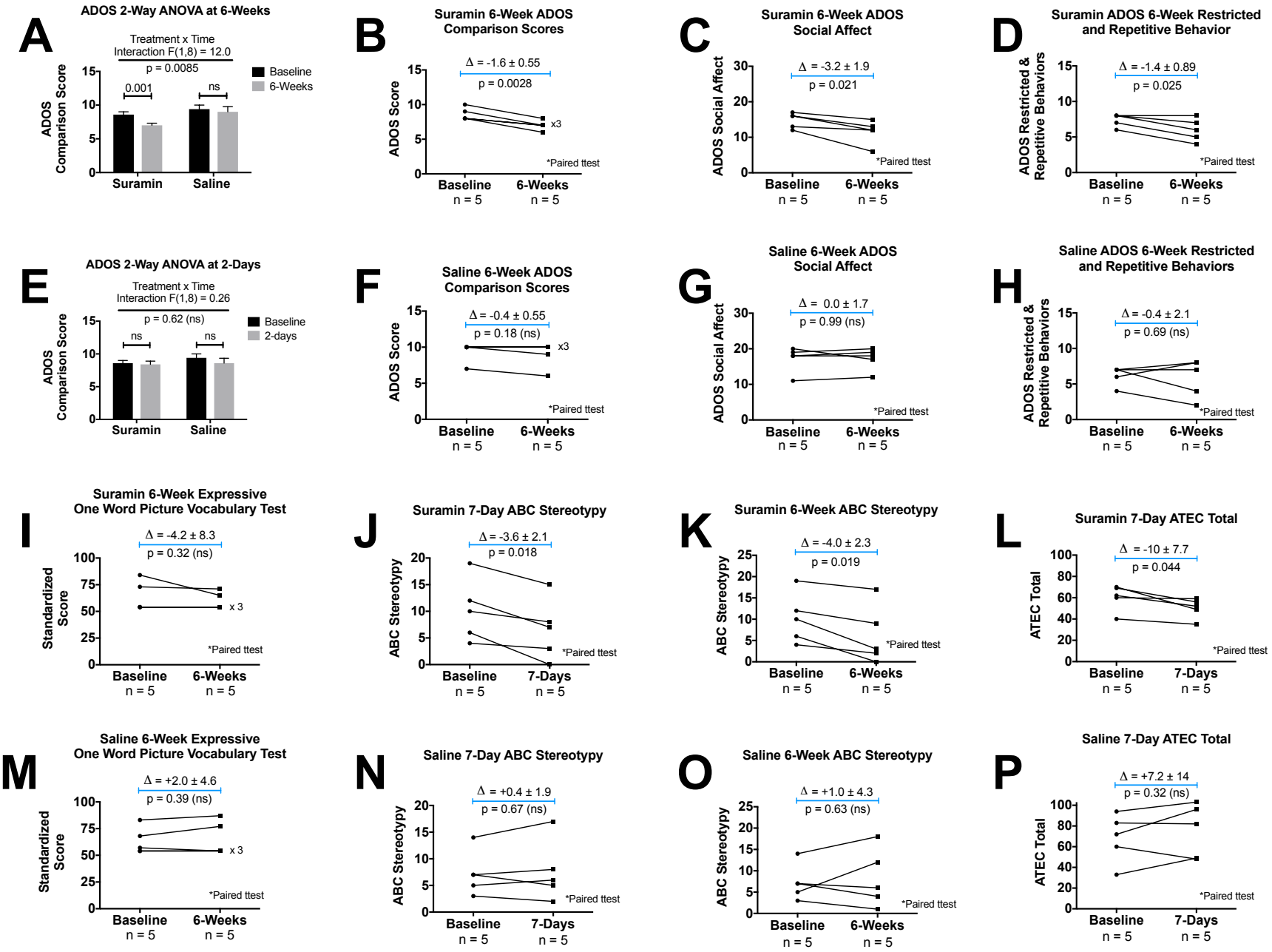


FIGURE S4 Outcomes, A-P

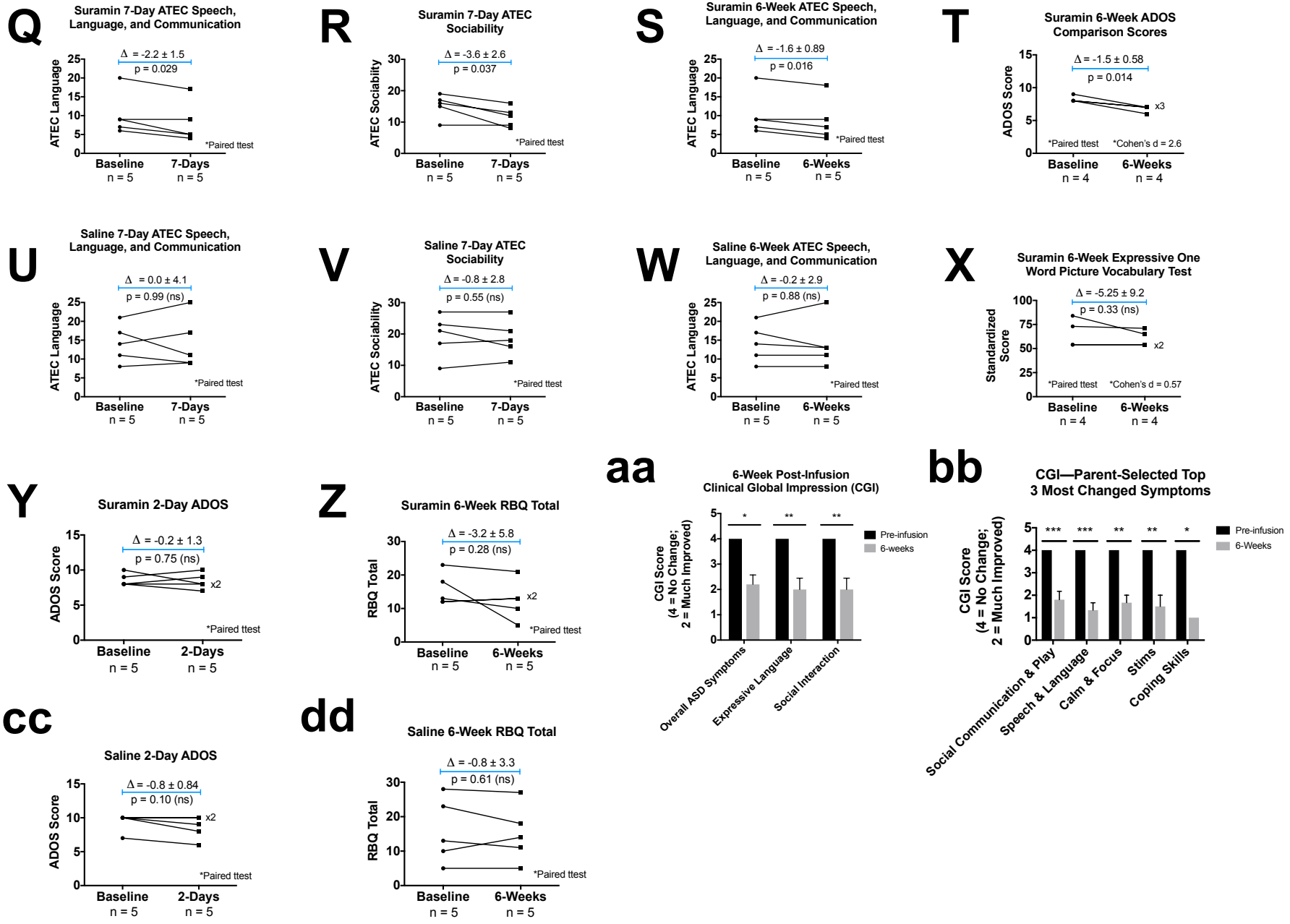


FIGURE S4 Outcomes, Q-dd