



RESEARCH ARTICLE

Placebo-controlled crossover assessment of mecamermin for the treatment of Rett syndrome

Heather M. O'Leary¹, Walter E. Kaufmann^{2,*}, Katherine V. Barnes¹, Kshitiz Rakesh¹, Kush Kapur¹ , Daniel C. Tarquinio³, Nicole G. Cantwell¹, Katherine J. Roche⁴, Suzanne A. Rose¹, Alexandra C. Walco¹, Natalie M. Bruck¹, Grace A. Bazin¹, Ingrid A. Holm^{5,6}, Mark E. Alexander^{5,7}, Lindsay C. Swanson¹, Lauren M. Baczewski⁴, Juan M. Mayor Torres^{1,8,9}, Charles A. Nelson III⁴ & Mustafa Sahin^{1,10,*} 

¹Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115

²Greenwood Genetic Center, Greenwood, South Carolina 29646

³Center for Rare Neurological Diseases, Norcross, Georgia 30093

⁴Division of Developmental Medicine, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115

⁵Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

⁶Division of Genetics and Genomics and the Manton Center for Orphan Disease Research, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115

⁷Department of Cardiology, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115

⁸Department of Information Engineering and Computer Science, University of Trento, Trento, Italy

⁹Department of Psychology, Stony Brook University, Stony Brook, New York 11794

¹⁰Translational Neuroscience Center, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Correspondence

Mustafa Sahin, Department of Neurology, Boston Children's Hospital, 300 Longwood Ave., Boston, MA 02115, USA.

Tel: 617-919-6258;

Fax: 617-730-0288;

E-mail: Mustafa.sahin@childrens.harvard.edu and

Walter E. Kaufmann, Greenwood Genetic Center, 106 Gregor Mendel Circle, Greenwood, SC 29646, USA. Tel: 864-672-6895; Fax: 864-388-1707;

E-mail: wkaufmann@ggc.org

Funding Information

The project was funded by the International Rett Syndrome Foundation/Rettsyndrome.org (Angel award 2907), the Translational Research Program at Boston Children's Hospital, Boston Children's Hospital Intellectual and Developmental Disabilities Research Center P30HD18655/U54 HD090255, Harvard Catalyst–The Harvard Clinical and Translational Science Center (National Institutes of Health Grant 1 UL1 RR 025758-01 and financial contributions from participating institutions), Autism Speaks, and Ipsen Biopharmaceuticals, who also provided mecamermin.

Received: 22 October 2017; Revised: 21 December 2017; Accepted: 21 December 2017

Abstract

Objective: To measure the efficacy of mecamermin (recombinant human insulin-like growth factor 1, rhIGF-1), for treating symptoms of Rett syndrome (RTT) in a pediatric population using a double-blind crossover study design. **Methods:** Thirty girls with classic RTT in postregression stage were randomly assigned to placebo or rhIGF-1 in treatment period 1 and crossed over to the opposite assignment for period 2 (both 20 weeks), separated by a 28-week washout period. The primary endpoints were as follows: Anxiety Depression and Mood Scale (ADAMS) Social Avoidance subscale, Rett Syndrome Behaviour Questionnaire (RSBQ) Fear/Anxiety subscale, Parent Target Symptom Visual Analog Scale (PTSVAS) top three concerns, Clinical Global Impression (CGI), Parent Global Impression (PGI), and the Kerr severity scale. Cardiorespiratory- and electroencephalography (EEG)-based biomarkers were also analyzed. **Results:** There were no significant differences between randomization groups. The majority of AEs were mild to moderate, although 12 episodes of serious AEs occurred. The Kerr severity scale, ADAMS Depressed Mood subscale, Visual Analog Scale Hyperventilation, and delta average power change scores significantly increased, implying worsening of symptoms. Electroencephalography (EEG) parameters also deteriorated. A secondary analysis of subjects who were not involved in a placebo recall confirmed most of these findings. However, it also revealed improvements on a measure of stereotypic behavior and another of social communication. **Interpretation:** As in the phase 1 trial, rhIGF-1 was safe; however, the drug did not reveal significant improvement, and some parameters worsened.

Annals of Clinical and Translational Neurology 2018; 5(3): 323–332

doi: 10.1002/acn3.533

ClinicalTrials.gov Identifier: NCT01777542.

Introduction

Rett syndrome (RTT) is a severe neurodevelopmental disorder associated with pathogenic variants in *MECP2*, which encodes the transcriptional regulator methyl CpG-binding protein 2 (MECP2).^{1–4} Rett syndrome (RTT) is the second most common cause of severe intellectual disability in females. It is characterized by normal early development followed by regression, leading to loss of spoken language and/or purposeful hand use, gait abnormalities, and development of stereotypic hand movements. Other common features include growth retardation, seizures, breathing abnormalities, vasomotor disturbances, and abnormal behavior.^{1–3,5–8} The current standard of care is symptomatic management, with no available treatments aimed at ameliorating the cardinal symptoms of the disorder.^{9–11}

Our initial understanding of the neurobiological bases of the disorder, primarily from tissue sample studies of children affected by RTT, served as the foundation for early drug trials.^{9,12} More recently, work on the role of MECP2 in synaptic maturation and maintenance and assessment of interventions in MECP2-deficient mice has resulted in potential therapeutic strategies.^{9,13–16} Prominent among these are compounds that modulate synaptic development and function, such as insulin-like growth factor 1 (IGF-1), which shares many features with brain-derived neurotrophic factor (BDNF), including activation of the AKT signaling pathway.^{17,18} Evaluation of IGF-1, and its active N-terminus peptide, in MECP2 mouse models, has rescued many RTT-like symptoms.^{19–21} These experimental findings have led to two phase 2 trials with the IGF-1 peptide analog trofinetide (NCT01703533 and NCT02715115) and one phase 1 trial with recombinant human IGF-1 (rhIGF-1) also termed mecasermin.²²

Mecasermin (rhIGF-1) is FDA-approved for the long-term treatment of growth failure in children with severe primary IGF-I deficiency.²³ In our phase 1 study in children with RTT, we demonstrated the safety of the drug that reached the CNS compartment and displayed a non-linear kinetics with greater distribution in the periphery.²² Furthermore, in a preliminary 20-week open-label assessment, we demonstrated improvements in cardiorespiratory parameters, such as an apnea index, and some neurobehavioral parameters, specifically measures of

anxiety and mood, which correlated with an EEG index of anxiety and depression. Thus, we initiated this study, a double-blind placebo-controlled crossover trial in 30 girls with RTT. We report our findings on safety and efficacy, including cognitive, behavioral, cardiorespiratory, EEG cortical biomarker, and other clinical severity endpoints.

Methods

Participants

Thirty girls between the ages of 2 and 10 years who met the 2010 diagnostic criteria for classic RTT had molecular documentation of a pathogenic *MECP2* variant, were in postregression stage of disease progression (postregression), were stable on current pharmacological treatments for at least 4 weeks and nonpharmacological treatments for at least 3 months, and reported a Rett Syndrome Behaviour Questionnaire (RSBQ) Fear/Anxiety subscale score of 4 or greater or an Anxiety Depression and Mood Scale (ADAMS) Social Avoidance subscale score of 6 or greater were enrolled at Boston Children's Hospital (BCH) between 2013 and 2015. Children taking antiepileptic medication were required to maintain a stable dose for at least 4 weeks prior to treatment with a stable presentation of seizure activity.

Figure 1 illustrates participant enrollment. Individuals were excluded if they had a scoliosis Cobb angle of 40 degrees or greater, a bone age greater than 11 years, cardiomegaly, previously used growth hormones, including IGF-1 or sex steroids, taken an investigational drug within 30 days of screening, were Tanner stage II breast development or later or had a chronic illness beyond that known to be associated with RTT. To ensure the reliability of our behavioral outcome measures, at least one caregiver was required to have English as their primary language. The study protocol was approved by the Institutional Review Boards at BCH and listed on *clinicaltrials.gov* (NCT01777542). Individual informed consent/assent was obtained before enrollment.

Study design and randomization

The study employed a double-blind, placebo-controlled crossover design (Fig. 2) and was divided into five parts:

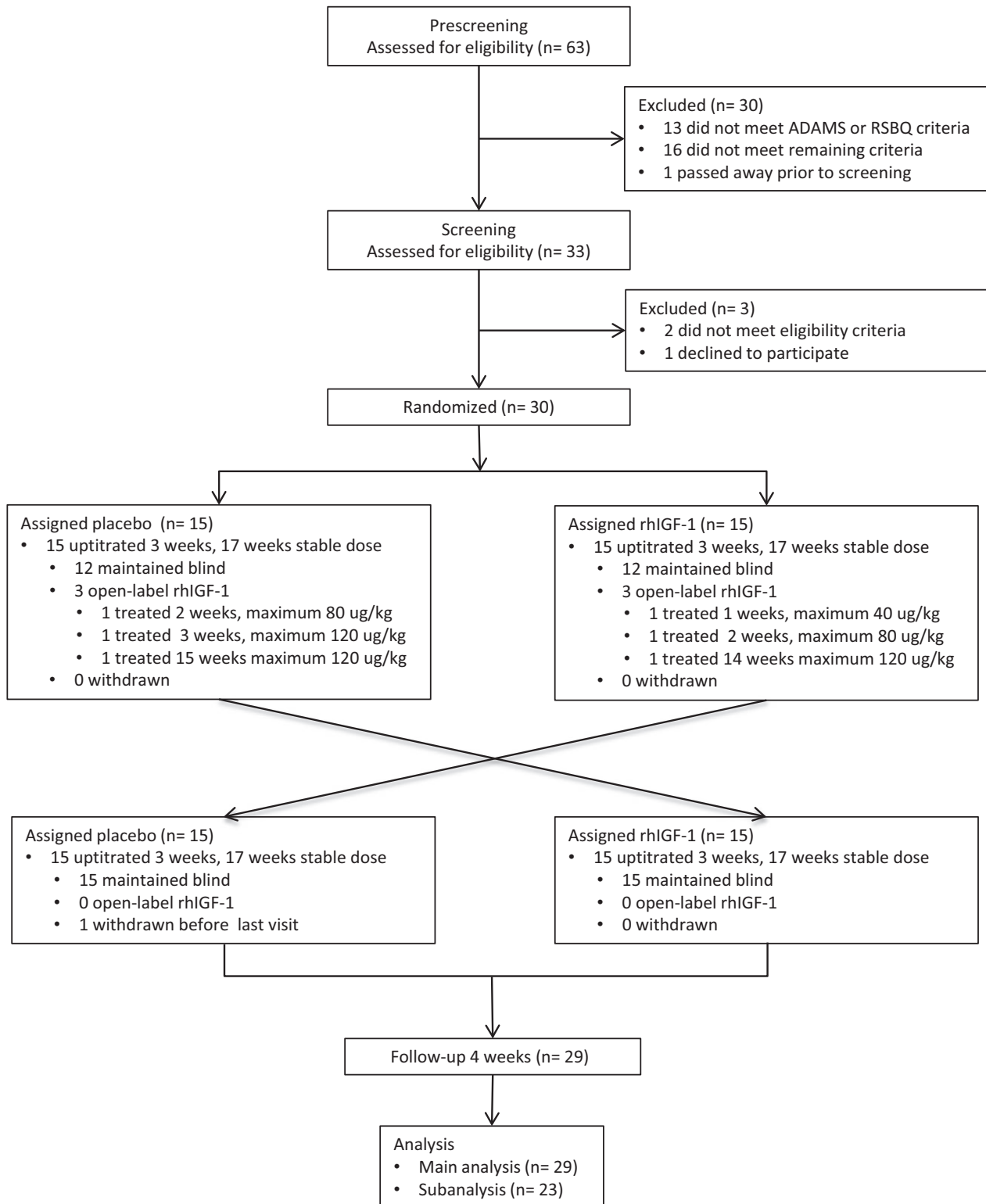


Figure 1. CONSORT-type flow diagram. Thirty participants enrolled with one dropout prior to the final visit. Open-label drug was administered to six participants during treatment period 1 due to a placebo recall. The intention to treat population of main analysis included 29 participants and secondary analysis, analysis of subjects who remained blinded to treatment during both periods, included 23 participants.

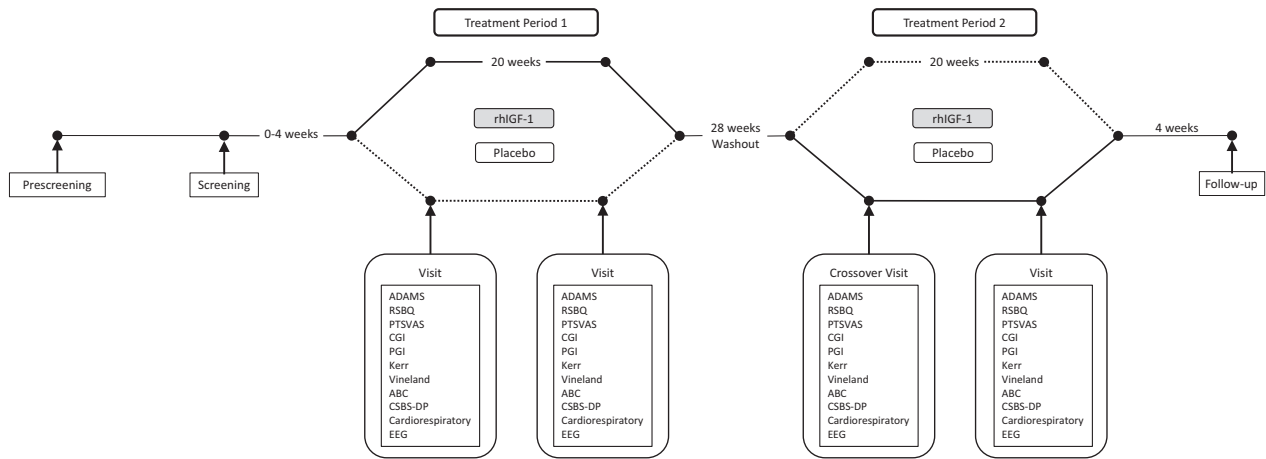


Figure 2. Diagram of the study design. The double-blind crossover trial included treatment period 1 (Tx1) and treatment period 2 (Tx2) blocks both with a length of 20 weeks. During the 28-weeks washout period, between the two treatment periods, none of the patients had a clinical visit or any dose of medication (rhIGF-1 or placebo).

screening, a 20-week treatment period 1, a 28-week washout period, a 20-week treatment period 2, and a 4-week follow-up phone interview. Enrolled participants were equally randomized to receive either rhIGF-1 or placebo for the first treatment period, thus 15 participants were randomized to receive rhIGF-1 and 15 were assigned to receive placebo. After the 28-week washout period, they crossed over to the opposite assignment.

Participants were administered injections of study drug, rhIGF-1 or placebo twice per day, separated by 12 ± 2 -h intervals. The injection volume of study drug was increased weekly to enable dose escalation for the first 3 weeks of the treatment period to equivalent volumes of 40, 80, and 120 $\mu\text{g}/\text{kg}$ of rhIGF-1, respectively. Subsequently, participants were administered the dose volume of study drug that corresponded to the 120 $\mu\text{g}/\text{kg}$ dose of rhIGF-1 for the remainder of each treatment period.

Due to concerns over the increasing pH of the placebo, investigators recalled placebo once during the first year of the study. To maintain the blind, the six participants who were in a treatment period 1 during the recall were given open-label rhIGF-1 regardless of treatment assignment. To allow for production of replacement placebo, the original washout period of 10 weeks was extended to 28 weeks. The flow diagram (Fig. 1) illustrates treatment assignment and open-label treatment.

Safety evaluations

Side effects were monitored through the Monitoring of Side Effects System (MOSES) via phone interview every 5 weeks during treatment. Caregivers were instructed to use an Accu-Chek[®] Aviva glucometer to monitor postinjection blood sugar levels to screen for hypoglycemia.

Urinalysis, complete blood count (CBC), a liver function panel, lipid profile, electrolyte panel, albumin, urea nitrogen, creatinine, and selected hormones in blood samples collected constituted the safety laboratory studies at the first and last visit of each treatment period. IGF-1, insulin, and glucose from blood samples were collected at each 10-week visit during treatment periods. IGF-1 values were monitored by designated personal who were not blinded to the treatment assignment. Laboratory assessments were complemented by height, weight, anthropometric measures, heart rate, blood pressure, and a 12-lead ECG collected during the clinical assessments. Scoliosis X-rays, bone density scans, and echocardiograms were performed to monitor progression of scoliosis, pubertal status, and cardiomegaly side effects.

Efficacy outcome measures

Outcome measures were chosen in part based on the results of our 20-week open-label assessment of efficacy and included measures derived from severity and global efficacy, behavior, cognitive and adaptive behavior, cardiorespiratory biomarkers, and cortical biomarkers assessments. Eight primary outcome measures were selected: ADAMS Social Avoidance subscale, RSBQ Fear/Anxiety subscale, Parent Targeted Symptoms Visual Analog Scale (PTSVAS) top three concerns, Clinical Global Impression Efficacy Index (CGI-EI),²⁴ Parent Global Impression Efficacy index (PGI-EI), and the Kerr (overall) severity scale. Secondary outcome measures were as follows: additional ADAMS subscales, additional RSBQ subscales, Aberrant Behavioral Checklist-Community (ABC-C) subscales, Mullen Scales of Early Learning (MSEL), Vineland Adaptive Behavior Scales-Second Edition (Vineland-II),

Communication and Symbolic Behavior Scales-Developmental Profile (CSBS-DP) subscales, and cardiorespiratory biomarkers. Exploratory outcome measures included cortical biomarkers, and a Visual Analog Scale focused on the RTT distinctive hand stereotypies and breathing (VAS-HS+B) that included hand stereotypies, hyperventilation, breath holding, cyanosis, and air/saliva expulsion.

Assessment schedule

Five visits were separated into 5-week intervals for each 20-week treatment period, totaling 10 study visits per participant. Visits 1 and 5 were at the start and end of the first treatment period, while 6 and 10 were at the start and end of the second treatment period. These visits incorporated a battery of assessments spanning 8 days and included all efficacy assessments, the MOSES and safety laboratories, and a clinical evaluation. No assessments were completed during the 28-week washout.

Cardiorespiratory biomarkers

Cardiorespiratory data were collected for 90–180 min in a quiet room while participants watched a movie of choice in a calm and wakeful state during three separate visits, across 7 days. Continuous chest respiratory inductance plethysmography and three-lead ECG signals were digitally sampled at 960 Hz using the BioRadio system (Great Lakes Neurotech, Cleveland, OH). Signals were low-pass filtered and peak detected, and measures were extracted using a Matlab (Mathworks, Natick, MA)-based software developed in-house. The software extracted apnea and hyperventilation indices, the mean and standard deviation of apnea length, respiratory cycle time, irregularity of respiratory cycles, expiratory time (Te), heart rate, and irregularity of heart rate.

Cortical biomarkers

Continuous resting state electroencephalography (EEG) data were collected for 5–10 min in an electrically shielded room while participants, calm and still, watched a movie of their choice. Data were collected using a 128-channel Hydrocel Geodesic Sensor Net System and a Net Amps 300 amplifier via Net Station software (all equipment and software from Electrical Geodesics, Inc., Eugene, OR), sampled at 1000 Hz, filtered, amplified, and referenced to the vertex (electrode Cz). In-house Matlab-based software was used to calculate frontal power spectral density binned into bandwidths previously described.²⁵ Alpha asymmetry scores were calculated by subtracting the average natural log alpha power (6–

13 Hz) on the left side from the natural log alpha power on the right side,^{26–29} with positive values corresponding to higher power in the right hemisphere but greater activation in the left hemisphere.

Statistical analyses

The primary statistical analysis examined differences in change scores. Change scores were calculated per participant by subtracting the change in each outcome variable during rhIGF-1 treatment from the change in each outcome variable during placebo treatment. Change in outcome variables for each participant during each treatment period was calculated as the difference between the value at the last visit from the value at the first visit of a given treatment period. We employed both the parametric *t*-test and the nonparametric Wilcoxon sign rank test. Sequence and period effects of treatment assignment were tested for all outcomes. The main analysis tested the average change scores against the null hypothesis. Statistical analysis was performed on all participants using the intention to treat principle (ITT). As part of sensitivity analysis, children who were not exposed to open-label drug during the placebo recall were analyzed in a subanalysis cohort. The primary outcomes were adjusted for multiple comparisons using a conservative Bonferroni's correction approach.

Results

A total of 63 girls were prescreened, with 34 qualifying for the screening visit but one passing away prior to screening. The main reason for prescreening failures was not meeting cutoffs on the RSBQ or ADAMS ($n = 13$); other reasons included bone age >11 years, scoliosis Cobb angle >40°, and recent loss of developmental skills. Of the 33 screened participants, two were excluded because they were in puberty as determined by physical examination and laboratory tests, and 31 were confirmed to be eligible. One eligible participant declined participation, and 30 participants were randomized for treatment, median (interquartile) age 5.54 (3.58) years and Kerr severity score 18 (8.00). Six of the 30 participants were treated with open-label rhIGF-1 during their first treatment period due to the placebo recall and one of the 30 participants dropped out of the study prior to completion. The flow diagram (Fig. 1) summarizes subject enrollment through analysis. Table 1 summarizes participant demographics, indicating no significant differences between randomization groups. Table 2 summarizes results for the primary outcome measures and significant changes in secondary and exploratory outcome measures. Only significant changes are reported below.

Table 1. Baseline characteristics and vitals.

	Sequence 1 median (IQR)	Sequence 2 median (IQR)	Total median (IQR)	P-value
Age	5.58 (4.25)	5.50 (2.83)	5.54 (3.58)	1
Ethnicity				0.2241
Hispanic, Latino, or Spanish origin	0.00 (0.00)	3.00 (20.00)	3.00 (10.00)	
Not Hispanic, Latino, or Spanish origin	14.00 (93.33)	12.00 (80.00)	26.00 (86.67)	
Refused	5.58 (6.67)	0.00 (0.00)	1.00 (3.33)	
Vitals				
Temperature	36.50 (0.50)	36.60 (0.40)	36.60 (0.40)	0.8669
Heart rate	94.00 (24.00)	98.00 (29.00)	96.00 (26.00)	0.9834
Respiratory rate	20.00 (8.00)	19.00 (6.00)	19.50 (6.00)	0.3443
Systolic BP	99.00 (9.00)	103.00 (22.00)	101.50 (11.00)	0.5064
Diastolic BP	65.00 (20.00)	69.00 (16.00)	68.00 (18.00)	0.8354
Height	110.13 (25.27)	110.07 (15.77)	110.10 (21.17)	0.5069
Weight	17.70 (6.23)	19.47 (10.03)	18.47 (6.73)	0.8195
FOC	48.50 (3.00)	49.00 (3.50)	48.50 (3.00)	0.8188
BMI	13.87 (3.73)	14.93 (4.40)	14.35 (3.38)	0.4306
QT	349 (26.0)	340 (50.0)	348 (34.0)	0.1273
QTc	446 (17.5)	437 (36.0)	441 (26.0)	0.683
Baseline measures				
ADAMS social avoidance	6.00 (3.00)	4.00 (6.00)	6.00 (5.00)	0.1219
RSBQ fear/anxiety	4.00 (3.00)	5.00 (4.00)	4.00 (3.00)	0.9831
Kerr severity scale	16.50 (7.00)	18.00 (10.00)	18.00 (8.00)	1
Apnea index (apneas/hour)	7.58 (8.75)	4.05 (5.84)	4.43 (7.07)	0.4448
Hyperventilation index (events/hour)	35.92 (28.07)	36.63 (19.86)	35.92 (21.33)	0.9131

Table 2. Average effects for the main analysis (all participants) and subanalysis (participants not involved in recall).

	All participants				No recall			
	n	IGF-1—placebo	P-value		n	IGF-1—placebo	P-value	
Primary								
ADAMS social avoidance	28	0.79 (2.54)	0.1138 ² ,0.0861 ³	w	22	0.32 (2.50)	0.5562 ² ,0.5133 ³	w
RSBQ fear/anxiety	28	0.43 (2.38)	0.3490 ² ,0.4712 ³	w	22	0.23 (2.14)	0.6230 ² ,0.7559 ³	w
PTSVAS symptom 1	28	-0.31 (3.85)	0.6735 ² ,0.9118 ³	i	22	-0.63 (3.16)	0.3565 ² ,0.6486 ³	i
PTSVAS symptom 2	28	0.64 (2.92)	0.2578 ² ,0.2386 ³	w	22	0.32 (2.89)	0.6110 ² ,0.5081 ³	w
PTSVAS symptom 3	28	0.25 (3.11)	0.6783 ² ,0.8419 ³	w	22	-0.03 (2.82)	0.9666 ² ,0.6946 ³	i
Clinical global impression	28	0.11 (0.63)	0.3753 ² ,0.5625 ³	w	23	0.17 (0.58)	0.1619 ² ,0.3125 ³	w
Parent global impression	25	-0.12 (1.01)	0.5593 ² ,0.4790 ³	i	20	-0.10 (1.06)	0.6775 ² ,0.6204 ³	i
Kerr severity	28	1.46 (3.77)	0.0494 ² ,0.0754 ³	w ¹	23	1.39 (3.76)	0.0900 ² ,0.1616 ³	w
Secondary								
ADAMS depressed mood	28	1.04 (2.71)	0.0535 ² ,0.0272 ³	w ¹	22	0.64 (2.72)	0.2845 ² ,0.1889 ³	w
ABC-C stereotypy	29	-1.21 (4.92)	0.1975 ² ,0.1684 ³	i	23	-1.91 (4.99)	0.0795 ² ,0.0558 ³	i ¹
CSBS-DP Social	28	2.04 (5.92)	0.0797 ² ,0.0882 ³	i	22	3.36 (5.49)	0.0091 ² ,0.0133 ³	i ¹
SD normal Te	29	0.02 (0.06)	0.0979 ² ,0.1083 ³	w	23	0.02 (0.06)	0.0932 ² ,0.0491 ³	w ¹
Mean HR	29	5.44 (10.00)	0.0067 ² ,0.0131 ³	u ¹	23	4.78 (10.56)	0.0411 ² ,0.0770 ³	u ¹
Exploratory								
Hyperventilation (VAS-HS+B)	28	1.64 (3.55)	0.0211 ² ,0.0111 ³	w ¹	22	1.84 (3.76)	0.0325 ² ,0.0164 ³	w ¹
Delta frontal power	17	0.23 (0.37)	0.0208 ² ,0.0110 ³	w ¹	14	0.27 (0.39)	0.0234 ² ,0.0166 ³	w ¹
Delta frontal relative power	17	0.04 (0.10)	0.1381 ² ,0.2633 ³	w	14	0.06 (0.09)	0.0340 ² ,0.0580 ³	w ¹
Beta frontal relative power	17	-0.03 (0.09)	0.1807 ² ,0.2069 ³	w	14	-0.05 (0.08)	0.0376 ² ,0.0353 ³	w ¹
Gamma frontal relative power	17	-0.04 (0.09)	0.1040 ² ,0.1324 ³	w ¹	14	-0.06 (0.08)	0.0191 ² ,0.0166 ³	w ¹

i, improvement; w, worsening; u, undefined.

¹Indicates P-value ≤0.05.²One sample or paired t-test.³Wilcoxon sign rank test.

Table 3. Adverse events (AEs).

	<i>n</i> events
AE category	
Cardiac	3
Dermatology	6
Gastrointestinal	14
Genitourinary	0
Hematology	0
Infectious	23
Metabolic	0
Musculoskeletal	5
Neurological	14
Psychiatric	3
Respiratory	11
Endocrine	3
Unspecified	12
Total AE	
Serious AEs	12
Nonserious AEs	82
AE severity	
Grade 1	27
Grade 2	48
Grade 3	18
Grade 4	1
Relationship to study drug	
Suspected	75
Not suspected	19
Expected	45
Not expected	49

Primary outcome measures

The Kerr clinical severity score increased in the main analysis, implying that during the study period, participants became overall more severely affected. However, this difference was no longer significant when the participants involved in the placebo recall were extracted. No other primary outcome measure changed.

Secondary outcome measures:

The entire cohort demonstrated increases in the ADAMS Depressed Mood subscale and heart rate, implying worsening of symptoms. When excluding participants involved in the placebo recall, only heart rate remained increased, and the standard deviation of expiratory time (T_e) was increased. Two behavioral measures showed improvements: scores on the ABC-C Stereotypy subscale significantly decreased and those on the CSBS-DP Social subscale significantly increased at a clinically meaningful level.³⁰

Exploratory outcome measures

VAS-HS+B hyperventilation increased in both sets of analyses (entire cohort and cohort without participants in

placebo recall), implying worsening of hyperventilation during the rhIGF-1 period. Average and relative frontal delta power increased in both sets of analyses. In contrast, both relative frontal beta power and relative frontal gamma power showed decreases. Overall, the spectral power findings suggest a worsening in EEG profiles on rhIGF-1.

Adverse events (AEs)

A total of 94 AEs were reported, the majority (80%) being mild or moderate (CTCAE 3.0 Grade 1 or 2). Infectious complaints, such as fever, otitis media, or sinusitis, were most common (24% overall). There were 12 serious AEs (SAEs) requiring hospitalization, many of which were recurring infections in the same individual. Table 3 summarizes the most common AEs and their frequency.

Discussion

The present intervention study was intended to examine the efficacy of rhIGF-1 for treating children affected by RTT, expanding on our previous phase 1 pharmacokinetic study of safety and open-label extension findings.²² The positive findings observed in our open-label extension, which included improvements in anxiety and mood, apnea index ($N = 9$ subjects analyzed), and EEG alpha asymmetry (an index of anxiety and depression; $N = 6$ subjects analyzed), were not confirmed in this study. Moreover, several efficacy parameters showed worsening. Nonetheless, one problem behavior measure and one communication/socialization measure improved, with the latter at a significant level. As in the phase 1 trial, the present phase 2 study confirmed the overall safety of rhIGF-1.

In general, outcome measures of severity and behavior did not change. While the Kerr severity scale and ADAMS Depressed Mood subscale worsened in the main analysis, changes were no longer significant in the subanalysis, analysis that excluded participants who were exposed to open-label rhIGF-1 during the placebo recall. The ADAMS Social Avoidance and the RSBQ Fear/Anxiety subscales also did not change. The ABC-C Stereotypy subscale showed a significant decrease in the subanalysis, although not clinically relevant.^{31,32} On the other hand, the significant increase in the CSBS-DP Social scores corresponded to a clinically meaningful level.³⁰ Altogether, severity and behavioral measures, whether clinician- or caregiver-reported, failed to demonstrate efficacy.

Biomarkers are of particular interest in severely neurologically impaired, nonverbal populations such as RTT due to limitations in verbal and motor functions. Changes in cardiorespiratory biomarkers reported in our open-label extension were highly significant and associated with a decrease in apnea. These autonomic biomarkers were

selected as sensitive surrogates of change in synaptic maturation. In this study, heart rate increased, but the respiratory measures did not change. The VAS demonstrated significant increases in severity of hyperventilation in both the main and subanalysis groups, but quantitative measures of hyperventilation and tachypnea did not support caregivers' reports. Mean heart rate was the only cardiorespiratory measure that demonstrated significant increase in both studies. While the correlation between heart rate and reduction in apnea was not explored, it is possible the two are associated and heart rate variability measures may be a more sensitive indicator of treatment response deserving further investigation.

Although the alpha frontal asymmetry was the main focus of the cortical biomarkers, it was unchanged in this study. However, multiple other EEG spectral measures changed in directions that suggest worsening. Significant increases in frontal delta power with decreases in beta and gamma power suggest an increased contribution of slow-wave activity following drug administration. These findings are consistent with reports of underlying cortical dysfunction related to various neurological disorders and encephalopathies, regardless of the underlying etiology.^{25,33,34} As these changes were seen in both main and secondary analyses, they represent relative strong findings.

There are multiple reasons for a predominantly negative (i.e., no change) drug trial. They include outcome measures with relatively low sensitivity/susceptible to placebo effect; inadequate dose/administration regimen; in crossover studies, carry forward effects (i.e., participants who were randomized to drug first may carry drug effects into placebo period diminishing the difference between treatment periods); low statistical power; and an ineffective drug. At present, we cannot rule out any of these possibilities. Few outcome measures have been validated based on measurement properties in RTT.¹¹ Among them are a recently reported CGI with RTT-specific anchors³⁵ and ADAMS Social Avoidance.⁵ Objective biomarkers are less susceptible to placebo effects or suboptimal reliability. Nonetheless, neither the apnea index nor alpha asymmetry replicated improvements observed in the open-label extension, although a significant increase in heart rate was present in both studies. Minimum apnea index was not part of the inclusion criteria; consequently, only 14 participants presented with a value high enough at visit 1 to demonstrate treatment efficacy. Likewise, only 17 of the 30 participants yielded analyzable EEG signal and only 13 showed a R > L alpha asymmetry at baseline. The lack of a significant decrease in apnea index and alpha asymmetry may be then attributed to low statistical power.

If endpoints were not an issue, could drug administration or study design affect the trial outcome? Subanalysis

suggests the drug-affected neurological function as long as appropriate drug-placebo comparisons were performed; however, this does not rule out the possibility that higher doses were necessary to influence certain CNS symptoms. Although the trial included uptitration, it targeted the maximum FDA-approved dose of mecasermin (120 µg/kg) and not a clinical improvement or a maximum tolerated dose. The nonlinear kinetics of rhIGF-1 also raises the possibility of long-term effects that, in the case of crossover trials, could be manifested as carry forward effect. Conversely, Pini and colleagues reported improvements in breathing that extend for the latter half of the 6-month treatment period that worsened after treatment cessation.³⁶ Another possibility is continuous administration is not the best approach for rhIGF-1's neurological effects. Pini and colleagues also showed significant decreases in mean amplitude difference of delta power between electrodes C4 and T4 in participants treated with a maximum open-label rhIGF-1 dose of 100 µg/kg for 20–24 weeks compared to untreated age-matched controls.³⁷ A follow-up study will focus on data analysis, including intermediate visits, to determine whether some rhIGF-1 effects are shorter term or could lead to carry forward.

In conclusion, in this study, administration of rhIGF-1 for 20 weeks at the maximum FDA-approved dose did not improve neurobehavioral symptoms or clinical apnea in girls with RTT. Although the safety profile of rhIGF-1 is adequate and replicated in the present trial, worsening of some endpoints could represent the natural evolution of the disorder or true negative drug effects. Nonetheless, rhIGF-1 kinetics, study design limitations, and other factors raise the possibility that two observed improvements could merit further investigation of the therapeutic potential of rhIGF-1 for some neurobehavioral manifestations of RTT.

Acknowledgments

We thank the children and caregivers who participated in the study. We are grateful to the Rett Syndrome Association of Massachusetts for their critical support. The project was funded by the International Rett Syndrome Foundation/Rettsyndrome.org (Angel award 2907), the Translational Research Program at Boston Children's Hospital, Boston Children's Hospital Intellectual and Developmental Disabilities Research Center P30 HD18655/U54 HD090255, Harvard Catalyst–The Harvard Clinical and Translational Science Center (National Institutes of Health Grant 1 UL1 RR 025758-01 and financial contributions from participating institutions), Autism Speaks, and Ipsen Biopharmaceuticals, who also provided mecasermin.

Author Contributions

Heather M. O'Leary, BS: design of cardiorespiratory data collection methods and outcome measures, writing of software to extract outcome measures from continuous data, acquisition of data, analysis and interpretation of data, and drafting and revision of manuscript for intellectual content.

Walter E. Kaufmann, MD: study PI, study concept and design, acquisition of data, analysis and interpretation of data, and drafting and revision of manuscript for intellectual content.

Katherine V. Barnes, BS: acquisition of data, analysis and interpretation of data, and revision of manuscript for intellectual content.

Kshitiz Rakesh, MS: analysis and interpretation of data, and revision of manuscript for intellectual content.

Kush Kapur, PhD: analysis and interpretation of data, and drafting and revision of manuscript for intellectual content.

Daniel C. Tarquinio, MS, DO: acquisition of data and revision of manuscript for intellectual content.

Nicole G. Cantwell, DO: acquisition of data and revision of manuscript for intellectual content.

Kate J. Roche, MD, PhD: acquisition of data, analysis and interpretation of data, and revision of manuscript for intellectual content.

Suzanne A. Rose, CPNP: acquisition of data and revision of manuscript for intellectual content.

Alexandra C. Walco, BS: acquisition of data and revision of manuscript for intellectual content.

Natalie S. Bruck, BS: acquisition of data and revision of manuscript for intellectual content.

Grace A. Bazin, BS: acquisition of data and revision of manuscript for intellectual content.

Ingrid A. Holm, MD, MPH: acquisition of data and revision of manuscript for intellectual content.

Mark E. Alexander, MD: acquisition of data and revision of manuscript for intellectual content.

Lindsay C. Swanson, MS: acquisition of data and revision of manuscript for intellectual content.

Lauren M. Baczewski, MA: acquisition of data, analysis and interpretation of data, and revision of manuscript for intellectual content.

Juan Manuel Mayor Torres, MS: acquisition of data and revision of manuscript for intellectual content.

Charles A. Nelson III, PhD: acquisition of data, analysis and interpretation of data, and drafting and revision of manuscript for intellectual content.

Mustafa Sahin, MD, PhD: study PI, acquisition of data, analysis and interpretation of data, and drafting and revision of manuscript for intellectual content.

Conflict of Interest

Dr. Sahin reports grants and personal fees from Roche and grants from Novartis, Pfizer, and LAM Therapeutics outside the submitted work. He also has served on the Scientific Advisory Board of Sage Therapeutics. Dr. Kaufmann reports funding from Anavex, AveXis, Biohaven, EryDel, GW Pharmaceuticals, Neuren, Newron Pharmaceuticals, Ovid, and nonfinancial support from Marinus outside the submitted work. The other authors have nothing to disclose.

References

1. Neul JL, Kaufmann WE, Glaze DG, et al. Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol* 2010;68:944–950.
2. Hagberg B. Clinical manifestations and stages of Rett syndrome. *Ment Retard Dev Disabil Res Rev* 2002;8:61–65.
3. Marschik PB, Kaufmann WE, Sigafoos J, et al. Changing the perspective on early development of Rett syndrome. *Res Dev Disabil* 2013;34:1236–1239.
4. Chahrouh M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. *Neuron* 2007;56:422–437.
5. Barnes KV, Coughlin FR, O'Leary HM, et al. Anxiety-like behavior in Rett syndrome: characteristics and assessment by anxiety scales. *J Neurodev Disord* 2015;7:30.
6. Julu POO. Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. *Arch Dis Child* 2001;85:29–37.
7. Mount RH, Charman T, Hastings RP, et al. The Rett Syndrome Behaviour Questionnaire (RSBQ): refining the behavioural phenotype of Rett syndrome. *J Child Psychol Psychiatry* 2002;43:1099–1110.
8. Mackay J, Downs J, Wong K, et al. Autonomic breathing abnormalities in Rett syndrome: caregiver perspectives in an international database study. *J Neurodev Disord* 2017;9:15.
9. Tarquinio DC, Kaufmann WE. Targeted treatments in Rett syndrome. In: R. J. Hagerman and R. I. Hendren, eds. *New developments in treatment for neurodevelopmental disorders: targeting neurobiological mechanisms*. pp. 129–158. New York, NY: Oxford University Press, 2014.
10. Percy AK. Progress in Rett syndrome: from discovery to clinical trials. *Wien Med Wochenschr* 2016;166:325–332.
11. Kaufmann WE, Stallworth JL, Everman DB, Skinner SA. Neurobiologically-based treatments in Rett syndrome: opportunities and challenges. *Expert Opin Orphan Drugs* 2016;4:1043–1055.
12. Percy AK. Rett syndrome: recent research progress. *J Child Neurol* 2008;23:543–549.
13. Guy J, Gan J, Selfridge J, et al. Reversal of neurological defects in a mouse model of Rett syndrome. *Science* 2007;315:1143–1147.

14. Kaufmann WE, Johnston MV, Blue ME. MeCP2 expression and function during brain development: implications for Rett syndrome's pathogenesis and clinical evolution. *Brain Dev* 2005;27(Suppl 1):S77–S87.
15. Robinson L, Guy J, McKay L, et al. Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. *Brain* 2012;135:2699–2710.
16. Khwaja OS, Sahin M. Translational research: Rett syndrome and tuberous sclerosis complex. *Curr Opin Pediatr* 2011;23:633–639.
17. Guan J, Mathai S, Liang HP, Gunn AJ. Insulin-like growth factor-1 and its derivatives: potential pharmaceutical application for treating neurological conditions. *Recent Pat CNS Drug Discov* 2013;8:142–160.
18. Itoh M, Ide S, Takashima S, et al. Methyl CpG-binding protein 2 (a mutation of which causes Rett syndrome) directly regulates insulin-like growth factor binding protein 3 in mouse and human brains. *J Neuropathol Exp Neurol* 2007;66:117–123.
19. Landi S, Putignano E, Boggio EM, et al. The short-time structural plasticity of dendritic spines is altered in a model of Rett syndrome. *Sci Rep* 2011;1:45.
20. Tropea D, Giacometti E, Wilson NR, et al. Partial reversal of Rett syndrome-like symptoms in MeCP2 mutant mice. *Proc Natl Acad Sci USA* 2009;106:2029–2034.
21. Castro J, Garcia RI, Kwok S, et al. Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett syndrome. *Proc Natl Acad Sci USA* 2014;111:9941–9946.
22. Khwaja OS, Ho E, Barnes KV, et al. Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome. *Proc Natl Acad Sci USA* 2014;111:4596–4601.
23. Backeljauw PF, Chernausek SD. The insulin-like growth factors and growth disorders of childhood. *Endocrinol Metab Clin North Am* 2012;41:265–282.
24. Guy W and National Institute of Mental Health (U.S.). Psychopharmacology Research Branch. Division of Extramural Research Programs., *ECDEU assessment manual for psychopharmacology*. Rev. 1976. ed. DHEW publication no (ADM) 76-338. 1976, Rockville, Md.: U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs. 603
25. Tierney AL, Gabard-Durnam L, Vogel-Farley V, et al. Developmental trajectories of resting EEG power: an endophenotype of autism spectrum disorder. *PLoS ONE* 2012;7:e39127.
26. Marshall PJ, Fox NA, G. Bucharest Early Intervention Project Core. A comparison of the electroencephalogram between institutionalized and community children in Romania. *J Cogn Neurosci* 2004;16:1327–1338.
27. Gabard-Durnam L, Tierney AL, Vogel-Farley V, et al. Alpha asymmetry in infants at risk for autism spectrum disorders. *J Autism Dev Disord* 2015;45:473–480.
28. Thibodeau R, Jorgensen RS, Kim S. Depression, anxiety, and resting frontal EEG asymmetry: a meta-analytic review. *J Abnorm Psychol* 2006;115:715–729.
29. Kagan J, Snidman N. Early childhood predictors of adult anxiety disorders. *Biol Psychiatry* 1999;46:1536–1541.
30. Wetherby AM, Woods J, Allen L, et al. Early indicators of autism spectrum disorders in the second year of life. *J Autism Dev Disord* 2004;34:473–493.
31. Erickson CA, Ray B, Maloney B, et al. Impact of acamprosate on plasma amyloid-beta precursor protein in youth: a pilot analysis in fragile X syndrome-associated and idiopathic autism spectrum disorder suggests a pharmacodynamic protein marker. *J Psychiatr Res* 2014;59:220–228.
32. Berry-Kravis E, Hagerman R, Visoosak J, et al. Arbaclofen in fragile X syndrome: results of phase 3 trials. *J Neurodev Disord* 2017;9:3.
33. Benz N, Hatz F, Bousleiman H, et al. Slowing of EEG background activity in Parkinson's and Alzheimer's disease with early cognitive dysfunction. *Front Aging Neurosci* 2014;6:314.
34. Smith SJ. EEG in neurological conditions other than epilepsy: when does it help, what does it add?. *J Neurol Neurosurg Psychiatry* 2005. 76(Suppl 2):ii8–ii12.
35. Neul JL, Glaze DG, Percy AK, et al. Improving treatment trial outcomes for Rett syndrome: the development of Rett-specific anchors for the clinical global impression scale. *J Child Neurol* 2015;30:1743–1748.
36. Pini G, Scusa MF, Congiu L, et al. IGF1 as a potential treatment for Rett syndrome: safety assessment in six Rett patients. *Autism Res Treat* 2012;2012:679801.
37. Pini G, Congiu L, Benincasa A, et al. Illness severity, social and cognitive ability, and EEG analysis of ten patients with Rett syndrome treated with Mecasermin (Recombinant Human IGF-1). *Autism Res Treat* 2016;2016:5073078.