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

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Abstract

Objective: To measure the efficacy of mecamaserin (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.

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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). 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. The current standard of care is symptomatic management, with no available treatments aimed at ameliorating the cardinal symptoms of the disorder.

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. 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. 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.

Evaluation of IGF-1, and its active N-terminus peptide, in MECP2 mouse models, has rescued many RTT-like symptoms. 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. Mecasermin (rhIGF-1) is FDA-approved for the long-term treatment of growth failure in children with severe primary IGF-1 deficiency. 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. 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:
Prescreening
Assessed for eligibility (n= 63)

Excluded (n= 30)
• 13 did not meet ADAMS or RSBQ criteria
• 16 did not meet remaining criteria
• 1 passed away prior to screening

Screening
Assessed for eligibility (n= 33)

Excluded (n= 3)
• 2 did not meet eligibility criteria
• 1 declined to participate

Randomized (n= 30)

Assigned placebo (n= 15)
• 15 uptitrated 3 weeks, 17 weeks stable dose
  • 12 maintained blind
  • 3 open-label rhIGF-1
    • 1 treated 2 weeks, maximum 80 ug/kg
    • 1 treated 3 weeks, maximum 120 ug/kg
    • 1 treated 15 weeks maximum 120 ug/kg
  • 0 withdrawn

Assigned rhiGF-1 (n= 15)
• 15 uptitrated 3 weeks, 17 weeks stable dose
  • 12 maintained blind
  • 3 open-label rhIGF-1
    • 1 treated 1 weeks, maximum 40 ug/kg
    • 1 treated 2 weeks, maximum 80 ug/kg
    • 1 treated 14 weeks maximum 120 ug/kg
  • 0 withdrawn

Assigned placebo (n= 15)
• 15 uptitrated 3 weeks, 17 weeks stable dose
  • 15 maintained blind
  • 0 open-label rhIGF-1
  • 1 withdrawn before last visit

Assigned rhiGF-1 (n= 15)
• 15 uptitrated 3 weeks, 17 weeks stable dose
  • 15 maintained blind
  • 0 open-label rhIGF-1
  • 0 withdrawn

Follow-up 4 weeks (n= 29)

Analysis
• Main analysis (n= 29)
• Subanalysis (n= 23)

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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.
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 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 μg/kg of rhIGF-1, respectively. Subsequently, participants were administered the dose volume of study drug that corresponded to the 120 μg/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 post-injection 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), 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),

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).
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.

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

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

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>No recall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>IGF-1—placebo</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>IGF-1—placebo</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>w</td>
</tr>
<tr>
<td>ADAMS social avoidance</td>
<td>28</td>
<td>0.79 (2.54)</td>
</tr>
<tr>
<td>RSBQ fear/anxiety</td>
<td>28</td>
<td>0.43 (2.38)</td>
</tr>
<tr>
<td>PTSSVAS symptom 1</td>
<td>28</td>
<td>-0.31 (3.85)</td>
</tr>
<tr>
<td>PTSSVAS symptom 2</td>
<td>28</td>
<td>0.64 (2.92)</td>
</tr>
<tr>
<td>PTSSVAS symptom 3</td>
<td>28</td>
<td>0.25 (3.11)</td>
</tr>
<tr>
<td>Clinical global impression</td>
<td>28</td>
<td>0.11 (0.63)</td>
</tr>
<tr>
<td>Parent global impression</td>
<td>25</td>
<td>-0.12 (1.01)</td>
</tr>
<tr>
<td>Kerr severity</td>
<td>28</td>
<td>1.46 (3.77)</td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td>w</td>
</tr>
<tr>
<td>ADAMS depressed mood</td>
<td>28</td>
<td>1.04 (2.71)</td>
</tr>
<tr>
<td>ABC-C stereotypy</td>
<td>29</td>
<td>-1.21 (4.92)</td>
</tr>
<tr>
<td>CSBS-DP Social</td>
<td>28</td>
<td>2.04 (5.92)</td>
</tr>
<tr>
<td>SD normal Te</td>
<td>29</td>
<td>0.02 (0.06)</td>
</tr>
<tr>
<td>Mean HR</td>
<td>29</td>
<td>5.44 (10.00)</td>
</tr>
<tr>
<td>Exploratory</td>
<td></td>
<td>w</td>
</tr>
<tr>
<td>Hyperventilation (VAS-HS+B)</td>
<td>28</td>
<td>1.64 (3.55)</td>
</tr>
<tr>
<td>Delta frontal power</td>
<td>17</td>
<td>0.23 (0.37)</td>
</tr>
<tr>
<td>Delta frontal relative power</td>
<td>17</td>
<td>0.04 (0.10)</td>
</tr>
<tr>
<td>Beta frontal relative power</td>
<td>17</td>
<td>-0.03 (0.09)</td>
</tr>
<tr>
<td>Gamma frontal relative power</td>
<td>17</td>
<td>-0.04 (0.09)</td>
</tr>
</tbody>
</table>

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

1Indicates P-value ≤ 0.05.

2One sample or paired t-test.

3Wilcoxon sign rank test.
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 (Te) 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.

Table 3. Adverse events (AEs).

<table>
<thead>
<tr>
<th>AE category</th>
<th>n events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>3</td>
</tr>
<tr>
<td>Dermatology</td>
<td>6</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>14</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>0</td>
</tr>
<tr>
<td>Hematology</td>
<td>0</td>
</tr>
<tr>
<td>Infectious</td>
<td>23</td>
</tr>
<tr>
<td>Metabolic</td>
<td>0</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>5</td>
</tr>
<tr>
<td>Neurological</td>
<td>14</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>3</td>
</tr>
<tr>
<td>Respiratory</td>
<td>11</td>
</tr>
<tr>
<td>Endocrine</td>
<td>3</td>
</tr>
<tr>
<td>Unspecified</td>
<td>12</td>
</tr>
<tr>
<td>Total AE</td>
<td>82</td>
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<tr>
<td>Serious AEs</td>
<td>12</td>
</tr>
<tr>
<td>Nonserious AEs</td>
<td>70</td>
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<tr>
<td>AE severity</td>
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<tr>
<td>Grade 1</td>
<td>27</td>
</tr>
<tr>
<td>Grade 2</td>
<td>48</td>
</tr>
<tr>
<td>Grade 3</td>
<td>18</td>
</tr>
<tr>
<td>Grade 4</td>
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</tr>
<tr>
<td>Relationship to study drug</td>
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</tr>
<tr>
<td>Suspected</td>
<td>75</td>
</tr>
<tr>
<td>Not suspected</td>
<td>19</td>
</tr>
<tr>
<td>Expected</td>
<td>45</td>
</tr>
<tr>
<td>Not expected</td>
<td>49</td>
</tr>
</tbody>
</table>

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 rate 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. 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. Among them are a recently reported CGI with RTT-specific anchors and ADAMS Social Avoidance. 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 titration, it targeted the maximum FDA-approved dose of mecamsermin (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. 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. 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.

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Author Contributions

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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.

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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.

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