Efficacy and safety of ganaxolone in patients with CDKL5 deficiency disorder: a randomized, double-blind, placebo-controlled, phase 3 trial

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SUMMARY

Background: CDKL5 deficiency disorder (CDD) is a rare, X-linked, developmental and epileptic encephalopathy characterized by severe global developmental impairment and early-onset, refractory seizures. Ganaxolone, an investigational neuroactive steroid, reduced seizure frequency in an open-label phase 2 trial. The current trial assessed the efficacy and safety of ganaxolone in patients with CDD-associated refractory epilepsy.

Methods: This was a randomized, double-blind, placebo-controlled, phase 3 trial conducted at 39 sites in eight countries. Patients ages 2–21 years with a pathogenic/likely pathogenic *CDKL5* variant and ≥16 major motor seizures (MMS; defined as bilateral tonic, generalized tonic-clonic, bilateral clonic, atonic/drop, or focal to bilateral tonic-clonic seizures) per month were eligible. After a 6-week prospective baseline period, patients were randomized via interactive web response system in a 1:1 ratio to oral adjunctive ganaxolone 63 mg/kg/day (≤28 kg) or 1800 mg/day (>28 kg) or placebo for 17 weeks. Patients/caregivers, investigators, trial staff, and sponsor (other than investigational product manager) were blinded to treatment. The primary endpoint was percentage change in 28-day MMS frequency (MMSF) averaged over the entire 17-week double-blind phase relative to baseline (Wilcoxon-rank sum test). Safety, including adverse events (AEs), was compared descriptively across treatment groups. This study is registered with ClinicalTrials.gov number NCT03572933.

Findings: The study was conducted between June 25, 2018 and July 2, 2020. One hundred and one patients (median age, 6 years) were randomized to ganaxolone (n=50) or placebo (n=51); all patients received a dose of study drug and were analyzed for efficacy and safety. One patient (ganaxolone group) did not have seizure frequency activity recorded in the baseline phase and was not included in the primary seizure frequency analysis. For the primary endpoint, patients in the ganaxolone group experienced a median reduction of 30·7% (IQR: 49·5 to 1·9) (from median [IQR] 28-day MMSF of 54·0 [31·3 to 147·3] to 45·0 [23·5 to 106·3]) compared with 6·9% (IQR: 24·1 to –39·7) in the placebo group

(p=0·0036) (from median [IQR] 28-day MMSF of 49·2 [18·7 to 120·0] to 55·5 [21·6 to 124·7]). The median difference was 27·1% (95% CI: 9·6, 47·9) over the 17-week double-blind phase. AEs occurred in 86% (n=43) and 88% (n=45) of patients in ganaxolone and placebo groups, respectively. Somnolence, pyrexia, and upper respiratory tract infections occurred in ≥10% of patients, with greater frequency in the ganaxolone group. Serious AEs occurred in 12% (n=6) and 10% (n=5) of patients in ganaxolone and placebo groups, respectively. In ganaxolone and placebo groups, two (4%) and four (8%) patients, respectively, discontinued the trial.

Interpretation: Ganaxolone significantly reduced CDD-associated seizures compared with placebo and was generally well tolerated. Results from the first controlled trial in CDD suggest a potential treatment benefit with ganaxolone in patients with CDD. Long-term treatment is being assessed in an open-label extension study.

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Research in context

Evidence before this trial

An unrestricted PubMed search of "[CDKL5] and [ganaxolone]" conducted on January 12, 2022 yielded no publications. The next PubMed search was of "CDKL5" only, restricting results to "clinical trial" publications. This search yielded three publications, of which two were non-interventional nor relevant to the present trial. The third publication was a randomized, double-blind, placebo-controlled study evaluating ataluren in patients with Dravet syndrome and CDKL5 deficiency disorder (CDD). Results showed no treatment difference between ataluren and placebo. A final PubMed search of "ganaxolone," also restricted to "clinical trial" publications, identified four publications reporting on clinical studies of ganaxolone treatment for epilepsy-related disorders. None of these studies evaluated the effect of ganaxolone in patients with CDD. A previous exploratory, open-label trial (NCT02358538) evaluated the safety and efficacy of ganaxolone as adjunctive therapy for several rare genetic epilepsies, including seven patients with CDD. Following a 26-week treatment period, the median change from baseline in 28-day seizure frequency (primary endpoint) among the seven patients was a decrease of 44·4%. Ganaxolone was generally well tolerated with no serious adverse events. These results provided proof-of-concept evidence of ganaxolone's anti-seizure activity in patients with CDD and contributed to the rationale for the phase 3 study presented herein.

Added value of this trial

The Marigold trial is the first phase 3, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of any anti-seizure treatment for CDD-associated refractory epilepsy. This trial evaluated ganaxolone, a novel neuroactive steroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors. Trial results show that, compared with placebo, adjunctive ganaxolone significantly reduced 28-day frequency of major motor seizures relative to placebo in

patients with CDD who had a history of early-onset seizures uncontrolled by ≥2 anti-seizure medications and ≥16 major motor seizures per month. Ganaxolone treatment was generally well tolerated, with a similar rate of treatment-emergent adverse events in ganaxolone and placebo groups (86% *vs* 88%, respectively).

Implications of all the available evidence

Adjunctive ganaxolone showed a favorable efficacy and safety profile in children and young adults with CDD, suggesting that ganaxolone could be a valuable treatment option for patients with refractory epilepsy. Data from the ongoing, open-label phase of this trial should provide additional insights regarding the extended use of ganaxolone in patients with CDD.

Introduction

Cyclin-dependent kinase-like 5 (CDKL5) is a protein that plays a critical role in brain development and function,[1,2] regulating neuronal proliferation, morphogenesis, and survival, as well as synaptic function, structure, and plasticity.[2-6] Pathogenic variants of *CDKL5* (*de novo*- or mosaicism-derived missense, nonsense, deletion, frameshift, and/or splicing mutations)[1,7-9] lead to the development of CDKL5 deficiency disorder (CDD), a rare, X-linked disorder with an incidence of approximately one in 40,000 live births and a female to male ratio of 4:1.[10,11] Although males are generally reported as experiencing more severe disease versus females,[12,13] results from a recent report suggest otherwise.[14] Additionally, cases of a milder phenotype have been reported in mosaic males.[11] Severity in females is also variable, possibly due to X-chromosome inactivation.[7,15]

CDD is a developmental and epileptic encephalopathy characterized by early-onset, refractory seizures and severe global developmental impairment; cortical visual impairment, sleep disturbances, and hypotonia are also common.[5,16,17] Seizures are most often the initial manifestation of the disorder, with a median time to onset of 4–6 weeks of age,[10,14,17,18] and with more than 90% of patients experiencing seizures within 3 months of birth.[16] Patients commonly have multiple seizures daily[16-18] and may experience differing seizure types over time. Epileptic spasms are the initial seizure type in nearly one quarter of patients.[5,14] Other presenting seizure types include tonic, focal, and generalized tonic-clonic.[5,14,15,18] Other seizure types can emerge over time, specifically seizures such as tonic spasms or hypermotor-tonic-spasms sequence (HTSS).[5,14,19] HTSS and HTSS-like seizures are characterized by multiple motor phases, including hypermotor, tonic, and spasm phases, with each phase lasting several seconds to minutes.[19] CDD-associated seizures are usually refractory to treatment with anti-seizure medications (ASMs), and when responsive to therapy, improvements are often short-lived (median, 6

months).[15,18,20-22] Thus, there is a considerable need for treatment that can durably decrease seizure burden (frequency, duration, and/or severity of seizures) in patients with CDD.

Pathogenic *CDKL5* variants can impair the excitatory/inhibitory neuronal balance due to alterations in glutamatergic and GABAergic mechanisms, including diminished GABAergic signaling.[23,24] Ganaxolone is a neuroactive steroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors to enhance GABAergic inhibitory tone.[25,26] Given that seizure activity was not a phenotype of preclinical CDD models until recently,[27,28] the potential utility of ganaxolone in CDD was suggested by its broad-spectrum activity in preclinical seizure models.[25,26,29,30] Ganaxolone was assessed clinically in CDD in an open-label, phase 2 trial (ClinicalTrials.gov NCT02358538) that included seven patients.[31] Patients dosed with ganaxolone up to 63 mg/kg/day in three divided doses experienced a 44·4% median reduction in 28-day seizure frequency during the 26-week treatment period in relation to baseline. Four patients progressed to the open-label extension of the study and continued to experience reduced seizure frequency out to 18 months.[32]

Given ganaxolone's unique mechanism of action, broad-spectrum anti-seizure activity in preclinical studies, and favorable preliminary phase 2 trial results, the phase 3 Marigold trial was conducted to compare the efficacy and safety of adjunctive ganaxolone compared with placebo in patients with CDD-associated refractory epilepsy.

Methods

Trial design

The trial (NCT03572933) was a randomized, placebo-controlled, phase 3 study conducted at 39 sites in eight countries (Australia, France, Israel, Italy, Poland, Russian Federation, United Kingdom, and United

States of America). It consisted of two parts, a double-blind phase followed by an open-label phase (supplemental figure 1), and was conducted in compliance with the International Conference on Harmonization Guidelines for Good Clinical Practice and applicable national and local regulatory requirements. The protocol was approved by the independent ethics committee/institutional review board at each participating site, and written informed consent was provided for all patients by their guardians or legal representatives prior to screening or at the screening visit.

Participants

Eligible patients included males and females 2–21 years of age with a molecularly confirmed pathogenic or likely pathogenic *CDKL5* variant, including mosaic variants. The lower limit of mosaicism detection was 10%. For all countries except France, genetic testing was performed and verified by a single testing facility (GeneDx, Gaithersburg, MD). For France, genetic mutations were confirmed by an approved French organization per the country's legislation. Other inclusion criteria included history of early-onset seizures uncontrolled despite the appropriate trial of ≥2 ASMs and ≥16 major motor seizures per 28 days (defined as bilateral tonic [sustained motor activity ≥3 seconds], generalized tonic-clonic, bilateral clonic, atonic/drop, or focal to bilateral tonic-clonic seizures) in each 4-week period of the 8-week historical seizure period before screening. Seizure type was classified by the physician using the International League Against Epilepsy's Classification of Epileptic Seizures and was verified by the independent Epilepsy Study Consortium. If seizures with multiple motor phases occurred, the seizure was classified as the most severe or prolonged phase. Hence, if a tonic seizure progressed into a spasm, it was classified as a tonic seizure. Patients were allowed to be on a stable regimen of up to four concomitant ASMs during the trial as long as the dosing regimens for these ASMs were stable for ≥1 month before screening, with no foreseeable change in dosing during the double-blind phase. Vagus nerve stimulation and ketogenic diet did not count toward the ASM limit but must have been consistent in the 3-month

period preceding screening. Key exclusion criteria included West syndrome or predominantly infantile spasm-type seizures; active central nervous system (CNS) infection, demyelinating disease, degenerative neurological disease, or CNS disease deemed progressive upon brain imaging; and abnormal liver function or significant renal insufficiency. Concomitant use of adrenocorticotropic hormone, prednisone (or other glucocorticoids), or moderate or strong inducers or inhibitors of CYP3A4/5/7 were not allowed, with the exception of moderate or strong inducer or inhibitor ASMs (eg, carbamazepine, phenytoin). Full inclusion and exclusion criteria are provided in supplemental table 1.

Randomization and masking

Eligible patients were randomized 1:1 centrally via an interactive web response system (IWRS) to receive either ganaxolone or placebo as adjunctive therapy to existing ASM(s). Each patient was assigned a unique number via IWRS at screening and then at randomization as determined by the randomization schedule, which was generated by the study's Clinical Research Organization (CRO), ICON plc (Dublin, Ireland). Ganaxolone and placebo were identical in taste and appearance and provided in bottles with unique numbers. Trial staff, patients, caregivers, investigators, and the sponsor (other than the investigational product manager) were blinded to treatment. The investigational product manager had contact with people involved in the trial, but had no role in the assessment.

Procedures

The first phase of the trial included an 8-week historical seizure period, a 6-week prospective baseline period, and a 17-week double-blind treatment period. Trial drug was administered three times daily with food as an oral suspension for 17 weeks. Ganaxolone (50 mg/mL) or matching placebo solution was titrated over 4 weeks up to a maximum dose of 63 mg/kg/day (≤28 kg) or 1800 mg/day (>28 kg) followed by 13 weeks of maintenance dosing. Following completion of the double-blind phase, patients

were allowed to enter the open-label phase of the trial, during which all patients could receive adjunctive ganaxolone until its regulatory approval or trial termination. As the open-label phase is ongoing, this report focuses exclusively on data from the double-blind phase.

Outcomes

This study used a primary efficacy measure inclusive of multiple seizure types, defined as major motor seizures (bilateral tonic [sustained motor activity ≥3 seconds], generalized tonic-clonic, bilateral clonic, atonic/drop, or focal to bilateral tonic-clonic seizures), as these included seizure types are the most common manifestations of epilepsy associated with CDD.[5,14] The primary endpoint was the percentage change from baseline in major motor seizure frequency (MMSF) during the 17-week double-blind phase expressed as a 28-day average. Baseline seizure frequency was established during the 6-week baseline period. Key secondary efficacy endpoints were the proportion of patients with ≥50% reduction from baseline in MMSF (responder analysis) and Clinical Global Impression of Improvement (CGI-I) scale score per caregiver and clinician assessment at the end of the 17-week double-blind phase. Trial drug effects on seizure frequency-related outcomes were assessed based on daily seizure frequency/type entries made by the patient's caregiver in an electronic diary (eDiary) throughout the double-blind phase. The CGI-I scale was used to assess the change in overall seizure control, behavior, safety, and tolerability before and after the initiation of trial treatment. Patients were rated by their caregiver and clinician on a 7-point Likert scale as follows: 1=very much improved, 2=much improved, 3=minimally improved, 4=no change, 5=minimally worse, 6=much worse, and 7=very much worse. Prespecified secondary efficacy endpoints included the Caregiver Global Impression of Change in Seizure Intensity/Duration (CGI-CSID) score, the Caregiver Global Impression of Change in Attention (CGI-CA) score, and the Caregiver Global Impression of Change (CGI-C – target behavior) score in parent/caregiver-identified behavioral target, and these CGI measures used the same 7-point Likert scale as described for the CGI-I. Safety was

evaluated throughout the trial. Adverse events (AEs) were coded to the Medical Dictionary for Regulatory Activities (version 23·0).

Additional prespecified subgroup and exploratory analyses included change from baseline to week 17 in 28-day MMSF by sex, plasma allopregnanolone sulfate levels at baseline (≤2·5 ng/mL, >2·5 ng/mL and <6·0 ng/mL, or ≥6·0 ng/mL), seizure type, and treatment phase (titration and maintenance).

Statistical analysis

Sample size estimation was based on data from the aforementioned open-label, phase 2 trial (NCT02358538) of adjunctive ganaxolone in females with rare genetic epilepsies, including CDD. Assuming that the standard deviation for the percentage change in 28-day seizure frequency was 44·5%, a sample size of 100 patients (50 per group) would have 92% power at a one-sided alpha of 0·025 to detect a between-group difference of 30% in the percentage change in 28-day MMSF. Statistical significance would be achieved with an estimated between-group difference of 18%. All patients who received ≥1 dose of trial treatment were included in the safety and efficacy analyses.

Seizure data was converted to a 28-day rate, calculated as the total number of seizures in the assessment period (eg, baseline, double-blind period) divided by the total number of days with seizure data, multiplied by 28. Formal hypothesis testing, along with calculation of p values, was pre-planned hierarchically for the primary endpoint and then for two key secondary endpoints sequentially, starting with the ≥50% responder rate, followed by the CGI-I scales. Comparison for statistical significance would end when the first non-significant result was encountered. The analysis of the primary endpoint, the percentage change in 28-day MMSF between baseline and the double-blind phase for ganaxolone *vs* placebo, was conducted using a Wilcoxon rank-sum test (with two-sided alpha of 0·05). The Hodges-Lehmann estimate of median difference was used to quantify the extent to which responses in the

ganaxolone group shifted from the placebo group. The first key secondary endpoint was the percentage of patients with a ≥50% reduction from baseline in primary seizure frequency, analyzed using Fisher's exact test. The next key secondary endpoints were the CGI-I scales (parent/caregiver and clinician) at the last scheduled visit in the 17-week double-blind treatment phase, analyzed using ordinal logistic regression, with treatment group as a fixed factor; the estimated odds ratios (OR) and corresponding 95% confidence intervals (CI) were generated.

Up to two interim efficacy analyses were planned using O'Brien-Fleming monitoring boundaries. These were to be conducted when 50 and 75 patients were at least 17 weeks post-randomization. Based on the hypothesis that the standard deviation for the percent change in 28-day seizure frequency was 44·5%, the boundary for terminating the study early because of a lack of benefit was an estimated treatment difference (ganaxolone – placebo) of –10% for the percent reduction in 28-day seizure frequency at 50 patients and 1% reduction for the estimated treatment difference at 75 patients. The boundary for terminating the study early because of benefit was a Z-value of 3·73 (with 1-sided nominal p-value=0·0001) at 50 patients and a Z-value of 3·03 (with 1-sided nominal p-value 0·0012) at 75 patients. When the standard deviation is 44·5%, this boundary was an estimated treatment difference of 47% reduction at 50 patients and 32% reduction at 75 patients. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC; version 9·4).

Role of the funding source

The sponsor, in collaboration with investigators and other experts, contributed to the design of the trial; data collection, analysis, and interpretation; verification of the data; and writing of this report. Authors had full access to all trial data, and the corresponding author had the final responsibility for the decision to submit this report for publication.

Results

The study was conducted between June 25, 2018 and July 2, 2020. One hundred and fourteen patients were screened for eligibility, of which 101 were randomized to adjunctive ganaxolone (n=50) or placebo (n=51) (figure 1). After the first interim efficacy analysis, the study was allowed to continue without modification. The second interim analysis was not performed because the assessment would have taken place after study enrollment was completed. There were no protocol violations that led to discontinuation during the study or had any meaningful impact on the integrity or ability to interpret the data. All patients received a dose of trial drug and were included in the intention-to-treat and safety analysis populations. Seventeen patients received trial drug by percutaneous endoscopic gastrostomy (10 on ganaxolone and 7 on placebo). One patient randomized to ganaxolone experienced seizures during the 6-week baseline period, but the frequency of those seizures was not recorded. Thus, all seizure-related efficacy endpoints, including the primary endpoint, were based on data from 100 patients (ganaxolone, n=49; placebo, n=51).

Baseline demographic and disease characteristics are shown in table 1. Of the 101 patients, 79% were female, and the median (interquartile range [IQR]) age was 6 years (3 to 10). Patients had previously received a median of seven (IQR: 5 to 10) ASMs and were on a median of two (IQR: 1 to 3) concomitant ASMs during the trial. During the 6-week baseline period, the median (IQR) 28-day MMSF in the ganaxolone and placebo groups was 54·0 (31·3 to 147·3) and 49·2 (18·7 to 120·0), respectively, with an estimated difference in MMSF of 12·0 (Hodges-Lehmann estimate of median difference, 95% CI: −8·4, 32·7).

At the end of the titration phase, 40 of 50 patients in the ganaxolone group and 42 of 51 patients in the placebo group achieved the target dose of 63 mg/kg/day or 1800 mg/day (dosing dependent on weight). A total of 95 patients (94%) completed the 17-week double-blind phase (figure 1). Two patients in the ganaxolone group discontinued the trial, one due to a treatment-emergent AE (TEAE; seizure) and one due to withdrawal of consent. Additionally, 1 patient in the ganaxolone group discontinued study drug due to an adverse event but continued in the study until the end of the double-blind phase. All four trial discontinuations in the placebo group were attributable to TEAEs (seizure, n=2; unresponsive to stimuli, n=1; abdominal pain/respiratory failure/sedation, all n=1).

At the end of the 17-week double-blind phase, the median (IQR) 28-day MMSF was 45·0 (23·5 to 106·3) in the ganaxolone group and 55·5 (21·6 to 124·7) in the placebo group. Compared with the 6-week baseline period, patients in the ganaxolone and placebo group experienced a median (IQR) reduction in MMSF of 30·7% (49·5 to 1·9) and 6·9% (24·1 to –39·7), respectively (p=0·0036). The Hodges-Lehmann estimate of median difference in responses between ganaxolone and placebo was 27·1% (95% CI: 9·6, 47·9).

Because the primary endpoint was met, formal statistical analysis of the first key secondary endpoint (≥50% responder rate) was permitted, according to the hierarchical gate-keeping procedure as previously described. The percentage of patients with ≥50% reduction between baseline and week 17 in MMSF was 24% (12/49) for ganaxolone and 10% (5/51) for placebo (p=0·0643), corresponding to a difference of 14.7% (95% CI: –4·7, 33·8) (figure 2). Seizure reductions in the ganaxolone group and placebo group along the entire MMSF response curve are shown in figure 2. No patient in the trial achieved 100% reduction (ie, seizure freedom). For the CGI-I caregiver-administered scale, 63% (30/48) of patients in the ganaxolone group were rated as minimally improved or better compared with 44%

(21/48) of patients in the placebo group (OR, 1·87; 95% CI: 0·89, 3·91). For the CGI-I clinician-administered scale, 54% (26/48) of patients in the ganaxolone group were rated as minimally improved or better compared with 42% (20/48) of patients in the placebo group (OR, 1·41; 95% CI: 0·68, 2·94). The median (IQR) change from baseline in percentage of major motor seizure-free days was 4·9 (0·0 to 15·6) for patients in the ganaxolone group and 0·2 (–3·0 to 15·2) for patients in the placebo group. Results for other secondary endpoints, including the CGI assessments, are shown in table 2 and supplemental figures 2 (CGI-I), 3 (CGI-CSID), 4 (CGI-CA), and 5 (CGI-C). Additionally, a higher proportion of patients in the ganaxolone group experienced minimal improvements or better on all secondary endpoint CGI's specific to seizure intensity and duration, attention, and selected target behavior. Percentage change from baseline in 28-day MMSF during the 17-week double-blind phase for sex, allopregnanolone sulfate levels, and seizure type subgroups, and for the titration and maintenance periods, are shown in supplemental table 2. The median (IQR) percentage reduction from baseline in 28-day seizure frequency for all seizure types combined was 19·1% (–8·6, 43·5) for ganaxolone and 8·9% (–29·1, 28·7) for placebo (Hodges-Lehmann estimate of median difference, 17·4%; 95% CI: –0·3, 36·4). During the titration period (weeks 1–4), the median (IQR) percentage reduction from baseline in 28-day MMSF was 35·1% (48·2 to 10·9) for ganaxolone and 13·9% (36·5 to –28·0) for placebo (Hodges-Lehmann estimate of median difference, 18·7%; 95% CI: 1·8, 34·8). The corresponding reductions in 28-day MMSF during the maintenance period (weeks 5–17) were 29·4% (65·8 to –1·0) and 6·5% (26·8 to –38·5) (Hodges-Lehmann estimate of median difference, 29·3%; 95% CI: 8·9, 51·5). Results when stratified by baseline allopregnanolone sulfate levels were consistent with those for all patients.

TEAEs were reported in 86% of patients (n=43) in the ganaxolone group and 88% of patients (n=45) in the placebo group (table 3), the majority of which were mild or moderate in severity. TEAEs reported in ≥10% of patients in either the ganaxolone or placebo group and more frequently with ganaxolone were

somnolence (36% [n=18] *vs* 16% [n=8] for ganaxolone and placebo groups, respectively); pyrexia (18% [n=9] *vs* 8% [n=4]), and upper respiratory tract infections (10% [n=5] *vs* 6% [n=3]). TEAEs leading to dose reduction or temporary discontinuation of trial drug were reported in 22% (n=11) of patients in the ganaxolone group and 16% (n=8) of those in the placebo group. TEAEs leading to dose reduction or temporary discontinuation of the trial drug reported in more than one patient overall were limited to somnolence (ganaxolone, n=4) and insomnia (placebo, n=2). No deaths were reported in either treatment group during the double-blind period.

Serious treatment-emergent adverse events (SAEs) were reported in 12% of patients (n=6) in the ganaxolone group and 10% of patients (n=5) in the placebo group. One SAE in the ganaxolone group, decreased oxygen saturation, was considered treatment-related. In the placebo group, one patient experienced episodes of hypotonia, unresponsiveness, or hypoxia that were considered related to study treatment.

There were no significant findings related to clinical laboratory evaluations; vital signs (including weight); physical, neurological, and developmental examinations; or ECG.

Discussion

This is the first randomized, placebo-controlled trial to evaluate a treatment for CDD-associated seizures. For the primary endpoint, there was a significantly greater reduction from baseline in 28-day MMSF with ganaxolone versus placebo (median reduction: 30·7% *vs* 6·9%; p=0·0036). Furthermore, anti-seizure effects were observed during the 4-week titration period (median reduction from baseline of 35·1% *vs* 13·9% in ganaxolone *vs* placebo groups, respectively) and they persisted through the 13-week maintenance period (median reduction from baseline: 29·4% *vs* 6·5%). A higher proportion of patients

on ganaxolone demonstrated improvements on all CGI scales assessing overall improvement, attention, target behavior, and seizure intensity and duration. These results are notable given that patients had a high seizure burden at baseline, with a median 28-day MMSF of 49·2 in the placebo group and 54·0 in the ganaxolone group at baseline, as well as having received a median of seven prior ASMs, reflecting their significant need for effective treatment of CDD-associated seizures.

The 6·9% reduction in 28-day MMSF observed in the placebo group is lower than placebo-response rates observed in other pediatric epilepsy studies.[33-35] One possible explanation for the lower placebo-response rate in this trial is the highly refractory nature of epilepsy in patients with CDD.[21,22] Another recent placebo-controlled trial evaluating ataluren in CDD found that patients in the placebo group had a 9% median reduction from baseline in motor seizure frequency.[36] Additionally, this trial included an 8-week historical seizure period (eDiary) which was used to determine eligibility rather than the prospective baseline on which seizure frequency reduction calculations were based. This may have reduced the influence of regression to the mean on response.

The rate of patients with ≥50% reduction from baseline in MMSF for ganaxolone (24% [n=12]) relative to placebo (10% [n=5]) did not achieve statistical significance. Therefore, testing for statistical significance was not performed for the subsequent key endpoints in the pre-specified hierarchical testing procedure.

Adjunctive ganaxolone oral suspension administered three times daily was generally well tolerated, with the majority of TEAEs categorized as mild or moderate in severity. The percentages of patients who had dose reductions or temporary discontinuations due to TEAEs were generally comparable between groups, and fewer than 5% of patients in the ganaxolone group discontinued treatment due to a TEAE. Other than somnolence, pyrexia, and upper respiratory tract infections, which were more common

among patients in the ganaxolone group, TEAEs generally occurred at a similar frequency in the ganaxolone and placebo groups. Importantly, there were no deaths during the double-blind phase. The safety profile of adjunctive ganaxolone in patients with CDD was consistent with that observed in the overall ganaxolone clinical development program.[26]

As with other randomized, placebo-controlled clinical trials of ASMs, there are a several limitations, including the relatively short treatment duration, the potentially confounding use of different concomitant ASMs and no formal assessment of concomitant ASM levels, and the inclusion of different geographical regions. The maintenance period of 13 weeks is similar to the 12-week maintenance most often used in trials of ASMs.[37] The relatively low number of patients enrolled in this trial is another limitation. CDD is a rare disorder, and in clinical trials investigating rare conditions, median participant enrollment is 61, with nearly 75% of completed clinical trials enrolling fewer than 100 patients.[38] Therefore, the Marigold trial population size is consistent with that in previous studies of other rare conditions. Data from the ongoing, open-label phase of this trial should provide additional insights regarding the extended use of ganaxolone in patients with CDD. Additional analyses are also underway to examine the effects of ganaxolone on quality of life and adaptive functioning.

In summary, patients with CDD commonly experience refractory seizures despite the use of existing ASMs, underscoring the need for new, evidence-based treatments. Results from this phase 3 trial provide clinical evidence that ganaxolone is generally well tolerated and effective in reducing the frequency of major motor seizures, supporting the potential for ganaxolone as a treatment option for patients with CDD-associated refractory epilepsy.

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Contributors

EMPK was a trial investigator; collected, had access to, and verified the data; and participated in the development, review, and approval of the manuscript. As corresponding author, **EMPK** had final responsibility for the decision to submit this report for publication.

SA contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

NB-B contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

TAB contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

JHC contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

STD contributed to trial design and data interpretation, was a trial investigator, and participated in the development, review, and approval of the manuscript.

HEO contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

NS contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

TRF contributed to trial design and conduct, data analysis and interpretation, and participated in the development, review, and approval of the manuscript.

AAA contributed to trial design, data analysis and interpretation, had access to and verified the data, and participated in the development, review, and approval of the manuscript.

MG contributed to trial conduct, data analysis and interpretation, had access to and verified the data, and participated in the development, review, and approval of the manuscript.

OD contributed to trial design, was a trial investigator, and participated in the development, review, and approval of the manuscript.

Declaration of Interests

EMPK has consulted for Marinus Pharmaceuticals, Inc., and participated in advisory boards for BioMarin, Inc. and Zogenix. **EMPK** had no prior relationship with Marinus Pharmaceuticals, Inc., until after the completion of the randomization portion of the current trial.

SA has received funding from GW Pharmaceuticals, Novartis, PTC Therapeutics, Boston Scientific, Nutricia, UCB, BioMarin, LivaNova, Medtronic, Desitin, Ipsen, CDKL5 UK, TSA, and the National Institute for Health Research.

NB-B has consulted for Roche, LivaNova, and PTC Therapeutics, and has received funding from GW Pharmaceuticals.

TAB has consulted for Taysha, Alcyone, Novartis/AveXis, Ovid, GW Pharmaceuticals, International Rett Syndrome Foundation, Takeda, Ultragenyx, and Marinus Pharmaceuticals, Inc.; has participated in clinical trials with Acadia, Ovid, GW Pharmaceuticals, Marinus Pharmaceuticals, Inc., and the Rett Syndrome Research Trust; has received research funding from the National Institutes of Health, the International Foundation for CDKL5 Research, Rocky Mountain Rett Association, GRIN2B Foundation, and Mallinckrodt. All remuneration has been made to his department.

JHC has acted as an investigator for studies with GW Pharmaceuticals, Stoke Therapeutics, Ovid, Zogenix, Vitaflo and the current trial with Marinus Pharmaceuticals, Inc.; has been a speaker and on advisory boards for Zogenix, Biocodex, UCB, and Nutricia; all remuneration has been paid to her department. **JHC** holds an endowed chair at UCL Great Ormond Street Institute of Child Health; grants from National Institute for Health Research, Engineering and Physical Sciences Research Council, Great Ormond Street Hospital Children's Charity, Epilepsy Research United Kingdom, the Waterloo Foundation, and the National Institute of Health Research Biomedical Research Centre at Great Ormond Street Hospital.

STD has consulted for Taysha, Neurogene, Ovid, and Marinus Pharmaceuticals, Inc.; has received speaker honoraria from BioMarin and Marinus Pharmaceuticals, Inc.; has received funding from the National Institutes of Health, International Foundation for CDKL5 Research, and Mila's Miracle Foundation; and serves on advisory boards for the nonprofit foundations SLC6A1 Connect, FamilieSCN2A, and Ring 14 USA.

HEO has consulted for Takeda, Ovid, Zogenix, FOXG1 Research Foundation, and Marinus Pharmaceuticals, Inc.; has served as site Principal investigator for a trial with Ovid and for the currently reported trial with Marinus Pharmaceuticals, Inc. **HEO** has funding from National Institute of Neurological Disorders and Stroke, the Loulou Foundation, the Manton Center for Rare Disease Research, and the International Foundation for CDKL5 Research for research on CDKL5 deficiency disorder.

NS has served on scientific advisory boards for GW Pharmaceuticals, BioMarin, Arvelle, Takeda, and Marinus Pharmaceuticals, Inc.; has received speaker honoraria from Eisai, BioMarin, LivaNova, and Sanofi; and has served as an investigator for Zogenix, BioMarin, UCB, Roche, and Marinus Pharmaceuticals, Inc.; and has received support for attending meetings from LivaNova.

TRF received consulting fees from Marinus Pharmaceuticals, Inc., for service on the scientific steering committee overseeing the design, conduct, and analysis of trials of ganaxolone in cyclin-dependent kinase-like 5 (CDKL5) deficient epileptic encephalopathy and in protocadherin 19 (PCDH19)–related epilepsy.

AAA is a salaried employee of Marinus Pharmaceuticals, Inc., and owns stock in the company.

MG is a salaried employee of Marinus Pharmaceuticals, Inc., and owns stock in the company.

OD receives grant support from the National Institute of Neurological Disorders and Stroke, National Institute of Mental Health, Multidisciplinary University Research Initiative, Centers for Disease Control and Prevention, and National Science Foundation. **OD** has equity, compensation, or both from the

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Data sharing statement

Anonymized data from this study, the study protocol, and statistical analysis plan are available at the time of publication. Requests should be made to Marinus Pharmaceuticals (Radnor, PA, USA), the company sponsoring the clinical development of ganaxolone for the treatment of CDKL5 deficiency disorder (medicalaffairs@marinuspharma.com). At the time of the request, the format and scope of the data to be disseminated will be determined by the authors and Marinus Pharmaceuticals. A data sharing agreement will need to be signed.

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References

1. Jakimiec M, Paprocka J, Smigiel R. CDKL5 deficiency disorder-a complex epileptic encephalopathy. *Brain Sci* 2020; **10**.
2. Pizzo R, Gurgone A, Castroflorio E, et al. Lack of Cdkl5 disrupts the organization of excitatory and inhibitory synapses and parvalbumin interneurons in the primary visual cortex. *Front Cell Neurosci* 2016; **10**: 261.
3. Fuchs C, Trazzi S, Torricella R, et al. Loss of CDKL5 impairs survival and dendritic growth of newborn neurons by altering AKT/GSK-3β signaling. *Neurobiol Dis* 2014; **70**: 53-68.
4. Kilstrup-Nielsen C, Rusconi L, La Montanara P, et al. What we know and would like to know about CDKL5 and its involvement in epileptic encephalopathy. *Neural Plast* 2012; **2012**.
5. Olson HE, Demarest ST, Pestana-Knight EM, et al. Cyclin-dependent kinase-like 5 deficiency disorder: clinical review. *Pediatr Neurol* 2019; **97**: 18-25.
6. Zhu YC, Xiong ZQ. Molecular and synaptic bases of CDKL5 disorder. *Dev Neurobiol* 2019; **79**: 8-19.
7. Bartnik M, Derwińska K, Gos M, et al. Early-onset seizures due to mosaic exonic deletions of CDKL5 in a male and two females. *Genet Med* 2011; **13**: 447-52.
8. Stosser MB, Lindy AS, Butler E, et al. High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. *Genet Med* 2018; **20**: 403-10.
9. Krishnaraj R, Ho G, Christodoulou J. RettBASE: Rett syndrome database update. *Hum Mutat* 2017; **38**: 922-31.
10. Symonds JD, Zuberi SM, Stewart K, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. *Brain* 2019; **142**.
11. Siri B, Varesio C, Freri E, et al. CDKL5 deficiency disorder in males: five new variants and review of the literature. *Eur J Paediatr Neurol* 2021; **33**: 9-20.

12. Fehr S, Downs J, Ho G, et al. Functional abilities in children and adults with the CDKL5 disorder. *Am J Med Genet A* 2016; **170**: 2860-9.

13. Fehr S, Leonard H, Ho G, et al. There is variability in the attainment of developmental milestones in the CDKL5 disorder. *J Neurodev Disord* 2015; **7**: 2.

14. Demarest ST, Olson HE, Moss A, et al. CDKL5 deficiency disorder: Relationship between genotype, epilepsy, cortical visual impairment, and development. *Epilepsia* 2019; **60**: 1733-42.

15. Archer HL, Evans J, Edwards S, et al. CDKL5 mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients. *J Med Genet* 2006; **43**: 729-34.

16. Fehr S, Wilson M, Downs J, et al. The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. *Eur J Hum Genet* 2013; **21**: 266-73.

17. Mangatt M, Wong K, Anderson B, et al. Prevalence and onset of comorbidities in the CDKL5 disorder differ from Rett syndrome. *Orphanet J Rare Dis* 2016; **11**: 39.

18. Bahi-Buisson N, Kaminska A, Boddaert N, et al. The three stages of epilepsy in patients with CDKL5 mutations. *Epilepsia* 2008; **49**: 1027-37.

19. Klein KM, Yendle SC, Harvey AS, et al. A distinctive seizure type in patients with CDKL5 mutations: hypermotor-tonic-spasms sequence. *Neurology* 2011; **76**: 1436-8.

20. Fehr S, Wong K, Chin R, et al. Seizure variables and their relationship to genotype and functional abilities in the CDKL5 disorder. *Neurology* 2016; **87**: 2206-13.

21. Frullanti E, Papa FT, Grillo E, et al. Analysis of the phenotypes in the Rett networked database. *Int J Genomics* 2019; **2019**: 6956934.

22. Müller A, Helbig I, Jansen C, et al. Retrospective evaluation of low long-term efficacy of antiepileptic drugs and ketogenic diet in 39 patients with CDKL5-related epilepsy. *Eur J Paediatr Neurol* 2016; **20**: 147-51.

23. Kadam SD, Sullivan BJ, Goyal A, Blue ME, Smith-Hicks C. Rett syndrome and CDKL5 deficiency disorder: from bench to clinic. *Int J Mol Sci* 2019; **20**.

24. Sivilia S, Mangano C, Beggiato S, et al. CDKL5 knockout leads to altered inhibitory transmission in the cerebellum of adult mice. *Genes Brain Behav* 2016; **15**: 491-502.

25. Carter RB, Wood PL, Wieland S, et al. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma-aminobutyric acid(A) receptor. *J Pharmacol Exp Ther* 1997; **280**: 1284-95.

26. Lattanzi S, Riva A, Striano P. Ganaxolone treatment for epilepsy patients: from pharmacology to place in therapy. *Expert Rev Neurother* 2021: 1-16.

27. Amendola E, Zhan Y, Mattucci C, et al. Mapping pathological phenotypes in a mouse model of CDKL5 disorder. *PLoS One* 2014; **9**: e91613.

28. Fallah MS, Eubanks JH. Seizures in mouse models of rare neurodevelopmental disorders. *Neuroscience* 2020; **445**: 50-68.

29. Gasior M, Ungard JT, Beekman M, Carter RB, Witkin JM. Acute and chronic effects of the synthetic neuroactive steroid, ganaxolone, against the convulsive and lethal effects of pentylenetetrazol in seizure-kindled mice: comparison with diazepam and valproate. *Neuropharmacology* 2000; **39**: 1184-96.

30. Saporito MS, Gruner JA, DiCamillo A, Hinchliffe R, Barker-Haliski M, White HS. Intravenously administered ganaxolone blocks diazepam-resistant lithium-pilocarpine-induced status epilepticus in rats: comparison with allopregnanolone. *J Pharmacol Exp Ther* 2019; **368**: 326-37.

31. ClinicalTrials.gov. Marinus Pharmaceuticals. A multicenter, open-label proof-of-concept trial of ganaxolone in children with PCDH19 female pediatric epilepsy and other rare genetic epilepsies.

ClinicalTrials.gov identifier: NCT02358538. https://www.clinicaltrials.gov/ct2/show/NCT02358538 (accessed April 28 2021).

32. Specchio N, Masuoka L, Aimetti A, Chez MG. Long-term, durable seizure frequency reduction in individuals with CDKL5 deficiency disorder (CDD) treated with ganaxolone. Abstract 3.283. American Epilepsy Society Annual Meeting, November 30-December 4, 2018, New Orleans, LA.
33. Thiele EA, Marsh ED, French JA, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2018; **391**: 1085-96.
34. Devinsky O, Cross JH, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *N Engl J Med* 2017; **376**: 2011-20.
35. Lagae L, Sullivan J, Knupp K, et al. Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet* 2019; **394**: 2243-54.
36. Devinsky O, King L, Bluvstein J, Friedman D. Ataluren for drug-resistant epilepsy in nonsense variant-mediated Dravet syndrome and CDKL5 deficiency disorder. *Ann Clin Transl Neurol* 2021; **8**: 639-44.
37. Perucca E. What clinical trial designs have been used to test antiepileptic drugs and do we need to change them? *Epileptic Disord* 2012; **14**: 124-31.
38. Rees CA, Pica N, Monuteaux MC, Bourgeois FT. Noncompletion and nonpublication of trials studying rare diseases: a cross-sectional analysis. *PLoS Med* 2019; **16**: e1002966.

Table 1: **Baseline demographic and disease characteristics**

	Ganaxolone (n=50)	**Placebo (n=51)**
Median age, years (IQR)	5·0 (3·0 to 10·00)	7·0 (4·0 to 11·0)
Sex, n (%)		
Male	11 (22)	10 (20)
Female	39 (78)	41 (80)
Ethnicity, n (%)		
Hispanic or Latino	4 (8)	6 (12)
Not-Hispanic or Latino	44 (88)	43 (84)
Unknown	1 (2)	1 (2)
Not reported	1 (2)	1 (2)
Race, n (%)		
White	46 (92)	47 (92)
Asian	2 (4)	3 (6)
Other	2 (4)	1 (2)
Major motor seizure types during the study, patient no. (%)[a]		
Bilateral tonic	35 (71)	39 (76)
Generalized tonic-clonic	24 (49)	20 (39)
Atonic/drop	9 (18)	12 (24)
Bilateral clonic	6 (12)	3 (6)
Focal to bilateral tonic-clonic	7 (14)	6 (12)
Median 28-day major motor seizure frequency, n (IQR)[a,b]	54·0 (31·3 to 147·3)	49·2 (18·7 to 120·0)
Median number of prior ASMs used, n (IQR)	7 (5 to 10)	7 (4 to 9)
Median number of concomitant ASMs, n (IQR)	2 (2 to 4)	2 (1 to 3)
Concomitant ASMs, n (%)		
Valproate	18 (36)	16 (31)
Levetiracetam	13 (26)	13 (25)
Clobazam	12 (24)	13 (25)
Vigabatrin	10 (20)	12 (24)

ASM=anti-seizure medication; IQR=interquartile range.

[a]One patient randomized to ganaxolone experienced seizures during the 6-week baseline period (seizure frequency not recorded).

[b]Determined during the 6-week baseline period.

Table 2: **Primary and secondary outcomes in the double-blind phase**

	Ganaxolone (n=50)	Placebo (n=51)	Ganaxolone–Placebo[a] or Odds Ratio[b] (95% CI)	p-value
Primary efficacy endpoint				
Percentage reduction from baseline to week 17 in 28-day major motor seizure frequency, n	49	51		
Median (IQR)	30·7 (49·5 to 1·9)	6·9 (24·1 to −39·7)	27·1 (9·6, 47·9)	0·0036[c]
Key secondary efficacy endpoints				
≥50% reduction from baseline in major motor seizure frequency, n	49	51		
n (%)	12 (24)	5 (10)	14·7 (−4·7, 33·8)	0·0643[d]
Clinical Global Impression of Improvement score at week 17, n	48	48		
Parent or caregiver rating				
Very much improved, n (%)	0	1 (2)		
Much improved, n (%)	13 (27)	7 (15)		
Minimally improved, n (%)	17 (35)	13 (27)		
No change, n (%)	14 (29)	22 (46)		
Minimally worse, n (%)	2 (4)	4 (8)		
Much worse, n (%)	2 (4)	1 (2)		
Very much worse, n (%)	0	0		
Minimally improved or better, n (%)	30 (63)	21 (44)	1·87 (0·89, 3·91)	—
Clinician rating, n (%)				
Very much improved, n (%)	0	0		
Much improved, n (%)	7 (15)	7 (15)		
Minimally improved, n (%)	19 (40)	13 (27)		
No change, n (%)	16 (33)	19 (40)		
Minimally worse, n (%)	2 (4)	9 (19)		
Much worse, n (%)	3 (6)	0		
Very much worse, n (%)	1 (2)	0		
Minimally improved or better, n (%)	26 (54)	20 (42)	1·41 (0·68, 2·94)	—
Secondary seizure control endpoints				
Change from baseline to week 17 in percentage of seizure-free days, based on major motor seizure types, n	49	50		
Median (IQR)	4·9 (0·0 to 15·6)	0·2 (−3.0 to 15·2)	1·7 (−2·7, 7·8)	—
Caregiver Global Impression of Change in Seizure	45	47		

Intensity/Duration score at week 17, n				
Very much improved, n (%)	2 (4)	1 (2)		
Much improved, n (%)	15 (33)	5 (11)		
Minimally improved, n (%)	11 (24)	11 (23)		
No change, n (%)	10 (22)	21 (45)		
Minimally worse, n (%)	3 (7)	5 (11)		
Much worse, n (%)	2 (4)	4 (9)		
Very much worse, n (%)	2 (4)	0		
Minimally improved or better, n (%)	28 (62)	17 (36)	2·56 (1·20, 5·45)	—
Secondary behavioral/neuropsychiatric endpoints				
Caregiver Global Impression of Change in Attention score at week 17, n	45	47		
Very much improved, n (%)	1 (2)	1 (2)		
Much improved, n (%)	2 (4)	7 (15)		
Minimally improved, n (%)	21 (47)	14 (30)		
No change, n (%)	18 (40)	23 (49)		
Minimally worse, n (%)	1 (2)	1 (2)		
Much worse, n (%)	1 (2)	1 (2)		
Very much worse, n (%)	1 (2)	0		
Minimally improved or better, n (%)	24 (53)	22 (47)	0·97 (0·45, 2·09)	—
Caregiver Global Impression of Change in parent or caregiver identified behavioral target score at week 17 (potential domains include sociability, communication, irritability, and hyperactivity), n	45	46		
Very much improved, n (%)	0	0		
Much improved, n (%)	4 (9)	6 (13)		
Minimally improved, n (%)	20 (44)	14 (30)		
No change, n (%)	19 (42)	22 (48)		
Minimally worse, n (%)	2 (4)	1 (2)		
Much worse, n (%)	0	2 (4)		
Very much worse, n (%)	0	1 (2)		
Minimally improved or better, n (%)	24 (53)	20 (43)	0·94 (0·44, 2·01)	—

CI=confidence interval; IQR=interquartile range.

[a]Hodges-Lehmann estimate of median difference (95% confidence interval).

[b]Ordinal logistic regression model.

[c]Wilcoxon rank-sum test.

[d]Fisher's exact test.

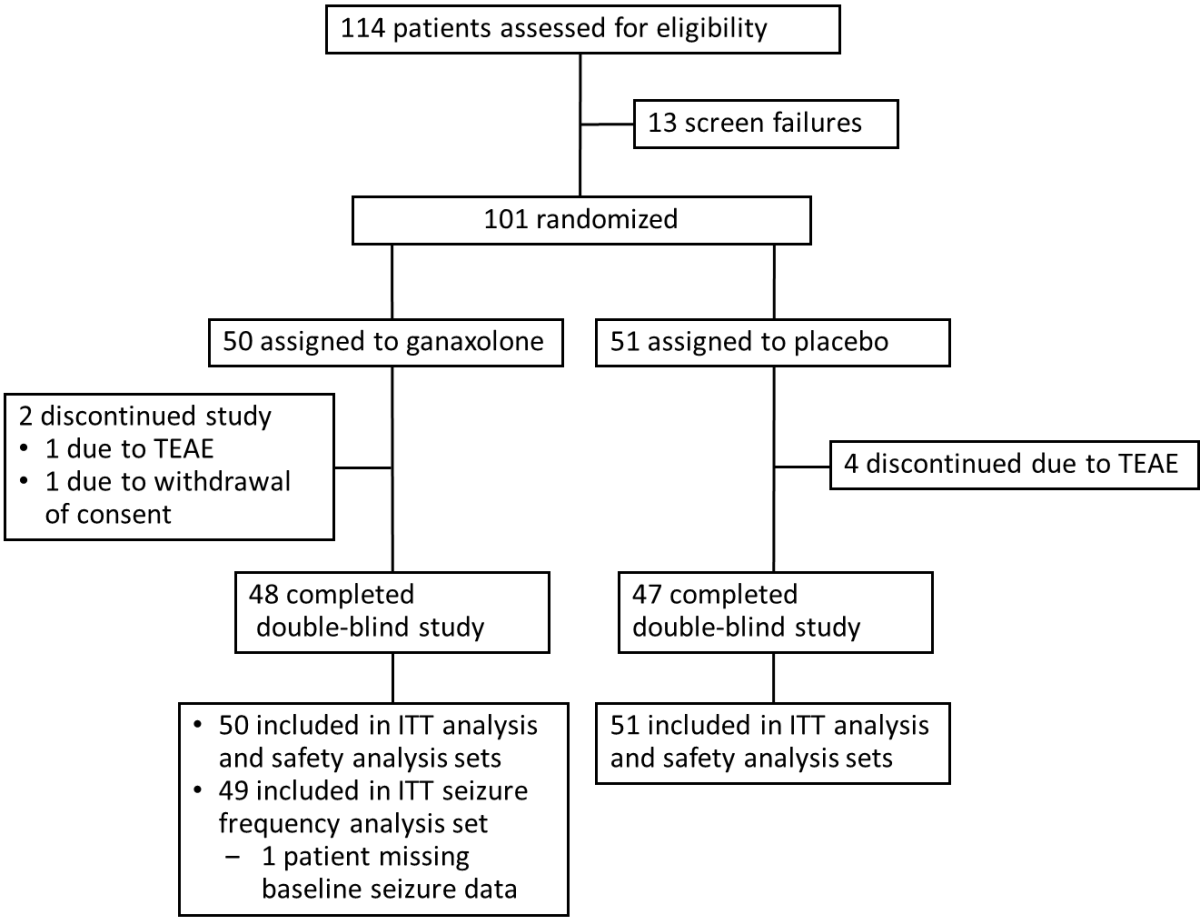
Table 3: Safety summary

Patients, n (%)	Ganaxolone (n=50)	Placebo (n=51)
Any TEAE[a]	43 (86)	45 (88)
Somnolence	18 (36)	8 (16)
Pyrexia	9 (18)	4 (8)
Seizure	7 (14)	9 (18)
Vomiting	5 (10)	10 (20)
Upper respiratory tract infection	5 (10)	3 (6)
Constipation	3 (6)	3 (6)
Salivary hypersecretion	3 (6)	1 (2)
Sedation	3 (6)	2 (4)
Ear infection	2 (4)	3 (6)
Rash	2 (4)	4 (8)
Rhinitis	2 (4)	4 (8)
Diarrhea	1 (2)	4 (8)
Respiratory tract infection viral	1 (2)	3 (6)
Urinary tract infection	1 (2)	3 (6)
Cough	0	3 (6)
Gastroesophageal reflux disease	0	3 (6)
Nasopharyngitis	0	5 (10)
Treatment-related TEAE[a]	35 (70)	22 (43)
Somnolence	17 (34)	3 (6)
Seizure	4 (8)	4 (8)
Constipation	3 (6)	0
Salivary hypersecretion	3 (6)	1 (2)
Sedation	3 (6)	2 (4)
Any serious TEAE	6 (12)	5 (10)
Bronchitis	1 (2)	0
Rhinovirus infection	1 (2)	0
Urinary tract infection	1 (2)	0
Pneumonia mycoplasmal	0	1 (2)
Pneumonia viral	0	1 (2)
Respiratory syncytial virus bronchiolitis	0	1 (2)
Oxygen saturation decreased	1 (2)	0
Food refusal	1 (2)	0
Pneumonia aspiration	1 (2)	0
Hypoxia	0	1 (2)
Faecaloma	0	1 (2)
Hypotonia	0	1 (2)
Seizure	0	1 (2)
Unresponsive to stimuli	0	1 (2)
TEAE leading to study drug discontinuation	2 (4)[b]	4 (8)

TEAE leading to dose reduction or temporary treatment discontinuation	11 (22)	8 (16)
TEAE leading to death	0	0

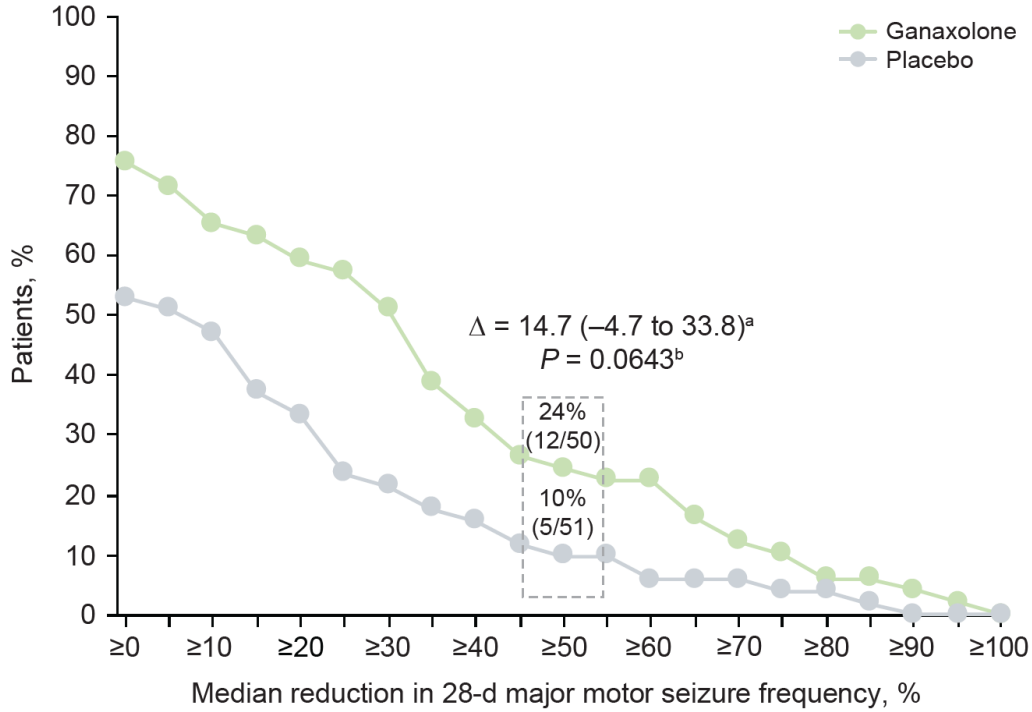
TEAE=treatment-emergent adverse event.
Patients could have more than one TEAE.
[a]Preferred terms reported in ≥5% of patients in either treatment group are presented.
[b]One additional patient in the ganaxolone group discontinued study drug due to a TEAE (somnolence), but remained in the study.

***Figure 1*: Trial profile**



TEAE=treatment-emergent adverse event.

Figure 2: **MMSF response curve including key secondary efficacy outcome**



MMSF= major motor seizure frequency.
First key secondary outcome was response rate (≥50% reduction in major motor seizure frequency between baseline and week 17). See supplemental figure 1 for second key secondary outcome, Clinical Global Impression Scale of Improvement (CGI-I) scores at week 17.
[a]Fisher's exact test.
[b]Difference (95% confidence interval).

SUPPLEMENTAL APPENDIX

Supplemental table 1: **Inclusion/exclusion criteria**

Inclusion criteria
• Patients must have (a) molecular confirmation of a pathogenic or likely pathogenic *CDKL5* variant, early onset, difficult to control seizures, and neurodevelopmental impairment are required. The principal investigator (PI) must review the results of the genetic analysis and confirm that gene mutation is likely to be the cause of the epilepsy syndrome. If the patient has a de novo variant of unknown significance (VUS) in the kinase domain of the *CDKL5*, parental testing is negative and meets all other inclusion criteria, then the subject can be included. Genetic mutations will be confirmed by the sponsor's chosen central laboratory. In France, genetic mutations may be confirmed by an approved French organization, in compliance with French legislation prior to Screening Visit 1. Patients must have (b) seizure onset by 1 year of age and (c) lack of independent ambulation by 2 years of age.
• Male or female patients aged 2 through 21 years, inclusive.
• Subject/parent or LAR willing to give written informed consent/assent, after being properly informed of the nature and risks of the study and prior to engaging in any study-related procedures.
• Failure to control seizures despite appropriate trial of 2 or more anti-seizure mediations at therapeutic doses.
• Have at least 16 seizures of primary seizure types: bilateral tonic (sustained motor activity ≥3 seconds), generalized tonic-clonic, bilateral clonic, atonic/drop or focal to bilateral tonic-clonic per 28 days in each 1-month period in the 2-month period prior to screening.
• Subject must be approved to participate by sponsor and/or designee (i.e., Epilepsy Consortium) after review of medical history, genetic testing, seizure classification, and historical seizure calendars.
• Participants should be on a stable regimen of 0–4 anti-seizure medications (ASMs, including moderate or strong inducer or inhibitor anti-seizure medications eg, carbamazepine, phenytoin, etc) for ≥1 month prior to the screening visit, without a foreseeable change in dosing for the duration of the double-blind phase. Vagus nerve stimulator (VNS), ketogenic diet, and modified Atkins diet do not count towards this limit but must be unchanged for 3 months prior to screening.
• Subjects with surgically implanted VNS will be allowed to enter the study provided that all of the following conditions are met: a. The VNS has been in place for ≥1 year prior to the screening visit; b. The settings must have remained constant for 3 months prior to the screening visit and remain constant throughout the double-blind phase; c. The battery is expected to last for the duration of the double-blind phase.
• Felbamate: The use of felbamate is allowed provided that the subject has been maintained on a stable dose of felbamate for > 6 months and has had stable liver function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) and hematology during the course of treatment, and is expected to remain constant throughout the double-blind phase.
• Parent/caregiver able and willing to maintain an accurate and complete daily electronic seizure calendar for the duration of the study.
• Able and willing to take investigational product with food 3 times daily. Ganaxolone must be administered with food.

- Sexually active female of childbearing potential must be using a medically acceptable method of birth control and have a negative quantitative serum β-human chorionic growth hormone (β-HCG) test collected at the initial screening visit. Childbearing potential is defined as a female who is biologically capable of becoming pregnant. A medically acceptable method of birth control includes intrauterine devices in place for at least 3 months prior to screening, surgical sterilization, or adequate barrier methods (eg, diaphragm and foam). An oral contraceptive alone is not considered adequate for the purpose of this study. Hormonal oral contraceptives must also be used when a condom is used. In subjects who are not sexually active, abstinence is an acceptable form.

Exclusion criteria

- Previous exposure to ganaxolone.
- Pregnant or breastfeeding.
- West Syndrome with hypsarrhythmia pattern on EEG or seizures predominantly of Infantile Spasm (IS) type; if EEG pattern/seizure type is uncertain, study inclusion should be reviewed and determined by the sponsor/sponsor delegate.
- Concurrent use of adrenocorticotropic hormone (ACTH), prednisone or other glucocorticoid is not permitted, nor use of moderate or strong inducers or inhibitors of CYP3A4/5/7. Moderate or strong inducer or inhibitor ASMs will be allowed (eg, carbamazepine, phenytoin, etc)
- Patients on ACTH, prednisone or other systemically (non-inhaled) administered steroids should be off the product greater than 28 days prior to screening. Concomitant PRN topical or intranasal steroids for dermatologic reactions and allergic rhinitis are allowed and do not warrant exclusion from the study.
- Subjects with a positive result on tetrahydrocannabinol (THC) or cannabidiol (CBD) test (via urine or plasma drug screen) at the screening visit, and a positive result on THC or CBD test (via plasma) at the baseline visit without prescription for Epidiolex (may go by another name in countries outside the United States) in epilepsy will be excluded from the study. Concomitant Epidiolex (CBD) use will be allowed in the double-blind phase provided the subject has been on a stable dose for at least 1 month prior to screening and is expected to remain on a stable dose without a foreseeable change for the duration of the double-blind phase. THC and/or CBD will be allowed in the open-label phase.
- Use of dietary supplements or herbal preparations are not permitted if subject has been using them consistently for less than 3 months prior to screening or does not plan on remaining on stable doses for the duration of the double-blind phase. Use of St. John's Wort is not permitted.
- Changes in ASMs within the last month prior to screening. All ASMs must be stable in dose for at least 1-month prior to screening unless otherwise noted.
- Have an active CNS infection, demyelinating disease, degenerative neurological disease, or CNS disease deemed progressive as evaluated by brain imaging (magnetic resonance imaging [MRI]).

- Have any disease or condition (medical or surgical; other than CDKL5) at screening that might compromise the hematologic, cardiovascular, pulmonary, renal, gastrointestinal, or hepatic systems; or other conditions that might interfere with the absorption, distribution, metabolism, or excretion of the investigational product, or would place the subject at increased risk.
- An AST (serum glutamic oxaloacetic transaminase [SGOT]) or ALT (serum glutamic pyruvic transaminase [SGPT]) greater than 3 times the upper limit of normal (ULN) at study entry. If AST or ALT increases > 3 times ULN during the study, subject should be followed with weekly laboratory repeat testing and continue in study if levels trending down. Subject will be discontinued if levels do not decline to under 3 x ULN.
- Total bilirubin levels greater than ULN at study entry. In cases of documented, stable medical condition (i.e., Gilbert's Syndrome) resulting in levels of total bilirubin greater than ULN, the medical monitor can determine if a protocol exception can be made. If total bilirubin increases to 1.5 x ULN or more during study, the subject will be discontinued.
- Subjects with significant renal insufficiency, estimated glomerular filtration rate (eGFR) < 30 mL/min (calculated using the Cockcroft-Gault formula, Pediatric GFR calculator or Bedside Schwartz), will be excluded from study entry or will be discontinued if the criteria is met post baseline.
- Have been exposed to any other investigational drug within 30 days or less than 5 half-lives prior to screening.
- Unwillingness to withhold grapefruit, Seville oranges or star fruit from diet during the entire clinical trial.
- Unwillingness to withhold alcohol throughout the entire clinical trial.
- Have active suicidal plan/intent or have had active suicidal thoughts in the past 6 months or a suicide attempt in the past 3 years.
- Known sensitivity or allergy to any component in the investigational product(s), progesterone or other related steroid compounds.
- Plasma allopregnanolone sulfate (Allo-S) levels ≥ 6.0 ng/ml at the screening visit.

β-HCG=β-human chorionic growth hormone; ACTH=adrenocorticotropic hormone; Allo-S=allopregnanolone sulfate; ALT=alanine aminotransferase; ASMs=antiseizure medications; AST=aspartate aminotransferase; CBD=cannabidiol; CNS=central nervous system; EEG=electroencephalogram; eGFR=estimated glomerular filtration rate; IS=infantile spasms; LAR=legally authorized representative; MRI=magnetic resonance imaging; PRN=as needed; SGOT=serum glutamic oxaloacetic transaminase; SGPT=serum glutamic pyruvic transaminase; THC=tetrahydrocannabinol; ULN=upper limit of normal; VNS=vagus nerve stimulator; VUS=variant of unknown significance.

Supplemental table 2: **Prespecified subgroup/exploratory analyses of median percentage change from baseline in 28-day seizure frequency by subgroup**

	Ganaxolone	Placebo	Ganaxolone–Placebo (95% CI)[a]
Sex[b]			
Female	−27·5% (n=38)	−10·2% (n=41)	−22·2[c] (-48·4, −1·4)
Male	−32·0% (n=11)	7·5% (n=10)	−42·1[c] (−95·2, −8·4)
Allopregnanolone sulfate[b]			
Patients with baseline levels ≤2.5 ng/mL	−25·4% (n=39)	−9·5% (n=37)	−21·0[d] (−47·3, 2·2)
Patients with baseline levels >2.5 and <6.0 ng/mL	−40·9% (n=5)	−3·5% (n=12)	−48·0[d] (−149·4, −16·8)
Patients with baseline levels ≥6.0 ng/mL[c]	−34·3% (n=4)	8·9% (n=2)	−47·9[d] (−83·1, 6·2)
Seizure type[b]			
All seizure types	−19·1% (n=49)	−8·9% (n=51)	−17·4[d] (−36·4, 0·3)
Bilateral tonic[e]	−30·7% (n=35)	−11·6% (n=39)	−16·8[d] (−39·5, 6·6)
Generalized tonic-clonic[e]	−34·5% (n=24)	13·4% (n=20)	−47·9[d] (−86·8, −3·5)
Atonic[e]	−80·5% (n=9)	−18·7% (n=12)	−49·2[d] (−108·7, 64·2)
Focal to bilateral tonic-clonic[e]	−60·1% (n=7)	121·5% (n=6)	−150·0[d] (−2445, −9·0)
Bilateral clonic[e]	−100% (n=6)	59·1% (n=3)	−159·1[d] (−488·5, −61·0)
Myoclonic	−41·9% (n=13)	−20·6% (n=13)	−28·4[d] (−118·0, 30·2)
Epileptic spasms	1·6% (n=12)	−3·7% (n=10)	12·6 (−67·8, 104·7)
Focal motor with intact awareness or altered awareness	−16·2% (n=10)	−52·6% (n=6)	36·4 (−43·5, 1110)
Absence	−49·9% (n=7)	−33·5% (n=2)	−16·5[d] (−61·4, 141·8)
Focal non-motor with altered awareness	−96·9% (n=3)	−66·9% (n=4)	−16·4[d] (−52·9, 67·2)
Treatment phase[f]			
4-week titration period	−35·1% (n=49)	−13·9% (n=51)	−18·7[d] (−34·8, −1·8)
13-week maintenance period	−29·4% (n=49)	−6·5% (n=51)	−29·3[d] (−51·5, −8·9)

Note: 6-week baseline period.

CI=confidence interval; MMSF=major motor seizure frequency.

[a]Hodges-Lehmann estimate of median difference (95% confidence interval).

[b]Median percentage change from baseline in 28-day major motor seizure frequency over 17 weeks.

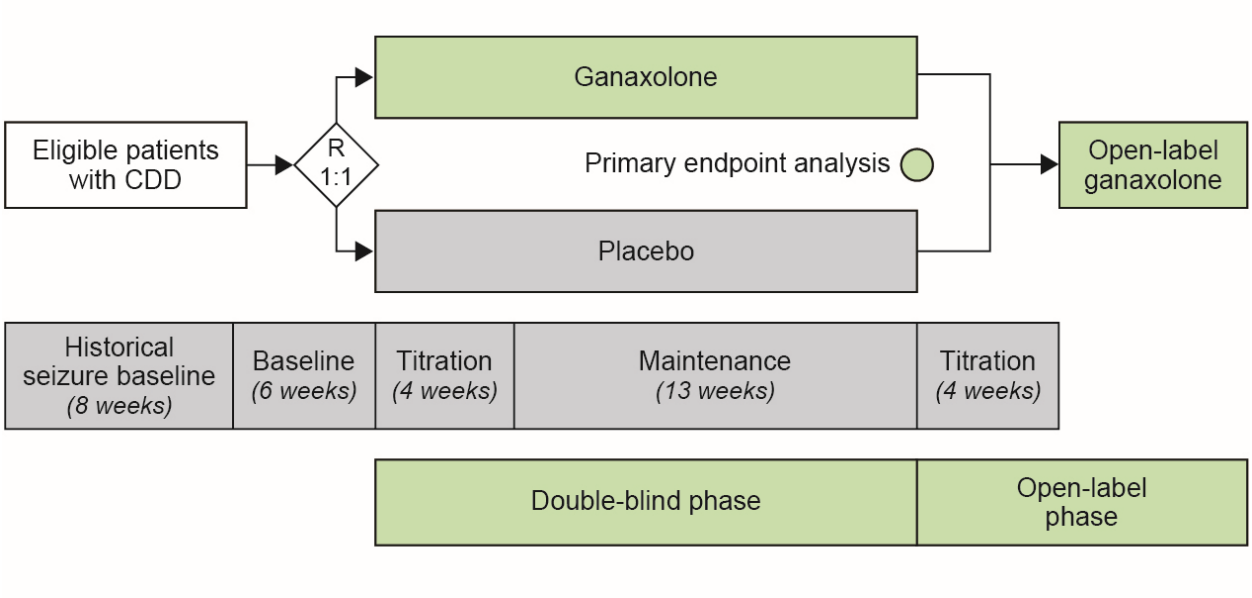
[c]After enrollment started, the protocol was amended to exclude patients with allopregnanolone sulfate levels ≥6.0 ng/mL at screening.

[d]Favors ganaxolone group.

[e]Indicates a major motor seizure included in the primary efficacy endpoint.

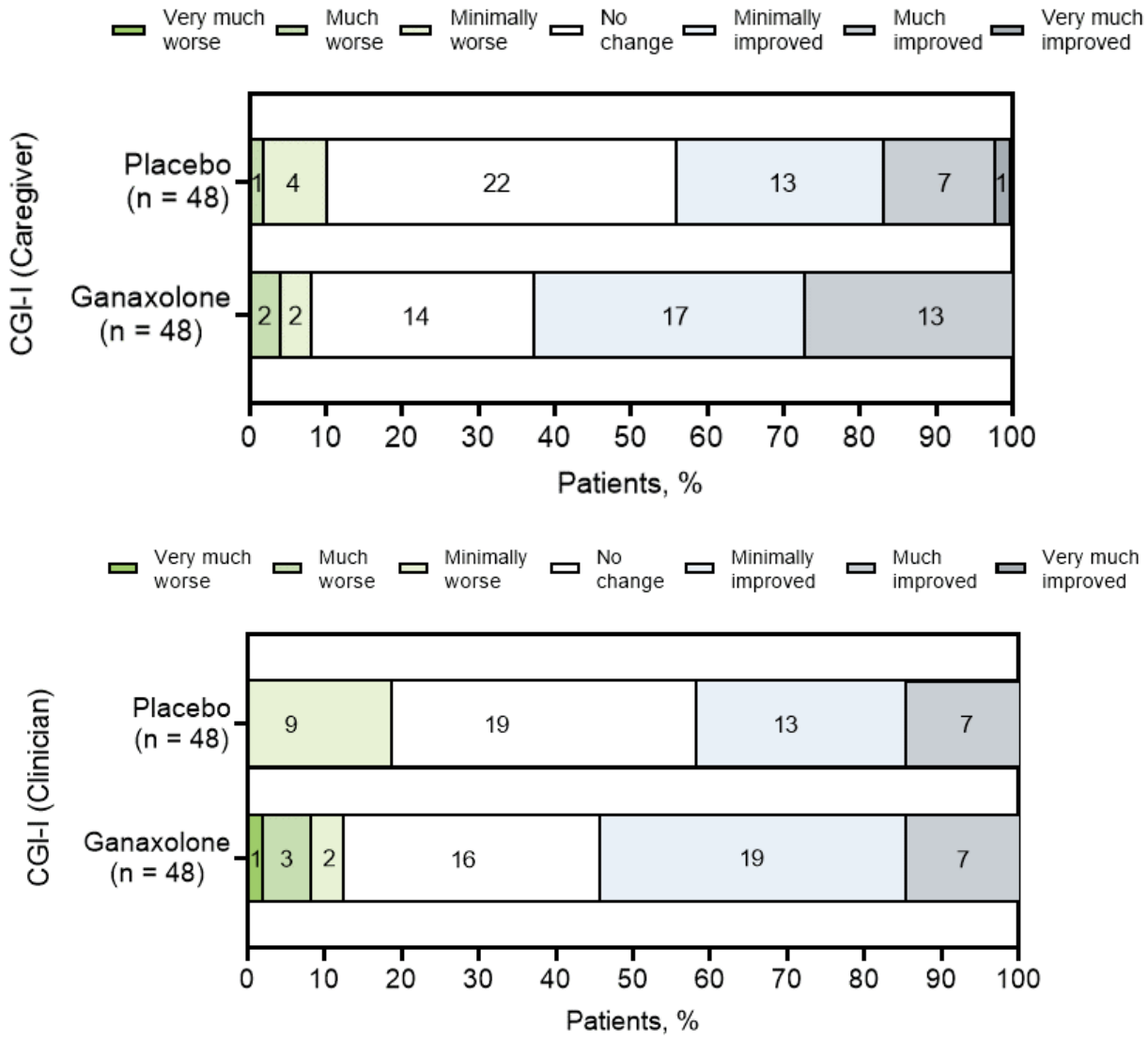
[f]Median percentage change from baseline in 28-day major motor seizure frequency at end of treatment phase.

Supplemental figure 1: **Trial design**



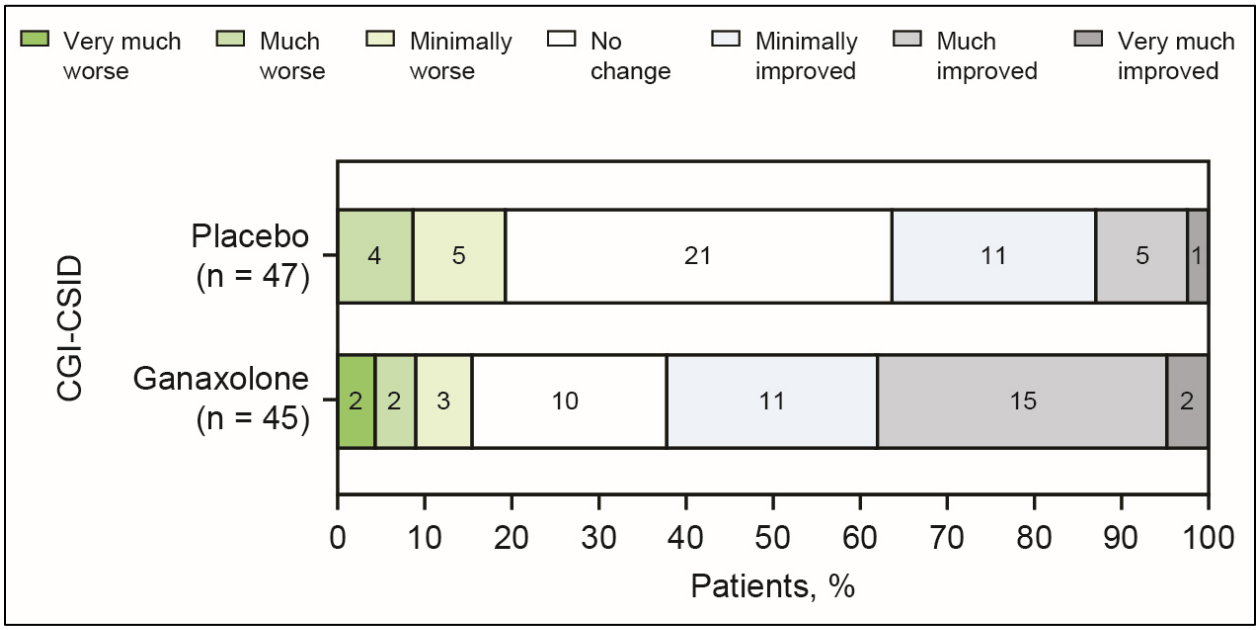
CDD=CDKL5 deficiency disorder; R=randomization.

Supplemental figure 2: **Clinical global impression of improvement (CGI-I) scores at week 17**



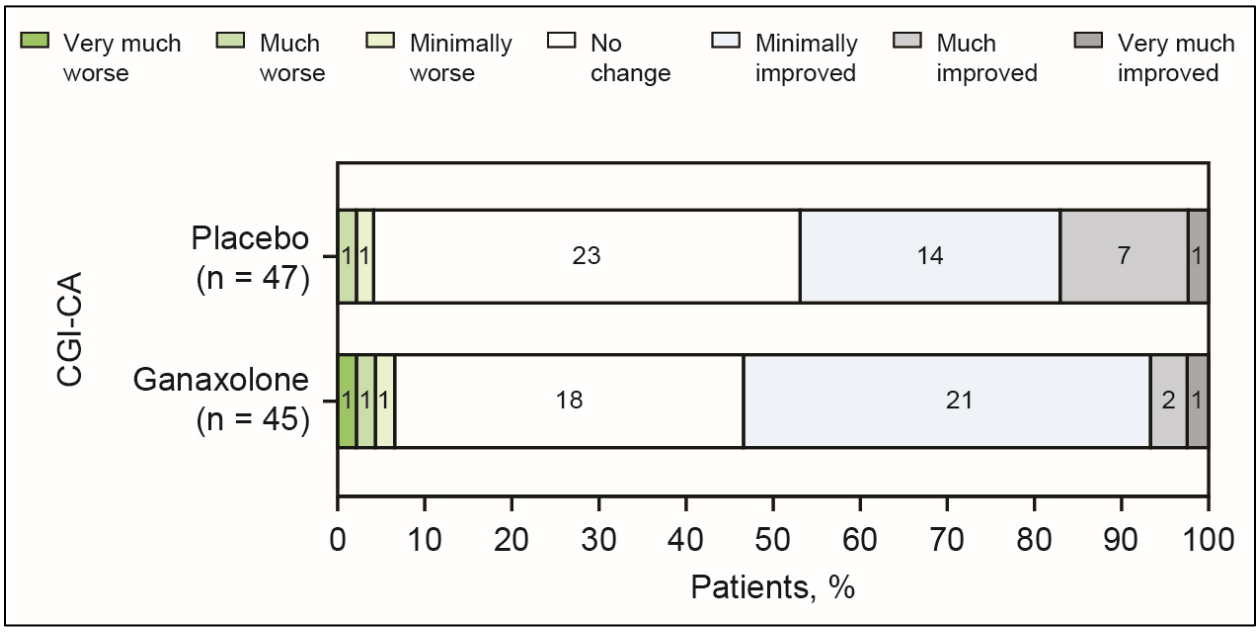
CI=confidence interval; OR=estimated odds ratio.
CGI-I scale score at week 17 per caregiver (OR, 1·87; 95% CI: 0·89, 3·91) and clinician (OR, 1·41; 95% CI: 0·68, 2·94) assessments for patients in the ganaxolone group compared with placebo.

Supplemental figure 3: **Caregiver Global Impression of Change in Seizure Intensity/Duration (CGI-CSID) score at week 17**



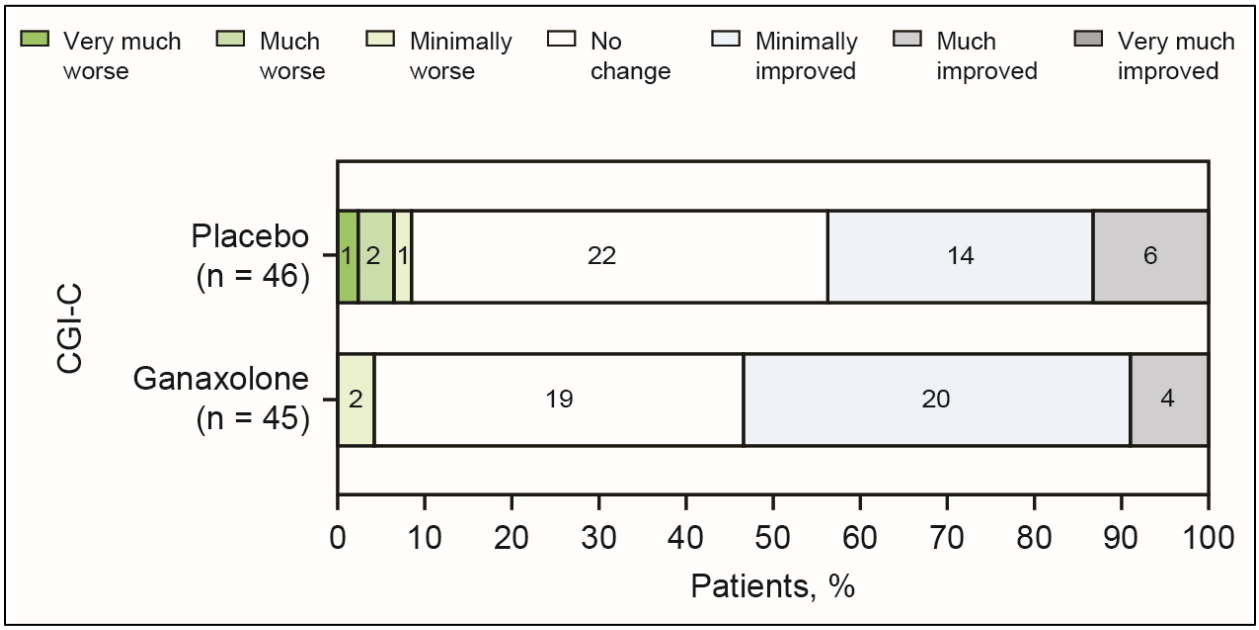
CI=confidence interval; OR=estimated odds ratio.
CGI-CSID score at week 17 (OR, 2·56; 95% CI: 1·20, 5·45) per caregiver assessment for patients in the ganaxolone group compared with placebo.

Supplemental figure 4: **Caregiver Global Impression of Change in Attention (CGI-CA) score at week 17**



CI=confidence interval; OR=estimated odds ratio.
CGI-CA score at week 17 (OR, 0·97; 95% CI: 0·45, 2·09) per caregiver assessment for patients in the ganaxolone group compared with placebo.

Supplemental figure 5: **Caregiver Global Impression of Change (CGI-C) in parent/caregiver identified target behavior score at week 17**



CI=confidence interval; OR=estimated odds ratio.
CGI-C score at week 17 (OR, 0·94; 95% CI: 0·44, 2·01) per caregiver assessment for patients in the ganaxolone group compared with placebo.