ZYN2-CL-016

A Randomized, Double-Blind, Placebo-Controlled Multiple-Center, Efficacy and Safety Study of ZYN002 Administered as a Transdermal Gel to Children and Adolescents with Fragile X Syndrome – CONNECT-FX

Clinical study Of caNNabidiol in childrEn and adolesCenTs with Fragile X (CONNECT-FX)

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IND Number:	130876
Sponsor:	Zynerba Pharmaceuticals Pty., Ltd. 2 Riverside Quay Southbank, VIC 3006 Australia Zynerba Pharmaceuticals, Inc. 80 W. Lancaster Ave., Suite 300 Devon, PA 19333 United States of America
Medical Monitors:	[redacted]

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1. INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure Edition 7 dated 22 May 2019 for ZYN002. I have read the ZYN2-CL-016.05 protocol dated 26 April 2020 and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Role in Study	Name	Email Address and Telephone Number
Responsible Physician	[redacted]	[redacted]

2. SYNOPSIS

Name of Sponsor/Company: Zynerba Pharmaceuticals Pty., Ltd. Zynerba Pharmaceuticals, Inc.
Name of Investigational Product (IP): ZYN002
Name of Active Ingredient: Cannabidiol (CBD)
Title of Study: A Randomized, Double-Blind, Placebo-Controlled, Multiple-Center, Efficacy and Safety Study of ZYN002 Administered as a Transdermal Gel to Children and Adolescents with Fragile X Syndrome - CONNECT-FX
Study Centers: Australia, Canada, New Zealand, and the United States of America
Study Period (years): Estimated date first patient enrolled: June 2018 Estimated date last patient enrolled: January 2020 Estimated date last patient completed: May 2020

Objectives:

Primary:

To evaluate the efficacy of ZYN002 administered as a transdermal gel formulation, for up to 12 weeks, in patients ages 3 to < 18 years, in the treatment of symptoms of Fragile X Syndrome (FXS). The primary endpoint is the change from Baseline to Week 12 in the Aberrant Behavior Checklist-Community FXS Specific (ABC-C_{FXS}) Social Avoidance subscale.

Secondary:

To further evaluate the efficacy of ZYN002 in the treatment of symptoms of FXS.

To evaluate the safety of ZYN002 in the treatment of symptoms of FXS.

To evaluate CBD, CBD metabolite and tetrahydrocannabinol (THC) exposure**.**

Methodology:

This is a randomized, double-blind, placebo-controlled, multiple-center study, to assess the efficacy and safety of CBD administered as ZYN002, a transdermal gel, for the treatment of child and adolescent patients with FXS. Male and female patients with FXS will be treated for 12 weeks with a [redacted] single-blind placebo lead-in preceding the 12-week double-blind treatment period. Approximately 204 male and female patients, ages 3 to < 18 years, will be randomized 1:1 to either trial drug or placebo. Randomization will be stratified by gender, weight category and region.

Site-specific supplemental protocols may be implemented in a subset of ZYN2-CL-016 patients.

Study Screening:

Prior to any Screening Procedures being performed, the parent/caregiver will provide written informed consent and, if applicable, the patient will provide assent. During the Screening Period, the site staff will review the eligibility criteria, review any medications including over-the-counter (OTC) medications the patient is taking, obtain the patient's medical history including their Fragile X Diagnosis, any seizure history and demographics, check their vital signs, perform an electrocardiogram (ECG), assess Tanner Stage, perform a physical and neurological exam and a skin assessment, obtain blood and urine for analysis, and administer assigned scales. Blood samples will be taken for hematology and chemistry testing, testosterone (T) testing (males only), pregnancy testing (if applicable) and Cytosine Guanine Guanine (CGG) repeat analysis. Patients will also have a blood sample drawn for plasma levels of CBD and THC testing, and adjunctive anti-epileptic drugs (AEDs) (for patients using AEDs). Urine will be collected for a urinalysis and drug screen. The scales administered include the ABC-C_{FXS}, Autism Diagnostic Observation Schedule®-2 (ADOS®-2) (ADOS®-2 will not be administered at Screening if it has been administered in the prior 6 months and the results are available), Clinical Global Impression-Severity (CGI-S), Columbia-Suicide Severity Rating Scale – Children's version (C-SSRS), Marijuana Withdrawal Checklist – Short Form (MWC, Behavior Checklist), Penn Physician Withdrawal Checklist (PWC-20) and the Vineland Adaptive Behavior Scales™ 3rd Edition (VABS-3).

The parent/caregiver will also be asked the following question "What are the five behavioral, emotional, or social problems that most impacted your son/daughter and his/her family in approximately the past year?" This is referred to as the "Qualitative Caregiver reported Behavioral Problems Survey" in the Schedule of Assessments, Table 3. Optionally, if an intelligence quotient (IQ) test was performed previously those results may be provided to the Sponsor.

[redacted] Single Blind Placebo Lead-In Period:

Following the Screening period, eligible patients will start a [redacted] Placebo Lead-In period. At Visit 2, parents/caregivers will be instructed on proper application of the study gel (active/placebo gel). [redacted]

Parents/caregivers will apply all trial drug to clean, dry, intact skin, thoroughly massaging it into the right and/or left upper arms and shoulders until the area is dry. The study gel will be rubbed in completely and must be dry prior to dressing. [redacted]

Parents/caregivers will use gloves supplied by the Sponsor to apply the study gel. If redness occurs at the application site, the parent (after consultation with the Investigator) may switch the application site temporarily to the upper thighs. Parents/caregivers will complete the Caregiver Global Impression of Severity scale at Visit 2.

12-Week Double Blind Treatment Period

At Visit 3, patients will be randomized to receive either ZYN002 or placebo. Patients will qualify for randomization if they continue to meet the inclusion criteria and none of the exclusion criteria for the study. Patients eligible for randomization will be either:

- Patients with an ABC-C_{FXS} [redacted] score [redacted] on Study Day 1 prior to randomization, who have [redacted] **OR**
- Patients with an ABC-C_{FXS} [redacted] score of [redacted] **with an** ABC-C_{FXS} [redacted] score [redacted] on Study Day 1 prior to randomization, who have [redacted]

All inclusion and exclusion criteria must be reviewed for eligibility for randomization.

In a blinded fashion, ZYN002-treated patients who weigh ≤ 35 kg will receive 125 mg CBD Q12H (every 12 hours) (± 2 hours); for a total daily dose of 250 mg CBD. Patients who weigh > 35 kg will receive 250 mg CBD Q12H (±2 hours); for a total daily dose of 500 mg CBD. All patients will remain on their assigned dose during the 12 weeks of the treatment phase of the study. Study visits will occur at Week 4 (Visit 4), Week 8 (Visit 5) and Week 12 (Visit 6). Patients who have successfully completed the 12 weeks of the double-blind study and have been at least 90% compliant with the trial drug and visits will have the option to enroll in an Open-Label Extension Study (OLE). A weekly Telephone Follow-Up call will occur for four weeks following the last dose of trial drug for patients who do not enter the OLE.

Patients and parents/caregivers will be required to visit the clinic at Week -2 (Visit 2), Study Day 1/Week 1 (Visit 3), Week 4 (Visit 4), Week 8 (Visit 5), and Week 12 (Visit 6) for vital signs, ECG, concomitant medication check, physical and neurological exams, Tanner Stage assessment, skin check examination, adverse event (AE) review including seizure assessment if applicable, and completion of the following questionnaires and scales: ABC-C_{FXS}, Anxiety Depression and Mood Scale (ADAMS), Clinical Global Impression-Improvement (CGI-I), CGI-S, C-SSRS, Pediatric Quality of Life Inventory™ – Family Impact (Family Impact PedsQL™), MWC and PWC-20.

In addition, parents/caregivers will complete a Caregiver Global Impression of Severity at Visit 3, and Visit 6/ET. Parents/caregivers will also complete a Caregiver Global Impression of Change at Visit 6/ET.

End of Study (EOS) / Early Termination Visit (ET):

Patients who have successfully completed through Week 12 of the protocol and have been at least 90% compliant with the trial drug and study visits will have the opportunity to continue in an OLE Study.

Patients not on AEDs who are not continuing in the OLE study or are prematurely discontinuing will complete the EOS procedures at Visit 6/ET. See Table 3 Schedule of Assessments for procedures at Visit 6/ET.

Patients taking concomitant AEDs who are not continuing in the OLE study or are prematurely discontinuing will taper down their study dose in the following manner at Visit 6/ET:

- For patients weighing ≤ 35 kg (250 mg daily dose), the dose of trial drug will be reduced, in a blinded manner, to a total daily dose of 125 mg ZYN002 or 2.98 g placebo gel each day for one week (one sachet each morning), after which time the patients will discontinue from the study and attend the EOS Visit. See Table 3 Schedule of Assessments for procedures at the EOS Visit. The parent/caregiver will be contacted weekly during the Taper Period and complete the MWC and PWC-20.
- For those patients > 35 kg (500 mg daily dose), the dose of trial drug will be reduced, in a blinded manner over two weeks: during the first week of taper the dose will be reduced to 125mg Q12H; (ZYN002 or placebo) (±2 hours); total daily dose of 250 mg, followed by a second week of taper from 250 mg total daily dose to a total daily dose of 125 mg ZYN002 or 2.98g placebo gel each day (one sachet each morning). After the taper, patients will discontinue from the study and attend the EOS Visit. See Table 3 Schedule of Assessments for procedures at the EOS Visit. The parent/caregiver will be contacted weekly during the Taper Period and complete the MWC and PWC-20.

[redacted]

Patients who do not enter the OLE study or who discontinue early will have a Telephone Follow-Up call weekly for four weeks following each patient's last dose of trial drug. The parent/caregiver will be contacted weekly via the telephone during the Follow-Up and complete the MWC and PWC-20.

Safety Monitoring:

Patient safety will be monitored during study visits using standard measures, including physical and neurological exams, Tanner Stage assessment, examination of skin at application sites for irritation, vital signs (including oral, infrared forehead or tympanic temperature), 12-lead ECGs, the C-SSRS, the MWC, the PWC-20, safety laboratory tests, urinalysis, seizure assessment and AE monitoring. Misuse, Abuse, and Diversion Drug Event Reporting System (MADDERS®) will be used to systemically capture and adjudicate abuse-related events. At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of blood samples and ECG's.

At Screening, patients will have an epilepsy diagnosis and seizure classification confirmed by the Investigator. The parents/caregivers of patients with a current epilepsy and/or seizure diagnosis will be asked at each study visit if there has been any increase in frequency or severity of the patient's seizures. If an increase in frequency or severity is noted, this will be recorded as an adverse event (AE) in the case report form (CRF).

Parents/caregivers will be provided a diary to complete a daily skin check examination. Every evening they will record the skin check score in the daily skin check diary. When skin redness is noted, parents/caregivers should apply the gel to a non-red area of the upper arms and shoulders. If the skin check score is higher than '2'(moderate erythema) at any time, the parent/caregiver will contact the study site to determine if an Unscheduled Visit is required. The Investigator will use discretion in suspending dosing for patients with a skin check score of '4' but will, in all cases, immediately (within 24 hours) complete an adverse event report and contact their study Clinical Research Associate (CRA) and the Zynerba Medical Monitor. A de-identified photograph of the skin finding (or area) of interest may be taken after consultation with and approval of the Sponsor. Digital photographs will be retained for information purposes only. In place of suspending the dosing due to skin irritation, the Investigator may instruct the parent/caregiver to temporarily change the application site to the upper thigh.

Blood Samples of AEDs, CBD, and THC:

Blood samples for plasma levels of CBD and THC will be collected at the Screening Visit, Visit 4, and Visit 6/ET. Plasma may also be analyzed for CBD metabolite concentrations. In addition, blood samples for adjunctive AED blood levels (for patients taking AEDs) will be collected at Screening, Visit 4, and Visit 6/ET. The time of blood sample collection, as well as the time of last AED and trial drug dose, will be recorded. Plasma samples will be analyzed [redacted] Plasma samples for adjunctive AEDs will be analyzed through a commercial laboratory.

Number of Patients (planned): Approximately 204 male and female patients will be randomized 1:1 to either trial drug or placebo.

Diagnosis and Criteria for Inclusion: Patients participating in this study will have a diagnosis of FXS through molecular documentation of Fragile X Mental Retardation 1 (FMR1) full mutation. No more than 25% of the patients screened will be females. Siblings may be screened and enrolled based upon Investigator discretion and discussion with the Medical Monitor. Patients must qualify based on meeting all of the inclusion and none of the exclusion criteria to be eligible to enroll.

Inclusion Criteria:

1. Male or female children and adolescents aged 3 to <18 years, at the time of Screening.
2. Judged by the Investigator to be in generally good health at Screening based upon the results of a medical history, physical examination, 12-lead ECG, and clinical laboratory test results. Laboratory results outside of the reference range must be documented as not clinically significant by both the Investigator and Sponsor.
3. Patients must have a diagnosis of FXS through molecular documentation of FMR1 full mutation.
4. Patients with an ABC-C_{FXS} [redacted] score of [redacted] at Screening **OR** patients with an ABC-C_{FXS} [redacted] score of [redacted] at Screening **with an** ABC-C_{FXS} [redacted] score [redacted].
5. Patients have a Clinical Global Impressions-Severity (CGI-S) score of at least '3' at Screening.
6. Patients with a history of seizure disorders must currently be receiving treatment with a stable regimen of one or two AEDs, or must be seizure-free for one year if not currently receiving AEDs.
7. Patients taking AEDs should be on a stable regimen for the four weeks preceding study Screening and taking no more than two.
8. Patients who are taking psychotropic medication(s) should be on a stable regimen of no more than two such medications for at least four weeks preceding study Screening and must maintain that regimen throughout the study. Psychotropic medications include (but are not limited to) antipsychotics, antidepressants, anxiolytics, and attention-deficit / hyperactivity disorder (ADHD) medications.
9. If patients are receiving non-pharmacological behavioral and/or dietary interventions, they must be stable and have been doing so for three months prior to Screening.
10. Patients have a body mass index between 12–30 kg / m^2 (inclusive).
11. Females of childbearing potential must have a negative serum pregnancy test at the Screening Visit and a negative serum or urine pregnancy test at all designated visits.
12. Patients and parents/caregivers agree to abide by all study restrictions and comply with all study procedures.
13. Patients and parents/caregivers must be adequately informed of the nature and risks of the study and give written informed consent (and assent if applicable) prior to Screening.
14. Parents/caregiver(s) must provide written consent to assist in trial drug administration.
15. In the Investigator's opinion, patients and parents/caregivers are reliable and willing and able to comply with all protocol requirements and procedures.

Exclusion Criteria:

Any of the following is considered criterion for exclusion:

1. Females who are pregnant, nursing, or planning a pregnancy; females of childbearing potential and male patients with a partner of childbearing potential who are unwilling or unable to use an acceptable method of contraception as outlined below for the duration of therapy and for three months after the last dose of study medication.

   Standard acceptable methods of contraception include abstinence or the use of a highly effective method of contraception, including hormonal contraception, diaphragm, cervical cap, vaginal sponge, condom, spermicide, vasectomy, or intrauterine device.
2. History of significant allergic condition, significant drug-related hypersensitivity, or allergic reaction to any compound or chemical class related to ZYN002 or its excipients.
3. Exposure to any investigational drug or device ≤ 30 days prior to Screening or at any time during the study.
4. Patient has alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin levels ≥ 2 times the upper limit of normal (ULN) or has alkaline phosphatase levels ≥ 3 times the ULN as determined from Screening safety laboratories.
5. Use of cannabis or any THC or CBD-containing product (aside from ZYN002) within three months of Screening Visit or during the study.
6. Patient has a positive drug screen, including ethanol, cocaine, THC, barbiturates, amphetamines (unless prescribed), benzodiazepines (except midazolam or comparable administered for blood draws and ECG collection), and opiates.
7. Patient is using the following AEDs: clobazam, phenobarbital, ethosuximide, felbamate, or vigabatrin.
8. Patient is using any strong inhibitor/inducer of CYP3A4 or sensitive substrate for CYP3A4 including but not limited to the following medications: midazolam (except single doses administered for the purposes of obtaining blood samples and ECG's), oral ketoconazole, fluconazole, nefazadone, rifampin, alfentanil, alfuzosin, amiodarone, cyclosporine, dasatinib, docetaxol, eplerenone, ergotamine, everolimus, fentanyl, halofantrine, irinotecan, lapatinib, levomethadyl, lumefantrine, nilotinib, pimozide, quinidine, ranolazine, sirolimus, tacrolimus, temsirolimus, toremifene, tretinioin, vincristine, vinorelbine, and St. John's Wort.
9. Patients may not be taking minocycline for 30 days prior to Screening or throughout the study.
10. Patients may not be taking any benzodiazepines (except single doses administered for the purposes of obtaining blood samples and ECG's) at screening or throughout the study.
11. Patient has an advanced, severe, or unstable disease that may interfere with the study outcome evaluations.
12. Patient is expected to initiate or change pharmacologic or non-pharmacologic interventions during the course of the study.
13. Patient has an acute or progressive neurological disease, psychosis, schizophrenia, or any psychiatric disorder or severe mental abnormalities (other than Fragile X Syndrome) that are likely to require changes in drug therapy or interfere with the objectives of the study or the ability to adhere to protocol requirements.
14. Patient has a positive result for the presence of HBsAg, HCV, or HIV antibodies.

15. Patient has known history of cardiovascular disease, advanced arteriosclerosis, structural cardiac abnormality, cardiomyopathy, serious heart rhythm abnormalities, coronary artery disease, cardiac conduction problems, exercise-related cardiac events including syncope and pre-syncope, risk factors for Torsades de pointes (TdP) (e.g., heart failure, hypokalemia, family history of Long QT Syndrome), or other serious cardiac problems.
16. Any clinically significant condition or abnormal findings at the Screening Visit that would, in the opinion of the Investigator, preclude study participation or interfere with the evaluation of the study medication.
17. Any skin disease or condition, including eczema, psoriasis, melanoma, acne, contact dermatitis, scarring, imperfections, lesions, tattoos, or discoloration, that may affect treatment application, application site assessments, or absorption of the trial drug.
18. History of treatment for, or evidence of, drug abuse within the past year.
19. Previous participation in a ZYN002 study.
20. Patient responds "yes" to Question 4 or 5 on the C-SSRS (Children) during Screening or at any time on study.

Investigational Product, Dosage, and Mode of Administration: ZYN002 gel, or Placebo gel, topical.

Placebo Lead-In Period:

During the single-blind Placebo Lead-In period patients ≤ 35 kg will receive placebo, applied Q12H (± 2 hours). Each application will consist of one sachet of placebo containing 2.98 g of gel (two sachets in total per day). Patients > 35 kg will receive placebo, applied Q12H (±2 hours). Each application will consist of two sachets of placebo containing 2.98 g of gel in each sachet (four sachets in total per day).

Treatment Period:

At Visit 3 patients will be randomized to either:

Treatment A (Trial Drug)

Patients ≤ 35 kg will receive 125 mg CBD applied Q12H (± 2 hours); total daily dose of 250 mg CBD. Each application will consist of one sachet of ZYN002 CBD 4.2% concentration, containing 2.98 g of gel. This dosing will continue for the duration of the study.

Patients >35 kg will receive 250 mg CBD applied Q12H (±2 hours); total daily dose of 500 mg CBD. Each application will consist of two sachets of ZYN002 CBD 4.2% concentration, each sachet containing 2.98 g of gel. This dosing will continue for the duration of the study.

OR

Treatment B (Placebo)

Patients ≤ 35 kg will receive placebo, applied Q12H (±2 hours). Each application Q12H (± 2 hours), will consist of one sachet of placebo, containing 2.98 g of gel. This dosing will continue for the duration of the study.

Patients > 35 kg will receive placebo, applied Q12H (±2 hours). Each application Q12H (±2 hours) will consist of two sachets of placebo, each sachet containing 2.98 g of gel. This dosing will continue for the duration of the study.

Duration of Treatment:

Parents/caregivers will apply trial drug twice daily for the [redacted] single-blind placebo lead-in period, followed by 12 weeks of randomized treatment twice daily. Following Week 12, patients on AEDs may have an additional one or two weeks of blinded treatment to taper off trial drug.

Criteria for Evaluation:

Efficacy:

Efficacy assessments are as indicated in Table 3 Schedule of Assessments.

The primary endpoint is the change from Baseline to Week 12 in the ABC-C_{FXS} Social Avoidance subscale score.

Key Secondary Endpoints Include:

a. Change from Baseline to Week 12 in ABC-C_{FXS} Irritability subscale score.
b. Change from Baseline to Week 12 in ABC-C_{FXS} Socially Unresponsive/Lethargic subscale score.
c. CGI-I at Week 12/ET.

Secondary Endpoints Include: Secondary Endpoints include:

a. Percent of patients who have ≥ 25% improvement from Baseline in ABC-C_{FXS} Social Avoidance subscale score at Week 12/ET.
b. Percent of patients who have ≥ 25% improvement from Baseline in ABC-C_{FXS} Irritability subscale score at Week 12/ET.
c. Change from Baseline in ABC-C_{FXS} Social Avoidance, Irritability, and Socially Unresponsive/Lethargic subscale scores at Weeks 4 and 8.
d. Change from Baseline in ABC-C_{FXS}, Stereotypy, Inappropriate Speech, and Hyperactivity subscale scores at Weeks 4, 8, and 12.
e. Change from Baseline to Weeks 4, 8, and 12 in ADAMS total score and subscale scores (Manic/Hyperactive Behavior, Depressed Mood, Social Avoidance, General Anxiety and Compulsive Behavior).
f. CGI-I at Weeks 4 and 8.
g. Percent of patients indicating improvement on the CGI-I (dichotomized) scale at Weeks 4, 8, and 12/ET.
h. Response rate for patients having both ≥ 25% improvement from Baseline in ABC-C_{FXS} Social Avoidance subscale score AND improved on CGI-I scale at Weeks 4, 8, and 12/ET.
i. Response rate for patients having both ≥ 25% improvement from Baseline in ABC-C_{FXS} Irritability subscale score AND improved on CGI-I scale at Weeks 4, 8, and 12/ET.
j. Change from Baseline in CGI-S at Weeks 4, 8, and 12/ET.
k. Change from Baseline in Family Impact PedsQL™ at Week 12/ET.

The efficacy population is defined as all patients who have taken at least one dose of double-blind study medication and have at least one post-randomization ABC-C_{FXS} assessment.

Safety Analyses:

Safety assessments will include collection of AEs including seizure assessment if applicable, physical and neurological examination, Tanner Stage assessment, 12-lead ECG, clinical laboratory assessments (hematology, chemistry, and urinalysis), vital signs, C-SSRS, MWC, PWC-20, and findings from the skin check examinations following treatment. All patients who receive at least one dose of randomized trial drug will be included in the safety analysis.

Plasma Concentrations of CBD, THC and AEDs:

Plasma concentrations for CBD, THC and concurrent AEDs will be summarized by randomized treatment group (active or placebo) and by Screening and treatment time points. Plasma may also be analyzed for CBD metabolite concentrations. [redacted]

Statistical Methods:

The sample size was estimated for the primary efficacy endpoint using Power Analysis and Sample Size Software (PASS [15]) (NCSS, 2017). Sample size requirements were based on a previous Phase 1/2 trial, ZYN2-CL-009, in patients with FXS and on published results in the literature with other studied treatments in this indication. A sample size of 102 patients per treatment group (204 patients total) is required for the detection of a 1.3-point difference (assuming a standard deviation of 2.8) in the ABC-C_{FXS} Social Avoidance subscale score between treatments (active versus placebo) using a 2-sided test at the 5% significance level with 90% power.

Descriptive statistics (mean, median, standard deviation, minimum, and maximum) for continuous data and number (n) and percentage (%) for categorical data will be presented for all efficacy and safety parameters.

All efficacy assessments will be summarized by randomized treatment group.

Unless otherwise stated, mixed effects models for repeated measures (MMRM) will contain fixed effects for treatment, visit, study site, region and treatment-by-visit interaction as factors as well as the continuous fixed covariate of baseline value for the dependent variable of interest.

Analysis of covariance (ANCOVA) models will contain fixed effects for treatment and study site and region with baseline value for the dependent variable of interest as a covariate.

For MMRM/ANCOVA, descriptive statistics will be presented by visit, which will include least-squares mean, least-squares standard errors, and p-values for between treatment group tests.

The statistical comparison of active versus placebo for the primary endpoint of change from baseline in ABC-C_{FXS} Social Avoidance scores will be based on MMRM. The same model used for the primary endpoint analysis will also be used for the key secondary endpoints of change from Baseline in ABC-C_{FXS} Irritability, and Socially Unresponsive/Lethargic subscale scores. The statistical comparison of active versus placebo for the key secondary endpoint of CGI-I will be based on Wilcoxon rank sum test.

Response rate for the secondary endpoint defined as the number of patients who have $\geq$ 25% improvement from baseline in ABC-C_{FXS} Social Avoidance subscale score will be compared using a chi-square test and binomial confidence intervals. Response rate for the secondary endpoint defined as the number of patients who have $\geq$ 25% improvement from baseline in ABC-C_{FXS} Irritability subscale score will be compared using a chi-square test and binomial confidence intervals. The statistical comparison for all secondary endpoints for changes from baseline in ABC-C_{FXS} and

ADAMS subscale scores will be based on MMRM. The statistical comparison of active versus placebo for the CGI-I at weeks 4 and 8 will be based on Wilcoxon rank sum test.

The response rates for the percent of patients improved using CGI-I, and for patients having both ≥ 25% improvement from Baseline in ABC- C_{FXS} Social Avoidance subscale scores AND improved on CGI-I scale will be compared using the chi-square test and binomial confidence intervals. The response rates for the percent of patients improved using CGI-I, and for patients having both ≥ 25% improvement from Baseline in ABC- CFXS Irritability subscale scores AND improved on CGI-I scale will be compared using the chi-square test and binomial confidence intervals.

The statistical comparison for active versus placebo for the Family Impact module of the Pediatric Quality of Life (PedsQL™) will be based on ANCOVA. The statistical comparison of active versus placebo for the CGI-S will be based on Cochran-Mantel-Haenszel test, adjusting for the baseline score as the covariate.

Results of exploratory analysis of the Caregiver Global Impression of Severity, the Caregiver Global Impression of Change, and the Qualitative Caregiver reported Behavioral Survey will be reported using descriptive statistics.

AEs will be tabulated by the actual treatment dose of trial drug received at the time of initiation of the adverse event and classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). Additionally, AEs will be tabulated overall (total number of AEs and total number of patients with AEs). Data from the MADDERS® will be summarized and presented separately from other AEs.

Symptoms of withdrawal will also be assessed by comparing change over time in the total score on the MWC and total score on the PWC-20 from Baseline to Week 12, weekly during the taper period, and weekly for four weeks after the patient stops study drug.

Vital signs assessments (actual and change from Baseline) taken at Baseline, Weeks 4, 8, and 12 will be summarized using descriptive statistics and presented by actual treatment group.

Safety laboratory and urinalysis assessments (actual and change from Baseline) will be summarized by actual treatment group.

ECGs (actual and change from Baseline) will be summarized by actual treatment group. ECG results including any clinically significant findings will be summarized at each study visit.

Application site check will be summarized using counts and percentages at each respective application site check score (‘0’, ‘1’, ‘2’, ‘3’, or ‘4’) by actual treatment group.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
2-AG	2-arachidonoylglycerol
5-HT_{1A}	Serotonin
AE	Adverse event
ABC-C_{FXS}	Aberrant Behavior Checklist-FXS factor analysis
ADAMS	Anxiety Depression and Mood Scale
ADHD	Attention-deficit / hyperactivity disorder
ADOS®-2	Autism Diagnostic Observation Schedule®-2
AEA	Anandamide
AED	Anti-epileptic drug
ALT	Alanine transaminase
ANCOVA	Analysis of covariance
API	Active Pharmaceutical Ingredient
AST	Aspartate transaminase
AUC	Area under the curve
BID	Twice Daily
BUN	Blood urea nitrogen
°C	Degrees Celsius
CB_1	Cannabinoid receptor type 1
CB_2	Cannabinoid receptor type 2
CBD	Cannabidiol
CFR	Code of Federal Regulations
CGG	Cytosine Guanine Guanine
CGI-I	Clinical Global Impression-Improvement
CGI-S	Clinical Global Impression-Severity

Table 2: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
CI	Confidence Interval
C_{max}	Maximum observed concentration
CMC	Chemistry, Manufacturing, and Control
CMH	Cochran-Mantel-Haenszel
CNS	Central nervous system
CONNECT-FX	**C**linical study **O**f ca**NN**abidiol in childr**E**n and adolesCen**T**s with **F**ragile **X**
CRA	Clinical Research Associate
CRF	Case report form
C-SSRS	Columbia-Suicide Severity Rating Scale
CTRA	Clinical Trial Research Agreement
CYP	Cytochrome P enzyme
DDI	Drug-drug interaction
DEA	Drug Enforcement Agency
DGL	Diacylglycerol lipase
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
e.g.	Exampli Gratia – 'for example'
EOS	End of Study
ET	Early Termination
et al.	Et alia – 'and others'
°F	Degrees Fahrenheit
FAAH	Fatty acid amide hydrolase
FABP	Fatty acid binding proteins
FAS	Full Analysis Set
FDA	Food and Drug Administration
FMR1	Fragile X Mental Retardation 1
FMRP	Fragile X Mental Retardation protein

Table 2: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
FXS	Fragile X Syndrome
g	Gram
GCP	Good clinical practice
GLP	Good laboratory practice
HBsAg	Hepatitis B Surface antigen
HCV	Hepatitis C virus
HDL	High density lipoprotein
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HRQOL	Health-related quality of life
ICF	Informed consent form
ICH	International Conference on Harmonization
i.e.	id est – 'that is'
IEC	Independent Ethics Committee
IP	Investigational Product
IQ	Intelligence Quotient
IRB	Institutional Review Board
IRT	Interactive Response System
IV	Intravenous
kg	Kilogram
KO	Knock-out
LDL	Low density lipoprotein
L	Liter
LS	Least-squares
LTD	Long-term depression

Table 2: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
MADDERS®	The Misuse Abuse and Diversion Drug Event Reporting System
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
MWC	Marijuana Withdrawal Checklist
MMRM	Mixed effects models for repeated measures
MRI	Magnetic resonance imaging
MS/MS	Tandem mass spectrometry
N	Number
ng	Nanogram
nm	Nanometers
NOAEL	No-observed-adverse-effect-levels
NONMEM	Nonlinear mixed effects model
OA	Osteoarthritis
OAE	Other significant adverse event
OLE	Open Label Extension
OR	Odds Ratio
OTC	Over-the-counter
PASS	Power Analysis and Sample Size
PedsQL™	Pediatric Quality of Life Inventory
PI	Principal Investigator The investigator who leads the study conduct at an individual study center. Every study center has a principal investigator.
PK	Pharmacokinetic
PND	Post-natal day
PWC-20	Penn Physician Withdrawal Checklist
Q12H	Every 12 hours

Table 2: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
RBC	Red blood cell
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SSRIs	Selective serotonin reuptake inhibitors
T	Testosterone
$t_{1/2}$	Half-life
TdP	Torsades de pointes
TEAE	Treatment emergent adverse event
THC	Δ9-tetrahydrocannabinol
UDS	Urine Drug Screen
ULN	Upper limit of normal
UK	United Kingdom
UV	Ultraviolet
VABS-3	Vineland Adaptive Behavior Scales™, Third Edition
VAS	Visual Analog Scale
VR1	Vanilloid type-1
vs	Versus
WBC	White blood cell
WDS	Withdrawal Discomfort Score

5. INTRODUCTION

5.1. Background

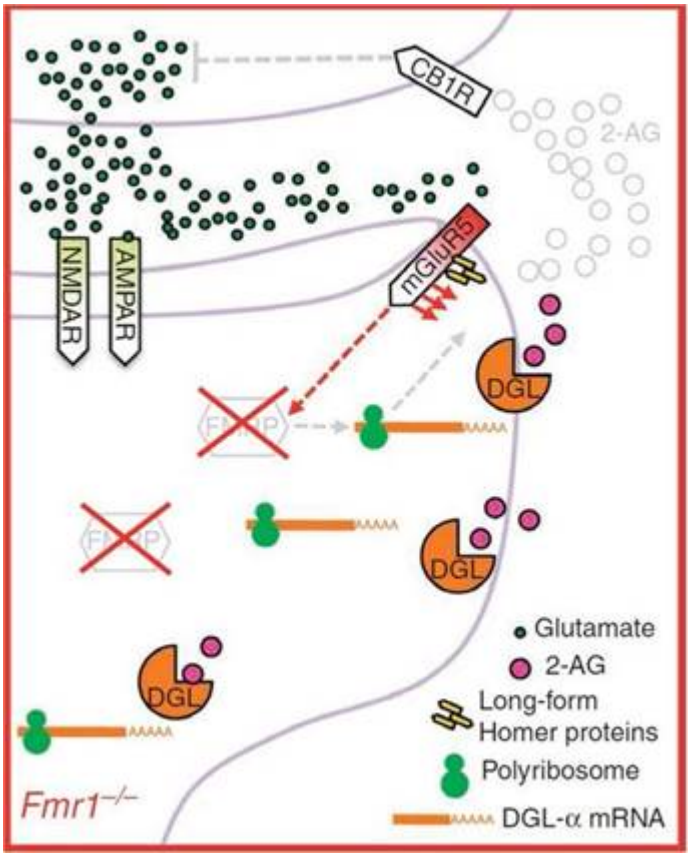
The Drug Product ZYN002 is a transdermal CBD gel. CBD is the primary non-euphoric cannabinoid contained in the *Cannabis sativa L.* plant. The CBD contained within ZYN002 is a pharmaceutically produced Active Pharmaceutical Ingredient (API) that is chemically identical to the CBD present in *Cannabis*.

Fragile X Syndrome (FXS) is a rare genetic condition caused by a mutation in the FMR1 gene (Haldeman-Englert 2013) located on the X chromosome. Mutations in the FMR1 gene, which silence the expression of the Fragile X mental retardation protein (FMRP), are characteristically found in patients with FXS. FMRP, a ribonucleic acid (RNA) binding protein, is important for normal synaptic function, synaptic plasticity, and the development of neuronal connections over time during brain maturation. The absence of FMRP in neurons accounts for many of the neuropsychiatric symptoms of Fragile X. Both males and females are at risk of developing symptoms linked to FXS. However, since the responsible gene is on the X chromosome, and only one changed (mutated) copy of the responsible gene is enough to cause the symptoms, FXS is inherited in an X-linked dominant pattern. In females (who have two X chromosomes), a mutation in one of the two genes can be sufficient to cause the disorder. In males (who have only one X chromosome), a mutation in the only copy of the gene can cause the disorder. While females are more likely to suffer from FXS, and are at increased risk of having a child with FXS, males typically exhibit more severe symptoms because of the X inactivation (Crawford et al. 2001).

FXS is characterized by many clinical symptoms, including anxiety (particularly social anxiety/avoidance), deficits in learning and cognition, sleep difficulties, seizures, social unresponsiveness and stereotypy (Lozano et al. 2015). These symptoms are typically managed by medications including melatonin and clonidine for sleep, selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines for anxiety, psychostimulants for hyperactivity and deficits in attention, and carbamazepine, valproic acid, and lamotrigine for seizures (Fragile X Clinical Research Consortium 2012). Unfortunately, many of these medications are ineffective for a number of patients with FXS and lead to multiple deleterious consequences (e.g., agitation, diarrhea, dizziness, nausea, vomiting, hair loss, memory, and sleep problems), some of which coincide with symptoms already experienced by patients with FXS (Fragile X Clinical Research Consortium 2012; Lozano et al. 2015). CBD is believed to have the potential for attenuating the loss of endogenous endocannabinoid signaling in FXS, allowing the FMRP protein deficiency inherent in FXS to be bypassed. Abnormalities seen in FXS appear to be rooted in dysregulation of the endocannabinoid pathways in the central nervous system. The endocannabinoid system consists of receptors in the brain and peripheral tissues that are involved in numerous physiological processes, as well as the endogenous cannabis-like ligands (endocannabinoids): anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The endocannabinoids bind to G-protein-coupled receptors, Cannabinoid receptor type 1 (CB_1) and Cannabinoid receptor type 2 (CB_2) (Mouslech and Valla 2009; Pacher et al. 2006) and modulate synaptic transmission throughout the central nervous system (Castillo et al. 2012; Ohno-Shosaku and Kano 2014). CB_1 receptors are abundantly expressed in the brain, and they are present at lower concentrations in a variety of peripheral tissues and cells. CB_2 receptors are expressed primarily in the immune and hematopoietic systems, as well as in the brain, pancreas, and bone (Pacher et al. 2006).

In patients with FXS, there is a reduction of endogenous stimulation of endocannabinoid receptors (see Figure 1). CBD has the capacity to interact with an FXS-compromised endocannabinoid system. Disruption of FMRP in FXS reduces the production of 2-AG, decreasing activation of CB_1 receptors in the central nervous system (Jung et al. 2012). CBD has been hypothesized to increase 2-AG availability (Elmes et al. 2015; Di Marzo and Maccarrone 2008), potentially attenuating or reversing one of the biological mechanisms of abnormal cellular function in FXS (Jung et al. 2012). Importantly, studies have shown that CB_1 protein expression is unaffected in FMR1 Knock Out (KO) mice, suggesting that the downstream elements of endocannabinoid signaling can be engaged even in the absence of FMRP (Zhang and Alger 2010).

Figure 1: Mechanism of Impaired Endocannabinoid Signaling in Fragile X



Legend: Disruption of FMRP (red X) in FXS reduces the production of 2-AG (clear circles), decreasing activation of CB1 receptors in the central nervous system. Loss of FMRP leads to a reduction of 2-AG production because the lack of FMRP expression disrupts diacylglycerol lipase (DGL) activity. (Jung et al. 2012)

In addition to the role of 2-AG, recent work has begun to highlight the potential importance of AEA in impacting social impairment as well as deficits in learning and memory among those with FXS. In an FMR1 KO mouse model of FXS, Qin and colleagues (2015) demonstrated that increased levels of AEA were associated with greater cognitive performance. Similarly, Wei et al. (2016) utilized a mouse model of FXS to show that increasing AEA activity resulted in improvements in social impairment. Much like its impact on 2-AG, CBD has been shown to increase levels of AEA (by binding to fatty acid binding proteins; FABP). FABPs are intracellular proteins that transport AEA to the catabolic enzyme fatty acid amide hydrolase (FAAH), an enzyme that breaks down AEA (Figure 4 in Bisogno et al. 2001; Table 3 in Pertwee

2008; Leweke et al. 2012; Elmes et al. 2015). Binding to FABP is thought to result in increased AEA availability and greater CB_1 activation.

Beyond providing benefit to FXS patients via increases in 2-AG and AEA availability, CBD may also affect synaptic plasticity. Mice lacking FMRP show alterations in synaptic plasticity, as evidenced by a reduction in long-term depression (LTD) of neuronal response properties in in vitro slice preparations (Zhang and Alger 2010). Preclinical data suggest that restoration of LTD in FMR1 KO mice requires activation of endocannabinoid receptors (Figure 4A in Zhang and Alger 2010). Therefore, it is hypothesized that CBD will increase synaptic plasticity in FXS, facilitating one of the basic cellular mechanisms thought to be associated with learning and improvements in cognition (Zhang and Alger 2010). Finally, more recent work has begun to identify deficits in GABA receptor expression among those with FXS. As FMRP has been shown in animal models to enhance expression of GABA receptors, the lack of FMRP among those with FXS has been associated with fewer GABA receptors (Lozano et al. 2015). Indeed, non-clinical studies in FMR1 KO mice have consistently shown down-regulation of the GABA system (Hare et al. 2014). As CBD acts as a positive allosteric modulator of GABA-A receptors (Bakas et al. 2017), CBD may act to enhance the binding affinity for GABA.

The existing literature combines to demonstrate that CBD may positively impact individuals with FXS through many mechanisms, including the endocannabinoid and GABA systems. While a number of drugs have been developed to target specific systems (e.g., GABA agonists), CBD may serve as a treatment that is likely to yield a multi-faceted benefit to individuals with FXS. CBD has not only been shown to be generally well tolerated relative to other treatments used in this population (Rohleder et al. 2016), but numerous studies have documented its benefits in terms of sleep quality (Carlini and Cunha. 1981; Chagas et al. 2014), anxiety (Bergamaschi et al. 2011; Viveros et al. 2005), cognitive impairment (Bergamaschi et al. 2011), and seizures (Friedman and Devinsky 2015). Anxiety symptoms are commonly associated with FXS. Both non-clinical (e.g., Blessing et al. 2015) and human (e.g., Bergamaschi et al. 2011) data have demonstrated the efficacy of CBD in the alleviation of anxiety symptoms. Reviews have highlighted anxiolytic effects of CBD in over 30 animal studies, using models for multiple types of anxiety (i.e., generalized anxiety models, stress-induced anxiety models, panic disorder models, and contextual fear conditioning models), as well as in a growing number of human studies, particularly in social anxiety. Several studies have explored potential mechanisms of action and demonstrated that the anxiolytic effects of CBD are likely due in large part to its effects on 5-HT_{1A} (serotonin).

5.2. Non-clinical Summary

To support the clinical development of ZYN002, Zynerba has relied upon:

- Data from non-clinical safety studies conducted by Zynerba with ZYN002, ZYN002 placebo gel, or CBD, the active ingredient in ZYN002,
- Publicly available information about the toxicity/safety of CBD and the excipients in ZYN002, and
- Clinical safety data generated by Zynerba with CBD and ZYN002.

Published non-clinical studies with CBD indicate that it does not produce adverse effects on nervous system, respiratory, or gastrointestinal function, although it does exhibit beneficial activity in some nervous system disorders (e.g., anticonvulsant and anxiolytic activity) and gastrointestinal disorders (e.g., anti-inflammatory activity). In a good laboratory practice (GLP)-

compliant study conducted by Zynerba, CBD did not affect cardiovascular system function even at an estimated plasma concentration (4350 ng/mL) that is [redacted] greater than the expected plasma maximum observed concentration (C_{max}) in patients over a 14-day period [redacted]. Based on this information, human subjects using ZYN002 are unlikely to experience adverse effects on nervous system, cardiovascular, respiratory, or gastrointestinal function.

ZYN002 was devoid of systemic toxicity in animals given topical doses applied to 10% of their body surface area daily for up to 26 weeks (rats) or twice a day for up to 13 weeks (minipigs).

[redacted]

- [redacted]
- [redacted]

- [redacted]
- [redacted]

[redacted]

- [redacted]
- [redacted]

Repeat-dose toxicity studies in rats and minipigs with ZYN002 have revealed no effects on endocrine systems or reproductive organs in either species. Published information suggests that CBD has the potential to affect reproductive function in animals, at least in males, possibly secondary to effects on sex hormone levels (Dalterio et al. 1984; Dalterio and deRooij, 1986).

Published information also suggests that administration of CBD to pregnant mice has the potential to affect development of offspring, at least to males (Dalterio et al. 1984; Dalterio and deRooij 1986).

CBD does not absorb UV or visible light over 290-700 nm (Hazekamp et al. 2005) and so ZYN002 does not present a potential phototoxicity hazard to human subjects.

[redacted]

5.2.1. Clinical Pharmacokinetics and Drug-Drug Interaction (DDI) Potential

Results from three completed Phase 1 studies, along with a population PK model, which includes results from ZYN2-CL-03, ZYN2-CL-04 and ZYN2-CL-05, have adequately described the PK of CBD following application of ZYN002 transdermal gel. Pharmacokinetic conclusions for these studies are as follows:

- [redacted]
- THC was not quantifiable in either plasma or urine.
- There is adequate animal NOAEL: human exposure ratios for AUC and C_{max}.

Population pharmacokinetic analyses indicate:

- [redacted]
- [redacted]

Because CYP3A4 and CYP2C19 are the major isoforms responsible for CBD metabolism, concomitant administration of drugs that inhibit these enzymes may result in higher exposure to CBD and drugs that induce these enzymes may result in lower exposure to CBD; therefore, strong inhibitors or inducers of CYP3A4 may increase or decrease the plasma concentrations of CBD and should be administered with caution.

As a potential perpetrator, CBD would not cause a clinically significant induction of CYP isoenzymes. However, CBD exhibited time-dependent inhibition of CYP2D6 and CYP1A2, which was reversible, and of CYP3A4, which was irreversible. Sensitive CYP3A4 substrates with a narrow therapeutic index or risk for severe toxicity should be avoided until this risk has been assessed with ZYN002. CBD did not inhibit human efflux and uptake transporters.

5.3. Clinical Summary

CBD studies completed with oral, inhaled, and intravenous (i.v.) formulations support a favorable tolerability and efficacy profile in several disease states, including topline data for ZYN002 in FXS. These efficacy and tolerability data provide a rationale for development of the transdermal delivery of synthetic CBD which is not subject to gastric acid degradation and first pass metabolism in the liver, and may achieve consistent blood levels for the treatment of child and adolescent patients with FXS.

5.3.1. Literature Review

Oral CBD has been clinically studied in healthy subjects and patients with a variety of conditions. The literature studies help to clarify the clinical potential of ZYN002 transdermal gel in the following areas:

- Most assessments have used a 600 mg oral dose of CBD, but subjects in several trials have been treated with oral CBD doses of 1200 mg or more (Zuardi et al. 2010; Matsuyama and Fu 1981), and one study employed a 1500 mg dose (Zuardi et al. 2006a). More recent epilepsy studies have titrated doses up to 25 – 50 mg/kg of oral doses in patients (age 1-30 years) (Devinsky et al. 2016; Devinsky et al. 2017). The mean CBD dose at 12 weeks was

22.9 mg/kg corresponding to a 732 mg oral dose for an average 10-year old patient. At the highest dose, Zynerba has studied a 780 mg daily dose at a CBD concentration of 4.2% (ZYN002 4.2%, 4.64 g twice a day).

Data from a recent randomized, double-blind, placebo-controlled trial of cannabidiol as add-on therapy in 171 patients with Lennox Gastaut syndrome, aged 2-55 years (mean of 15 years) supported the efficacy seen in the open-label study (Thiele et al. 2016). Patients received an oral solution of cannabidiol [Epidiolex®, GW Pharmaceuticals, London, UK; 100 mg per mL sesame oil-based solution] titrated to a dose of 20 mg/kg/day or placebo in two divided doses. A median reduction in total seizures of 45% in the CBD group versus 15% in the placebo group reported. Drop seizures were reduced by a median of 49% in the CBD group compared to 20% in the placebo group. The most common adverse events (>10% of cannabidiol-treated patients) were similar to those seen in the open-label study and included diarrhea, somnolence, decreased appetite, pyrexia, and vomiting.

Data supportive of the open-label results in patients in Dravet syndrome has also been reported from a recent randomized, double-blind, placebo-controlled trial of 120 children and young adults (mean age of 9.8 years, range 2.3 to 18.4 years) (Devinsky et al. 2017). Patients received an oral solution of cannabidiol [Epidiolex®, GW Pharmaceuticals, London, UK; 100 mg/mL sesame oil-based solution] titrated to a dose of 20 mg/kg/day or placebo in two divided doses. The primary endpoint was the change in convulsive-seizure frequency over a 14-week treatment period, as compared with a four-week baseline period. The median frequency of convulsive seizures per month decreased from 12.4 to 5.9 with cannabidiol, as compared with a decrease from 14.9 to 14.1 with placebo (adjusted median difference between the cannabidiol group and the placebo group in change in seizure frequency, -22.8 percentage points; 95% confidence interval [CI], -41.1 to -5.4; p=0.01). The percentage of patients who had at least a 50% reduction in convulsive-seizure frequency was 43% with cannabidiol and 27% with placebo (OR: 2.00; 95% CI; 0.93 to 4.30; p=0.08). The frequency of total seizures of all types was significantly reduced with cannabidiol (p=0.03), but there was no significant reduction in non-convulsive seizures. Adverse events that occurred more frequently in the cannabidiol group than in the placebo group included diarrhea, vomiting, fatigue, pyrexia, somnolence, and abnormal results on liver function tests.

Long-term exposure: — The 600 mg oral dose of CBD has been monitored in multiple long-term treatment situations. In at least seven studies, study periods of 3 months have been used (Martin-Santos et al. 2012; Bhattacharyya et al. 2012; Winton-Brown et al. 2011; Fusar-Poli et al. 2010; Bhattacharyya et al. 2010; Fusar-Poli et al. 2009, Borgwardt et al. 2008), and several patients have taken CBD for 4.5 months (Cunha et al. 1980). The 25 mg/kg oral doses were studied for 3 months in an open-label expanded access trial (Devinsky et al. 2016).

Psychoactive effects: — Studies of the potential psychoactive effects associated with CBD have not been widely reported until recently. Previous reports suggest the absence of psychoactive effects whether CBD is administered intravenously (Perez-Reyes et al. 1973) or orally (Englund et al. 2013; Martin-Santos et al. 2012; Bhattacharyya et al. 2012; Bhattacharyya et al. 2010; Zuardi et al. 2009). And pre-treatment with oral CBD 600 mg has been shown to inhibit the psychosis and cognitive impairment associated with intravenous (IV) THC 1.5 mg (Englund et al. 2013; Bhattacharyya et al. 2010). A recent placebo-controlled study with Epidiolex showed high rates of somnolence (36%), diarrhea (31%), fatigue (20%), vomiting (15%), and lethargy (13%) (Devinsky et al. 2017). These effects could be due to drug, underlying disease, potential conversion of oral CBD to THC or a combination of these factors. Previous work has shown that

in the presence of acidic reagents, CBD isomerizes to tetrahydrocannabinol (Gaoni and Mecoulam 1966). In simulated gastric fluid, CBD converts to Δ9-tetrahydrocannabinol, 9-α-hydroxy-hexahydrocannabinol and 8-hydroxy-iso-hexahydrocannabinol. All have psychoactive activity (Merrick et al. 2016; Watanabe et al. 2007).

Effects on vital signs or clinical lab tests: — CBD-treated subjects in clinical studies have shown no treatment-related effects on key vital sign indicators, including blood pressure and heart rate (Martin-Santos et al. 2012; Hallak et al. 2011; Fusar-Poli et al. 2009; Borgwardt et al. 2008; Zuardi et al. 1993a; Consroe et al. 1991; Zuardi et al. 1982, and Perez-Reyes et al. 1973), as well as electrocardiography (Guy and Flint 2003; Carlini and Cunha 1981; Cunha et al. 1980). Guy and Flint (2003) performed cardiac monitoring continually from pre-dose to four hours post-dose along with pre- and post-dose ECG. Heart rate, PR interval, QT interval and QRS width were evaluated pre- and post-dose. Elevated transaminases (ALT or AST) without association of drug induced liver injury have been identified in epilepsy studies of oral CBD in children and young adults (Devinsky et al. 2016; Devinsky et al. 2017; Bebin et al. 2017; Devinsky et al. 2018). Most cases were associated with the concomitant use of valproic acid and resolved with continued treatment or with decreasing the dose of valproic acid, CBD or discontinuation of CBD.

No association with response inhibition: — Findings from functional magnetic resonance imaging (MRI) and behavioral studies show that CBD modulates function in regions not usually implicated in response inhibition. In terms of clinical sequelae, these data help to explain why CBD does not impair motor or cognitive performance and has anxiolytic effects (Borgwardt et al. 2008).

5.3.2. ZYN002 Phase 1 studies

Three Phase 1 (ZYN2-CL-01, ZYN2-CL-02, and ZYN2-CL-08) studies investigating ZYN002 (CBD) administered via a transdermal delivery system have been conducted in healthy subjects and patients with epilepsy. In addition, one placebo study (ZYN2-CL-06) was conducted to evaluate the skin tolerability of the excipients in the ZYN002 formulation.

These efficacy and tolerability data provide a rationale for development of a transdermal delivery of synthetic CBD which is not subject to first pass metabolism and may achieve consistent blood levels for the treatment of patients with FXS and epilepsy.

A summary of the safety results from the Zynerba studies further supports the development of ZYN002. Four Phase 1 safety and tolerability study results as follows:

- ZYN2-CL-01, a single rising-dose study in healthy subjects (n=32) and patients with epilepsy (n=10) receiving ZYN002 (50, 100, 125, and 250 mg) or placebo showed that ZYN002 was safe and well tolerated at all doses. The incidence of treatment-emergent adverse events (TEAEs) associated with ZYN002 was similar to placebo in healthy volunteers. There were no serious adverse events (SAEs), no clinically significant changes in ECGs, vital signs or clinical laboratory results. ZYN002 had good skin tolerability, and there was no post-dosing erythema at 24, 48, 72, and 96 hours.
- ZYN2-CL-02, a seven-day repeat application, multiple rising dose study of healthy subjects (n=24) receiving ZYN002 (200, 250, 500 mg/day), and patients with epilepsy (n=12), receiving 500 mg/day showed that ZYN002 was safe and well tolerated at all doses. One subject receiving placebo discontinued due to an SAE, a device related infection (catheter) not related to trial drug. Most TEAEs were mild in intensity and there was only one severe

TEAE of back pain in a healthy subject administered ZYN002 500 mg. Most TEAEs were considered related to trial drug and either resolved or were resolving. Application site disorders were the most frequently reported TEAEs for both healthy subjects and epilepsy patients. The most frequently reported TEAEs were application site dryness and application site pruritus. Headache was the most frequently reported TEAE that was not associated with the application site. There were no clinically significant changes in ECGs or vital signs.

- [redacted]

- ZYN2-CL-08, a 14-day repeat application study in healthy subjects (n=42) receiving ZYN002 (394.8, 500, and 504 mg/day) or placebo showed that ZYN002 was safe and well tolerated at all doses. Most TEAEs were mild in intensity. Most TEAEs were considered not related to trial drug and either resolved or were resolving at the time of database lock. No SAEs were reported and no subjects discontinued from the study due to a TEAE. Headache and upper respiratory tract infection were the most frequently reported TEAEs and occurred in similar incidence for both ZYN002 and placebo. Application site dryness, application site pain, and application site pruritus were the next frequently reported TEAEs.

In the completed clinical pharmacology studies, ZYN002 was not associated with any impairment in critical areas of cognitive functioning often impacted by central nervous system (CNS) drugs, including divided attention and working memory and focused attention after single or repeat doses of ZYN002 at doses up to 250 mg as a single or BID dose. Assessments of psychological health also demonstrated no changes in depression and anxiety symptoms, or positive and negative affect, following administration of ZYN002.

5.3.3. ZYN002 Phase 2A Studies

Two Phase 2A studies have completed in patients with epilepsy (ZYN2-CL-03) and osteoarthritis (OA) (ZYN-CL-005).

- ZYN2-CL-03, a randomized, double-blind, placebo-controlled, multiple-dose study was conducted at 10 sites in Australia and 4 sites in New Zealand, to assess the efficacy and safety of ZYN002 administered as a transdermal gel to patients with focal epilepsy. The results showed ZYN002 to be safe and well tolerated with a safety profile consistent with previous studies. A total of 188 patients were randomized to receive either 195 or 390 mg/day of ZYN002 or placebo for 12 weeks. AEs were reported by 26 of 63 (41.3%) of placebo-treated patients, 31 of 63 (49.2%) of patients randomized to 195 mg/day of ZYN002 and 32 of 62 (51.6%) of patients randomized to 390 mg/day of ZYN002. For each dosing group, the majority of AEs were mild to moderate in intensity. The TEAE that occurred more frequently in patients on drug than those on placebo and occurred in at least 2% of patients included: nausea, vomiting, abdominal pain, diarrhea, fatigue, application site dryness, application site pruritus, nasopharyngitis, urinary tract infection, thermal burn (secondary to seizure related accident), decreased appetite, headache, ataxia, anxiety, and oropharyngeal pain.

- ZYN2-CL-005, a randomized, double-blind, placebo-controlled, multiple center, multiple dose study, was conducted at 10 sites in Australia, to assess the efficacy and safety of a 12 week repeat application in patients diagnosed with OA of the knee. The results showed ZYN002 to be safe and well tolerated with a safety profile consistent with previous studies. A total of 320 patients were randomized to receive ZYN002 at 250 or 500 mg/day or placebo for 12 weeks. Patients experiencing at least one treatment-emergent adverse event were similar between those on trial drug (n=106, 50%) and those on placebo (n=45, 42%). The TEAEs that occurred more frequently in patients on drug than those on placebo and occurred in at least at 2% of patients included: headache, dizziness, application site dryness, application site reaction, and application site pain.

5.3.4. Ongoing ZYN002 Clinical Studies

Two clinical studies are ongoing:

- ZYN2-CL-009 is an ongoing open-label trial to assess the safety and efficacy of ZYN002 administered as a transdermal gel to children and adolescents with FXS. The trial is being conducted at three investigative sites in Australia. The 12-week treatment period includes a 6-week titration period, after which the patient is to remain on their maintenance dose over the next 6 weeks of treatment. Following completion of the first 12 weeks of treatment, patients have the option of continuing into an extension phase of the trial, which allows for them to receive trial drug for up to 24 additional months. The last patient is expected to complete the extension phase in September 2019.

  Twenty-two patients were screened for the study and 20 were enrolled. Of the 20 patients, 18 completed the 12 weeks of open-label treatment and 13 of those 18 patients continued into the extension phase of the study. Currently, 12 patients are ongoing in the extension phase.

  Initial results of the 12-week treatment period include:

  Safety Results**:** Seventeen patients reported 33 TEAEs in this open-label trial. The majority of TEAEs were mild (25 [76%]) or moderate in intensity (8 [24%]) and were considered unrelated to treatment with CBD (25 [76%]). There were no SAEs reported, and 1/20 patients (5%) discontinued treatment due to a TEAE.

  The most common treatment emergent adverse event was mild-moderate gastroenteritis (6 [18%]), not related to trial drug and resolved during the study period. This was followed by upper respiratory tract infection, viral infection, influenza, otitis media, and tonsillitis (7 [21%], not related to trial drug and all resolved during the study period. One patient (129-003) developed a moderate application rash 35 days after starting trial drug. Trial drug was withheld one day and the application site was changed to the thighs for 6 days while the rash resolved. The rash did not recur. This patient also had a high eosinophil count, considered probably related to the skin rash. Upon a repeat laboratory test, the eosinophil count had decreased to just above normal. This patient currently is in the extension phase of the study.

  Other adverse events considered possibly related included symptoms of FXS (e.g., sensorial hyperactivity, nightmares, increased bedwetting and increase in self stimulatory talk).

  There were no clinically significant changes in vital signs, ECG, clinical laboratory results (other than eosinophilia noted above), and no reports of suicidal ideation for any patient throughout the trial.

Efficacy Results**:** Eighteen patients completed the efficacy assessments for this study through Week 12. Validated clinician- and caregiver-rated FXS scales were used to measure Baseline symptom severity and changes in symptoms between Baseline and Week 12 of the treatment period in this open-label trial.

The primary endpoint was the change from Baseline to Week 12 in the total score of the ADAMS, a caregiver-rated scale. Compared to the Baseline total score, the ZYN002 treated patients had a 45.81% reduction ($p<0.0001$) in the ADAMS Total Score. Furthermore, ZYN002 treated patients had statistically and clinically significant improvement compared to Baseline in all but one of the ADAMS subscales (i.e., Manic/Hyperactive Behavior, Social Avoidance, General Anxiety, and Compulsive Behavior) at Week 12. A significant change was not observed for the Depressed Mood subscale of the ADAMS. For other assessments, changes representative of clinically meaningful changes were observed, including in the ABC-FXS, PARS-R, and Visual Analog Scale (VAS).

ZYN2-CL-004 is an ongoing open-label continuation study to allow patients with focal epilepsy who completed ZYN2-CL-03 to continue to receive ZYN002. The primary objective is to assess the long-term safety and tolerability of ZYN002 in adult epilepsy patients over an 18-month period. The secondary objective is to evaluate efficacy in this population. Patients had to complete the 12 weeks of study treatment on protocol ZYN2-CL-03. ZYN002 is being administered as a transdermal gel with all patients starting on ZYN002 at doses equal to or higher than those used in the blinded study – CBD 195 mg every 12 hours (Q12 H) (±2 hours) (390 mg daily), with the option that after Month 1 to either increase or reduce the dose of ZYN002. The dose may be increased to 292.5 mg Q12H (±2 hours) (585 mg daily). After one month at the 585 mg daily dose, the Investigator has the option to increase the dose to 390 mg Q12H (± 2 hours) (780 mg daily). Of the patients who completed the blinded 12-week phase, 171 (98%) enrolled in the open-label extension study. Results from patients who had completed 3 (n=169) and 6 (n=63) months of treatment as of August 15, 2017, showed an approximate seizure reduction ranging from 30% (Month 3) to 65% (Month 6). The un-blinded use of ZYN002 for an additional three and six months appeared to result in clinically meaningful seizure reductions and was not due to an increase or addition of other AEDs.

6. TRIAL OBJECTIVES AND PURPOSE

6.1. Primary Objective

To evaluate the efficacy of ZYN002 administered as a transdermal gel formulation, for up to 12 weeks, in patients ages 3 to < 18 years, in the treatment of symptoms of Fragile X Syndrome. The primary endpoint is the change from Baseline to Week 12 in the ABC-C_{FXS} Social Avoidance subscale score.

6.2. Secondary Objectives

To further evaluate the efficacy of ZYN002 in the treatment of symptoms of FXS.

To evaluate the safety of ZYN002 in the treatment of symptoms of FXS.

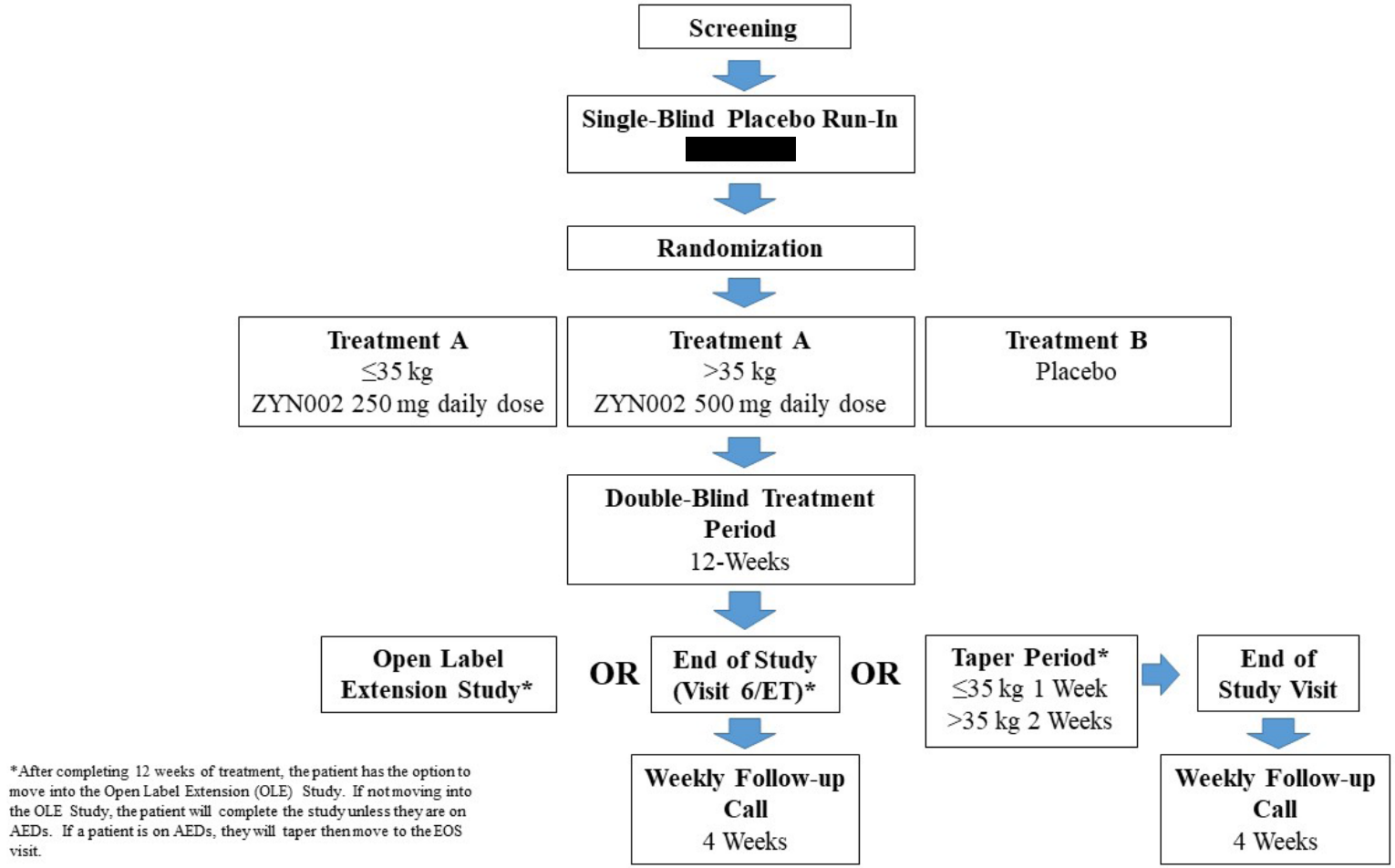
To evaluate CBD, CBD metabolite and THC exposure**.**

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

This is a randomized, double-blind, placebo-controlled, multiple-center study, to assess the efficacy and safety of CBD administered as ZYN002, a transdermal gel, for the treatment of child and adolescent patients with FXS. Male and female patients with FXS will be treated for 12 weeks with a [redacted] single-blind placebo lead-in preceding the 12-week double-blind treatment period. Approximately 204 male and female patients, ages 3 to < 18 years, will be randomized 1:1 to either trial drug or placebo to attain a sample size of 102 subjects in each treatment arm. Randomization will be stratified by gender, weight category and region.

Figure 2: Study Design



7.2. Number of Patients

Approximately 204 male and female patients will be randomized 1:1 to either trial drug or placebo. No more than 25% of the patients screened will be females.

7.3. Dose Rationale

[redacted]

7.4. Dose Schedule

Following the Screening period, eligible patients will start a single-blind Placebo Lead-In period for [redacted]. The placebo will be applied Q12H (± 2 hours). For patients ≤ 35 kg, each application will consist of one sachet of placebo containing 2.98 g of gel (two sachets in total per day). For patients > 35 kg, each application will consist of two sachets of placebo containing 2.98 g of gel in each sachet (four sachets in total per day).

At Visit 3, patients will be randomized to receive either ZYN002 or placebo. In a blinded fashion, ZYN002 treated patients who weigh ≤ 35 kg will receive 125 mg CBD Q12H (± 2 hours); total daily dose of 250 mg CBD. Patients who weigh > 35 kg will receive 250 mg CBD Q12H (±2 hours); total daily dose of 500 mg CBD. All patients will remain on their assigned dose during the 12 weeks of the treatment phase of the study. Study visits will occur at Week 4 (Visit 4), Week 8 (Visit 5), and Week 12 (Visit 6). Patients who have successfully completed the 12 weeks of the double-blind study will have the option to enroll in an OLE study. Patients not taking AEDs and not continuing in the OLE study will complete their EOS procedures at Visit 6/ET. Patients on AEDs who are not continuing in the OLE study will taper off trial drug and then have their EOS Visit. Telephone Follow-Up calls will occur during the taper period and weekly for all patients who do not enter the OLE study for four weeks post the last dose of trial drug. At Visit 2, parents/caregivers will be instructed on proper application of the study gel (active/placebo gel). [redacted] Parents/caregivers will apply all trial drug to clean, dry, intact skin, thoroughly massaging it into the right and/or left upper arms and shoulders until the area is dry. The study gel will be rubbed in completely and must be dry prior to dressing. [redacted] The application site should be covered to minimize sun exposure when going outside during the day. Parents/caregivers will

use gloves supplied by the Sponsor to apply the study gel. If redness occurs at the application site, the parent (after consultation with the Investigator) may switch the application site temporarily to the upper thighs.

7.5. Criteria for Study Withdrawal

Each parent/caregiver and patient has the right to withdraw the patient or his/herself from the study at any time without prejudice. If a parent/caregiver or patient withdraws the patient or his/herself from the study, the reason(s) must be stated in the CRF, and a final evaluation of the patient should be performed.

The Investigator may discontinue any patient's participation if he or she feels it is necessary for any reason during the study. The Investigator and Sponsor may discontinue any patient's participation for any reason including: any adverse event, a positive endorsement on Question 4 or 5 of the C-SSRS, clinically significant worsening in Fragile X symptoms, adverse change in any laboratory test, or failure to comply with the protocol. Samples for a post-study laboratory profile and follow-up safety exams should be obtained as soon after patient discontinuation as possible.

All effort will be made to ensure that the ET procedures will be completed at the time of discontinuation.

Patients who discontinue after randomization and before study completion may be replaced at the Sponsor's discretion.

7.6. Study Assessments

7.6.1. Overview of Study Assessments

Study procedures will be performed as summarized in the study schematic presented in Table 3. As patients as young as 3 years old will be enrolled in this study, parents/caregivers will play a significant role in the clinical trial. Most of the procedures will need to be performed by the parent/caregiver, including trial drug application and routine site visits (monthly) as required per the study protocol. Parents/caregivers should make every attempt to bring the patient to their scheduled visits within the window allowed by the protocol. If there are visits where it is impossible for a patient to attend the site visit, the parent/caregiver should contact the Investigator to determine if an exception can be made for a research nurse to visit the patient at home.

During the Screening Period, the site staff will review the eligibility criteria of the patient, review any medications including OTC medications the patient is taking, obtain the patient's medical history including their Fragile X Diagnosis, any seizure history and demographics, check their vital signs, perform an ECG (may be collected at either Screening or Visit 2), assess Tanner Stage, perform a physical and neurological exam and a skin assessment, obtain some blood and urine for analysis, and administer assigned scales. Blood samples will be taken for hematology and chemistry testing, T testing (males only), pregnancy testing (if applicable), and CGG repeat analysis. Patients will also have a pre-dose blood sample drawn for plasma levels of CBD and THC testing, and AEDs testing (for patients using AEDs). Urine will be collected for a urinalysis and drug screen. The scales administered include the ABC-C_{FXS}, ADOS-2® (ADOS®-2 will not be administered at Screening if it has been administered in the prior 6

months and the results are available), CGI-S, C-SSRS, MWC, PWC-20, VABS-3. The parent/caregiver will also be asked the following question "What are the five behavioral, emotional, or social problems that most impacted your son/daughter and his/her family in approximately the past year?" This is referred to as the "Qualitative Caregiver reported Behavioral Problems Survey". Optionally, if an IQ test was performed previously, those results may be provided to the Sponsor.

Patients and parents/caregivers will be required to visit the clinic at Week -2 (Visit 2), Study Day 1/Week 1 (Visit 3), Week 4 (Visit 4), Week 8 (Visit 5), and Week 12 (Visit 6) for vital signs, ECG (may be collected at either Screening or Visit 2), concomitant medication check, physical and neurological exams, Tanner Stage assessment, skin check examination, AE review including seizure assessment if applicable, and completion of the following questionnaires and scales: ABC-C_{FXS}, ADAMS, CGI-I, CGI-S, C-SSRS, Family Impact PedsQL ™, MWC, PWC-20.

In addition, parents/caregivers will complete a Caregiver Global Impression of Severity at Visit 2, Visit 3, and Visit 6/ET. Parents/caregivers will also complete a Caregiver Global Impression of Change at Visit 6/ET.

Patients who have successfully completed the 12 weeks of the double-blind study will have the option to enroll in an OLE study. Patients who do not enter the OLE study or who discontinue early will have a Telephone Follow-Up call weekly for four weeks following each patient's last dose of trial drug. The parent/caregiver will be contacted weekly via the telephone during the Follow-Up and complete the MWC and PWC-20.

Site-specific supplemental protocols may be implemented in a subset of ZYN2-CL-016 patients.

Table 3: Schedule of Assessments

	Screening	Placebo Lead-In	Double-Blind Treatment							
	Screening Visit [a] (Week -4)	Visit 2 Placebo Lead-In ([redacted] [b]	Visit 3 (Study Day 1) Randomization [c]	Visit 4 (Week 4)	Visit 5 (Week 8)	Visit 6 (Week 12) / Early Termination (ET)	Taper Period [d, e]	End of Study Visit (EOS)	Weekly Telephone Follow-Up [e] (Four Weeks)	Un-scheduled Visit(s)
	Days [redacted] to Day -[redacted]	Days [redacted] to Day [redacted]	Day 1 (± 3 days)	Day 28 (± 3 days)	Day 56 (± 3 days)	Day 84 (± 3 days)	(One-Two Weeks)	(± 3 days after Taper Period)	(± 3 days)	Skin Check Follow-up
Informed consent	X									
Review eligibility criteria	X	X	X							
Medical history & demographics	X									
Concomitant Meds	X	X	X	X	X	X		X	X	X
Complete physical and neurological examination [f]	X									
Targeted Physical and Neurological Examination						X		X		
Tanner Stage assessment [g]	X					X				
Vital signs [h]	X	X	X	X	X	X		X		X
12-lead ECG[i]	X			X		X		X		
Laboratory tests and urinalysis	X			X		X		X		
CGG Repeat Analysis	X									
Serum / urine pregnancy test [j]	X			X	X	X		X		
Urine Drug Screen (UDS)	X									
Fragile X Diagnosis	X									
Trial drug application [k]		X	X	X	X		X			
CBD, THC and AED Blood Samples [l]	X			X		X				
Qualitative Caregiver reported Behavioral Problems Survey	X									
Aberrant Behavior Checklist-FXS Specific (ABC-C_{FXS})	X	X	X	X	X	X				

	Screening	Placebo Lead-In	Double-Blind Treatment							
	Screening Visit [a] (Week -4)	Visit 2 Placebo Lead-In ([redacted] [b]	Visit 3 (Study Day 1) Randomization [c]	Visit 4 (Week 4)	Visit 5 (Week 8)	Visit 6 (Week 12) / Early Termination (ET)	Taper Period [d, e]	End of Study Visit (EOS)	Weekly Telephone Follow-Up [e] (Four Weeks)	Un-scheduled Visit(s)
	Days [redacted] to Day - [redacted]	Days [redacted] to Day [redacted]	Day 1 (± 3 days)	Day 28 (± 3 days)	Day 56 (± 3 days)	Day 84 (± 3 days)	(One-Two Weeks)	(± 3 days after Taper Period)	(± 3 days)	Skin Check Follow-up
Anxiety, Depression and Mood Scale (ADAMS)		X	X	X	X	X				
Clinical Global Impression-Severity (CGI-S)	X	X	X	X	X	X				
Clinical Global Impression-Improvement (CGI-I)			X	X	X	X				
Caregiver Global Impression of Severity		X	X			X				
Caregiver Global Impression of Change						X				
Family Impact PedsQL™		X	X			X				
Autism Diagnostic Observation Schedule® (ADOS®-2) [m]	X									
Vineland Adaptive Behavior Scales™ (VABS-3)	X									
Columbia-Suicide Severity Rating Scale (C-SSRS)	X	X	X	X	X	X	X	X	X	X
Marijuana Withdrawal Checklist -Short form (MWC)	X					X	X	X	X	
Penn Physician Withdrawal Checklist (PWC-20)	X					X	X	X	X	
Skin assessment exam [n]	X	X								
Skin irritation check [o]		X	X	X	X	X		X		X
Skin irritation check diary (daily) [o]		X	X	X	X	X	X	X		X
Adverse events	X	X	X	X	X	X	X	X	X	X
IQ Test Results (optional) [p]	X									

Table 3 Footnotes

a. The Screening visit procedures can be split into multiple visits for those patients whom the Investigator determines it would be difficult for the patient to undergo all Screening assessments on one day. The first part of the Screening procedures will include eligibility criteria.
b. If Visit 2 is split into multiple days, ABC-C_{FXS}, ADAMS, CGI-S and Family Impact PedsQL™ should be performed on the same day.
c. Patients will receive blinded study medication for the month between scheduled visits. Medication dispensed at Visit 3 will include either four weeks of placebo OR four weeks of 125 mg Q12H OR four weeks of 250 mg Q12H.
d. For Patients taking concomitant AEDs and not continuing in the OLE or are discontinuing prematurely: At Visit 6/ET, patients ≤ 35 kg will be reduced, in a blinded manner, total daily dose of 125 mg, over one week. For patients > 35 kg, receiving 500 mg daily dose, the dose of trial drug will be reduced in a blinded manner over two weeks. During the first week of taper, the dose will be reduced to 125 mg Q12H; (ZYN002 or placebo) (±2 hours); total daily dose of 250 mg, followed by a second week of taper from 250 mg total daily dose to total daily dose of 125 mg. After the one- or two-week taper, patients will discontinue from the study and attend EOS Visit.
e. The Weekly Telephone Follow-Up calls will occur during the taper and weekly for four weeks post patient's last dose of trial drug for those patients not continuing on to the open-label extension study.
f. Complete physical and neurological exam including height and weight at screening. Targeted physical and neurological exam at Visit 6/ET.
g. Tanner Stage assessment performed on adolescent patients 10 to less than 18 years of age or earlier if clinically indicated by onset of menarche or other signs of precocious puberty.
h. Vital signs include blood pressure, heart rate, respiratory rate, oral, infrared red forehead or tympanic temperature and will be recorded after the patient has been sitting for at least 5 minutes. Vital signs will be taken prior to blood draws. No food or drink is allowed 30 minutes before vital sign assessment.
i. The ECG scheduled for collection at Screening may be completed at the Screening Visit **or** Visit 2.
j. A serum pregnancy test will be completed at screening for females of childbearing potential. A serum or urine pregnancy test will be completed at Visits 4, 5, and 6 for females of childbearing potential.
k. At Visit 2, all patients will receive placebo for [redacted]. At Visit 3, patients will be randomized to receive placebo or ZYN002. No trial drug will be administered on the patient's last day of study. If the patient is continuing into the OLE study, they will receive the first dose of OLE trial drug on the last day of the ZYN2-CL-016 study. On days of study visits, the trial drug will be applied at the study site during the visit.
l. Blood will be collected for CBD and THC samples and AED plasma samples (for patients taking AEDs) pre-AED dose at Screening, pre-dose at Visit 4, and Visit 6/ET. Only patients currently taking AEDs will have blood samples for AED levels drawn. Time of the last trial drug dose (if applicable) and AED dose (if applicable) will be recorded. Time of blood collection will also be recorded.
m. ADOS®-2 will not be administered at Screening if it has been administered in the prior 6 months and the results are available.
n. Right and left upper arms and shoulders will be examined to determine there are no imperfections, lesions, or discolorations where trial drug could be applied.
o. A complete skin irritation check/ examination of the patient's application sites for irritation will be completed daily by parent/caregivers and at pre-dose by the site staff at every visit through the study and any unscheduled visits for skin irritation follow-up. When redness exists, efforts will be made to apply the gel to a non-red area of the shoulders and upper arms. If the skin check score is higher than '2' (moderate erythema) at any time, the caregiver will contact the site to determine if an unscheduled visit is required. If redness occurs at the application site, the parent (after consultation with the Investigator) may switch the application temporarily to the upper thighs. A de-identified photograph of the skin finding (or area) of interest may be taken after consultation with and approval of the Sponsor.
p. Please provide results of patient's IQ Test if this is available and is current within the past 5 years.

7.6.2. Informed Consent

Signed informed consent will be obtained at Screening from parent/caregivers and if applicable assent will be obtained from patients where developmentally appropriate. The informed consent form (ICF) will be signed by the parent/caregiver and the assent by the patient (if applicable) before any study procedures are undertaken. Details about how the ICF will be obtained and documented are provided in Section 17.3.

7.6.3. Fragile X Diagnosis

Patients must have a diagnosis of FXS through molecular documentation of FMR1 full mutation, at Screening.

7.6.4. Optional IQ Test Results

If the patient has had an IQ Test within the past 5 years, the Investigator may choose to include this at Screening. If this is not available, the Investigator should indicate this in the CRF.

7.6.5. Demographics

At Screening, patient demographic information will be collected and recorded in the CRF. No more than 25% of the patients screened will be females.

7.6.6. Medical History

A complete medical history will be obtained for each patient at Screening.

7.6.7. Vital Signs

Vital sign determinations, including sitting blood pressure, heart rate, respiratory rate, and oral, infrared forehead or tympanic body temperature, will be recorded at each study visit and any unscheduled visits. Vital signs will be recorded after the patient has been sitting for at least 5 minutes. No food or drink is allowed 30 minutes before vital sign assessment. Vital signs will be assessed prior to dosing and will be taken before any blood sample collection at all visits.

7.6.8. Concomitant Medication Review

Medication (prescription and OTC) use will be completed at Screening. A review of patient concomitant medication will be performed at all study visits and any unscheduled visits.

7.6.9. Adverse Event Review

A review of AEs will be performed at all study visits and any unscheduled visits.

Detailed information regarding AEs can be found in Section 12.2.

7.6.10. Complete and Targeted Physical and Neurological Examinations

A complete physical and neurological examination, including height and weight will be performed at the Screening Visit. A brief targeted physical (including heart, lungs, abdomen, extremities, and body weight) and neurological examination (mental status, gait/cerebellar testing, extraocular movements, and reflexes with additional areas depending on patient) will be performed at Visit 6/ET and the EOS Visit (if applicable). Any clinically significant changes will be documented.

Patient weight will be collected with minimal clothing (e.g., no coats, shoes, jumpers, or jackets).

A complete physical examination will only be performed at Visit 6/ET if considered clinically relevant.

7.6.11. Tanner Stage Assessment

The pubic hair growth (both sexes), genital (males only) and breast (females only) development of all adolescent patients (i.e., 10 to less than 18 years of age at the time at entry, or earlier if clinically indicated by onset of menarche or other signs of precocious puberty) will be assessed using Tanner Staging (Appendix 21.1). The assessments will be made by physical examination at Screening and Visit 6/ET. Patients will be assigned a value under each category of Pubic Hair Growth (both sexes), Genitals (male patients only), and Breasts (female patients only). Once a patient reaches a score of '5', the examination need not be performed again.

7.6.12. Electrocardiogram

A 12-lead resting ECG will be obtained at Screening (may be collected at either Screening or Visit 2), Visit 4, Visit 6/ET, and the EOS Visit (if applicable). As applicable, ECGs will be conducted pre-dose, within 60 minutes of trial drug application. A qualified physician will interpret, sign, and date the ECGs. Only clinical interpretations (normal, abnormal but not clinically significant, or abnormal and clinically significant) will be recorded in the CRF. ECGs will be reviewed by a central, independent reader and abnormal ECGs will be reviewed by the Zynerba Medical Monitor.

At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of ECG's.

7.6.13. Clinical Laboratory Testing

All blood samples will be collected and handled in accordance with the instructions from the central laboratory. For collection of laboratory samples, patients should fast for approximately eight hours prior to having blood drawn for blood laboratory analysis.

All clinically significant abnormal laboratory test results will be followed to a satisfactory resolution. Instructions regarding the collection, processing, and shipping of these samples will be provided by the laboratory chosen for this study.

Samples will be collected based on Table 3 Schedule of Assessments. Routine laboratory tests (clinical chemistry and hematology) and urinalysis will be collected at Screening, Visit 4, Visit 6/ET, and the EOS Visit (if applicable).

At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of blood samples.

7.6.13.1. Screening, Visit 4, Visit 6/ET and EOS Laboratory Assessments

Table 4: Laboratory Assessments

Laboratory Testing	Screening	Visit 4, Visit 6/ET, EOS
Chemistry		
Glucose	X	X
Total bilirubin	X	X
serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST)	X	X
serum glutamic pyruvic transaminase/alanine transaminase (SGPT/ALT)	X	X
Alkaline Phosphatase	X	X
blood urea nitrogen (BUN)	X	X
Creatinine	X	X
Amylase	X	X
Total Protein	X	X
Uric Acid	X	X
Sodium	X	X
Chloride	X	X
Bicarbonate	X	X
Potassium	X	X
Calcium	X	X
Phosphorus	X	X
Albumin	X	X
Triglycerides	X	X
Cholesterol: low density lipoprotein (LDL)	X	X
Cholesterol: high density lipoprotein (HDL)	X	X
Testosterone (males only)[a]		
Total	X	X
Free	X	X
Hematology		
White blood cell (WBC) with differential count	X	X
Red blood cell (RBC)	X	X
Hematocrit	X	X
Hemoglobin	X	X
Platelet Count	X	X
Hep & HIV[b]		
HBsAg	X	
HCV	X	
HIV	X	
Urine [c]		
Specific Gravity	X	X

Laboratory Testing	Screening	Visit 4, Visit 6/ET, EOS
pH	X	X
Protein	X	X
Glucose	X	X
Ketones	X	X
Bilirubin	X	X
Blood	X	X
Leukocyte esterase	X	X
Nitrite	X	X
Drug Screen [b]	X	

a. Testosterone should be collected at the same time of day throughout the study.
b. Hep & HIV testing and Drug Screen performed at Screening Visit Only.
c. Urine microscopic analysis if indicated.

7.6.14. Serum and Urine Pregnancy Tests

A serum pregnancy test will be performed for female patients of childbearing potential at the Screening Visit. A serum or urine pregnancy test will be performed for female patients of childbearing potential on Visit 4, Visit 5,Visit 6/ET, and the EOS Visit (if applicable). Any patient who becomes pregnant will be excluded or discontinued from the study, as applicable. The Investigator will advise on further medical attention, should this be necessary. The Investigator will ask for the patient's or caregiver's consent to follow the progress of the pregnancy and the birth and the health of the child. In the event of a positive pregnancy test in a female participant, where the caregiver may not be aware of sexual activity, the parent/caregiver will be notified of a confirmed positive pregnancy test. The Investigator will refer the patient and her parent(s)/caregiver to an appropriate obstetrician/gynecologist with relevant expertise for further management, following discussion of family planning and counseling options.

7.6.15. Skin Assessment Examination

At Screening and Visit 2, the shoulders and upper arms will be examined to determine there is no skin disease or condition, including eczema, psoriasis, melanoma, acne, contact dermatitis, scarring, imperfections, lesions, tattoos, or discoloration, that may affect treatment application, application site assessments, or absorption of the trial drug.. If there is irritation on the shoulders and upper arms, an assessment of the upper thighs will be made to determine if the upper thighs can be an alternative application site.

7.6.16. Skin Irritation Check Examination

Parents/caregivers will use a diary to complete a daily skin irritation check examination (Appendix 21.2). This diary will be reviewed at each study visit including any unscheduled visits. Every evening, they will record the skin irritation check score in the daily skin irritation check diary.

A complete skin check examination will be conducted by the Investigator at each study visit starting with Visit 2, including Unscheduled Visits and the End of Study Visit (if applicable) for skin redness follow-up.

When skin redness is noted, parents/caregivers should apply the gel to a non-red area of the shoulders and upper arms. If the skin irritation check score is higher than '2' at any time, the parent/caregiver will contact the study site to determine if an Unscheduled Visit is required. The Investigator will use discretion in suspending dosing for patients with a skin irritation check score of '4' but will, in all cases, immediately (within 24 hours) complete an adverse event report and contact their study CRA and the Zynerba Medical Monitor. A de-identified photograph of the skin finding (or area) of interest may be taken after consultation with and approval of the Sponsor. Digital photographs will be retained for information purposes only. If redness occurs at the application site, the parent (after consultation with the Investigator) may switch the application temporarily to the upper thighs.

Refer to Table 5 for the Skin Irritation Check Scale to be used for skin irritation examinations.

Table 5: Skin Irritation Check Scale

Score	Definition
0	No erythema
1	Minimal erythema
2	Moderate erythema with sharply defined borders
3	Intense erythema with or without edema
4	Intense erythema with edema and blistering/erosion

7.6.17. Blood Samples for AEDs, CBD and THC Levels

Blood samples for plasma levels of CBD and THC will be collected in all patients at the Screening Visit, Visit 4, and Visit 6/ET. Plasma may also be analyzed for CBD metabolite concentrations. In addition, pre-dose blood samples for adjunctive AED blood levels (for patients taking AEDs) will be collected at Screening and Visit 4, and Visit 6/ET. The times of blood sample collection, as well as the times of last dose (AED and trial drug) will be recorded. Plasma samples will be analyzed by [redacted] Plasma samples for adjunctive AEDs will be analyzed through a commercial laboratory.

At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of blood samples.

7.6.18. Blood Samples for CGG Analysis

Blood samples will be collected during the Screening Visit (although not required for inclusion) for analysis of CGG repeat size, as well as methylation status. Whole blood will be collected and stored by the Central Laboratory at -20°C and will be batch shipped periodically to the analytical laboratory selected by the Sponsor.

At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of blood samples.

7.6.19. Assessments of FXS Symptomatology

The same site rater should be used for all clinician-reported assessments across all visits. The same parent/caregiver should be used for all parent/caregiver-reported assessments across all visits but at minimum Visits 3 and 6/ET.

7.6.19.1. Aberrant Behavior Checklist-Community FXS Specific (ABC-C_{FXS})

The Aberrant Behavior Checklist- Community, 2nd Edition, will be scored using the FXS-specific factoring system (ABC-C_{FXS}). The ABC-C_{FXS} will be completed by the parent/caregiver, with support from the site staff, at Screening, Visit 2, Visit 3, Visit 4, Visit 5, and Visit 6/ET. The ABC-C_{FXS} asks responders to rate behaviors from "0 - not at all a problem" to "3 - the problem is severe in degree" across 58 questions. Use of the checklist has been validated in a variety of clinical populations, including in FXS. Scores will be analyzed using the FXS-specific factor structure such that 55 of the items resolve into 6 subscales (Irritability, Socially Unresponsive/Lethargic, Social Avoidance, Stereotypy, Hyperactivity, and Inappropriate Speech; Sansone et al. 2012).

7.6.19.2. Autism Diagnostic Observation Schedule® (ADOS®-2)

The ADOS (Western Psychological Services) is a semi-structured assessment of communication, social interaction and play or imaginative use of materials for individuals suspected of having Fragile X or other pervasive developmental disorders from 12 months through adulthood (Lord et al. 2000). The ADOS®-2 consists of various activities to allow the observation of social and communication behaviors related to the diagnosis of pervasive developmental disorders. The ADOS®-2 includes five modules for use with different age groups and language level. In this version, a Toddler Module has been included. For this patient population, the appropriate module will be utilized based on age and language level. Each subject will be administered one module. The assessment is administered by a certified rater and requires 35 to 40 minutes to complete. The ADOS®-2 is a diagnostic tool and this information will be used to document the presence of autism or other pervasive developmental disorders. This assessment will only be administered at sites with qualified individuals based on the publisher's criteria. The ADOS®-2 will be completed at Screening unless it has been administered within the prior 6 months and the results are available.

7.6.19.3. Anxiety, Depression, and Mood Scale (ADAMS)

The Anxiety, Depression, and Mood Scale will be completed by the parent/caregiver, with support from the site staff, at Visit 2, Visit 3, Visit 4, Visit 5, and Visit 6/ET. The ADAMS will be used as a comprehensive assessment of anxiety, depression, and mood among the FXS patients. The ADAMS is comprised of 28 items, which are rated on a scale of "0 - not a problem" to "3 - severe problem." The ADAMS yields a total score as well as five subscale scores: "Manic/Hyperactive Behavior," "Depressed Mood," "Social Avoidance," "General Anxiety," and "Compulsive Behavior." The ADAMS has been validated in clinical FXS populations, and has demonstrated good internal consistency and test-retest reliability. Interrater reliability has been shown to be satisfactory (Esbensen et al. 2003).

7.6.19.4. Clinical Global Impressions Scale-Severity and Improvement

The Clinical Global Impression Scales, Severity (CGI-S) and Improvement (CGI-I), are commonly used in clinical trials (Leigh et al. 2013) as they allow the clinician to utilize the history from the caregiver and incorporate the score into a clinical rating for the severity of symptoms. CGI-S will be assessed at Screening, Visit 2, Visit 3, Visit 4, Visit 5, and Visit 6/ET to judge the severity of the symptoms of FXS at the onset of and throughout the study. CGI-I will be assessed at Visit 3, Visit 4, Visit 5, and Visit 6/ET.

CGI-S is a 7-point scale that requires the clinician to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis. Considering total clinical experience, a patient is assessed on severity of mental illness at the time of rating 1 - normal, not at all ill; 2 - borderline mentally ill; 3 - mildly ill; 4 - moderately ill; 5 - markedly ill; 6 - severely ill; or 7 - extremely ill.

CGI-I is a 7-point scale that requires the clinician to assess how much the patient's illness has improved or worsened relative to a Baseline state at the beginning of the intervention and rated as: 1 - very much improved; 2 - much improved; 3 - minimally improved; 4 - no change; 5 - minimally worse; 6 - much worse; or 7 - very much worse. Information from both the clinician and the parent/caregiver history are incorporated into a clinical rating.

7.6.19.5. Caregiver Global Impression of Severity and Change Scales

Using a Caregiver Global Impression of Severity scale, parents/caregivers will be asked to complete a [redacted] scale rating the problems their child may be having with [redacted], and the child's behavior overall at Visit 2, Visit 3, and Visit 6/ET. Using the Caregiver Global Impression of Change scale, parents/caregivers will be asked at Visit 6/ET to complete [redacted] for the same questions asked in the Caregiver Global Impression of Severity scale.

7.6.19.6. Family Impact PedsQL™ (Pediatric Quality of Life Inventory)

The Family Impact PedsQL™ includes evaluations that are completed by the parent/caregiver. The scale will be completed at Visit 2, Visit 3, and Visit 6/ET. The newly developed PedsQL™ Family Impact Module is a 36-item scale, designed to measure the impact of pediatric chronic health conditions on parents and the family. The PedsQL™ Family Impact Module measures

parent self-reported physical, emotional, social, and cognitive functioning, communication, and worry. The Family Impact Module also measures parent-reported family daily activities and family relationships (Varni et al. 2004).

7.6.19.7. Vineland Adaptive Behavior Scales™, Third Edition (VABS-3)

The VABS-3 (Sparrow et al. 2016) was designed to assess adaptive functioning from birth to age 90. The VABS-3 includes a comprehensive structured interview administered to a parent/caregiver, within which four key domains are assessed: Communication, Daily Living Skills, Socialization, and Motor Skills, as well as Maladaptive Behavior (i.e., internalizing and externalizing). The scale has been successfully used in clinical trials of pharmaceuticals in children and adolescents with FXS (Berry-Kravis et al. 2017). The Adaptive Behavior Composite (a total score), the Communication, Daily Living Skills, and Socialization domains, as well as Maladaptive Behavior, will be evaluated in the present study. The VABS-3 will be completed at Screening to assess baseline adaptive functioning.

7.6.20. Columbia Suicide Severity Rating Scale (C-SSRS) (Children's Version)

The C-SSRS (Children's version) is to be completed at Screening, all study visits and any unscheduled visits. The C-SSRS assessment will be conducted only if the patients are of an appropriate age (6 years or older) and capable of understanding and answering the questions in the Investigator's opinion. For patients under the age of 6 or who are not capable of understanding and answering the questions in the Investigator's opinion, the Investigator will consider the trend in ABC-Irritability when assessing the risk of self-harm for an individual patient. Note that any completed suicide or suicidal attempt will be collected as an SAE.

7.6.21. Marijuana Withdrawal Checklist - Short form (MWC, Behavior Checklist)

The 15-item Marijuana Withdrawal Checklist – Short Form (Budney et al. 1999; 2003) retrospectively assesses cannabis withdrawal symptoms experienced during the past week. The symptoms included in the MWC consists of validated cannabis withdrawal symptoms: depressed mood, irritability, nervousness/anxiety, restlessness, increased aggression, increased anger, nausea, decreased appetite, stomach pains, shakiness/tremulousness, sweating, sleep difficulty, strange/wild dreams, craving to smoke marijuana, and headaches. The clinician will interview the parent/caregiver (or patient, if applicable), to rate each of the 15 symptoms on a four-point Likert-type scale (0=none, 1=mild, 2=moderate, 3=severe). A composite Withdrawal Discomfort Score (WDS) is calculated as the sum of the MWC items known to be valid, reliable cannabis withdrawal symptoms (American Psychiatric Association 2013).

The MWC is to be completed at Screening, Visit 6/ET, during the Taper Period, the EOS Visit, and during the four-week Weekly Telephone Follow-up calls.

7.6.22. Penn Physician Withdrawal Checklist (PWC-20)

The Penn Physician Withdrawal Checklist (PWC-20; Rickels et al. 2008) is a 20-item instrument developed to assess anxiolytic discontinuation symptoms. The clinician will interview the parent/caregiver (or patient, if applicable) to rate the presence/severity of 20 common anxiolytic withdrawal symptoms (e.g., insomnia, diarrhea, weakness) on a four-point Likert-type scale

ranging from 0 ("Not present") to 3 ("Severe"). Individual item scores are summed to yield a total score, with higher scores indicative of more severe withdrawal symptoms.

The PWC-20 is to be completed at Screening, Visit 6/ET, during the Taper Period, the EOS Visit, and during the four-week Weekly Telephone Follow-up calls.

7.7. End of Study (EOS) Visit and Early Termination (ET) Visit

Patients, who have successfully completed through Week 12 of the protocol and have been at least 90% compliant with the trial drug and visits, have the opportunity to continue in an OLE Study.

Patients not on AEDs who are not continuing in the OLE study or are prematurely discontinuing will complete the EOS procedures at Visit 6/ET. See Table 3 Schedule of Assessment for procedures at Visit 6/ET.

Patients taking concomitant AEDs who are not continuing in the OLE study or are prematurely discontinuing will taper down their study dose in the following manner at Visit 6/ET:

- For patients weighing ≤ 35 kg (250 mg daily dose), the dose of trial drug will be reduced, in a blinded manner, to a total daily dose of 125 mg ZYN002 or 2.98 g placebo gel each day for one week (one sachet each morning), after which time the patients will discontinue from the study and attend the EOS Visit. See Table 3 Schedule of Assessments for procedures at the EOS Visit. The parent/caregiver will be contacted weekly during the Taper Period and complete the MWC and PWC-20.
- For those patients > 35 kg (500 mg daily dose), the dose of trial drug will be reduced, in a blinded manner over two weeks: during the first week of taper the dose will be reduced to 125mg Q12H; (ZYN002 or placebo) (±2 hours); total daily dose of 250 mg, followed by a second week of taper from 250 mg total daily dose to a total daily dose of 125 mg ZYN002 or 2.98g placebo gel each day (one sachet each morning). After the taper, patients will discontinue from the study and attend the EOS Visit. See Table 3 Schedule of Assessments for procedures at the EOS Visit. The parent/caregiver will be contacted weekly during the Taper Period and complete the MWC and PWC-20.

[redacted]

Patients who do not enter the OLE study or who discontinue early will have Telephone Follow-Up calls weekly for four weeks following each patient's last dose of trial drug. The parent/caregiver will be contacted weekly via the telephone during the Follow-Up and complete the MWC and PWC-20.

8. SELECTION AND WITHDRAWAL OF PATIENTS

Patients participating in this study will have a diagnosis of FXS through molecular documentation of FMR1 full mutation. No more than 25% of the patients screened will be females. Siblings may be screened and enrolled based upon Investigator discretion and discussion with the Medical Monitor (See section 8.3.1). Patients must qualify based on completing all the inclusion criteria and none of the exclusion criteria to be eligible to enroll.

8.1. Patient Inclusion Criteria

1. Male or female children and adolescents aged 3 to <18 years, at the time of Screening.
2. Judged by the Investigator to be in generally good health at Screening based upon the results of a medical history, physical examination, 12-lead ECG, and clinical laboratory test results. Laboratory results outside of the reference range must be documented as acceptable by both the Investigator and Sponsor.
3. Patients must have a diagnosis of FXS through molecular documentation of FMR1 full mutation.
4. Patients with an ABC-C_{FXS} [redacted] score of [redacted] at Screening **OR** patients with an ABC-C_{FXS} [redacted] score of [redacted] at Screening **with an** ABC-C_{FXS} [redacted] score [redacted].
5. Patients have a CGI-S score of at least '3' at Screening.
6. Patients with a history of seizure disorders must currently be receiving treatment with a stable regimen of one or two AEDs or must be seizure-free for one year if not currently receiving AEDs.
7. Patients taking AEDs should be on a stable regimen for the four weeks preceding study Screening and taking no more than two.
8. Patients who are taking psychotropic medication(s) should be on a stable regimen of no more than two such medications for at least four weeks preceding study Screening and must maintain that regimen throughout the study. Psychotropic medications include (but are not limited to) antipsychotics, antidepressants, anxiolytics, and ADHD medications.
9. If patients are receiving non-pharmacological behavioral and/or dietary interventions, they must be stable and have been doing so for three months prior to Screening.
10. Patients have a body mass index between 12–30 kg / m^2 (inclusive).
11. Females of childbearing potential must have a negative serum pregnancy test at the Screening Visit and a negative serum or urine pregnancy test at all designated visits.
12. Patients and parents/caregivers agree to abide by all study restrictions and comply with all study procedures.
13. Patients and parents/caregivers must be adequately informed of the nature and risks of the study and give written informed consent (and assent if applicable) prior to Screening.
14. Parents/caregiver(s) must provide written consent to assist in trial drug administration.

15. In the Investigator's opinion, patients and parents/caregivers are reliable and willing and able to comply with all protocol requirements and procedures.

8.2. Patient Exclusion Criteria

Any of the following is considered criterion for exclusion:

1. Females who are pregnant, nursing, or planning a pregnancy; females of childbearing potential and male patients with a partner of childbearing potential who are unwilling or unable to use an acceptable method of contraception as outlined below for the duration of therapy and for three months after the last dose of study medication.

   Standard acceptable methods of contraception include abstinence or the use of a highly effective method of contraception, including hormonal contraception, diaphragm, cervical cap, vaginal sponge, condom, spermicide, vasectomy, or intrauterine device.
2. History of significant allergic condition, significant drug-related hypersensitivity, or allergic reaction to any compound or chemical class related to ZYN002 or its excipients.
3. Exposure to any investigational drug or device ≤ 30 days prior to Screening or at any time during the study.
4. Patient has ALT, AST, or total bilirubin levels ≥ 2 times the ULN or has alkaline phosphatase levels ≥ 3 times the ULN as determined from Screening safety laboratories.
5. Use of cannabis or any THC or CBD-containing product (aside from ZYN002) within three months of Screening Visit or during the study.
6. Patient has a positive drug screen, including ethanol, cocaine, THC, barbiturates, amphetamines (unless prescribed), benzodiazepines (except midazolam or comparable administered for blood draws and ECG collection), and opiates.
7. Patient is using the following AEDs: clobazam, phenobarbital, ethosuximide, felbamate, or vigabatrin.
8. Patient is using any strong inhibitor/inducer of CYP3A4 or sensitive substrate for CYP3A4 including the following medications: midazolam (except single doses administered for the purposes of obtaining blood samples and ECG's), oral ketoconazole, fluconazole, nefazadone, rifampin, alfentanil, alfuzosin, amiodarone, cyclosporine, dasatinib, docetaxol, eplerenone, ergotamine, everolimus, fentanyl, halofantrine, irinotecan, lapatinib, levomethadyl, lumefantrine, nilotinib, pimozide, quinidine, ranolazine, sirolimus, tacrolimus, temsirolimus, toremifene, tretinioin, vincristine, vinorelbine, and St. John's Wort.
9. Patients may not be taking minocycline for 30 days prior to Screening or throughout the study.
10. Patients may not be taking any benzodiazepines (except single doses administered for the purposes of obtaining blood samples and ECG's) at screening or throughout the study.
11. Patient has an advanced, severe, or unstable disease that may interfere with the study outcome evaluations.

12. Patient is expected to initiate or change pharmacologic or non-pharmacologic interventions during the course of the study.
13. Patient has an acute or progressive neurological disease, psychosis, schizophrenia or any psychiatric disorder or severe mental abnormalities (other than Fragile X Syndrome) that are likely to require changes in drug therapy or interfere with the objectives of the study or the ability to adhere to protocol requirements.
14. Patient has a positive result for the presence of HBsAg, HCV, or HIV antibodies.
15. Patient has known history of cardiovascular disease, advanced arteriosclerosis, structural cardiac abnormality, cardiomyopathy, serious heart rhythm abnormalities, coronary artery disease, cardiac conduction problems, exercise-related cardiac events including syncope and pre-syncope, risk factors for Torsades de pointes (TdP) (e.g., heart failure, hypokalemia, family history of Long QT Syndrome), or other serious cardiac problems.
16. Any clinically significant condition or abnormal findings at the Screening Visit that would, in the opinion of the Investigator, preclude study participation or interfere with the evaluation of the study medication.
17. Any skin disease or condition, including eczema, psoriasis, melanoma, acne, contact dermatitis, scarring, imperfections, lesions, tattoos, or discoloration, that may affect treatment application, application site assessments, or absorption of the trial drug.
18. History of treatment for, or evidence of, drug abuse within the past year.
19. Previous participation in a ZYN002 study.
20. Patient responds "yes" to Question 4 or 5 on the C-SSRS (Children) during Screening or at any time on study.

8.3. Randomization Criteria

After the Baseline Period, patients will qualify for randomization if they continue to meet the inclusion criteria and none of the exclusion criteria for the study. Patients eligible for randomization will be either:

- Patients with an ABC-C_{FXS} [redacted] score [redacted] on Study Day 1 prior to randomization, who have [redacted], **OR**
- Patients with an ABC-C_{FXS} [redacted] score of [redacted] **with an** ABC-C_{FXS} [redacted] score [redacted] on Study Day 1 prior to randomization, who have [redacted]

All inclusion and exclusion criteria must be reviewed for eligibility for randomization.

8.3.1. Sibling Options

Siblings may be screened and enrolled based upon Investigator discretion and discussion with the Medical Monitor. After the discussion with the Medical Monitor and confirming eligibility, the Investigator has three options:

Option 1: Siblings may be randomized at the same time and participate in the study (i.e., concurrent).

Option 2: One sibling is randomized, completes the 12 weeks of treatment, at which point the next sibling is randomized (i.e., sequential).

Option 3: Only one sibling is allowed to be enrolled and randomized. No other siblings are allowed to participate.

8.4. Patient Withdrawal Criteria

Each parent/caregiver and patient has the right to withdraw the patient or him/herself from the study at any time without prejudice. If a parent/caregiver or patient withdraws the patient or him/herself from the study, the reason(s) must be stated in the CRF, and a final evaluation of the patient should be performed.

The Investigator may discontinue any patient's participation if he or she feels it is necessary for any reason during the study. The Investigator and Sponsor may discontinue any patient's participation for any reason including: any adverse event, a positive endorsement on Question 4 or 5 of the C-SSRS, clinically significant worsening in Fragile X symptoms, adverse change in any laboratory test, or failure to comply with the protocol. All efforts will be made to follow-up adverse events until resolution.

Samples for a post-study laboratory profile and follow-up safety exams should be obtained as soon after patient discontinuation as possible.

All effort will be made to ensure that the End of Study procedures will be completed at the time of discontinuation.

9. TREATMENT OF PATIENTS

9.1. Description of Trial Drug

ZYN002 is a pharmaceutically manufactured CBD in a clear permeation-enhanced gel formulation. The drug product will be supplied as a transdermal gel and will be contained in a sachet. The gel will be applied to clean, dry, intact skin of the shoulders and upper arms or on a case by case basis the upper thighs. The drug product concentration is 4.2%. Dosing volume will be one (1) or two (2) sachets of 4.2% per day.

Placebo will consist of a clear gel similar in appearance and texture to ZYN002. The placebo will be supplied as a transdermal gel and will be contained in a sachet. The gel will be applied to clean, dry, intact skin of the shoulders and upper arms or on a case by case basis the upper thighs. Each application will consist of one or two sachets of placebo containing 2.98 g of gel applied Q12H (±2 hours).

SINGLE-BLIND PLACEBO LEAD-IN PERIOD

During the single-blind Placebo Lead-In period ([redacted]) patients ≤ 35 kg will receive placebo, applied Q12H (± 2 hours). Each application will consist of one sachet of placebo containing 2.98 g of gel (two sachets in total per day). Patients > 35 kg will receive placebo, applied Q12H (±2 hours). Each application will consist of two sachets of placebo containing 2.98 g of gel in each sachet (four sachets in total per day).

STUDY DAY 1, RANDOMIZATION

At Visit 3 (Study Day 1, Randomization) patients will be randomized to either:

Treatment A (Trial Drug)

Patients ≤ 35 kg will receive 125 mg CBD applied Q12H (± 2 hours); total daily dose of 250 mg CBD. Each application will consist of one sachet of ZYN002 CBD 4.2% concentration, containing 2.98 g of gel. This dosing will continue for the duration of the study.

Patients > 35 kg will 250 mg CBD applied Q12H (±2 hours); total daily dose of 500 mg CBD. Each application will consist of two sachets of ZYN002 CBD 4.2% concentration, each containing 2.98 g of gel. This dosing will continue for the duration of the study.

OR

Treatment B (Placebo)

Patients ≤ 35 kg will receive placebo, applied Q12H (± 2 hours). Each application (Q12H; ± 2 hours) will consist of one sachet of placebo, containing 2.98 g of gel. This dosing will continue for the duration of the study.

Patients > 35 kg will receive placebo, applied Q12H (± 2 hours). Each application (Q12H; ±2 hours) will consist of two sachets of placebo, each containing 2.98 g of gel. This dosing will continue for the duration of the study.

9.2. Concomitant Medications

Because CYP3A4 and CYP2C19 are the major isoforms responsible for CBD metabolism, concomitant administration of drugs that inhibit these enzymes may result in higher exposure to

CBD and drugs that induce these enzymes may result in lower exposure to CBD; therefore, strong inhibitors or inducers of CYP3A4 may increase or decrease the plasma concentrations of CBD and should be administered with caution.

As a potential perpetrator, CBD would not cause a clinically significant induction of CYP isoenzymes. However, CBD exhibited time-dependent inhibition of CYP2D6 and CYP1A2, which was reversible, and of CYP3A4, which was irreversible. Sensitive CYP3A4 substrates with a narrow therapeutic index or risk for severe toxicity should be avoided until this risk has been assessed with ZYN002. CBD did not inhibit human efflux and uptake transporters.

Refer to the following site for examples of strong inhibitors/inducers of CYP 3A4 and CYP 3A4 substrates with narrow therapeutic indexes:

https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.

9.2.1. Concomitant Medications Allowed

At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of blood samples and ECG's.

Patients may take hormonal contraception and AEDs (not in the exclusion criteria) during study participation. Other prescription or over-the-counter medications may be taken as approved in advance by the Investigator and recorded in the CRF.

AEDs may be added or discontinued as clinically indicated but patients on prior AEDs must remain on at least one concomitant AED in addition to ZYN002. Only AEDs not listed in the exclusions may be used.

9.2.1.1. Psychotropic Medications

Patients may only take one medication each to treat psychosis, anxiety or ADHD but may not take more than two medications total to treat all three conditions.

Psychotropic medications include (but are not limited to) antipsychotics, antidepressants, anxiolytics, and ADHD medications.

Since there are no medications with an indication specific to Fragile X Syndrome, treatment is symptomatic and consists of medications approved for other indications. The following clarifies which drug classes should be considered as psychotropic medications as they relate to inclusion number 8:

- Antipsychotics
- Antidepressants
- Anxiolytics (including buspirone or anticonvulsants prescribed for anxiety)
- Stimulants (e.g. methylphenidate)

Patients should be taking no more than two of the medications in the above classes and be on a stable regimen for four weeks prior to screening.

For the purposes of the protocol, the following sedative medications, when used as a single, bedtime dose – need not be considered one of the two allowed psychotropic medications:

- Clonidine
- Guanfacine
- Melatonin
- Diphenhydramine or other sedating antihistamine medications

Clonidine or guanfacine administered in the morning or more frequently than once daily at bedtime would, however, be counted as one of the two permitted psychotropic medications. There are certain medications used for their sedative properties that may have more profound or long-lasting psychotropic effects, so these would need to be considered as one of the two permitted psychotropic medications, e.g.:

- Quetiapine
- Trazadone
- Amitriptyline, or other tricyclic antidepressants

Additionally, metformin need not be considered as a psychotropic medication for purposes of inclusion criterion number 8.

9.2.2. Concomitant Medications Not Allowed

The following medications are not allowed: midazolam*****, oral ketoconazole, fluconazole, nefazadone, rifampin, alfentanil, alfuzosin, amiodarone, cyclosporine, dasatinib, docetaxol, eplerenone, ergotamine, everolimus, fentanyl, halofantrine, irinotecan, lapatinib, levomethadyl, lumefantrine, nilotinib, pimozide, quinidine, ranolazine, sirolimus, tacrolimus, temsirolimus, toremifene, tretinioin, vincristine, vinorelbine and St. John's Wort.

Patients may not take any benzodiazepines at screening or throughout the study*****.

***** Except single doses administered for the purposes of obtaining blood samples and ECG's.

9.3. Treatment Compliance

The Investigator will keep a current and accurate inventory of all clinical supplies received from the Sponsor. Any deviations from the protocol will be recorded.

All parents/caregivers will be provided with a sufficient supply of trial drug for the patient during their site visit. A Replacement kit(s), containing the patient's assigned trial drug, will also be supplied at each visit, as needed during the study. The Replacement kit(s) are to be returned at each study visit so that drug accountability can be performed. The Replacement kits are to be used in situations where a visit is outside the visit window and the patient required additional trial drug prior to a visit and/or trial drug is inadvertently lost/damaged/destroyed. Parents/caregivers will bring the used and unused sachets, in the appropriate study kit box (normal study supply kit and the Replacement kit), to the site at each study visit. The site will perform drug accountability (if all sachets were completely or partially utilized) at each study visit and record compliance for the previous visit.

10. TRIAL DRUG MATERIALS AND MANAGEMENT

10.1. Trial Drug

ZYN002 Transdermal Synthetic Cannabidiol Gel is a clear transdermal gel containing 4.2 % CBD for topical application.

Placebo is an identical clear transdermal gel without the CBD.

10.2. Trial Drug Packaging and Labeling

ZYN002 drug product and placebo will be packaged in sachets.

Study supplies will be labeled with a computer-generated label, which will include the following information:

- Protocol Number
- Intended Use
- Storage Conditions
- Labeled: Keep out of reach of children
- Identification Manufacturer/Sponsor

10.3. Trial Drug Storage

Trial drug and placebo are to be stored between 15°C - 25°C / 59°F - 77°F.

10.4. Trial Drug Preparation

Trial drug or placebo will be applied directly from the sachets as received.

10.5. Administration

At Visit 2 Placebo Lead-In, parents/caregivers will be instructed on proper application of the study gel (active/placebo gel). [redacted] Parents/caregivers will apply all trial drug to clean, dry, intact skin, thoroughly massaging it into the right and/or left upper arms and shoulders until the area is dry. The study gel will be rubbed in completely and the skin must be dry prior to dressing. [redacted] The application site should be covered to minimize sun exposure when going outside during the day. Parents/caregivers will use gloves supplied by the Sponsor to apply the study gel. If redness occurs at the application site, the parent (after consultation with the Investigator) may switch the application temporarily to the upper thighs.

10.6. Trial Drug Accountability

Parents/caregivers will bring the used sachets in a plastic bag, provided by the site, at each study visit. Any unused sachets will be returned in their box at each study visit to the clinic. The site will perform drug accountability (if all sachets were completely or partially utilized) at each study visit and record trial drug compliance since the previous visit. The number of used and unused sachets returned by the parent/caregiver will be counted and recorded on the appropriate CRF page.

10.7. Trial Drug Handling and Disposal

The site will place all used returned sachets in the plastic bag in the kit box labeled with the appropriate patient information. For drug accountability purposes the patient number, initials and Visit number will be written on the outside of the study kit box.

The study monitor will confirm the number of unused sachets of trial drug with the research facility and coordinate return or disposal of the used and unused supplies.

11. ASSESSMENT OF EFFICACY

Efficacy assessments are as indicated in Table 3 Schedule of Assessments.

11.1. Primary Endpoint

The primary endpoint is change from Baseline to Week 12 in the ABC-C_{FXS} Social Avoidance subscale score.

11.2. Key Secondary Endpoints

a) Change from Baseline to Week 12 in ABC-C_{FXS} Irritability subscale score.

b) Change from Baseline to Week 12 in ABC-C_{FXS} Socially Unresponsive/Lethargic subscale score.

c) CGI-I at Week 12/ET.

11.3. Secondary Endpoints

a) Percent of patients who have ≥ 25% improvement from Baseline in ABC-C_{FXS} Social Avoidance subscale score at Week 12/ET.

b) Percent of patients who have ≥ 25% improvement from Baseline in ABC-C_{FXS} Irritability subscale score at Week 12/ET.

c) Change from Baseline in ABC-C_{FXS} Social Avoidance, Irritability, and Socially Unresponsive/Lethargic subscale scores at Weeks 4 and 8.

d) Change from Baseline in ABC-C_{FXS} Stereotypy, Inappropriate Speech, and Hyperactivity subscale scores at Weeks 4, 8, and 12.

e) Change from Baseline to Weeks 4, 8, and 12 in ADAMS total score and subscale scores (Manic/Hyperactive Behavior, Depressed Mood, Social Avoidance, General Anxiety and Compulsive Behavior).

f) CGI-I at Weeks 4 and 8.

g) Percent of patients indicating improvement on the CGI-I (dichotomized) scale at Weeks 4, 8, and 12/ET.

h) Response rate for patients having both ≥ 25% improvement from Baseline in ABC-C_{FXS} Social Avoidance subscale score AND improved on CGI-I scale at Weeks 4, 8, and 12/ET.

i) Response rate for patients having both ≥ 25% improvement from Baseline in ABC-C_{FXS} Irritability subscale score AND improved on CGI-I scale at Weeks 4, 8, and 12/ET

j) Change from Baseline in CGI-S at Weeks 4, 8, and 12/ET.

k) Change from Baseline in Family Impact PedsQL^{TM} at Week 12/ET.

11.4. Method of Treatment Assignment or Randomization

Patients will be randomly assigned to treatment according to a computer generated randomization scheme. Randomization will be coordinated centrally through an interactive response system (IRT). The system will provide patient identification numbers at Screening, which are subsequently linked to the treatment assignments at randomization. Following completion of all evaluations at Visit 3, patients who meet all eligibility requirements and randomization criteria will be randomly allocated to active or placebo treatment groups using a 1:1 allocation ratio. Randomization will be stratified by gender (Male vs. Female), weight (≤ 35 kg vs. > 35 kg) and region (North America vs. non-North America).

11.5. Breaking the Blind

This is a double-blind study. During the conduct of the study, the patient, Investigator and study personnel at each center, and the Sponsor or its designated personnel directly involved in the clinical study will remain blinded to study treatment. The Investigator will not be provided with the randomization scheme. The randomization scheme will be maintained within the IRT. The Investigator should contact the Medical Monitor to discuss individual situations prior to breaking the blind.

If a medical emergency occurs and a decision regarding the patient's condition/treatment requires knowledge of the treatment assignment, the study blind may be broken for the specific patient via the IRT; the Investigator will immediately notify the Medical Monitor of the situation. The date, time, and reason for un-blinding must be documented in the source document and in the appropriate section of the CRF. Additionally, the documentation received from the IRT indicating the blind break must be retained in a secure manner, in the patient’s source documents.

12. ASSESSMENT OF SAFETY

12.1. Safety Parameters

Safety assessments will include collection of AEs including seizure assessment if applicable, physical and neurological examination, Tanner stage assessment, 12-lead ECG, clinical laboratory assessments (hematology, chemistry, and urinalysis), vital signs, C-SSRS, MWC, PWC-20, and findings from the skin irritation checks following treatment. MADDERS® will be used to systemically capture and adjudicate abuse-related events. All patients who receive at least one dose of randomized trial drug will be included in the safety analysis.

At the discretion of the investigator, patients will be allowed a short acting sedative (such as midazolam) to assist with the collection of blood samples and ECG's.

12.2. Adverse and Serious Adverse Events

Throughout the study, the Investigator will monitor each patient for evidence of drug intolerance and for the development of clinical and/or laboratory evidence of an AE. An AE assessment will be made by the Investigator on a routine basis at each study visit. In order to standardize the approach to assessing the occurrence of AEs, the Investigator should make a judgement as to any change in condition or AEs that were not present before trial drug administration when he obtains the patient's response to how they are feeling. Patients having AEs will be followed until they return to normal or become stabilized.

Investigators and study staff will be trained to recognize abuse, misuse and addiction. They will be instructed to document all cases in which study drug is taken in a manner that deviates from the protocol, is unaccounted for, or is used by anyone other than the study participant. In addition, MADDERS® will be used to systematically capture and adjudicate abuse-related events in this study (Treister et al. 2016).

All AEs that occur during the course of the study (starting at the time the informed consent is signed) must be reported in detail on the appropriate CRFs and patient's source document record and on any other report form required by national law. All efforts will be made to follow-up events until resolution.

12.2.1. Definition of Adverse Events

12.2.1.1. Adverse Event (AE)

An AE is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition (e.g., diabetes, congestive heart failure, rheumatoid arthritis, psoriasis) that occurs at any time after signing of the ICF whether or not it is considered to be related to treatment. Worsening of an existing medical condition is when a condition present at the time of signing of the ICF (e.g., cancer, diabetes, gout) becomes more severe, more frequent, or increased in duration during the study. Hospitalizations for pretreatment conditions (e.g., elective cosmetic procedures) or surgeries that were planned before entry into the study are not considered AEs. AEs will be collected from the signing of the ICF through the 4th week of the weekly follow-up phone call.

The parents/caregivers of patients with a current epilepsy and/or seizure diagnosis will be asked at each study visit if there has been any increase in frequency or severity of the patient's seizures. If an increase in frequency or severity is noted, this will be recorded as an AE in the CRF.

The Investigator is responsible for reviewing laboratory test results and determining whether an abnormal value represents for the patient a change from the time of signing of the ICF. In general, abnormal laboratory findings without clinical significance (based on the Investigator's judgement) should not be recorded as AEs. Clinically significant changes occurring after the signing of the ICF are considered AEs; however, the reported adverse event should include the underlying diagnosis or resulting clinical sequelae. Patients having clinically significant AEs will be followed until they return to normal or become stabilized.

12.2.1.1.1. Application site irritation scores

12.2.1.1.2. Pregnancy

If a patient or partner of a patient becomes pregnant during or after exposure to the trial drug (within three months of trial drug discontinuation) received in this study, the Investigator will immediately discontinue the patient from the study (if patient is still on study) and contact the Sponsor or designee. The Investigator will complete the Sponsor's (or designee's) Clinical Pregnancy Notification Form and alert the Sponsor within two days of learning of the pregnancy. Diligent efforts will be made to determine the outcome for all pregnancy exposures in the clinical trial. Information on the status of the mother and the child will be forwarded to the Sponsor.

Generally, follow-up will occur within six to eight weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported. Both maternal and paternal exposure will be collected. For exposure involving the female partner of a male patient, the necessary information must be collected from the patient, while respecting the confidentiality of the partner.

Pregnancy itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even though the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The date of onset of the adverse event will be collected. Also, the date of the resolution of the adverse event will be collected.

12.2.1.2. Serious Adverse Event (SAE)

Any adverse event that results in one or more of the following is considered an SAE.

1. Death
2. Life Threatening - The patient was at risk of death at the time of the event. It does not refer to the hypothetical risk of death if the adverse event were more severe or were to progress
3. In-patient hospitalization (admission or prolongation of existing hospitalization). Planned hospitalizations to treat a pre-existing condition or an inpatient stay required by the protocol is not considered to be an SAE unless otherwise specified in the study protocol. If there is any doubt whether the hospitalization constitutes an SAE, it should be treated as serious and reported to the Sponsor.
4. Persistent or significant disability / incapacity - Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions. This includes the inability to work. This is not intended to include transient interruptions of daily activities.
5. Congenital abnormality or birth defect - Any structural abnormality in patient offspring that occurs after intrauterine exposure to treatment.
6. Other Medically Important Events - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon medical judgement, they may jeopardize the patient or may require intervention to prevent one of the outcomes listed above.

12.2.1.3. Other Adverse Event (OAE)

OAEs will be identified by the study Medical Monitor during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the patient from the study, will be classified as OAEs. For each OAE, a narrative may be written and included in the Clinical Study Report.

12.3. Recording Adverse Events

Adverse events spontaneously reported by the patient/parent/caregiver and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. Clinically significant changes as judged by the Investigator in laboratory values, blood pressure, and pulse will be reported as AEs. Abnormal values that constitute an SAE or lead to discontinuation of administration of trial drug must be reported and

recorded as an AE/SAE. Information about AEs/SAEs will be collected from the signing of the ICF until the last Telephone Follow-Up call. The AE term should be reported in standard medical terminology when possible.

For each AE, the Investigator will evaluate and report the onset date, resolution date, intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

The relationship of the adverse event to the trial drug will be assessed using the following definitions:

- Related (An adverse event has a strong temporal relationship to trial drug or recurs on re-challenge, and another etiology is unlikely).
- Not Related (An adverse event is due to underlying or concurrent illness or effect of another drug and is not related to the trial drug (e.g., has no temporal relationship to trial drug or has a much more likely alternative etiology). The alternative etiology will be recorded in the CRF.

Intensity will be assessed according to the following scale:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 12.2.1.2. An AE of severe intensity may not be considered serious.

Should a pregnancy occur, it must be reported and recorded on the Sponsor's (or designee's) pregnancy form. Pregnancy itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even though the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

12.4. Reporting Adverse Events

If any protocol defined expedited event, or serious AE occurs whether related to trial drug or not, the Investigator must notify the Sponsor within 24 hours by telephone or email.

24 Hour SAE Telephone: [redacted]

Email: [redacted]

All SAEs (related and unrelated) will be recorded from the signing of the ICF until the last Telephone Follow-Up call. Any SAEs considered related to the investigational product and discovered by the Investigator at any time after the study should be reported. All SAEs must be

reported to Zynerba within one business day of the first awareness of the event. The Investigator must complete, sign and date the SAE form, verify the accuracy of the information recorded on the SAE form with the corresponding source documents, and send a copy by e-mail to Zynerba.

Additional follow-up information, if required or available, should all be e-mailed to Zynerba within one business day of receipt and this should be completed on a follow-up SAE form and placed with the original SAE information and kept with the appropriate section of the CRF and/or study file.

Zynerba is responsible for notifying the relevant regulatory authorities of certain events. It is the Investigator's responsibility to notify the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

13. ASSESSMENT OF PHARMACOKINETICS

13.1.1. Blood Levels of AED, CBD and THC

13.1.1.1. Blood Sample Collection

[redacted] Plasma samples will be shipped to the central laboratory. The samples will be stored until the central laboratory batch ships them to the analytical laboratory chosen by the Sponsor.

An inventory of the samples shipped will accompany the package.

13.1.1.2. Sample Analysis

Plasma samples will be analyzed [redacted]

Plasma samples for adjunctive AEDs will be analyzed through a commercial laboratory.

All analyses will be completed to GLP standards. Results will be provided in a separate bioanalytical report.

14. STATISTICS

A Statistical Analysis Plan (SAP) will be prepared after the protocol is approved. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives.

14.1. Sample Size Determination

The sample size was estimated for the primary efficacy endpoint using Power Analysis and Sample Size Software (PASS 15) (NCSS, 2017). Sample size requirements were based on a previous Phase 1/2 trial, ZYN2-CL-009, in patients with FXS and on published results in the literature with other studied treatments in this indication. [redacted]

14.2. Analysis Populations

The safety analysis set will include all enrolled patients who receive at least one dose of trial drug (which includes placebo run-in).

The randomized safety analysis set will include all patients who receive at least one dose of randomized trial drug. The randomized safety analysis will be the primary population for safety analyses. The treatment group assignment for this population will be the treatment actually received.

The full analysis set (FAS) will include all patients in the randomized safety analysis set who have both a baseline and a post-baseline ABC-C_{FXS} assessment. The FAS set will serve as the primary efficacy study population. The treatment group assignment for analysis will be the treatment the patient was randomized to.

The PK population will include all patients who have at least one sample collected during treatment and analyzed for determination of the CBD and THC concentrations.

14.3. Efficacy Analysis

Descriptive statistics (mean, median, standard deviation, minimum, and maximum) for continuous data and number (n) and percentage (%) for categorical data will be presented for all efficacy and safety parameters.

All efficacy assessments will be summarized by randomized treatment group.

14.3.1. Primary Efficacy Analyses

Null Hypothesis:

The null-hypothesis to be tested is that there is no difference in effect between the active treatment group and placebo in the change from Baseline to Week 12 in the ABC-C_{FXS} Social Avoidance subscale.

The change from Baseline in the ABC-C $_{FXS}$ Social Avoidance subscale will be analyzed using MMRM. The MMRM will contain fixed effects for treatment, visit, study site, region and treatment-by-visit interaction as factors as well as the continuous fixed covariate of Baseline value for the dependent variable of interest. For the MMRM model, descriptive statistics will be presented by visit, which will include least-squares mean, least-squares standard errors of the mean, least-squares meant treatment difference (active-placebo), 95% confidence intervals around the least-squares (LS) mean treatment difference and p-values for between treatment group tests. MMRM will model the data specifying an unstructured variance-covariance structure. Details will be presented in the SAP for situations where convergence is not met using the unstructured variance-covariance structure.

14.3.2. Key Secondary Efficacy Analyses

The key secondary endpoint, change from Baseline to Week 12 in the ABC-C_{FXS} Irritability, and ABC-C_{FXS} Socially Unresponsive/Lethargic subscale score, will be analyzed using the same MMRM approach as the primary endpoint.

The CGI-I at Week 12/ET will be analyzed using the Wilcoxon rank sum test.

The response rate for the percent of patients who have ≥ 25% improvement at Week 12/ET from Baseline in the ABC-C_{FXS} Irritability, and ABC-C_{FXS} Social Avoidance subscale score will be compared using the chi-square test and binomial confidence intervals.

14.3.3. Secondary Efficacy Analyses

All secondary endpoints including change from Baseline in ABC-C_{FXS} Social Avoidance, Irritability, Inappropriate Speech, Stereotypy and Hyperactivity subscales scores and changes from Baseline in the ADAMS total score and subscale scores, will also be analyzed using the same MMRM approach as the primary endpoint.

The statistical comparison of active versus placebo for the CGI-I at weeks 4 and 8 will be based on Wilcoxon rank sum test. The statistical comparison of active versus placebo for the CGI-S will be based on a covariate adjusted Cochran-Mantel-Haenszel (CMH) test, adjusting for the baseline score as the covariate.

A patient will be considered as "improved" on the CGI-I scale if they are rated as "1-very much improved" or "2-much improved". All other ratings, including missing values, will be considered "not improved" (Guy 1976; Berry-Kravis et al. 2017). The response rates for the percent of patients improved using CGI-I , and for patients having both ≥ 25% improvement from Baseline in ABC- C_{FXS} Social Avoidance subscale score AND improved on CGI-I scale will be compared using the chi-square test and binomial confidence intervals.

Change from Baseline in Family Impact PedsQL^{TM} will be analyzed using ANCOVA. ANCOVA models will contain fixed effects for treatment, region and study site with Baseline value for the dependent variable of interest as a covariate.

For MMRM/ANCOVA, descriptive statistics will be presented by visit, which will include LS mean, LS standard errors, LS mean treatment difference (active-placebo) and associated 95% confidence interval (CI) and p-values for between treatment group tests.

14.3.4. Exploratory Analysis

Results of exploratory analysis of the Caregiver Global Impression of Severity, the Caregiver Global Impression of Change, and the Qualitative Caregiver reported Behavioral Survey will be reported using descriptive statistics.

14.4. Safety Analysis

AEs will be tabulated by the actual treatment dose of trial drug received at the time of initiation of the adverse event and classified by system organ class and preferred term using MedDRA. Additionally, AEs will be tabulated overall (total number of AEs and total number of patients with AEs).

MADDERS® will be used in this study to systematically capture and adjudicate abuse-related events. (Treister et al. 2016). Data from the MADDERS® will be summarize and presented separately from other AEs.

Symptoms of withdrawal will also be assessed by comparing change over time in the total score on the MWC and total score on the PWC-20 from Baseline to Week 12/ET, weekly during the taper period and weekly for four weeks after the patient stops study drug.

Vital signs assessments (actual and change from Baseline) will be summarized using descriptive statistics and presented by actual treatment group.

Safety laboratory and urinalysis assessments (actual and change from Baseline) will be summarized by actual treatment group.

ECGs (actual and change from Baseline) will be summarized by actual treatment group. ECG results including any clinically significant findings will be summarized at each study visit.

Application site irritation check will be summarized using counts and percentages at each respective site check score (‘0’, ‘1’, ‘2’, ‘3’, or ‘4’) by actual treatment group.

14.5. Pharmacokinetic Analysis

Plasma concentrations for CBD, THC and concurrent AEDs will be summarized using descriptive statistics and presented by randomized treatment group (active or placebo) and by Screening and Treatment time points. [redacted]

14.6. Interim Analysis

There is no planned interim analysis for this study.

14.7. Adjustments for Multiplicity

15. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

15.1. Study Monitoring

Before an investigational site can enter a patient into the study, a representative of Zynerba will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of Zynerba or its representatives. This will be documented in a Clinical Study Agreement between Zynerba and the Investigator.

During the study, a monitor from Zynerba or representative will have regular contact with the investigational site, for the following:

- Provide information and support to the Investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed.
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (e.g. clinic charts).
- Record and report any protocol deviations not previously sent to Zynerba.
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to Zynerba and those SAEs that met criteria for reporting have been forwarded to the IRB/IEC.

The monitor will be available between visits if the Investigator(s) or other staff needs information or advice.

15.2. Audits and Inspections

Authorized representatives of Zynerba, a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of a Zynerba audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. The Investigator should contact Zynerba immediately if contacted by a regulatory agency about an inspection.

15.3. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

The Investigator must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval, and all materials approved by the IRB/IEC for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

16. QUALITY CONTROL AND QUALITY ASSURANCE

Original patient records such as research facility records and laboratory reports should be available at each site for source document review by Sponsor personnel. Source document review is the verification of the information recorded on CRFs with that recorded in the original patient records. In this study, source document review of specific types of information will be conducted for all patients.

To ensure compliance with Good Clinical Practices and all applicable regulatory requirements, Zynerba, or its representative, may conduct a quality assurance audit. Please see Section 18.1 for more details regarding the audit process.

17. ETHICS

17.1. Independent Ethics Committee or Institutional Review Board

GCP requires that the clinical protocol, any protocol amendments, the Investigator's Brochure, the ICF, and all other forms of patient information related to the study (e.g., advertisements used to recruit patients) and any other necessary documents be reviewed by an IEC/IRB. IEC/IRB approval of the protocol, ICF and patient information and/or advertising, as relevant, will be obtained prior to the authorization of drug shipment to a study site. Any amendments to the protocol will require IEC/IRB approval prior to implementation of any changes made to the study design.

17.2. Ethical Conduct of the Study

The study will be conducted in accordance with the Declaration of Helsinki and GCP guidelines. Each Investigator will complete the Statement of Investigator form (FDA 1572 form). The Investigator is responsible for reporting to the IEC/IRB modifications, safety updates, amendments, and deviations of the protocol that impact on patient safety.

At appropriate intervals, the clinical monitor will visit the site during the clinical study and assure that the Investigator's obligations are being fulfilled. Per GCP requirement for confirmatory proof of patient files**,** a copy of all records must be retained with the files of the PI for a minimum of 15 years. These records include the Confidential Follow-up Forms and other documents such as ICFs, laboratory reports, and other source documents, drug accountability forms, IEC/IRB approvals, protocols, and CRFs.

17.3. Patient Information and Informed Consent

The study protocol and ICF (and assent if applicable) must be approved by the Investigator's IEC/IRB and a copy of the approved ICF (and assent if applicable) must be supplied to the Sponsor. The parent/caregiver will be asked to read the consent form. If the parent/caregiver decides that the patient should participate in the study, the parent/caregiver will be asked to sign and date the form as evidence of consent and as the Legalized Authorized Representative. Each parent/caregiver must voluntarily sign and date a consent form before the patient participates in this study. It is the obligation of the Investigator or their representative to explain the nature of the study to the parent/caregiver. The Investigator will document in the patient's medical chart that the parent/caregiver has signed an ICF to participate in an investigational trial, a copy of the ICF will be given to the parent/caregiver, and the original should be retained with the patient's study records.

Patient names will remain confidential. Only the patient number, patient initials, and birth date will be recorded in the CRF. The parent/caregiver will give explicit permission for representatives of the regulatory authorities and the IRB/IEC to inspect the patient's medical records to verify the information collected. Parents/caregivers will be informed that all protected health information and clinical data are saved in a confidential manner.

All study data are confidential with restricted access. Information made available for inspection will be handled in the strictest confidence and in accordance with all state, local, and federal data

protection/privacy laws, including, without limitation, the Health Insurance Portability and Accountability Act (HIPAA).

All parents/caregivers of patients in the United States will provide written authorization to disclose protected health information either as a part of the written ICF or as a separate authorization form. The authorization will contain all required elements specified by the FDA 45 Code of Federal Regulations (CFR) 164. The parent/caregiver will be informed that the authorization does not expire. The parent/caregiver will be informed they can revoke this authorization at any time by giving written notice. If the parent/caregiver revokes authorization the patient will not be permitted to continue in the study. The revoking of authorization cannot be considered retroactive to data already collected under an existing authorization. Individual patient medical information obtained during this study is confidential and its disclosure to third parties, other than those mentioned in this section, is strictly prohibited. In addition, medical information obtained during this study may be provided to the patient's personal physician or to other appropriate medical personnel when required in connection with the patient's continued health and welfare.

The Investigator will maintain a personal patient identification list (patient and enrollment numbers with the corresponding patient names) to enable records to be identified.

18. DATA HANDLING AND RECORDKEEPING

18.1. Inspection of Records

The Sponsor will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, trial drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

Source documents are defined as original documents, data and records. This may include hospital records, clinical and office charts, laboratory data/information, evaluation checklists, pharmacy dispensing and other records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

In addition, the Investigator will permit trial-related audits, IEC/IRB review, and regulatory inspection(s), providing direct access to source data documents.

18.2. Retention of Records

The Investigator must maintain all documentation relating to the study for a period of 15 years from study completion. If it becomes necessary for the Sponsor or the Regulatory Authority to review any documentation relating to the study, the Investigator must permit access to such records.

19. PROTOCOL MODIFICATIONS IMPLEMENTED DURING THE COVID-19 PANDEMIC

Due to the pandemic of Coronavirus (COVID-19), adjustments to the ZYN2-CL-016 protocol have been implemented, where necessary, and only at the time of the pandemic. These changes may remain in place until such time that restrictions for travel and attendance at study visits are lifted. The specific modifications are described below.

19.1. Changes to Allow for Remote Patient Visits

During the COVID-19 pandemic, when patient and parent/caregiver travel is restricted and/or investigative sites have restricted patient visits, remote patient study visits will be permitted. Zynerba has developed patient packets for the sites, corresponding to each remote visit, that will allow for many (but not all) of the study procedures to be performed remotely by trained site staff. Those assessments that can be performed remotely include ABC-CFXS, ADAMS, CGI-S, CGI-I, Caregiver Global Impression of Change, Caregiver Global Impression of Severity, Family Impact Peds QLTM, C-SSRS, MWC, PWC-20, skin assessment exam (if using Skype, Facetime, etc.), daily skin diary, check on concomitant medications and check on adverse events. As with onsite visits, the clinician or site staff conducting the remote visit will read the caregiver the rating instructions before ratings are completed. The site will witness the parent/caregiver completing the assessments when video visits are conducted. The parent/caregiver will return the completed assessments, along with the skin irritation diary, to the investigational site, using pre-printed shipping materials. The site will then upload the completed questionnaires to the electronic system. Safety assessments will all be conducted with the parent/caregiver on the phone or via video call.

19.2. Changes to Allow for Investigational Product (IP) Shipment from Investigative Site to Parent/Legal Guardian

[redacted]

19.3. Changes to Allow for Courier Return of Investigational Product from Parent/Legal Guardian to an Approved Depot

[redacted]

19.4. Changes to Allow Patients to Attend Local Laboratory Facilities to Obtain Blood Collection for Safety Laboratory Analyses

To help ensure patient safety during the COVID-19 pandemic when onsite visits cannot be conducted, Zynerba has arranged for trial participants to be seen at local laboratories when a study visit requires a blood collection for safety laboratories. Each of these local centers will have a work specifications worksheet, which describes the analyses to be completed and the process to follow for the Zynerba clinical trial participants.

The Principal Investigator will complete a requisition for the patient to visit a local facility for sample collection. The local laboratory facility will collect the blood sample, analyze, and report results to the Principal Investigator. Site staff will be able to recommend the facility in closest proximity to the patient.

19.5. Shifting of Some Safety Assessments to the Next Onsite Visit

Since some of the patient visits will be completed remotely, several of the assessments normally done at an in-person visit will be shifted to the next onsite visit that a patient can attend. These include ECG; targeted physical/neurological examinations, including Tanner stage assessments, if applicable; and vital signs. Although the Principal Investigator will be able to check in on the

well-being of the patient at remote visits, and assess for any new/increasing adverse events, the ECG, vital signs and targeted physical and neurological examinations will be deferred until the next visit on site. Although local laboratories can be used for safety assessments, if a patient cannot travel to the closest local laboratory facility, the safety labs will be deferred to the next study visit.

20. PUBLICATION POLICY

All information concerning ZYN002 and the Sponsor's operations, such as ZYN002 patent applications, formulas, manufacturing processes, basic scientific data, or formulation information, supplied by the Sponsor and not previously published, is considered confidential information.

The information developed during the conduct of this clinical study is also considered confidential and will be used by the Sponsor in connection with the development of ZYN002. This information may be disclosed as deemed necessary by the Sponsor. To allow for the use of the information derived from this clinical study and to ensure complete and thorough analysis, the Investigator is obligated to provide the Sponsor with complete test results and all data developed in this study.

This confidential information shall remain the sole property of the Sponsor, shall not be disclosed to others without the written consent of the Sponsor, and shall not be used except in the performance of this study.

Notwithstanding the foregoing, the rights, duties and obligations of Sponsor, PI and other Investigators involved in the study concerning the publication of the methods, results of, and conclusions from the study shall be subject to the applicable provisions of the clinical trial research agreement entered into between Sponsor and as applicable, the institutions, study sites and/or Investigators who undertake to perform the study. At this time, Sponsor has not definitively determined whether it will publish study results. However, under the terms of the Australia Medicines Standard Clinical Trials Research Agreement (CTRA) (the "Standard CTRA"), the New Zealand Association of Clinical Research standardized Clinical Trial Research Agreement ("sCTRA"), and Sponsor's template Clinical Trial Research Agreement for use with institutions, study sites and/or Investigators who undertake study activities in the United States ("Sponsor CTRA"), even if Sponsor determines not to publish study results, Investigators shall have the right to publish such results so long as they comply with terms of the Standard CTRA, sCTRA or Sponsor CTRA, as applicable.

21. LIST OF REFERENCES

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