CLINICAL PROTOCOL

A Phase II, Placebo-Controlled, Randomized, Double-Blind Study to Assess the Safety, Tolerability and Efficacy of terpenes-enriched CBD-predominant cannabis oil, Administered to Pediatric Subjects with Autism Spectrum Disorder (ASD)

Protocol Number: 0336-24-SZMC

Investigational Product: Terpenes-enriched cannabis oil T1/C28

Protocol Version: September 1, 2025, Version 3.0

ClinicalTrials.gov Identifier: NCT07199218

Prepared by:	Title	Date	Signature
Adi Aran, MD	Principal Investigator	01/09/2025	

Revision History – After recruitment of first patient

Version	Author	Date	Description

Short Synopsis

Title	A Phase 2, Placebo-Controlled, Randomized, Double-Blind Study to Assess the Safety, Tolerability and Efficacy of terpenes-enriched cannabis oil T1/C28, Administered to Pediatric Subjects with Autism Spectrum Disorder (ASD)
Short Title	A placebo-controlled trial of terpenes-enriched CBD-predominant cannabis oil in children with ASD
Running Head	Terpenes-enriched CBD oil in ASD
Protocol Number	0336-24-SZMC
Study Center	Shaare Zedek Medical Center, Jerusalem, Israel
CinicalTrials.gov Identifier	NCT07199218
- **Registration Date**	September 29, 2025
Ethic Approval Date	May 25, 2025
Trial Commencement	TBD
Planned Number of Participants	78
Study duration per participant	Placebo-controlled phase: 8 weeks; After completing the first 8-week period, participants who received placebo will receive terpenes-enriched CBD, and participants who received the study drug will continue with the same treatment for another 8 weeks.
Study Intervention	Cannabidiol (CBD; 7.2mg/kg/d); Tetrahydrocannabinol (THC; 0.257mg/kg/d); and Terpenes (0.5mg/kg/d). Study intervention will be administered separately **as an add-on** to any existing treatment.
Primary outcome	ASD-associated behavioral problems, using the Aberrant Behavior Checklist-Irritability Subscale (ABC-I) score - change from baseline to week 8.

Contents

A Placebo-Controlled Trial of Terpenes-Enriched, Cannabidiol-Predominant Cannabis Oil in Children with ASD

Background: Autism spectrum disorder (ASD) encompasses a wide range of neurodevelopmental disorders with diverse etiologies and symptoms. Efforts to develop a single pharmacological treatment for the core symptoms of ASD have been unsuccessful. Approved antipsychotic medications for managing associated irritability often result in substantial metabolic side effects. Cannabidiol (CBD), a non-psychoactive cannabinoid, targets multiple neuronal and glial pathways to reduce excitation and inflammation. In contrast, tetrahydrocannabinol (THC), a psychoactive cannabinoid, directly activates the endocannabinoid system, which is impaired in ASD, and decreases social anxiety and hostility. Preliminary trials in children with ASD have demonstrated the efficacy of a CBD-predominant formulation combined with low-dose THC. Preclinical data suggest that efficacy may be further enhanced by enriching cannabinoid compounds with selected terpenes, which activate CB1 and CB2 receptors both directly and by modulating THC interactions at these receptors.
Objective: To assess the impact of terpenes-enriched, CBD-predominant, cannabis oil on children with ASD in a randomized, double-blind, placebo-controlled trial.
Specific aims:
The primary aim is to assess the effect of the study drug compared to placebo on behavioral problems, using the Aberrant Behavior Checklist-Irritability Subscale (ABC-I) score - change from baseline to week 8.
The main secondary aim is to assess the effect of the study drug compared to placebo on core ASD symptoms using the Vineland ™ Adaptive Behavior Scales (3rd edition, VABS3)-Socialization Domain Score, and the Social Responsiveness Scale 2nd edition (SRS-II) total score - change from baseline to week 8.
Other secondary aims include: (1) To assess the tolerability and safety of the study drug compared to placebo, using detailed questionnaires, physical examination, blood count, and liver enzyme tests. (2) To assess global change in ASD-associated symptoms since baseline using the Clinical Global Impression- Improvement (CGI-I) and the Caregiver Global Impression of Change (CGIC) assessments – at week 8. (3) To assess changes in the quality of life, sleep parameters, and gastrointestinal problems using the Quality of Life in Autism Questionnaire (QOLA) – Parent version, the Children's Sleep Habits Questionnaire (CSHQ) and a modified gastrointestinal sign and symptoms inventory (GISSI) - change from baseline to week 8. (4) To compare the effect of CBD-predominant, cannabis oil (study drug) with terpenes-enriched CBD, using the above assessments - change from baseline to week 8 and week 17.
Study design: In this phase 2, randomized, double-blind, placebo-controlled, parallel-group study,78 children with ASD and behavioral problems, will be randomized (in a 1:1 ratio) to receive either placebo or a terpenes-enriched CBD-predominant cannabis oil (CBD 7.2mg/kg/d; THC 0.257mg/kg/d; Total terpenes 0.5mg/kg/d). The treatment will be given as an add-on to any existing treatment, for 8 weeks. The treatment dose will be up-titrated over 8days. After completing the first 8-week period, participants who received placebo will receive terpenes-enriched pure CBD, and participants who received the study drug will continue with the same treatment for another 8 weeks.
Inclusion criteria: A diagnosis of ASD according to the DSM-5 criteria; Age 4-12 years; Bodyweight between 12.5kg and 57.5kg; CGI-Severity score ≥ 4; ABC-I score ≥ 18; and SRS-II total T score ≥ 66.
Exclusion criteria: Seizures or changes in anticonvulsive therapy within the last 16 weeks; Treatment with cannabinoids or any change in the pharmacological or behavioral treatment in the

4 weeks prior to randomization; Participants or caregivers are unlikely (in the investigator's opinion) to comply with all trial requirements including blood tests.
Significance: Previous studies suggest that CBD-rich cannabis oil (CBD:THC ratio = 20:1) reduces disruptive behavior and improves core symptoms in children with ASD. However, THC use remains controversial. While whole-plant extracts may enhance benefits via the "entourage effect" (synergy of cannabinoids and terpenes), cannabinoid isolates offer precise and consistent dosing. Enriching cannabinoid isolates with selected terpenes preserves the entourage effect and enables reducing THC dosage while ensuring both efficacy and consistency.

Introduction

There is no established pharmacological treatment for the core symptoms of autism spectrum disorder (ASD), persistent deficits in social communication, and repetitive, restrictive patterns of behavior.[1] Risperidone and aripiprazole have been approved by the U.S. Food and Drug Administration (FDA) to treat comorbid irritability[2] but these medications often cause obesity and metabolic syndrome.[2,3] Additionally, Up to 80% of children with ASD also have sleep disorders.[4,5] Cannabinoid therapy with various THC to CBD ratios is being increasingly used to alleviate sleep disorders regardless of cause. However, currently there is insufficient evidence to support this line of treatment.[6-9]

Cannabinoids, have been assessed before to treat core and comorbid symptoms in children with ASD with mixed results.[10]

The partial effects demonstrated in these studies can be attributed to the heterogeneous and multifactorial etiology of ASD which should probably better be addressed with polytherapy.

The current study aims to assess the combined impact of cannabidiol (CBD), terpenes and low-dose THC which target multiple key biochemical hubs in antioxidative, anti-inflammatory, and cytoprotective pathways.[10]

We hypothesize that combined treatment with these 3 compounds will lead to significantly higher improvement in disruptive behavior and core autistic symptoms compared with placebo in children with ASD.

The endocannabinoid system is a cell-signaling system composed of the cannabinoid receptors, their endogenous ligands [*endocannabinoids, mainly* anandamide (AEA) and 2-AG], transporters, and enzymes that produce and degrade the endocannabinoids.[11]

Studies in animal models suggest a reduced endocannabinoid tone in ASD.[12-16] Stimulation of the endocannabinoid system[12-17] have improved social deficits in some models. Additionally, children with ASD have lower peripheral endocannabinoid levels.[16,18,19]

The main components of the cannabis plant (phytocannabinoids) are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC activates the type-1 cannabinoid receptor (CB_1R) – the main cannabinoid receptor in neurons (Figure 1). THC is psychoactive (causes a 'high' feeling) and can lead to anxiety, addiction, cognitive decline, motivational loss, and psychosis.[20] CBD, on the other hand, is a negative allosteric modulator of the CB_1R which decreases the effects of CB_1R agonists such as THC. Nevertheless, CBD can activate the endocannabinoid system by decreasing the reuptake of the endocannabinoids (AEA and 2-AG) and the degradation of AEA (Figure 1). CBD is not psychoactive and has a relatively high toxicity threshold.[20] It also appears to have anxiolytic, antipsychotic, antiepileptic, and neuroprotective properties that may be mediated through receptors such as serotonin 5-HT_{1A}, TRPV1, GPR55, $GABA_A$, D2, and PPARγ, and by inhibiting adenosine reuptake (Figure 1).[21-25]

Besides its direct effects on multiple receptor targets in neurons, CBD has prominent direct and indirect antioxidant and immunomodulatory effects[26-33] that were extensively studied in microglia[34-37] (Figure 2). Immune system dysregulation has a direct impact on numerous neurodevelopmental processes.[38] In ASD, immune dysregulation includes differential monocyte, macrophage, and microglia responses,[39-44] and abnormal cytokine, and T cell levels.[38,45,46]

Additionally, in most of the ASD cases, there are indications of mitochondrial function impairments including lactic acidemia, abnormal lactate: pyruvate ratios, and accumulation of alanine in the plasma and urine indicating defects in oxidative phosphorylation.[47-49]

Hence, the immunomodulatory and antioxidative effects of CBD and its effects on the endocannabinoid system and various other receptors could be beneficial in ASD.

Previously, we demonstrated in a placebo-controlled trial that a CBD-rich cannabis extract (with a CBD-to-THC ratio of 20:1) can reduce disruptive behaviors associated with ASD and improve core autistic symptoms with acceptable tolerability.[50] However, due to the potential risks of THC,

particularly in children with neuropsychiatric disorders, ongoing and emerging placebo-controlled studies are focusing on the effects of CBD isolates devoid of THC.[51]
Nevertheless, animal studies,[17] preliminary evidence from randomised studies,[10] and our clinical experience suggest that CBD alone has significantly lower efficacy. Recent preliminary clinical and preclinical studies indicate that efficacy might be enhanced by enriching CBD-dominant compounds with specific terpenes. These terpenes can activate CB1 and CB2 receptors directly or modulate THC interactions with these receptors.[52-57]
In the current study, we aimed to evaluate the efficacy, safety, and tolerability of terpene-enriched, CBD-predominant cannabis oil with a CBD-to-THC ratio of 28:1

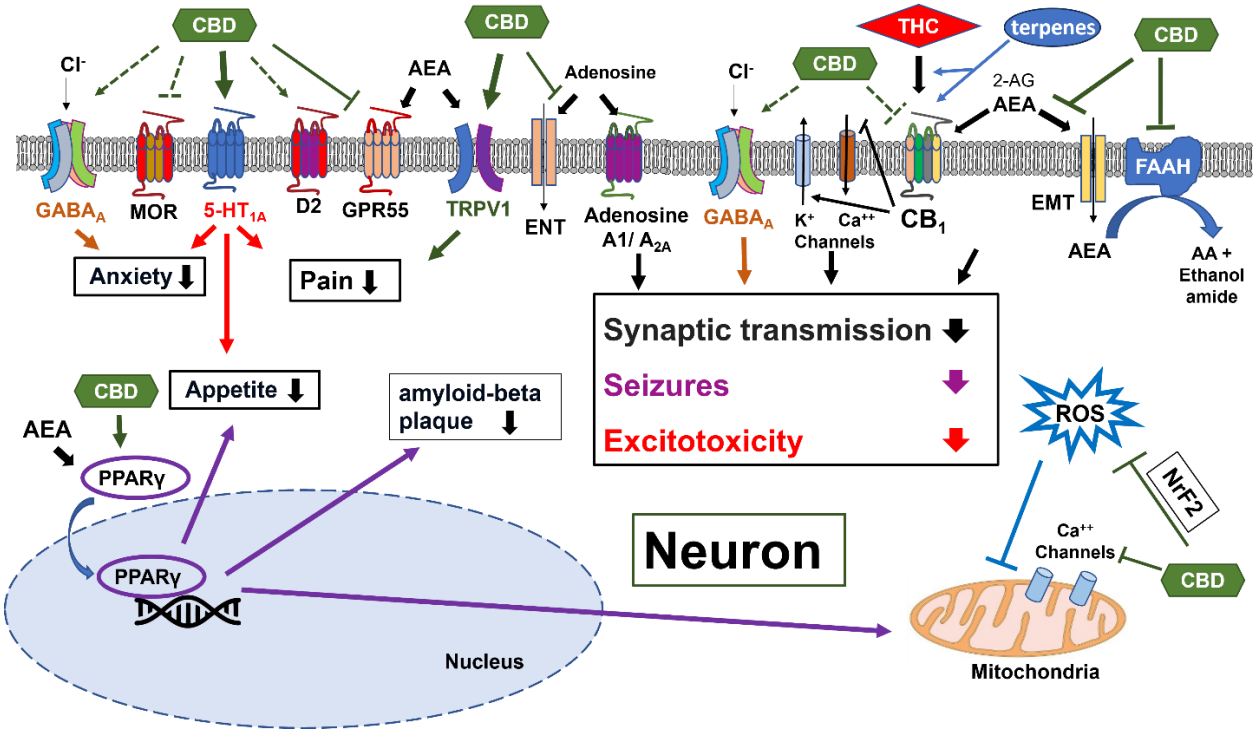


Figure 1: *The main cannabinoid receptor in neurons is the Cannabinoid receptor type-1 (CB_1R). Activated CB_1R increases outflow of potassium and decreases inflow of calcium reducing synaptic transmission. THC directly activates the CB_1R. Specific terpenes directly activate the CB_1R and increase CB_1R activation by THC. CBD is a negative allosteric modulator of the CB_1R. However, CBD can activate the endocannabinoid system by inhibiting the endocannabinoid membrane transporter (EMT) and the degradation of anandamide (AEA) through Fatty acid amide hydrolase (FAAH). This, in turn, increases the levels of the endocannabinoids AEA (a main agonist of CB_1R) and 2-Arachidonoylglycerol (2-AG). Other neuronal effects of CBD are mediated through full agonism at 5-HT_{1A} serotonin receptors and TRPV1 channel reducing anxiety and pain; Partial agonism at D2 dopamine receptors increasing emotional regulation; A negative allosteric modulation of MOR reducing opioid drug abuse and relapse; and Agonism of the nuclear PPARγ receptors increasing expression of cytoprotective enzymes. CBD also has direct antioxidative effects.*
D2: dopamine receptor 2; ENT: equilibrative nucleotide transporter; 5-HT_{1A}: 5-hydroxytryptamine1A receptor; GPR55: G protein-coupled receptor 55; MOR: μ opioid receptor; PPARγ: peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; TRPV1: transient receptor potential vanilloid 1.

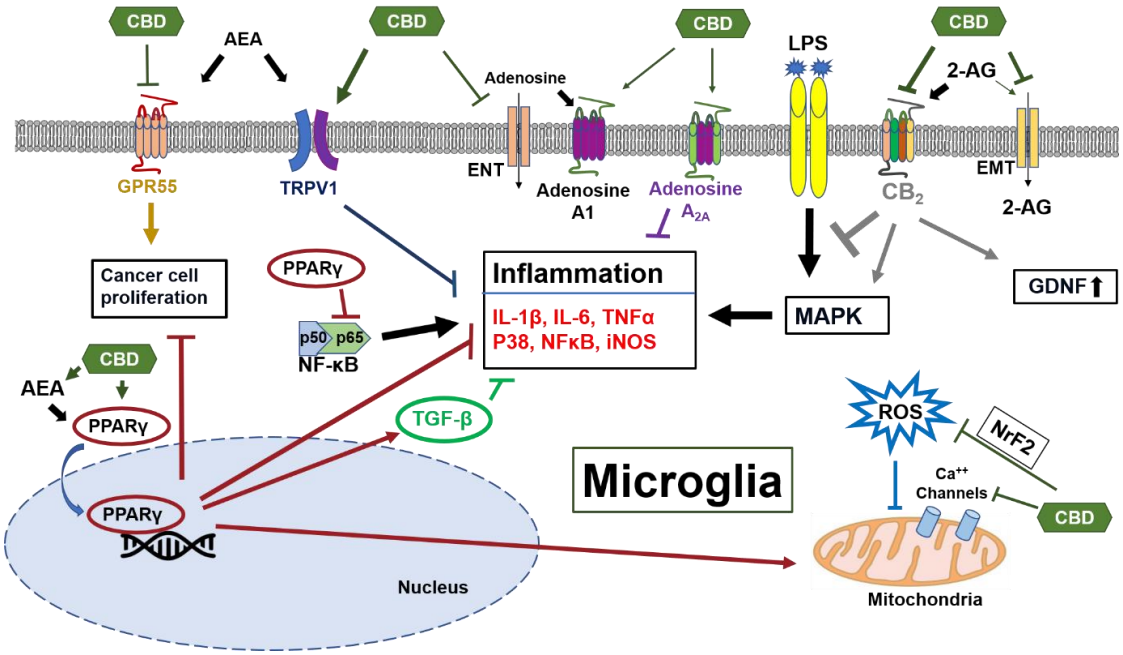


Figure 2: *Molecular targets of CBD in microglia. CBD has anti-inflammatory and antioxidative effects in the microglia which are mostly not related to the cannabinoid receptors.*
CBD is a full agonist of adenosine A_2A, and the TRPV1 cell membrane receptors and the nuclear PPARγ receptor – all convey anti-inflammatory effects. PPARγ is a ubiquitin E3 ligase that leads to proteasomal degradation of the p65 subunit of the proinflammatory transcription factor nuclear factor kappa B (NF-κB). In the nucleus, PPARγ reduces the expression of proinflammatory cytokines and increases the expression of anti-inflammatory and anti-oxidative mediators. CBD also has direct antioxidative effects. The main cannabinoid receptor in microglia is Cannabinoid receptor type-2 (CB_2R). Expression of CB_2R is upregulated in reactive microglia. The main effect of the CB_2R is anti-inflammatory. While initially activating the proinflammatory mediators of the mitogen-activated protein kinase (MAPK) family, CB_2R prevents further activation of MAPK by powerful triggers such as lipopolysaccharide (LPS). CBD has a weak affinity to the CB_2R (mostly as an inverse agonist). However, CBD can activate the endocannabinoid system through the CB_2R by inhibiting the endocannabinoid membrane transporter (EMT) which leads to reuptake inhibition of 2-Arachidonoylglycerol (2-AG) the main agonist of CB_2R.
ENT: equilibrative nucleotide transporter; GDNF: a glial cell line-derived neurotrophic factor; GPR55: G protein-coupled receptor 55; PPARγ: peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha; TRPV1: transient receptor potential vanilloid 1

Working hypothesis and expected significance:

In the proposed study, we will assess the effects of CBD and low-dose THC isolates, enriched with selected terpenes, on alleviating disruptive behavior and core symptoms in children with ASD. Combination therapy is anticipated to achieve higher efficacy compared to monotherapy and is a standard of care in many medical disorders, including cancer and cardiovascular disease. CBD and terpenes are plant-derived compounds with high safety and tolerability, possessing proven anti-inflammatory, antioxidative, and mitochondrial-supportive properties. These compounds have shown promising preliminary efficacy in ASD trials.
THC directly activates the endocannabinoid system, which has been shown to be impaired in ASD. Due to concerns regarding THC safety in children, we will use a lower THC dose in this study (0.27 mg/kg/day) compared to our previous study, where a dose of 0.5 mg/kg/day was used. We hypothesize that this combination of compounds will lead to significantly greater improvements in disruptive behavior and core autistic symptoms compared to placebo. The use of cannabinoid isolates enriched with terpenes, rather than whole-plant extracts (which can vary by batch), will ensure consistency of treatment and allow for a substantial increase in the dose of terpenes, the safest component of the formulation.
Despite the efficacy of atypical antipsychotics (mainly risperidone) in treating behavioral problems in individuals with ASD,[58-60] approximately 40% of patients experience drug-refractory aggression, self-injurious behavior, and severe tantrums.[61] Furthermore, risperidone and other atypical antipsychotics are associated with substantial adverse effects, such as sleepiness and weight gain. Consequently, many patients continue to suffer from disruptive behaviors that significantly impact their daily functioning and place a heavy burden on their caregivers.
Even a minor improvement in the severity of ASD core and associated symptoms can have a profound impact on the lives of countless families and reduce public health costs.

Methods

Study design

This is a phase 2, randomized, double-blind, placebo-controlled, parallel-group study, involving 78 children with ASD and behavioral problems, age 4-12 years. Participants who will pass screening will be randomized (in a 1:1 ratio) to receive either placebo or terpenes-enriched CBD rich cannabis oil (CBD: 3.6 mg/kg twice daily, terpenes: 0.25mg/kg twice daily, and THC: 0.128 mg/kg twice daily). The treatment will be given orally as an add-on to any existing treatment, for 8 weeks. The treatment dose will be up-titrated over 8 days.
After completing the first 8-week period, participants who received placebo will receive pure CBD (3.6 mg mg/kg twice daily enriched with terpenes (0.25mg/kg twice daily), and participants who received the study drug will continue with the same treatment for another 8 weeks of the second phase. (Figure 3).

First phase

Every 4 days during the titration phase and every 2 weeks thereafter, parents or primary caregivers will rate adverse events and document: any use of medical services, any change in the doses of the study intervention and concomitant medications, any change in the regular diet and intake of food supplements, and any change in the home or school routine or in the behavioral or emotional treatments.
Additionally, at each study visit, physicians will assess physical examination and BMI, and parents or primary caregivers will rate behavioral symptoms on the Aberrant Behavior Checklist-Community (ABC-C) – Irritability subscale (ABC-I) and ASD core symptoms on the Social Responsiveness Scale – second edition (SRS-2).

Other assessments at the onset and end of the first phase:

1. Parents will rate their child's: (a) *Communication, Socialization and maladaptive behavior* using the Vineland Adaptive Behavior Scales (VABS-III; a structured interview); (b) *behavior* using the emotion dysregulation inventory (EDI) and the multidimensional assessment of disruptive behavior (MAP-DB); (c) *severity of core symptoms* using the autism impact measure (AIM); (d) *sleep habits* using the Children's Sleep Habits Questionnaire (CSHQ); (d) *health-related quality of life of themselves* using the Quality of Life in Autism Questionnaire (QOLA) – Parent version; and (e) *gastrointestinal symptoms* the gastrointestinal symptoms a modified gastrointestinal sign and symptoms inventory (GISSI) and executive functions using the Behavior Rating Inventory of Executive Function (BRIEF).
2. Complete blood count (CBC), liver transaminases (LTs), and total bilirubin.
3. Plasma levels of 7-carboxy-CBD (the major circulating metabolite of CBD.
4. Possible biomarkers of treatment response (exploratory outcomes) including serum endocannabinoid (eCB) levels, relative mRNA expression levels of components of the eCB system in whole blood, RNA-sequencing of whole blood samples (transcriptome), plasma proteome, and gut microbiota

Additionally, at the end of the first phase physicians and caregivers will rate the global change in ASD-associated symptoms using the Overall Function Clinical Global Impression- Improvement (CGI-I) and the Caregiver Global Impression of Change (CGIC) assessments. Parents or primary caregivers will complete an Assessment of Blinding Questionnaire.

Second phase: Assessments at the end of the second phase will include: CGI-I and CGIC (global change in ASD-associated symptoms); adverse events; ABC-I, EDI, and MAP-DB (behavioral symptoms); SRS-2 and AIM (ASD core symptoms); CSHQ (sleep habits); BRIEF (executive functions), QOLA (quality of life); modified GISSI (gastrointestinal symptoms) and Assessment of Blinding Questionnaire.

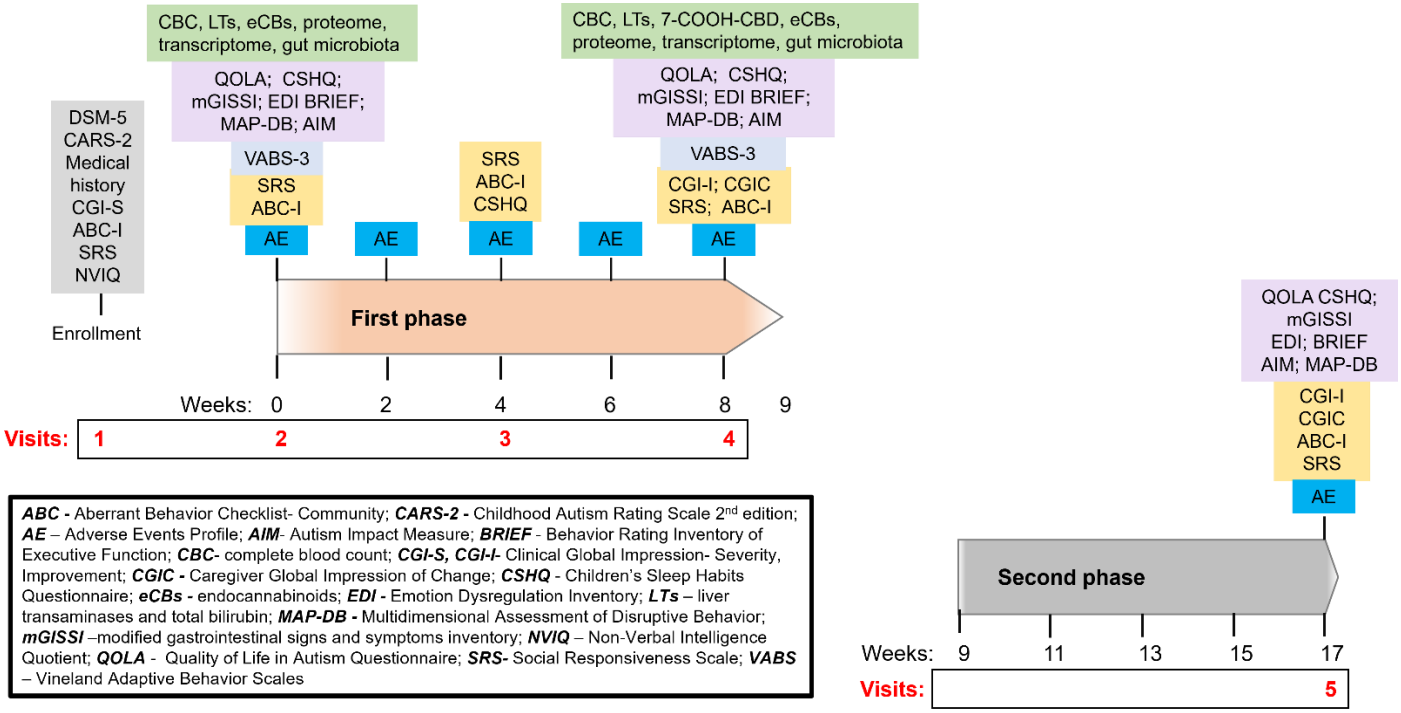


Figure 3: Study design

Participants

The study will be conducted at Shaare Zedek Medical Center, Jerusalem, Israel. Written informed consent will be obtained from a parent or legal guardian of participants after approval by the local institutional review board.

Inclusion criteria are:

- Children aged 4 to 12 years.
- A diagnosis of ASD based on the Diagnostic and Statistical Manual of Mental Disorders [Fifth Edition; DSM-V], confirmed by CARS-2 assessment.
- Moderate or greater ASD-associated symptoms as measured by a rating of moderate or higher (≥4) on the Overall Function Clinical Global Impression–Severity (CGI-S).
- Aberrant Behavior Checklist-Irritability Subscale (ABC-I) score of 18 or higher.
- Social Responsiveness Scale 2nd edition (SRS-II) total score of 66T or higher.

Exclusion criteria are:

- Bodyweight below 12.5kg or above or equal 57.5 kg.
- Current or past diagnosis of heart failure, drug addiction, schizophrenia, psychosis, bipolar disorder PTSD or MDD or diagnosis of schizophrenia in a first degree relative.
- A seizure or change in the antiepileptic medications in the 4 months prior to randomization.
- A physical exam and laboratory results are out of the normal range for individuals with ASD, including a significant impairment in heart, liver, or renal function.
- Any change in the pharmacological or behavioral treatment or change in the home or school settings (other than school holidays) in the 4 weeks prior to randomization or planned changes during the study.
- Cannabinoid treatment in the 4 weeks prior to randomization.
- Predicted low compliance to the study procedures (such as blood tests).
- Concurrent use of opiates or alcohol.

Criteria for termination of participation:

- The Principal Investigator believes that continued participation endangers the participant.
- Upon a decision of the regulatory bodies: IRB or MOH.
- Upon request of one of the legal guardians

Terms for termination of participation: After termination of participation – participants will continue to receive the same treatments they received before termination, excluding the study treatment. These treatments will not be affected in any way by their decision to terminate their participation in the study

Procedures

Screening: ASD diagnosis will be established using a structured assessment of DSM-5 criteria together with a Childhood Autism Rating Scale, Second Edition (CARS-2) total score ≥30. Caregivers will complete a medical history questionnaire covering demographic information, potential etiological factors (perinatal, genetic, imaging), comorbidities (epilepsy, allergies, immune-mediated disorders, sleep disturbances), autistic regression, diet, nutritional supplements, and behavioral treatments. Other assessments will include the CGI-S, ABC-I, SRS, and NVIQ (as detailed below). ADOS-2 - module, date and calibrated severity score will be documented.
Blood samples- (15 ml) will be collected at 0 and 8 weeks.

- Complete blood count (CBC), liver transaminases (LTs) and total bilirubin will be measured as part of the safety analyses.
- Plasma levels of 7-carboxy-CBD[62] will be assessed to determine individual bioavailability of CBD. At 2-month, blood will be collected 3-4 hours post treatment administration.
- Serum endocannabinoids (ECs)[16,19] and relative mRNA expression levels (real-time PCR) of components of the eCB system in whole blood,[16] RNA-sequencing of whole blood samples (transcriptome),[63] and plasma proteome,[64] will be assessed to explore possible markers of treatment response.

Stool samples will be collected at 0 and 8 weeks.

- Gut microbiota was demonstrated to be associated with ASD as well as symptom severity in multiple studies.[65-67]

Study treatment:
Participants will receive terpenes-enriched CBD-predominant cannabis oil for oral administration (8.96 mg CBD and 0.32 mg THC per drop, T1/C28, Bazelet, Israel) or matched placebos: an oil with similar appearance, taste and smell for 8 weeks (first phase).
The target dose is: CBD- 7.2mg/kg/d, THC- 0.257mg/kg/d, and terpenes - 0.5mg/kg/d.

The initiation dose will be 0.1 drops/kg twice daily: morning and afternoon (6-9 hours apart) for 4 days (1.8mg CBD/kg/d); followed by 0.2 drops/kg twice daily (3.6mg CBD/kg/d) for 4 days and 0.4 drops/kg twice daily (7.2mg/kg/d) thereafter.

Second phase: At the end of the first 8-week phase participants will gradually stop the treatment they received in the first phase over 1 week: 0.2 drops/kg twice daily for 3 days; followed by 0.1 drops/kg twice daily for 4 days.
Participants who received placebo in the first phase will receive terpenes-enriched CBD isolate, and participants who received the study drug will continue with the same treatment for another 8 weeks.
The titration in the second phase will be like the first 8-week phase: at week 9: 0.1 drops/kg twice daily for 4 days (1.8mg CBD/kg/d); followed by 0.2 drops/kg twice daily (3.6mg CBD/kg/d) for 4 days and 0.4 drops/kg twice daily (7.2mg/kg/d) until week 17. At week 17, participants will gradually stop the treatment over 1 week: 0.2 drops/kg twice daily for 3 days; followed by 0.1 drops/kg twice daily for 4 days.

Based on previous studies the safety of the study treatment (in the target dose) is very high. If the LTs (at 0 or 8 weeks) will be between 2 to 3 times higher than the upper limits of the norm (ULN) we will not change the target doses and repeat the tests after 2 weeks. If the LTs are more than 3 times higher than the ULN we will decrease the target dose of the CBD by 50% and repeat the tests after 2 weeks. If the repeated LTs are less than 2 times higher than the ULN we will increase the daily CBD dose to 5mg/kg.

Study visits
On-site visits: 4 visits: Screening (visit #1), Baseline (visit #2), 4 weeks (visit #3), 8 weeks (visit #4 - end of the first phase) and 17 weeks (visit #5 - end of the second phase). Phone visits to document compliance and adverse events: Three visits at study onset- (days 4, 8, and 12) and another 3 visits on weeks 6, 11, and 13. The PI may decide to hold the third meeting on Zoom

Outcomes and measures:

Baseline evaluations (at screening):
Childhood Autism Rating Scale – Second Edition (CARS2):[68] CARS-2 is a validated tool for screening and classifying ASD, based on direct behavioral observation across 15 items (14 specific domains such as communication, body use, and response to stimuli, plus one global severity rating). Each item is scored from 1 (normal) to 4 (severely abnormal). Total scores of 30–36.5 indicate mild to moderate ASD, and scores >36.5 indicate severe ASD. The High-Functioning Form (CARS2-HF) is used for children ≥6 years with IQ >80, while the Standard Form (CARS2-ST) is used for all others.

Nonverbal Intelligence Quotient (NVIQ): NVIQ will be assessed using Raven's Colored Progressive Matrices (RPM), an untimed measure of problem-solving and abstract reasoning. The RPM consists of colored pattern sets (A, Ab, B) of increasing difficulty to assess observational and fluid intelligence. Test–retest reliability is typically >0.80. The RPM is generally considered to provide a more accurate estimate of NVIQ in children with ASD compared to the WISC or WPPSI.[69-71]

Outcome Measures
ABC-I: The ABC[72,73] is a caregiver-completed questionnaire with 58 items, organized into five subscales. The ABC-I (irritability) subscale consists of 15 items that measure the emotional and behavioral symptoms of ASD, including aggression toward others, deliberate self-injuriousness, temper tantrums, depressed mood and rapidly changing moods. The ABC-I has been accepted as the gold standard for measuring irritability in medication trials for ASD[74] and therefore, we will use it as the primary endpoint in this study. Individual items are rated from 0 (never a problem) to 3 (severe problem).

VABS-III [75]: The VABS is a semi-structured caregiver interview designed to assess functional skills in three domains: Communication, Socialization, and Daily Living Skills. It also contains a short Maladaptive Behavior Domain. The Adaptive Composite Score estimates overall adaptive behavior. Vineland items are scored based on an open-ended question as 0 (behavior not performed), 1 (performed sometimes), or 2 (performed on a regular basis). The instrument provides Standard scores (mean = 100, SD = 15) and Age Equivalent scores

SRS (Hebrew version) [76,77]: The SRS is a 65-item caregiver or teacher questionnaire with five subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Autistic Mannerisms. Items are rated on a 4-point Likert scale (0–3, total 0–195), with higher scores indicating greater impairment. Subscales yield T-scores; a Total T-score of 60–75 indicates mild to moderate impairment, and >75 indicates severe impairment. Caregivers will complete the SRS based on behavior during the previous 6 weeks. The SRS is widely used in ASD research. [78-83]

CGI [84]: this is a 7-point scale designed to measure the severity of illness (CGI-S) and, an overall improvement from baseline (CGI-I). This measure has been used in many clinical trials in ASD.[58,85,86] Scores in the severity scale range from 1 (normal) through to 7 (amongst the most severely ill patients); in the improvement scale, scores range from 1 (very much improved) through 4 (unchanged) to 7 (very much worse).

CGIC: this is a 7-point scale designed to measure the overall improvement from baseline. This measure is completed by the primary caregivers and is similar to the CGI-I

Autism Impact Measure (AIM)[87,88]: This is a 41-item caregiver-reported questionnaire designed to characterize core symptoms of ASD in individuals aged 3–18 years

Multidimensional Assessment of Preschool Disruptive Behavior (MAP-DB)[89]: This 40-item caregiver-reported questionnaire assesses behavior across four domains: Temper Loss, Noncompliance, Aggression, and Low Concern for Others. It is available in three age-specific versions: 3-5 years, 6-8 years, and 9-10+ years.

Emotion dysregulation inventory (EDI)[90]: This 30-item caregiver-reported questionnaire assesses observable signs of poor emotion regulation in children with autism.

Behavior Rating Inventory of Executive Function (BRIEF)[91]: this is an 88 items Hebrew validated questionnaire, consists of 8 subscales, grouped into two index scores—Behavioral Regulation Index and Metacognition Index. Raw scores are converted into age- and sex-normed T scores.

Children's Sleep Habits Questionnaire (CSHQ)[92]: this is a 33 items Hebrew validated questionnaire. A higher score reflects more significant sleep disturbances (range: 33–99, a score of ≥41 classifies a "poor sleeper). The CSHQ has eight subscales: bedtime resistance; sleep onset delay; sleep duration; sleep anxiety; night waking; parasomnias; sleep-disordered breathing; and daytime sleepiness. This questionnaire has been used extensively in previous research in ASD

Quality of Life in Autism Questionnaire (QOLA) – Parent version:[93]The QOLA is a 48-item tool with two subscales to measure quality of life in parents of children with ASD. Part A (QoL subscale) includes 28 items assessing parents' overall quality of life, with scores ranging from 28 to 140 (higher scores indicate greater QoL). Part B (impact of ASD symptoms subscale) includes 20 items evaluating how problematic a child's ASD-related difficulties are for the parent, with scores ranging from 20 to 100 (higher scores indicate fewer problems).

Modified gastrointestinal signs and symptoms inventory (GISSI): This 15-item questionnaire was adapted to this study based on the GISSI-17,[94] a brief parent-report screen for common gastrointestinal problems in children with ASD.

Adverse events (AE) will be monitored using a 31-item modification of the Dosage Record Treatment Emergent Symptom Scale. Recent health concerns, use of medical services, and change in study treatment dose and concomitant medications will be recorded as well. BMI, pulse rate, and blood pressure will be assessed at each clinic visit. Reports of new adverse events or worsening of previously reported events will be rated mild (present, but not persistent or requiring intervention), moderate (requiring intervention), or severe (posing a severe problem and requiring immediate intervention). Hospitalization will be documented as a serious adverse event. The PI will systematically evaluate all AEs for timing, biological plausibility, alternative causes, dose–response relationship, and prior knowledge to determine relatedness to the study drug, categorized as: not related, unlikely, possibly, probably, or definitely related.

Table 1: Primary, secondary, and exploratory outcomes

Primary outcome measure: Change in the Aberrant Behavior Checklist-Irritability Subscale (ABC-I) score from Baseline to week 8.

Main secondary outcome measures:
1) Change from baseline to week 8 in the Vineland ™ Adaptive Behavior Scales (VABS3)-Socialization Domain Score
2) Change from baseline to week 8 in the Social Responsiveness Scale (SRS-II) total raw score.

Other secondary outcome measures:
1) Number of participants with severe, moderate, and mild treatment-emergent adverse events during the 8-week first phase.
2) Number of participants with clinically significant abnormal values in the CBC, liver transaminases, or total bilirubin or clinically significant abnormal values in the vital signs or physical examination at week 8
3) Change from baseline to week 8 in the BMI
4) Clinical Global Impressions-Improvement since baseline at week 8.
5) Caregiver Global Impression of Change (CGIC) since baseline at week 8.
6) Change from baseline to week 8 in the Vineland ™ Adaptive Behavior Scales (VABS3)-Communication and maladaptive behavior Domains Scores
7) Change from baseline to week 8 in the emotion dysregulation inventory (EDI)
8) Change from baseline to week 8 in the multidimensional assessment of disruptive behavior (MAP-DB)
9) Change from baseline to week 8 in the autism impact measure (AIM).
10) Change from baseline to week 8 in the Quality of Life in Autism Questionnaire (QOLA) – Parent version – part A: parents' overall perception of their QOL- subscale score; and (c) part B: impact of ASD symptoms on parents' QOL- subscale score
11) Change from baseline to week 8 in the Children's Sleep Habits Questionnaire (CSHQ)
12) Change from baseline to week 8 in the gastrointestinal signs and symptoms inventory.
13) Change from baseline to week 8 in the Behavior Rating Inventory of Executive Function
14) Global Impressions-Improvement since baseline at week 17
15) Caregiver Global Impression of Change (CGIC) since baseline at week 17.
16) Change from baseline to week 17 in the Vineland ™ Adaptive Behavior Scales (VABS3)-Communication and maladaptive behavior Domains Scores
17) Change from baseline to week 17 in the emotion dysregulation inventory (EDI)
18) Change from baseline to week 17 in the multidimensional assessment of disruptive behavior (MAP-DB)
19) Change from baseline to week 17 in the autism impact measure (AIM).
20) Change from baseline to week 17 in the Quality of Life in Autism Questionnaire (QOLA) – Parent version – part A: parents' overall perception of their QOL- subscale score; and (c) part B: impact of ASD symptoms on parents' QOL- subscale score
21) Change from baseline to week 17 in the CSHQ -total score
22) Change from baseline to week 17 in the gastrointestinal signs and symptoms inventory.
23) Change from baseline to week 17 in the BRIEF -Global Executive Composite (GEC) -T score

Exploratory outcomes

1) Correlations between 'markers of CBD' at 8weeks (Plasma levels of 7-carboxy-CBD) and treatment outcomes, in the active treatment group.
2) Correlations between 'biomarkers' at baseline [markers of the endocannabinoid system, transcriptome, proteome, and gut microbiota] and treatment outcomes, in the treatment group.
3) Correlations between change in 'biomarkers' from baseline to 8 weeks and treatment outcomes, in the treatment group, in the placebo group, and in the entire cohort.
4) Correlations between 'child characteristics' at baseline [age, sex, level of function (DSM-5 levels of support required, CARS, VABS, CGI-S, ABC-I, SRS, NVIQ), etiological factors, comorbidities, history of autistic regression, diet, behavioral treatments and impact of high fever on social deficits status], and treatment outcomes, in the treatment group.
5) Correlations between 'child characteristics' at baseline and 'biomarkers' at baseline.
6) Effects of the treatment compared to placebo on 'biomarkers' - change from baseline to week 8.
7) Correlations between plasma levels of 7-carboxy-CBD at 8 weeks and change in 'biomarkers' from baseline to 8 weeks, in the treatment group.
8) Correlations between levels of 7-carboxy-CBD at 8 weeks, and 'biomarkers' at 8 weeks, in the treatment group.
9) Correlations between 'biomarkers' at baseline and levels of 7-carboxy-CBD at 8 weeks, in the treatment group.
10) Correlations among 'biomarkers' [markers of the endocannabinoid system, transcriptome, proteome, and gut microbiota]: at baseline (entire cohort), at 8 weeks (active treatment group, placebo group, and entire cohort), and when combining data from baseline and 8 weeks (entire cohort).
11) Correlations between each 'biomarker' and each behavioral assessment score (i.e., symptom severity) at baseline (entire cohort), at 8 weeks (active treatment group, placebo group, and entire cohort), and when combining data from baseline and 8 weeks (entire cohort).
12) Longitudinal changes in gut microbiota in the study group and in the placebo group and correlations with longitudinal changes in the behavioral assessments in these time points.
13) Cross-sectional correlations between gut microbiota and behavioral assessments at baseline (entire cohort) and at week 8 (active treatment group, placebo group, entire cohort) and when combining all time points (entire cohort).
14) Correlations between treatment outcomes in the placebo group ('placebo response') and the family characteristics: 'child characteristics' (age, IQ, severity of core symptoms, experience with previous oral treatments and perceived ability to understand the study goals) and 'parent characteristics' (age, education, and parental expectations and believes regarding pharmacological and natural treatments as assessed by the parental expectations and believes 29-item questionnaire).
15) Correlations between gut microbiota and gastrointestinal symptoms as assessed by the 30-item Health State Questionnaire (adverse events questionnaire) – when combining all time points (entire cohort).
16) Correlations among all assessments including: 'treatment outcomes', 'child characteristics', and 'parent characteristics' (e.g., child QOL, parent QOL, severity of ASD symptoms, etc.).
17) Correlations between 'child characteristics', 'treatment outcomes', and 'biomarkers' assessed in this study, and polymorphism in genes that compose the endocannabinoid system and the 'methionine and methylation cycle' for participants in whom this data will be available (genetic assessments will not be performed as part of this study).

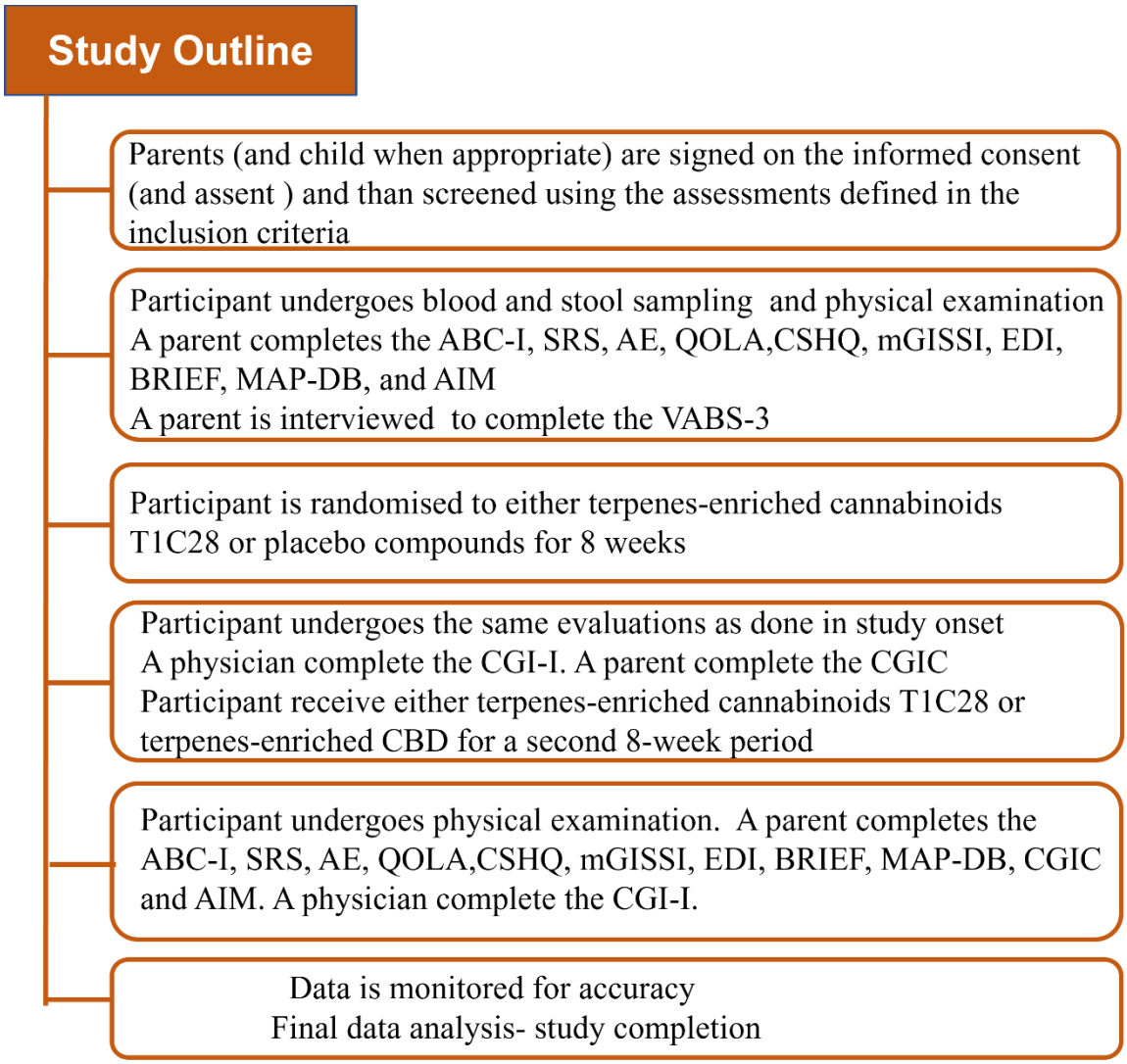
Table 2: Assessment Schedule during the first phase

Visit Number	**1** Screening	**2** Baseline	**a**	**b** *End of Titration*	**c**	**3** 4W	**d**	**4** 8W
Day Number (Visit Window)	**-90 to 1**	**1**	**4±2**	**8±2**	**12±2**	**28±3**	**42±3**	**56±3**
Informed consent/assent	X							
Eligibility criteria[$]	X	X						
Demographics & Medical history	X							
ASD criteria - DSM-5	X							
- CARS-2	X							
Overall severity – CGI-S	X							
Behavioral problems: ABC-I	X	X				X		X
Core symptoms: SRS-2	X	X				X		X
Nonverbal IQ	X							
Vital signs	X	X						X
Physical exam (including BMI)	X	X						X
Concomitant Medications	X	X	X	X	X	X	X	X
Parent expectations and believes		X						
Adverse Events		X	X	X	X	X	X	X
Compliance with Intervention			X	X	X	X	X	X
Blood sampling*		X						X
Gut microbiota		X						X
Socialization – VABS-3		X						X
QOLA; EDI; AIM		X						X
BRIEF, MAP-DB, GI problems		X						X
Sleep Habits – CSHQ		X				X		X
Physician impression of overall change - CGI-I								X
Caregiver impression of overall change – CGIC								X
Assessment of Blinding Questionnaire								X

[$] A diagnosis of ASD based on DSM criteria and CARS-2, core symptoms cannot be explained by the level of intellectual disability (NVIQ), age 4-12 years, weight 12.5-57.49 kg, CGI-S ≥4, ABCI-I score ≥18, SRS-2 total score ≥ 66, predicted compliance with study requirements including blood tests and tolerability to taste and smell of the study interventions.

[*] CBC, liver transaminases, total bilirubin; Plasma levels of 7-carboxy-CBD; Serum endocannabinoid levels, relative mRNA expression levels of components of the eCB system in whole blood (real-time PCR), RNA-sequencing of whole blood samples, and plasma proteome.

Figure 4: Study Flow Chart



Statistical Analyses

A detailed statistical plan is provided in the Statistical Analyses Plan (SAP).

Based on previously reported placebo effects on ABC-I scores in other ASD trials[74,95,96] and allowing for a slightly greater placebo effect due to higher expectations associated with cannabis treatment, we estimate that participants assigned to the placebo group will exhibit a mean 20% reduction from baseline ABC-I scores. Based on our preliminary results, we assume a small-to-medium effect size (0.35) for the study treatment—terpenes-enriched cannabis oil (T1/C28). Consequently, a sample size of 34 participants per group is required to achieve 80% power, using a mixed model of repeated measures (MMRM). To account for early withdrawals, an additional 15% will be recruited, resulting in a total of 39 participants per group.
This sample size is expected to provide sufficient power to detect differences in the primary outcome. However, an adaptive design with interim analysis by an external, non-blinded biostatistician will determine the final sample size.
For secondary and exploratory efficacy analyses, nominal p-values will be presented for informational purposes only.
The primary report will focus on odds ratios (OR) and 95% confidence intervals (CI).
Additionally, a secondary analysis will evaluate the proportion of participants achieving the minimal clinically important difference (MCID) using a chi-square test or logistic regression analysis to compare proportions between groups. The analysis of the Vineland Adaptive Behavior Scales, Third Edition (VABS-III), will utilize Growth Scale Values (GSV), with adjustments for participants transitioning between age categories during the study.
All efficacy analyses will be based on an intent-to-treat (ITT) population, defined as all randomized subjects who receive at least one dose of the study medication and have post-baseline efficacy data available. For the primary efficacy outcome and key secondary outcomes, a per-protocol (PP) population will also be defined, consisting of ITT participants who substantially adhere to the study protocol.
The primary analysis assumes that ABC-I scores are missing at random (MAR), an assumption deemed reasonable for this study. Missing data are unlikely to differ systematically from observed values for unknown reasons.
The primary endpoint will be assessed using a Mixed Model for Repeated Measures (MMRM), implemented in SAS (PROC MIXED), R (lme4 package), or equivalent statistical software.
Sensitivity analyses will include per-protocol analysis and may utilize multiple imputations to confirm the robustness of findings
Secondary analyses will be conducted using the same methodology as the primary ABC-I endpoint for each treatment sequence.

Data Management and Confidentiality

Identifiers- We will use a mathematic manipulation on the participants' ID number (Xor with a 4-digit secret code) to assign a unique 'participant number'. We will use the participant number to avoid exposing personally identifiable information (PII). Only the Principal Investigator and data coordinating manager will have the list connecting the identified data with the participant number. The list will be kept in a locked desktop computer protected by two passwords and in a locked safe in Shaare Zedek Medical Center. Representatives of the IRB and MOH may have access to this identified list upon specific request.

Confidentiality: To protect the privacy of human subjects and maintain the confidentiality of study data we will use a strict separation between identified and non-identified data.
Identified data: consent forms and contact information will not contain 'participant number'. It will be kept in a locked cabinet at Shaare Zedek Medical Center- SZMC. Electronic files of contact information and scans of consent forms will be kept in a locked desktop computer protected by two passwords.
ADOS-2 assessments (if performed) will be videotaped (with legal guardians' consent) to allow evaluation by two independent ratters. Files will be kept in external hard disks at a locked safe. All video files will be completely removed within 12 months after study completion.
All other data, records, and biological samples will be captured and stored un-identified, using the participant number as the sole identifier.
Hard copies of all outcome questionnaires and assessment tools will be stored un-identified (participant number only) in locked cabinets at the study site (SZMC). Biological samples (sera, plasma, and stool samples) will be stored un-identified (participant number only) in locked freezers at the study site. Identified and non-identified data will be kept in different rooms. Only the study PI and the data coordinating manager will have access to study records, data, and specimens. Representatives of the IRB, and MOH are eligible to review study records.

Data capture, verification, and disposition: Study data will be captured using paper and pencil forms that will not contain identified data. Forms will be uploaded to the web application REDCap. REDCap is a secure web application for building and managing online surveys and databases. It can be used to collect any type of data including 21 CFR Part 11, FISMA, and HIPAA-compliant environments. REDCap supports online and offline data capture for research studies and the REDCap Consortium is composed of thousands of active institutional partners in over one hundred countries.
All data (hard copy and electronic) will be stored for 15 years.

Sharing study results: The results of all baseline assessments (CARS-2, nonverbal IQ), blood work, and other data regarding study participation (including study assignments and individual results) will be shared with the primary care providers.

Procedures Description:

The principal investigator (PI) for the clinical investigation is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported in the eCRFs and in all other reports during the clinical investigation

The Site Clinical Coordinator for the clinical investigation is responsible for:
1 Maintaining all of the study-related files: This includes maintaining all logs such as:

- Ethics Approvals and all communications with the ethic committees (such as protocol amendments and serious adverse events reports)
- Protocol Deviation Log
- Subject Informed Consent (SIC) Signature Log
- Investigation Team Log

2 Correct filling of the eCRFs based on source documentation and live documentation
3 Maintaining contact with study subjects, and coordinating study visits

At the start of the clinical study, the Clinical Coordinator will make sure that all study files for the following:

- IRB/EC/Helsinki Committee Documents.All IRB Correspondence is on file.The study staff are IRB approved prior to performing any study procedures.Adverse events and deviation procedures for reporting to IRB per current guidelines are in place.All versions of the IRB protocols and informed consent forms are on file.Signed Good Clinical Practice compliance form, financial disclosure and CV for the PI and sub investigators.

Other documents:

- CVs for all study staff are on file and updated within the last 2 years
- Medical licenses for the Sub-Investigators are on file and updated prior to the expiration
- Delegation Log is updated and procedures for updating the log with new staff are in place
- All other essential documents have been prepared and completed as appropriate

Before, during, and at the close of the study period, study documentation will be monitored by the Clinical Coordinator for the following:

- Informed Consent Form (ICF)
    - Ensure that subject identification is on all pages of the ICF, if applicable
    - Documentation of subject receiving a copy of the consent form is present
    - Clinic note documents informed consent process
    - The parents or a legal guardian of the subject and study representative signed and dated the consent form for their self
    - Note to file completed for any informed consent deviations.
    - Ensure a valid (current version date) copy of the consent form was used

Protocol:

- Confirm that the study staff is conducting the study in compliance with the protocol approved by IRB/CE/Helsinki Committee and are trained in Good Clinical Practices (GCP).
- The protocol deviations (exceptions and violations) are documented in the subject chart and reported to IRB/CE/Helsinki Committee as required.
- Ensure any deviations are logged in the form *'Protocol Deviations'*

Source Documents:

- Review subject charts to ensure accuracy, completeness, and legibility of the data
- Any correction made to the source documents is dated, initialed, and explained. The original entry should not be obscured.
- The protocol-specific source documents are on file.
- Source documents are completed in ink or electronically.
- Note to files are generated for missing or incomplete data, and to explain any discrepancies or additional comments.

Case Report Forms:

- Ensure that the data reported on the CRF is consistent with the source documents.
- Discrepancies between the source documents and eCRF are explained in a note to file or captured in a comment in the eCRF.

Biological samples
Biological samples will be labeled with participant numbers and stored in locked freezers. The legal guardians will decide in the consent form if the samples will be kept after the termination of the study for possible use in further studies or be destroyed upon termination of the study.

Blood samples
Blood (6 ml in tubes with procoagulant, 6 ml in tubes with EDTA, and 3 ml in Tempus™ Blood RNA Tube) will be collected at the baseline (9-11 AM) and at 2-month (on 9:00- 11:00 AM, 3-4 hours post treatment administration).
Assessments will include:

- Plasma levels of 7-carboxy-CBD
- Serum endocannabinoid levels
- Relative mRNA expression levels of components of the eCB system (CB1R, CB2R, NAPE-PLD, FAAH, DAGL, and MAGL) normalized to GAPDH in whole blood.
- RNA-sequencing of whole blood samples (transcriptome), and plasma proteomics will be performed pre and post-treatment to discover treatment-responsive biomarkers which correlate with behavioral improvement.

Stool samples
Stool samples will be collected using OMNIgene GUT sample collection kit (DNA Genotek, Canada). This kit homogenizes the sample and stabilizes the DNA for up to 60 days at room temperature. Participants will receive the kits in advance: a first kit during the screening visit, a second kit during the 2nd or 3rd. Participants will be instructed to use the kits within 2 weeks prior to their next visit (baseline, 8 weeks) and bring them with them to the next visit. Stool samples will not be collected if the participant had received any antibiotic treatment during the 4 weeks prior to the sampling day. Any antibiotic treatment, special diets, and use of probiotics during the 4 to 8 weeks prior to the sampling day - will be documented.

Informed Consent Process

Subjects' legal guardians interested in having their children participate in the clinical investigation will be screened by the participating physician.
Subjects' legal guardians will then be given a copy of the Patient Informed Consent with ample time to read and review the document and ask questions before signing.
Subjects will meet with one of the investigators to discuss investigational participation and any questions they may have regarding the investigation, the investigational device, and the informed consent.
The informed consent will be signed by the legal guardian, during the meeting with one of the participating investigators.
A photocopy of the signed patient informed consent form will be provided to the legal guardian prior to the procedure.
Subjects requesting to withdraw consent will be disqualified from the investigation at no cost or consequence

All subjects in this study are pediatric subjects (4-12) with ASD and are therefore unable to sign an informed consent. A legal guardian will sign the informed consent. Assent form will be obtained from any participant who is able to give it.

Vulnerable Population

This clinical investigation will be conducted on pediatric (4-12 years old) ASD populations. Vulnerable patients' legal guardians will be responsible for patient informed consent.

Data and Safety Monitoring Board (DSMB)

An independent Data and Safety Monitoring Board (DSMB) will oversee the conduct of the trial. The DSMB is responsible for monitoring study progress, including data quality, recruitment, retention, and the overall risk–benefit balance. It will also consider external developments relevant to participant safety and research ethics. The DSMB will review interim analyses as needed, although no formal stopping rules are defined for this study, and will ensure that all trial data remain confidential. The DSMB will provide regular reports to the Bazelet Group regarding participant safety and trial progress, and will make recommendations on whether the study should continue as planned, be modified, or be terminated. In addition, the DSMB will advise the Bazelet Group on issues related to study conduct, enrollment, or data integrity. The DSMB will conclude its responsibilities upon completion of follow-up for the final participant.

Randomization and blinding

Eligible subjects will be assigned an enrollment number in sequential order beginning with 01. Allocation to the treatment arm will be based on a randomization list. A randomization scheme will be generated by an external unblinded biostatistician. The blocked randomization will be performed using SAS statistical software V9.4 via the plan procedure with a fixed block size. The randomization scheme and lists will be sent to the study site. The randomization list will include sequential numbers and assigned 4-letter codes. Randomization will be kept at a 1:1 ratio. The randomization list will be encrypted by password and will be sent prior to first dosing to the principal investigator.
The study active treatments as well as the placebo treatments will be packed in similar bottles and packages and will have similar appearance, taste, and smell.

CBD-predominant cannabis oils and matched placebo will be prepared, bottled, and labeled by the Bazelet Group (Or Akiva, Israel). CBD, terpenes, and THC (when applicable), together with a taste and odor mixture, will be dissolved in olive oil to yield a concentration of 28% CBD (280 mg/g, equivalent to 256 mg/mL, or 8.96 mg per 0.035-mL drop) and 9 mg THC/mL (0.32 mg per 0.035-mL drop). An international company (dsm-firmenich) designed the taste and odor mixture to ensure that all three treatments (study drug, terpene-enriched CBD oil, and placebo) are indistinguishable.

The code key for the active/ placebo labels will be kept by the external biostatistical until study-end. Neither the principal investigator nor any other team member or individual will have access to the codes until study-end.

To assess the blinding state, caregivers, participants (when applicable) and researchers will complete an Assessment of Blinding Questionnaire at the end of the randomized study.

Study Interventions

CBD-predominant cannabis oil (CBD 3.6 mg/kg twice daily; THC 0.128mg/kg twice daily, and terpenes 0.25mg/kg twice daily) will be administered orally **as an add-on** to any existing treatment.

Dose Justification

The target dose of 7.2mg CBD/kg/d and 0.257mg THC/kg/d divided into 2 daily doses is based on the results of the randomized study that was completed recently[50] in which the target dose was 10mg CBD/kg/d and 0.5mg THC/kg/d and on our clinical experience in recent years in more than 400 children. Notably, ongoing clinical trials of CBD for children with ASD use 3-20mg/kg/d, and most are using 10mg/kg/d.[51] The safety of plant-derived CBD isolate at doses of 10-50mg/kg/d was demonstrated in multiple placebo-controlled studies in children with epilepsy and lower doses are associated with fewer AEs.[97-101] At a dose of 7.2mg/kg/d we expect no treatment related adverse events.

Administration

CBD rich oil will be administered by drops containing 0.035 ml / 8.96 mg CBD each.
The CBD dose will be up-titrated over 8 days:
Days 1-4: 0.1 drops per Kilogram of Bodyweight (rounded number)– twice daily (1.8 mg/kg/d)
Days 5-8: 0.2 drops per Kilogram of Bodyweight (rounded number – twice daily (3.6 mg/kg/d)
Days 9-56: 0.4 drops per Kilogram of Bodyweight (rounded number – twice daily (7.2 mg/kg/d)

The CBD dose will be down-titrated at the end of the randomized study over 7 days:
Days 57-60: 0.2 drops per Kilogram of Bodyweight (rounded number – twice daily (3.6 mg/kg/d)
Days 61-64: 0.1 drops per Kilogram of Bodyweight – twice daily (1.8 mg/kg/d)

On day 65, the parents or legal guardian will decide if the treatment will be discontinued or be switched to a second treatment (either terpenes enrich CBD isolate or CBD-predominant oil) at the same dose for 4 days followed by 0.2 drops per Kg X 2/d for another 4 days and 0.4 drops/kg X2/d until the end of the second phase.

Amendments to the Protocol

The study coordinator will ensure that
1 Amendments to the Clinical Investigation Plan are documented, using the form 'Amendment and Revision Sheet'
2 Approval is documented, using form 'Clinical Investigation Document Approval Log''
The amended protocol is sent to all investigators and the EC/IRB/Helsinki Committee after approval

Deviations from the Protocol

Neither the Principal Investigator nor any of the sub-investigators are authorized to deviate from the protocol, except as specified in 4.5.4 b of ISO 14155:2011 and 21 CFR 812.150 (a)(4), specifically requests for deviations, and reports of deviations, if the deviation affects subject's rights, safety, and wellbeing, or the scientific integrity of the clinical investigation.
Under emergency circumstances, deviations from the protocol to protect the rights, safety, and well-being of human subjects may proceed without prior approval of the sponsor and the EC/IRB/Helsinki Committee. Such deviations shall be documented and reported to the sponsor and the EC/IRB/Helsinki Committee as soon as possible.
The research coordinator will prepare the EC/IRB /Helsinki Committee Notifications letter using the EC Notification Form if any reportable serious deviations or adverse events were recorded and send them to the Ethics Committee/Institutional Review Board/Helsinki Committee and Principal Investigator, according to this protocol.
All notifications will be carried out within the time frames specified by local regulatory authorities.

Statements Of Compliance

1	This clinical investigation will be conducted in accordance with ethical principles stated in the Declaration of Helsinki and in compliance with all national and local regulatory requirements for the safety and protection of human subjects.
2	This clinical investigation will be implemented only upon receiving all final notification/authorizations from local and national regulatory and Helskinki/EC/IRB authorities, as appropriate.
3	This clinical investigation will comply with any additional requirements set forth by the Helsinki/EC/IRB and/or regulatory authorities.
4	This clinical investigation provides clinical study medical insurance for study participants as required by local and national regulatory authorities.
5	This protocol was written in accordance with the ISO 14155:2011 Standard for Clinical investigation of medical devices for human subjects – Good Clinical Practice

Early Termination or Suspension of the Clinical Investigation

Grounds for suspension or premature termination of the clinical investigation include but are not limited to:

- Non-compliance of the investigational site personnel to the protocol and or investigational procedures
- Serious Adverse Events that demonstrate danger to the patient population
- Unethical performance on the part of any of the investigators
- Failed audit of investigation with serious flaws to study design, performance or outcomes
- Business/Financial issues that cannot be resolved
- Other unforeseen events that give the PI reasonable doubts to the safety of the participants which cannot be resolved in a reasonable time

Publication Policy

The results of this clinical investigation may be published in a mainstream peer-reviewed professional journal as per Chapter I. of the agreement between the Shaare Zedek Medical Center and the Sponsor

Adverse Events

An **adverse event** is any undesirable or unintentional or unexpected event (sign, symptom, illness, abnormal laboratory value, or other medical events) that occurs during the course of the study, whether or not considered related to the study interventions. This definition includes events related to the investigational treatments and also includes events related to the procedures involved.

Regardless of the relationship to the investigational treatments, all moderate to serious adverse events occurring from the time that the informed consent is signed must be recorded in the patient's CRF.

A **mild adverse event** is one that the symptom does not influence performance, require drug treatment or prevent the patient from carrying on with normal life activities.
A **moderate adverse event** is one that the symptoms make the patient uncomfortable and causes some impairment to normal life activities and require treatment for symptom(s).
A **serious adverse event** is an adverse event that:

- Led to death
- Led to a serious deterioration in the health of the subject, that either resulted in
    - A life-threatening illness or injury, or
    - A permanent impairment of a body structure or body function, or
    - In-patient or prolonged hospitalization, or
    - Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function

In the event of an emergency resulting from the clinical investigation, all emergency treatment will be provided at no cost to the patient. Clinical Investigation Insurance has been provided to ensure all costs be covered if necessary.

The principal investigator will notify the sponsor immediately (within 24 hours) of any serious adverse event. All reports to the appropriate authorities will be made by the principal investigator as set forth in the standard clinical practices outlined in the 21 CFR (§ 812.150(a)(1), § 812.46(b) and § 812.150(b)(1))/ MEDDEV 2.7/3 Dec 2010 and ICH-GCP Guidelines.

Adverse Events will be reported and treated as per the standard clinical practices outlined in the 21 CFR (§ 812.150(a)(1), § 812.46(b) and § 812.150(b)(1))/ MEDDEV 2.7/3 Dec 2010 and ICH-GCP Guidelines

Anticipated adverse events include rare insomnia, irritability, and intolerance of the taste and smell.

Appendix 1 – non-generic questionnaires

The Hebrew version of the following questionnaires appears in the investigator brochure.

Cannabinoid treatment in children with ASD – Demographic questionnaire

Date: _____ GUID: _____ Informant: Mothe / Father / Guardian

1. **Has your child regressed in his/her development or communication?** No/Yes
   Please describe at which age and the nature of the regression: _____
   _____

2. **When your child has a high temperature, does she/ he have a substantial improvement in communication?** No/Yes (Please specify) _____
   _____

3. **Has your child had a genetic testing?** No/Yes
   CMA (chip) - No/Yes /unknown; **Exome sequencing** - No/Yes /unknown

4. **Does your child have a genetic diagnosis?** No/Yes _____

5. **Does your child suffer from a syndromic ASD?** (Such as Down syndrome or Angelman syndrome, or substantial deficits in motor function or cardiac problems, or unusual facial features) – No/Yes (Please specify _____

6. **Does your child have a sibling or an uncle with ASD (even mild)?** No/Yes (Please specify) _____

7. **Does your child have a sibling or an uncle with intellectual disability?** No/Yes (Please specify) _____

8. **Birth week**: _____: Normal / 35-36/ 32-34/ 29-31/ 23-28

9. **Birth weight:** _____ Appropriate for Gestational Age /
   Small for Gestational age/ Large for Gestational age

10. **Curse of delivery:** Normal / Cesarean / Vacuum/ Forceps, **APGAR**: _____

11. **Was your child transferred to NICU after the labor?** No / Yes-less than 24 hours / 2-7 days / 8-13 days / 2-4 weeks / longer than 4 weeks _____

12. **Was you child ever hospitalized in ICU:** No/Yes (Please specify)
    _____

13. **Was there a serious problem in pregnancy or childbirth or a serious illness / accident in the first two years of life? (**Severe complications of prematurity, meningitis or encephalitis with prolonged hospitalization in intensive care, drowning or suffocation, severe accident) No / Yes_____

14. **How old was your child when he was first diagnosed with ASD?** _____

15. **How old was your child when he/she started attending a special education program?**
    _____

16. **Did your child suffer (for more than 6 months) from any of the following problems?** (Please mark all that applies)
Significant behavioral difficulties / Anxiety/ Significant sleep disorder / ADHD / Epilepsy / High sensitivity to noise, smell or touch /Unusual seeking of stimulations: smell, touch, sight / Low motor skills / GI problems / Food allergy / Food sensitivity

17. **Does your child have any other chronic diseases?** No/Yes _____

18. **Has your child ever taken any medications on a regular basis (for more than a month)**? No/Yes (Please mark all that applies)

Antipsychotics: Neuleptil (Pericyazine) / Rispond, Risperdal (Risperidone) / Abilify, Ariply (Aripiprazole) / Entumine (Clothiapine) / Zyprexa (Olanzapine) / Promethazine (Prothiazin) / Seroquel (quetiapine) / Ziprasidone (Geodon) /
Nozinan (levomepromazine) / clozapine

Stimulants: Ritalin, Concerta, Focalin / Attent, Vyvanse

SNRI: Atomic, Strattera

alpha-2 agonists: Clonirit (clonidine), Intuniv (Guanfacine)

Mood stabilizers: Depalept (Valproic acid) / Tegratol, Timonil (Carbamazepine)

Benzodiazepines: Rivotril, Clonex (Clonazepam) / Xanax (alprazolam)

SSRIs: Cipralex, Lexapro (escitalopram), Prozac, Prizma, Flutine (fluoxetine), Favoxil (fluvoxamine), Seroxat (paroxetine), Lustral, Serenada, Zoloft (sertraline)

Other: _____

19. **Is your child currently taking any medications on a regular basis?** No/Yes

1.Name: _____ daily dose _____ since _____
2.Name: _____ daily dose _____ since _____
3.Name: _____ daily dose _____ since _____
4.Name: _____ daily dose _____ since _____
5.Name: _____ _____ daily dose _____ since _____

20. **Has your child ever taken any nutritional supplements (for more than a month)?** No/Yes (Please mark all that applies)

Vitamins: Folic Acid / Folinic Acid / B12 / B6 / Vitamin D
Minerals: Magnesium / Iron / Calcium
Other: Carnitine (L-carnitine, Acetyl carnitine) / Sulforaphane, Glucoraphanin
Other: Melatonin / Probiotics/ Omega – 3
Other: _____

21. **If any of the mentioned nutritional supplements were discontinued in the past 3 months, please specify which and when**: _____

22. **Has your child received antibiotics in the past two months**?
No/Yes (Please specify Name, daily dosage and for how long) _____
_____

23. **Has your child currently taking any nutritional supplements?** No/Yes

1.Name: _____ daily dose _____ since _____
2.Name: _____ daily dose _____ since _____
3.Name: _____ daily dose _____ since _____
4.Name: _____ daily dose _____ since _____
5.Name: _____ daily dose _____ since _____

24. **Please specify the amount of cow milk (not dairy products but actual milk) that your child consumes per day:**
Not at all / Up to half a glass / about one glass / about two glasses / 3 glasses or more

25. **Is your child currently on a special diet?** (Gluten-free / Low Gluten / Lactose free / Low Carbs (modified Atkins)
No/Yes (Please specify Which and for how long) _____
_____

26. **Has your child ever taken medical Cannabis / CBD / Cannabis products?**
No/Yes (Please specify for how long he/she took it and when was it discontinued)
    1. Licensed Medical Cannabis which mostly contains CBD (20:1, 15:3, 24:0, 28:1) for _____ months. Discontinued _____ years and _____months ago.
    2. Medical Cannabis which mostly contains THC (such as: night oil, Erez, Alaska) for _____ months. Discontinued _____ years and _____months ago.
    3. Other: _____ for _____ months. Discontinued _____ years and _____months ago.

27. **Is your child currently receiving behavioral/emotional therapy?** No/Yes
    1. _____. Hours per week: _____. Since _____
    2. _____. Hours per week: _____. Since _____
    3. _____. Hours per week: _____. Since _____
    4. _____. Hours per week: _____. Since _____
    5. _____. Hours per week: _____. Since _____

28. **Please specify all the schools/ education programs your child attended:**

Age _____. Program: _____
Age _____. Program: _____
Age _____. Program: _____
Age _____. Program: _____
Age _____. Program: _____
Age _____. Program: _____
Age _____. Program: _____

29. **Parents education (years)**: Mother: _____ Father: _____

30. **Parents occupation**: Mother: _____ Father: _____

Health State Questionnaire (modified Dosage Record Treatment Emergent Symptom Scale)

Date: _____ GUID: _____ Informant: Mothe / Father / Guardian

During the last 2 weeks (or 4 days, if at study onset), has your child had any of the problems listed?
If you didn't mark 'No', please indicate if the problem was mild, moderate, or severe. If you think that this problem is related to the study treatment, please mark it as well.

	Health Problem	**No**	**Rarely**	**Sometimes**	**Always**	**mild**	**moderate**	**Severe**	**Treatment related**
1	Dizziness								
2	Drowsiness								
3	Restlessness								
4	Aggression								
5	Increased tantrums								
6	Emotional liability								
7	Hair loss								
8	Rash								
9	Blurred vision								
10	Nausea								
11	Vomiting								
12	Abdominal pain								
13	Concentration problem								
14	Dry mouth								
15	Tremors								
16	Headache								
17	Insomnia								
18	Involuntary movements								
19	Depression								
20	Memory problems								
21	Constipation								
22	Loss of appetite								
23	Gain of appetite								
24	Diarrhea								
25	Nasal congestion								
26	Sweating								
27	Weakness								
28	Increased salivation								
29	Increased anxiety								
30	Confusion								
31	Suicidal thoughts								

During the last 2 weeks (or 4 days, if at study onset) Please indicate:
Any other important health problems: _____
Any use of medical services: _____
Any change in the study treatment dose: _____
Any change in the regular medications: _____
Any change in the regular diet or intake of food supplements (including probiotics): _____
Any change in the home or school routine or in the behavioral/ emotional treatments: _____

Do you think the current treatment is helpful: Yes / No
If you answered Yes, please specify in what way: _____

Parental expectations and beliefs

The following table presents various statements. Please indicate to what extent do you agree with each statement (your answers will have no effect on your participation in the study)

#	Statements	Strongly Agree	Quite agree	Not so agree	Not at all agree
1	When I give my child medicine (for example to relieve pain) he/she usually understands that the treatment may help him/her.				
2	My child can understand that the treatment given in the study may improve his / her behavior.				
3	My child can understand that the treatment given in the study may improve his / her communication difficulties.				
4	My child's condition in the last month is worse compared to his average condition				
5	It is hard for me to deal with my child's behavioral difficulties (I am losing patience)				
6	When I am calm and patient my child is usually calmer too				
7	I believe that the treatment given in the study may **greatly** improve my child's communication difficulties.				
8	I believe that the treatment given in the study may **greatly** improve my child's behavioral difficulties				
9	I personally know a family that treatment similar to the one given in the study - helped her a lot.				
10	In the last year, my child received natural or medicinal therapy that helped him/ her with his/ her behavioral difficulties.				
11	In the last year, my child received natural or medicinal therapy that helped him/ her with his/ her communication difficulties.				
12	In the last year my child received natural or medicinal treatment for the behavioral difficulties which was discontinued due to side effects				
13	My child is relatively sensitive to side effects of medications				

Questions for the child participating in the study

- What do you see in the picture?
- Why do you think the child is taking medicine? (Maybe his tummy hurts and they want to help him feel better, maybe he has trouble behaving nicely and they want to help him)
- How can the medicine help the child?
- Do you sometimes have trouble behaving nicely?
- We want to give you a treatment that will help you feel calmer and behave more nicely. Do you think that will help you?
- We also believe the treatment can help you with your communication, like talking, understanding, and playing with others. Do you think that will help you?

Rank (from 1-10):
A. The child's ability to understand that the medicine given to him will improve his behavioral difficulties _____
B. The child's expectations that the treatment will help with his behavior problems _____

Assessment of Blinding Questionnaire

1) **Please select the option that best reflects your beliefs regarding the treatment that your child received in the study**:
   A) I am convinced that the treatment was 'real treatment'
   B) It seems to me that the treatment was 'real treatment'
   C) It seems to me that the treatment was 'placebo'
   D) I am convinced that the treatment was 'placebo'
   E) I really do not know

2) **If you chose A, B, C, or D, please tell us why you chose this option (you can choose more than one option)**
   A) Due to the appearance, smell, or texture of the treatment. please specify: _____
   _____
   B) Due to the effectiveness of the treatment
   C) Due to the inefficiency of the treatment
   D) Due to the side effects of the treatment. Please indicate which side effects: _____
   _____
   E) I have such a feeling but I cannot point to the reason

Gastrointestinal signs and symptoms inventory (modified GISSI-17)

Participant research ID_____

Your relationship to this child: ☐Mother ☐Father ☐Other: _____

Most of the questions on this form are about THE LAST SIX WEEKS.
Please put a check (V) in the box that best describes your child.

In the last 6 weeks, has your child experienced any of the following gastrointestinal (tummy) symptoms:

	Yes	No	Unsure
1. Abdominal (belly) pain	☐	☐	☐
2. Nausea	☐	☐	☐

3. **In the last 6 weeks,** how often did your child usually have BMs?
   a. ☐ Less than once a week
   b. ☐ 1-2 times a week
   c. ☐ 3-6 times a week
   d. ☐ Once a day
   e. ☐ 2-3 Times a day
   f. ☐ More than 3 times a day
   g. ☐ Unsure

4. **In the last 6 weeks**, what were your child's BMs usually like?
   a. ☐ Very hard
   b. ☐ Hard
   c. ☐ Not too hard and not too soft
   d. ☐ Very soft or mushy
   e. ☐ Watery
   f. ☐ Unsure

In the last 6 weeks, did your child:	Yes	No	Unsure
5. Appear to feel pain when having a BM?	☐	☐	☐
6. Have to rush to the bathroom for a BM?	☐	☐	☐

In the last 6 weeks, has your child:	Yes	No	Unsure
7. Spit up ≥ 2x per day?	☐	☐	☐
8. Experienced retching?	☐	☐	☐
9. Tilted his/her head to the side and arched back?	☐	☐	☐

In the last 6 weeks, has your child missed activities due to:	**Yes**	**No**	**Unsure**
10. pain and/or discomfort?	☐	☐	☐
11. Vomiting	☐	☐	☐
12. problems with BMs	☐	☐	☐
13. **In the last 6 weeks**, did your child push his abdomen with his/her hands or your hands, push his/her abdomen against or lean forward over furniture?	☐	☐	☐
14. **In the last 6 weeks**, did your child choke, gag, cough, or sound wet during or after swallowing or with meals?	☐	☐	☐
15. **In the last 6 weeks**, has your child started to refuse many foods that he or she would eat in the past?	☐	☐	☐

Appendix 2

Placebo-controlled clinical trials of cannabinoid therapy in ASD with published results

Study	Treatment	Target daily dose	Participants & design	Primary outcome/s	Key Secondary outcomes	Results
Aran et al. [50]	CBD-rich cannabis extract, [1] CBD:THC ratio = 20:1; Pure CBD&THC, [2] CBD:THC = 20:1	10mg CBD **per kg** of body weight, divided into 3 daily doses	N=150; age 5-21 years; Cross-over; Two 12-week periods	Behavioral improvement at week 12: 1. CGI-I [3] 2. HSQ-ASD [4]	1. Social Responsiveness Scale (SRS-2) 2. Autism Parental Stress Index (APSI) 3. Safety	Both cannabinoids were superior to placebo in 2 out of 4 assessments: CGI-I [3] and SRS-2. No severe adverse events
Silva et al. [102]	CBD-rich cannabis extract [5] CBD:THC ratio = 9:1	Variable dose: 1.0-11.6 mg CBD (**total**) divided into 2 daily doses	N=60; age 5-11 years; Parallel groups; 12 weeks	Primary Vs Secondary outcomes were not specified. **1.** A semi-structured interview prepared by the authors. **2.** Autism Treatment Evaluation Checklist (ATEC). **3.** Childhood Autism Rating Scale (CARS)		Superior to placebo in 3 out of 9 symptoms assed by the interview but not on ATEC or CARS

[1] BOL-DP-O-01-W, a whole-plant cannabis extract containing CBD and THC at a 20:1 ratio

[2] BOL-DP-O-01, purified CBD + purified CBD at a 20:1 ratio

[3] CGI-I: Clinical Global Impression-Improvement was used to measure improvement in disruptive behaviors by incorporating anchoring instructions related to behavioral difficulties.

[4] HSQ-ASD: Home Situations Questionnaire-ASD, a parent-rated measure of noncompliant behavior in children with ASD

[5] CBD-rich cannabis extract containing CBD and THC at a 9:1 ratio, supplied at a very low concentration (5mg/mL) by the Associação Brasileira de Apoio Cannabis Esperança (ABRACE)

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