Statistical Analysis Plan (SAP)

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)

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SAP revision history

Version	Author	Date	Description

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1 Introduction

1.1 Background and rationale

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.[1] Approved antipsychotic medications for managing associated irritability often result in substantial metabolic side effects. [2,3] Cannabidiol (CBD), a non-psychoactive cannabinoid, targets multiple neuronal and glial pathways to reduce excitation and inflammation.[4-11,12-15] In contrast, tetrahydrocannabinol (THC), a psychoactive cannabinoid, directly activates the endocannabinoid system, which is impaired in ASD, and decreases social anxiety and hostility. [16] Preliminary trials in children with ASD have demonstrated the efficacy of a CBD-predominant formulation combined with low-dose THC.[17] 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.[18-23]
The present study is designed to assess the combined impact of CBD, terpenes, and low-dose THC. We hypothesize that this combination will lead to significantly greater improvements in disruptive behaviors and core autistic symptoms compared with placebo.

1.2 Objectives

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 using the Clinical Global Impression- Improvement (CGI-I) and the Caregiver Global Impression of Change (CGIC) assessments – change from baseline to week 8. (3) To assess changes in the quality of life, sleep parameters, and gastrointestinal problems using the Pediatric Quality of Life Inventory (PedsQL), 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, terpene-enriched cannabis oil (study drug) and terpenes-enriched CBD oil, using the above assessments - change from baseline to week 8 and week 17.

2 Study Methods

2.1 Trial design

This is a phase II, 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-predominant 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 (3.8 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.

2.2 Randomization

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.

2.3 Sample Size

The planned sample size is 78 participants (39 per arm). This calculation, including assumptions about expected effect size and dropout rate, is described in detail in Protocol 0336-24-SZMC, Section 13 (Statistical Considerations).

2.4 Framework

This trial is designed as a **superiority trial**. The primary hypothesis is that terpene-enriched CBD-predominant cannabis oil will be superior to placebo in reducing irritability symptoms in children with ASD, as measured by change in ABC-I score from baseline to week 8.

2.5 Statistical interim analysis and stopping guidance

This study incorporates an adaptive design with interim analysis by an external, non-blinded biostatistician. Final sample size will be determined based on that interim analysis

An independent Data and Safety Monitoring Board (DSMB) will review the interim analyses as needed, although no formal stopping rules are defined for this study, and will ensure that all trial data remain confidential.

2.6 Timing of Analyses

The final statistical analysis will be conducted after completion of follow-up for the last randomized participant, once all data through week 17 have been collected.

2.7 Timing of outcome assessments

On-site visits: 5 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.

3 Statistical Principals

3.1 Confidence intervals and *P* values

Hypothesis testing, unless otherwise indicated, will be 2-sided and performed at the 5% significance level. When confidence intervals (CI) are presented, they will be 2-sided with a confidence coefficient of 95%.

No adjustments for multiplicity will be made for the secondary efficacy analyses in this study. Nominal p-values will be presented for informational purposes only.

3.2 Adherence and Protocol deviations

3.2.1 Adherence

Adherence is defined as the proportion of study doses actually taken during the randomized phase (Days 1–56). Adherence will be assessed from caregiver reports collected at prespecified phone/Zoom visits (Days 4, 8, 12,42) and at on-site visits.

Overall duration of exposure in days will be computed for each randomized subject using the following equation:

End date of study treatment – start date of study treatment +1

Summary statistics for treatment compliance will be displayed by arm (n, mean, median, standard deviation, minimum, and maximum).

3.2.2 Deviations from the Protocol

Neither the Principal Investigator nor any sub-investigator is authorized to deviate from the protocol, except as permitted under section 4.5.4(b) of ISO 14155:2011 and 21 CFR 812.150(a)(4). Specifically, protocol deviations may only be made or reported if they affect a subject's rights, safety, or well-being, 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.

3.3 Analysis populations

3.3.1 Safety Population

The Safety population is defined as all enrolled subjects who received at least one dose of study medication (including placebo). Safety analyses will be performed using the Safety population and subjects will be analyzed according to the treatment received in each part.

3.3.2 Intent to Treat Population (ITT)

ITT population will include all randomized subjects regardless of receiving study drug or being assessed in study measurements.

If all randomized subjects receive at least one dose of study drug, the ITT population and the safety population will be equal, and will be reported as one.

3.3.3 Per Protocol Population (PP)

The PP population will include all randomized subjects who completed the study according to protocol.

The primary efficacy analysis will be conducted on the intention-to-treat (ITT) population, defined as all randomized participants analysed according to their assigned treatment arm, regardless of adherence or protocol deviations. A supportive secondary analysis will be performed on the per-protocol (PP) population, consisting of participants who complete the study without major protocol deviations, in order to assess the robustness and consistency of the results.

4 Trial Population

4.1 Screening data and Eligibility

Screening will occur at Visit 1 (Day −90 to Day −1). Eligibility assessments include DSM-5 diagnostic confirmation with CARS-2, CGI-S, ABC-I, SRS-II, medical history, vitals/exam, and labs, as outlined in the protocol schedule of activities. We will report screening data to describe the representativeness of the randomized cohort as follows:

- **CONSORT-style flow:** numbers screened, eligible, consented, randomized, and not randomized, with reasons for screen failure.
- **Reasons for screen failure** (tabulated counts and %): did not meet inclusion thresholds (e.g., ABC-I <18, SRS-II <66, CGI-S <4, age/weight out of range), met exclusion criteria (e.g., recent seizures/antiepileptic change, recent cannabinoid use, clinically significant abnormal labs, prohibited comedications), declined consent, logistical reasons.
- **Descriptive comparison**: age and sex distributions in screened vs randomized participants, to comment on representativeness.

Eligibility criteria (full detail in **Protocol 0336-24-SZMC**) are summarized here for analysis clarity.

Inclusion criteria

- Age 4–12 years.

- ASD diagnosis per DSM-5, confirmed by CARS-2.
- CGI-S ≥4 (moderate or greater severity).
- ABC-I ≥18.
- SRS-II total T ≥66.

Exclusion criteria

- Weight <12.5 kg or ≥57.5 kg.
- Current/past heart failure, drug addiction, schizophrenia/psychosis/bipolar/PTSD/MDD, or schizophrenia in a first-degree relative.
- Seizure or change in antiepileptic medication in the 4 months before randomization.
- Clinically significant abnormal physical exam or labs (e.g., heart, liver, renal function).
- Change in pharmacologic/behavioral treatment or home/school setting within 4 weeks prior to randomization, or planned changes during the study.
- Cannabinoid treatment within 4 weeks prior to randomization.
- Predicted low compliance with study procedures.
- Concurrent opiates or alcohol use.

4.2 Withdrawal/ Follow-up

Withdrawal will be classified at four levels:

1. **From intervention** – participant discontinues study drug (reasons will be documented)
2. **From follow-up** – participant stops attending visits/assessments (lost to follow-up)
3. **Withdrawal of consent** – participant/caregiver withdraws consent for further participation (reasons will be documented)
4. **Protocol-mandated discontinuation** – Investigator/sponsor stops intervention and/or follow-up (e.g., safety reasons, ineligibility discovered later).

Withdrawal status (and reason, if provided) will be captured at the time of discontinuation and/or at the next scheduled contact (phone/Zoom or on-site), using the visit schedule and windows defined in the protocol. Compliance and adverse events are collected repeatedly at these visits, enabling precise dating of discontinuation and last contact.

Reasons will be recorded and coded into prespecified categories: adverse event/tolerability, lack of efficacy, caregiver decision/burden, non-adherence, intercurrent illness/procedure, protocol-specified ineligibility discovered post-randomization, logistical/administrative, and other.

Presentation will include:

- **CONSORT flow**: numbers screened, randomized, treated, completed, withdrawn (by level and reason), and analysed.

- Tables by treatment arm: counts (%) for each withdrawal level (intervention only; follow-up; consent withdrawal), timing and reason category.

4.3 Baseline patient characteristics

Demographics variables and baseline characteristics will be summarized for the Safety population. Demographic and baseline data will be presented overall and by treatment sequence.

Demographic variables include age, sex, ethnicity, height, weight, and body mass index (BMI) collected at the Screening Visit.

Baseline characteristics include the following:

- DSM-5 levels of support required
- CARS-2
- VABS-3
- CGI-S,
- ABC-I
- SRS
- NVIQ
- Etiological factors
- Comorbidities
- History of autistic regression
- Diet
- Behavioural treatments
- Impact of high fever on social deficits status
- Concomitant medications

Data from continuous variables will be tested for normal distribution. Summaries of continuous variables that are normally distributed will be presented as means and standard deviations or medians and inter quartiles for skewed data. Categorical variables will be presented as frequencies and percentages.

5 Analysis

5.1 Outcomes

Primary endpoint

ABC-I: The ABC[24,25] 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[26] 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) and the ABC-I total is the sum of the 15 items (range 0–45; higher = worse).

Assessed at baseline, Week 4, Week 8, and Week 17; the primary endpoint is the change in ABC-I from baseline to Week 8.

Main secondary outcome measures

VABS-III [27]: 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.

Assessed at baseline and Week 8; analysed as change from baseline to Week 8.

SRS (Hebrew version) [28,29]: this 65-item caregiver or teacher questionnaire contains five subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Autistic Mannerisms, which respectively measure the ability to recognize social cues, the ability to interpret social cues, the ability to use expressive verbal and nonverbal language skills, the ability to engage in social-interpersonal behaviors, and the tendency to display stereotypical behaviors and restricted interests characteristic of autism. Each item is rated on a four-point Likert scale, (0 to 3, total scores range from 0 to 195) with a higher score indicating greater impairment. Each subscale can be converted into T-score. The SRS Total T-score reflects the sum of the T-scores for the five scales. SRS T-scores between 60 and 75 indicate a mild to moderate social impairment whereas scores above 75 indicate a "severe" level. The caregivers will complete the SRS based on the participant's behavior over the previous 6 weeks. The SRS has been used extensively to assess behavioral problems in ASD research [30-35]

Assessed at baseline, Week 4, Week 8, and Week 171; analysed as change from baseline to Week 8.

Other secondary outcome measures:

CGI-I: The CGI [36] 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.[37-39] 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).

CaGIC: 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)[40,41]: 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)[42]: 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)[43]: This 30-item caregiver-reported questionnaire assesses observable signs of poor emotion regulation in children with autism.

Behavior Rating Inventory of Executive Function (BRIEF)[44]: 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)[45]: 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

Pediatric Quality of Life Inventory (PedsQL)[46]*:* The PedsQL™ is a validated tool for measuring health-related quality of life (HRQOL) in children aged 2–18 years with acute or chronic health conditions, as well as in healthy individuals. Different formats exist, including parent proxy reports and age-specific versions, which use identical items with age-appropriate language. This study will use the parent proxy report of the PedsQL 4.0 Generic Core Scales, which assess physical, mental, social health, and role functioning.

Quality of Life in Autism Questionnaire (QOLA) – Parent version[47]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,[48] a brief parent-report screen for common gastrointestinal problems in children with ASD.

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[49] 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)[50,51] and relative mRNA expression levels (real-time PCR) of components of the eCB system in whole blood,[51] RNA-sequencing of whole blood samples (transcriptome),[52] and plasma proteome,[53] 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.[54-56]

Assessment of the other secondary outcomes is detailed within the protocol

5.2 Statistical Methods

The primary endpoint, change in ABC-I from baseline to Week 8, will be analysed under a superiority framework using a mixed model for repeated measures (MMRM). The model will include fixed effects for treatment (study drug vs placebo), visit (Baseline, Week 4, and Week 8), and the treatment-by-visit interaction, with a subject-level random effect. The primary contrast is the adjusted difference between arms at Week 8, reported as a least-squares (LS) mean difference with two-sided 95% confidence interval and p-value.

Secondary analyses will be conducted using the same methodology as for the primary ABC-I endpoint.

Sensitivity analyses will include per-protocol analysis and may utilize multiple imputation to confirm the robustness of findings.

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.

Data from continuous variables will be tested for normal distribution. Summaries of continuous variables that are normally distributed will be presented as means and standard deviations or medians and inter quartiles for skewed data. Categorical variables will be presented as frequencies and percentages. If distributions are not normal, rank-based approaches will be applied.

5.3 Missing data

The primary analyses will use mixed models for repeated measures, which incorporate all available observations under a missing at random (MAR) assumption and do not require single imputation. The primary analysis assumes that ABC-I scores are MAR, an assumption deemed reasonable for this study. Missing data are unlikely to differ systematically from observed values for unknown reasons. We will describe the extent and timing of missing data by arm and visit, including numbers and percentages missing for each endpoint and known reasons. To assess robustness to departures from MAR, we will perform multiple imputation as a sensitivity analysis.

5.4 Harms

All subjects who enter the study will be assessed for safety. Safety will be monitored by structured monthly parental reports, immediate intercurrent reports of exceptional phenomena, physical examination, detailed interview, and adverse event (AE) assessment at each visit.

5.4.1 Adverse Events

AEs will be monitored using a 31-item modification of the Dosage Record and Treatment Emergent Symptom Scale. Recent health concerns, use of medical services, and changes in study treatment dose and concomitant medications will be recorded. BMI, pulse rate, and blood pressure will be assessed at each clinic visit. New AEs or worsening of previously reported AEs 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 Principal Investigator will evaluate all AEs for timing, biological plausibility, alternative causes, dose–response relationship, and prior knowledge to determine relatedness to the study drug as not related, unlikely, possibly, probably, or definitely related. AEs will be reported and managed according to 21 CFR (§812.150(a)(1), §812.46(b), §812.150(b)(1)), MEDDEV 2.7/3 (Dec 2010), and ICH-GCP guidelines. Anticipated AEs include rare insomnia, irritability, and intolerance of the taste and smell. An overview of the incidence and frequency of AEs will be presented by treatment condition in the Safety population.

5.4.2 Blood samples

Complete blood count, liver transaminases and total bilirubin will be measured as part of the safety analyses.

5.5 Statistical software

SAS (PROC MIXED), R (lme4 package), or equivalent statistical software.

6 References

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