AWARD NUMBER: W81XWH-17-1-0253

TITLE: Cannabidivarin (CBDV) Versus Placebo in Children with Autism Spectrum Disorder (ASD).

PRINCIPAL INVESTIGATOR: Eric Hollander, MD

CONTRACTING ORGANIZATION: Albert Einstein College of Medicine, Inc., Bronx, NY

REPORT DATE: **August 2021**

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
August 2021	annual	01Aug2020-31Jul2021

4. TITLE AND SUBTITLE
Cannabidivarin (CBDV) versus placebo in children with Autism Spectrum Disorder

5a. CONTRACT NUMBER
W81XWH-17-1-0253

5b. GRANT NUMBER
AR160104

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The CDC currently estimates 1 in 54 children have Autism Spectrum Disorder (ASD). Children with ASD have problems with social communication, irritability, repetitive behaviors, impulsivity, temper tantrums, and a high caregiver burden. The only medications approved by the FDA for symptoms of ASD are aripiprazole and risperidone. Both of these are used to reduce irritability but have short-term and long-term side effects. This double-blind randomized placebo-controlled study will test the efficacy and safety of a new medication, cannabidivarin (CBDV), to treat autism in children ages 5-18. CBDV is non-psychoactive, is derived from the cannabis plant, but has effects opposite to THC. Approximately 100 patients will be enrolled at Montefiore and NYU sites during the duration of the study. The study lasts up to 16 weeks, and during this time, patients will undergo 9 study visits. Mood, social and cognitive functions will be assessed by the means of research questionnaires. All adverse effects will be reported. We will assess the effects of CBDV versus placebo on irritability and social functions in patients with ASD.

In year 3 of the project we have adapted study protocol to the COVID-19 pandemic situation making 5 out of 9 visits of study participants remote, and received acknowledgement from ORP HRPO that these changes do not require HRPO approval prior to implementation. During year 4 of this project, we continued our efforts in screening and enrolling patients both at AECOM-MMC site and NYU site and obtained No cost Extension (NCE) to conduct a study from the Department of Defense.

Study is ongoing. Prior to COVID-19 pandemic onset, we have projected a total of 84 patients enrolled by end of Q4 year 4, at the two sites, including AECOM/MMC and NYU (consisting of 16 subjects to be enrolled at year 2, i.e. 8 subjects at each site and 34 subjects to be enrolled at year 3, i.e. 17 subjects at each site; and 34 subjects to be enrolled at year 4, i.e. 17 subjects at each site). Total number of subjects screened at AECOM/MMC site is 31 to date. Therefore 31 subjects were screened, 17 subjects have completed the study, 1 is active, 3 are screen failures, 6 have dropped out from the study and 4 are waiting to be randomized. Total number of subjects screened at NYU site is 9 to date. Therefore 9 subjects were screened, 2 subjects have completed the study, 1 is active, 4 are screen failures and 1 is waiting to be randomized.

15. SUBJECT TERMS

None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		37	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

TABLE OF CONTENTS

1.INTRODUCTION:

The CDC currently estimates 1 in 54 children have Autism Spectrum Disorder (ASD). Children with ASD have problems with social communication, irritability, repetitive behaviors, impulsivity, temper tantrums, and a high caregiver burden. The only medications approved by the FDA for symptoms of ASD are aripiprazole and risperidone. Both of these are used to reduce irritability but have short-term and long-term side effects. This double-blind randomized placebo-controlled study will test the efficacy and safety of a new medication, cannabidivarin (CBDV),to treat autism in children ages 5-18. CBDV is non-psychoactive, is derived from the cannabis plant, but has effects opposite to THC. Approximately 100 patients will be enrolled at Montefiore and NYU sites during the duration of the study. The study lasts up to 16 weeks, and during this time, patients will complete 9 study visits (due to COVID-19 Pandemic 5 out of 9 visits of study participants are remote). Mood, social and cognitive functions will be assessed by the means of research questionnaires. All adverse effects will be reported. We will assess the effects of CBDV versus placebo on irritability and social functions in patients with ASD.

2. KEYWORDS:

Cannabidivarin, Autism spectrum Disorders, irritability, cannabinoids, autism, repetitive behaviors

3. ACCOMPLISHMENTS:

What were the major goals of the project?

	Timeline	**AECOM/Monte**	**NYU**
Major Task 1: Preparatory Stage	Months		
Subtask 1: Prepare and Submit Regulatory Documents			
Coordinate with Sites and GW Pharma for clinical trial agreements (CTAs) submission	1-4	100%, 11/16/2018	100%, 08/09/2019
Coordinate with Sites and GW Pharma for nondisclosure agreements (NDAs).	1-4	06/03/2016	06/03/2016
Coordination with GW Pharma and other sites for the continued work on the submission or re-submission of an Investigational New Drug (IND) application to the U.S. Food and Drug Administration. The Investigators plan to submit the IND application prior to study start so that approval is underway when the funding period begins.	1-4	IND application submitted on 01/19/2018. IND may proceed status granted on 04/02/2018. Annual IND report receipt acknowledged on 07/31/2019 and 09/29/2020.	Same status as at AECOM/ MMC
Refine eligibility criteria, exclusion criteria, screening protocol	1-4	100%	100%
Finalize consent form & human subjects protocol	1-4	100%	100%
Finalize recruitment materials	1-4	100%	100%
Coordinate with Sites for the US Army Medical Research and Material Command Office of Research Protections (ORP) and the Human Research Protection Office (HRPO) Submission	1-4	100%	100%
Coordinate with Sites for IRB protocol submission	1-4	100%	100%
Coordinate with Sites for CTSA sponsored Clinical Research Center (CRC) approval	1-4	100%	100%
Submit amendments, adverse events and protocol deviations as needed	As needed	As needed	As needed

Coordinate with Sites for annual IRB report for continuing review	Annually	Reapproved on 10/8/2020.	Reapproved on 03/09/2021.
Milestone Achieved: Local IRB approval at AECOM/Monte and NYU	4	05/15/2018	05/02/2018
Milestone Achieved: HRPO and ORP approval for protocol	4	05/25/2018	2/5/2019
Subtask 2: Training of Study Staff			
Coordinate with Sites for Coordinator training	4-6	100%, 06/01/2018	100%, 06/01/2018
Coordinate with Sites for training Raters until 100% concordance	4-6	100%, 06/01/2018	100% 06/01/2018
Complete Regulatory Binders	4-6	100%	100%
Create Manual of Operations	4-6	100%	100%
Milestone Achieved: Research staff trained and Regulatory procedures set up	4-6	06/01/2018	06/01/18
Subtask 3: Facilitate Communication Between Sites and Staff			
Coordinate with Sites bi-weekly meetings to review study goals, recruitment and enrollment	1-48	100%	100%
Milestone Achieved: Bi-weekly meetings completed throughout study	1-48	Study is ongoing	Study is ongoing
Subtask 4: Assessments and Surveys Preparation			
Finalize assessment measurements	4-6	100%	100%
Order all assessments and distribute across sites	4-6	100%	100%
Prepare Patient Study Binders including surveys and source documents	4-6	100%	100%
Milestone Achieved: All study materials prepared for patient enrollment	4-6	100%	100%
Subtask 5: Receipt of Study Drug			
Develop protocol with pharmacy for storage and dispensing of study drug/placebo and randomization protocol	1-4	08/01/2018	08/01/2018
Work with GW Pharma to ship study drug and placebo to sites	6	***Drug shipped by GW to the US depot on 2/5/19; received at the US depot on 02/15/19; received at AECOM/MMC on 03/20/19.***	***Drug shipped by GW to the US depot on 2/5/19; received at the US depot on 02/15/19. NYU site received the drug supply on 11/19/2019***
Milestone Achieved: Receipt of Study Drug and Placebo	6	Achieved	Achieved
Subtask 6: Data Management Preparation			
Coordinate with Sites for flow chart for study steps, data collection and database requirements	4-6	100%	100%
Develop and Maintain Electronic Database at AECOM	4-48	100%, maintenance is ongoing	100%, maintenance is ongoing
Milestone Achieved: Electronic database prepared and maintained throughout study	4-48	Study is ongoing	Study is ongoing
Major Task : Participant Recruitment and Enrollment			
Subtask 1: Begin Subject Recruitment			
Contact patients in site databases	6-42	100%	100%

Advertise using internet and radio advertisements and study flyers	6-42	99%, study is ongoing	99%, study is ongoing
Milestone Achieved: All patients in databases contacted	6	100%, ongoing	100%, ongoing
Milestone Achieved: 1st Advertisements are placed	6	100%, study is ongoing	100%, study is ongoing
Subtask 2: Conduct Study			
Consent and screen potential patients and review I/E criteria (n=100)	6-42	Ongoing	Ongoing
Evaluate and assign eligible participants to one of the two randomized groups	6-42	Ongoing	Ongoing
Participants complete assigned condition over 12 weeks	6-42	Ongoing	Ongoing
Assess all participants at the appropriate study visits	6-42	Ongoing	Ongoing
Milestone Achieved: Study Begins	6	April 12 2019	October 23 2019
Milestone Achieved: 1st Patient Consented and Enrolled	6	April 12 2019	January 14 2020
Milestone Achieved: Last Patient Consented and Enrolled	42	Ongoing	Ongoing

What was accomplished under these goals?
Major Task 1: Preparatory Stage.

Subtask 1: Prepare and Submit Regulatory Documents.

Coordinate with Sites and GW Pharma for Clinical Trial Agreements (CTA) Submission.

We regularly discussed all contract-related issues with Dr. Dhanonjoy C. Saha, Director of Office of Grant Support at AECOM/MMC. Furthermore, we conducted a series of conference calls for CTA negotiation and revisions with Deirdre Flaherty, Head of Pipeline Programs at GW Pharma, which occurred on 9/11/17, 10/20/17 and 10/23/17.

On 01/22/2018 we conducted a meeting between Dr. Eric Hollander (AECOM/MMC), Dr. Vera Nezgovorova (AECOM/MMC), Deirdre Flaherty (GW Pharma) and James Ryan (GW Pharma Contract Specialist) to finalize terms of statistical support for the project and discuss study drug dispense questions. Same day, a revised draft of the CTA was received for review by Dr. Dhanonjoy C. Saha, Director of Office of Grant support at AECOM/MMC. On 02/07/2018 we conducted a meeting with Attorney Laura Wilson-Youngblood, Assistant Counsel at AECOM/MMC, to review the terms before she could proceed for further negotiation of final details with GW Pharma.

On 03/16/18 a call between Attorney Laura Wilson-Youngblood, Assistant Counsel at AECOM/MMC, Deirdre Flaherty (GW Pharma), James Ryan (GW Pharma Contract Specialist), Dr. Eric Hollander (AECOM/MMC) and Dr. Vera Nezgovorova (AECOM/MMC) occurred to further negotiate details of the CTA with GW Pharma with an emphasis on Steering Committee charter development. It was decided that AECOM/MMC will proceed with CTA signature with GW Pharma, and a subcontract will be than issued between AECOM/MMC and NYU to document the terms of collaboration for this study. Subcontract between AECOM/MMC and NYU is currently being finalized.

Following that call, CTA updated by GW Pharma was reviewed by Einstein legal team, which included Lara Jean Ancona, Esq., Partner at Garfunkel Wild firm. Feedback on breaking the blind for the analyses; rights and liabilities of GW Pharma and feedback on publications committee has been incorporated. CTA and Steering Committee Charter were submitted for GW Pharma review on 03/30/18. To further address final terms of the agreement, a call between Dr. Volker Knappertz (Chief Medical Officer at GW Pharma), Deirdre Flaherty (GW Pharma) and Dr. Eric Hollander (AECOM/MMC) occurred on 05/03/18, where key points of the CTA were reviewed prior to its execution by legal teams on both sides. On 05/10/2018 Einstein legal team, comprising Attorney Laura Wilson-Youngblood, Assistant Counsel at AECOM/MMC and Lara Jean Ancona, Esq., Partner at Garfunkel Wild firm, had a call with James Ryan (GW Pharma Contract Specialist) to finalize terms of the CTA. Revisions to the contract in regards to IP and data analysis provisions were incorporated by GW Pharma contract department and Einstein legal team and sent to GW Pharma contract division for approval on 06/13/2018. GW Pharma contract division completed review of the revised contract terms on 07/18/2018. Dr. Eric Hollander (AECOM/MMC), Dr. Orrin Devinsky (NYU) and Dr. Volker Knappelz (Chief Medical Officer at GW Pharma) had a call on 07/20/2018 to discuss IP provisions for the CTA finalization. Einstein legal team had a series of calls with Cynthia Clark, attorney from Greenwich Biosciences (US subsidiary of GW Pharmaceuticals) on 07/18/2018, 08/07/2018, 08/20/2018 and 08/28/2018 to discuss IP provisions. Following these calls a budget was prepared to reflect additional funds that would be provided by GW to cover the creation of a study specific EDC (Electronic data capture solution), additional staff and pharmacy support, and for safety labs requested by the FDA. Budget was prepared for AECOM/MMC and NYU sites and submitted on 09/12/2018. On September 26 2018 Dr. Eric Hollander (AECOM/MMC), Casara Jean Ferretti (AECOM/MMC), Dr. Vera Nezgovorova (AECOM/MCC) had a call with GW Pharma financial team to review the submitted budget. Additional follow-up communication, which included calls and emails in regards to budget justification were conducted bi-weekly during October 2018 between AECOM/MMC team and GW Pharma team. On 11/16/18 GW Pharma finalized approval of the additional funding support, and the CTA contract between AECOM/MMC and GW Pharma was signed by GW Pharma. An amended contract between AECOM/MMC and GW was executed on 03/20/2019. Subcontract between AECOM/MMC and NYU was signed on 08/09/2019. An additional amendment for the contract between AECOM/MMC to reflect data management costs supplementation by GW was signed on 01/16/2020. Amendment to the contract between AECOM/MMC and GW Pharma was signed on 03/24/2021. Research subaward agreement amendment between AECOM/MMC and NYU was signed on 04/06/2021.

Coordinate with Sites and GW Pharma for Nondisclosure Agreements (NDA).

Two-way confidentiality agreement between Montefiore Medical Center and GW Pharma has been signed as of June 3 2016, and the copy of the executed CDA is on file.

Coordinate with GW Pharma and other sites for the continued work on the submission or resubmission of an Investigational New Drug (IND) Application to the U.S. Food and Drug Administration.

During current reporting period, we obtained a study May proceed letter from an Investigational New Drug (IND) Application to the U.S. Food and Drug Administration.

This work was preceded by a series of email communications and phone calls organized by Kevin Hong (AECOM/MMC)) and Karen Twigden (GW Pharma), which took place on 7/26/17, 7/28/17, 8/7/17, 8/10/17, and 8/16/17. Dr. Hollander and Kevin Hong organized and participated in a meeting with Dr. Orrin Devinsky, Latoya King, and Anjanette Burns at NYU to review logistic obstacles and regulatory challenges associated with IND submission. Following that meeting on 9/11/2017, Dr. Hollander and GW Pharma agreed to delay IND application submission. It was suggested to wait until release of Edition 6 of the Investigator's Brochure (IB), as it would include updated pharmacokinetics and dosage regimen data pertinent to the upcoming IND submission. Upon release of IB edition 6 by GW Pharmaceuticals in preparation of our IND submission, we conducted a series of meetings with Dr. Michelle Wellborn (GW Pharma consultant) and Deirdre Flaherty (GW Pharma), which occurred on 11/22/2017, 11/27/2017 and 01/17/2018. Investigational New Drug Application to the U.S. Food and Drug Administration was submitted by us on 01/19/2018. It was received into Neurology Division Office on 02/01/2018 with assigned IND # 138371. Study May Proceed letter from Department of Health and Human services, Food and Drug Administration, Division of Psychiatry Products, Office of Drug Evaluation I, Center for Drug Evaluation and Research was received on 04/02/18 with non-hold comments. These non-hold clinical and clinical pharmacology comments were incorporated in the study protocol. Annual IND report was submitted on 07/22/2019 and its receipt was acknowledged on 07/31/2019. Annual IND report was submitted on 09/21/2020, and its receipt was acknowledged on 09/29/2020.

BNE application submission.

On 10/9/17, Dr. Eric Hollander and Kevin Hong consulted Dr. Sheryl Haut, Director of the Adult Epilepsy Program and Chief of Neurology Service at AECOM/MMC, who had worked with a Cannabidiol containing compound from GW Pharma in the past to inquire about unanticipated challenges for the study implementation and were advised on importance of tandem submission to the Institutional Review Board and to the New York State Department of Health, Bureau of Narcotic Enforcement (BNE) for the timely BNE inspection scheduling and application processing.
We submitted application to the New York State Department of Health, Bureau of Narcotic Enforcement (BNE) for Class 7 Individual Researcher and Class 9 Importer licenses approvals in tandem with our IRB submission. Our BNE application was received as of 11/7/2017. Soon after AECOM/MMC Investigational Drug Service Pharmacy received BNE inspection for GW Pharma safe, which was approved. On 01/16/2018 after submitting conditional IRB approval notice for AECOM/MMC site, we were advised by Michele Mulloy, Regulatory Compliance Section Manager, Bureau of Narcotic Enforcement (BNE), that we would need to provide final unconditional IRB approval letter and IND approval letter from the FDA prior to the BNE final review and approval. Following FDA study May Proceed letter receipt on 04/02/18, we received an IRB approval letter without IND clause, which satisfied Bureau of Narcotic Enforcement (BNE) requirements. Case number for BNE inspection was assigned. As we were moving forward with BNE inspection, we were advised that an independent safe designated only for Dr. Hollander study would be required as a condition for BNE approval. Reconditioned Amsec Plate Steel Safe Model 2230 (right swing) UL-TL-30 1 Group 1R radiological lock: SG560 UL group 1 lock, SGD550 dial w/ 10" spindle; SGR167 ring, SC, SPKL was successfully ordered and installed on 04/16/18. Inspector Johnson from the BNE completed site and safe inspection on 04/18/18. BNE review was completed on 04/25/18. Dr. Hollander received Class 7 Individual Researcher and Class 9 Importer licenses approvals on 05/11/2018. Renewal of BNE Class 7 license application to engage in a controlled substance activity was submitted by Dr. Hollander on 02/03/2020 and was approved on 03/13/2020. BNE Class 7 license is effective from April 25 2020 to April 25 2022.

DEA application submission.

We communicated with Terrance Woodworth (GW Pharma consultant) and were advised to submit DEA application upon submission of our IND application prior to the IND approval by the FDA. Thus on 02/09/2018 we submitted DEA Schedule I application, which included the following items:
-DEA application form 225
-Certification that an IND application was submitted
-PI signed and dated CV
-Cover Letter
-Application Fee
-Copy of conditionally IRB approved study protocol
-IND acknowledgement letter
BNE approval and IND approval by the FDA are required for the subsequent DEA approval. IND approval by the FDA was received on 04/02/2018. However, in order to expedite DEA approval, we scheduled DEA Inspection on site. Inspector Rivera completed DEA inspection at AECOM/MMC on 04/11/18. Additional DEA inspection to approve reconditioned Amsec Plate Steel Safe Model 2230 (right swing) UL-TL-30 1 Group 1R radiological lock: SG560 UL group 1 lock, SGD550 dial w/ 10" spindle; SGR167 ring, SC, SPKL was conducted by Inspector Rivera on 05/18/2018. DEA Schedule I license was received on June 26 2018. Upon DEA annual renewal requirements, it was renewed 09/05/2018 and 09/18/2019 and was valid through 10/31/2020. As of March 20, 2020, DEA formally notified GW/Greenwich Biosciences

Inc. that they considered GW's products, including CBDV and GWP42006 to be non-controlled substances. Recently, DEA also published this information in the Federal Register. We also received a memo from GW/Greenwich Biosciences Inc. on June 8 2020, which was acknowledged by Einstein IRB, #1 East on 06/11/2020 with reference #065366.

Since the CBDV used in the study is no longer a controlled substance, there is no further need for Dr. Hollander to have the DEA Schedule I Researcher registration i.e. to extend his registration.

Finalize consent form and human subjects protocol.

After programmatic review and subsequent follow-up communications with Dr. Stan Niu, Science Officer at the Department of Defense, requested changes in the protocol have been incorporated. After a conference call on 10/20/17 between Dr. Eric Hollander (AECOM/MMC), Dr. Orrin Devinsky (NYU), Dr. Volker Knappertz (Chief Medical Office at GW Pharma), Deirdre Flaherty (GW Pharma), James Ryan (Contract specialist at GW Pharma), Dr. Vera Nezgovorova (AECOM/MMC), Kevin Hong (AECOM/MMC), Danya Schlussel (AECOM/MMC) and Dr. Bonnie Taylor (AECOM/MMC), language was added to eligibility criteria regarding parent consent and child assent; exclusion criteria were specified regarding history of drug abuse. As the protocol was finalized, Dr. Hollander deemed that these additions were not substantive, and that they would not affect study recruitment process or statistical power.

Following a meeting on 11/22/17 between Dr. Eric Hollander (AECOM/MMC), Dr. Orrin Devinsky (NYU), Deirdre Flaherty (GW Pharma), Dr. Terrance Woodworth (GW Pharma), Dr. Michelle Wellborn (GW Pharma), Dr. Kenneth Sommerville (GW Pharma) Dr. Vera Nezgovorova (AECOM/MMC), Danya Schlussel (AECOM/MMC) and Dr. Bonnie Taylor (AECOM/MMC), language was added to human subjects protocol to include safety assessments. It was concluded that Columbia Suicide Severity Rating Scale will be administered in the beginning of each study visit. Following this meeting schedule of events was updated to include safety labs. As an additional statistical analysis not affecting statistical power, age of subject was proposed to be a covariate. Appendix A on P450 Drug Interactions, Flockchart table and Appendix B on U91A9 or U9T2B7 potential interactions were added to the human subjects protocol. Informed consent was revised to include language specifying potentials benefits to the study participant. IDSMC (Independent Data Safety Monitoring Committee) charter was developed and its members were confirmed. As the protocol and informed consent were finalized, Dr. Hollander deemed that these additions were not substantive, and that they would not affect study recruitment process or statistical power.

The following changes were made to the study protocol and ICF following HRPO ORP comments received on 02/13/18, and were IRB approved on 03/28/18:

1. Protocol: Section L: As required by the Human Research Protections Office (HRPO)/U.S. Army Medical Research and Materiel Command (USAMRMC) the role and responsibilities of the Research Monitor have been modified and now include: May discuss the protocol with the investigators, interview subjects, and consult with others outside the study about the research; Shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB can assess the Monitor's report; Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official; Is required to review all unanticipated problems involving risks to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor must comment on the outcomes of the event or problem and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and report of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO.
2. Protocol: Section C7: As required by the Human Research Protections Office (HRPO)/U.S. Army Medical Research and Materiel Command (USAMRMC), the protocol must describe how participation in the research is intended to be beneficial to each subject participating in the study and the benefit(s) must apply to both the treatment and placebo groups. This section has been modified accordingly.
3. Consent Form: Page 7: The “Benefits” section of the ICF has been modified according to #2 above

Following non-substantial changes were made and approved by Einstein IRB#1, East on 12/13/2018 with reference #047991:

Addition of Investigator Brochure Edition 7, September 18.

Amendment to the study protocol:
1. change of version of VABS-II scales to VABS-3

2. addition of MERS scale
3. per FDA non-hold comments:
a) Laboratory safety studies were added to Week 2 study visit to identify any abnormality occurring early in the study drug exposure.

b) As it was recommended to monitor blood levels during the titration phase as well, to ensure safe use of the background medications that are mainly metabolized by CYP2C19, CYP3A4, UGT1A9, or UGT2B7, we will monitor for plasma drug levels of VPA, Lamotrigine, Oxcarbazepine, Phenytoin and Clobazam if applicable to any patient. Other medications may be monitored on a patient by patient basis, if there are safety concerns suspected to be related to a drug-drug interaction.
c) language was added, that the exclusion of medications that are potent inhibitors or inducers of CYP2C9 is not required.
d) language was added, that study drug is administered with food consistently throughout the study.
e) protocol wording on section C1 (page 10) and H (page 25) was amended to indicate that only patients with ABC-I≥18 will be included rather that refer to 'stratified for marked irritability' and 'stratified for ABC-I≥18'.
f) the protocol text for 'Analysis in regard to ABC-SW' is amended to specify more clearly the planned analysis.
4. Addition of GW Pharmaceuticals as additional funding source.

Amendment to informed consent:
1. Change of VABS-II to VABS-3 scales (as new version of VABS appeared).
2. Addition of GW Pharmaceuticals as additional funding source.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 03/27/2019 with reference # 051122:

1. Adding second location for the CRC (1300 Morris park ave) at 1572 form.
2. Changes in the protocol:
a) removing line "module 4 will be used in this study" for ADOS-2 scale on page 17 of the protocol
b) removing Social Responsiveness Scale (SRS) from baseline assessments (p. 20 of the protocol)
c) adding Dr. J. Battaglia as research monitor (p.26 of the protocol).

On 04/08/2019 first IDSMC meeting has occurred, and following that meeting was decided to add sesame oil allergy as an exclusion criterion to the study protocol on page 12.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 04/16/2019 with reference # 051762:
addition of sesame oil allergy as an exclusion criteria to the study protocol on page 12.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 10/04/2019 with reference #056458:
1. Addition of IB version 7.1. Summary of changes table is provided at page 79 of IB. Major updates included are final clinical data from a Phase 1 pharmacokinetic (PK) trial and a Phase 2 trial in focal seizures, pharmacology, absorption, distribution, metabolism and excretion (ADME), and toxicology studies. The cut-off for IB edition 7 was 31 July 2018 and included information from Development Core Safety Information (DCSI; cut-off 27 September 2018).
2. Changes in the study protocol:
  1) change in exclusion criteria 11 to reflect liver dysfunction manifested by > 2 X UNL values of AST or ALT
  2) liver function tests to be done at week 2 and week 8.
  3) addition of the MediData RAVE electronic data capture (EDC) system developed by Bioforum Data Masters.
3. Changes to ICF:
  1) addition of a new pager number
  2) use of finger prick technology for blood draw at week 2 and week 8
4. change to the assent:
  addition of word finger to the section "Will it hurt to be in the study".

Following non-substantial changes were made and approved by Einstein IRB#1, East on 10/17/2019 with reference #057064:
We have added a line to the ICF saying patients will have the option of choosing either needle draw or finger prick for week 2 and week 8 lab work based on their comfort level/ preference

Following non-substantial changes were made and approved by Einstein IRB#1, East on 11/13/2019 with reference #057577.
Changes on page 17, Section VI: Adding a line at the end of the paragraph to specify that the MERS will be completed only for subjects who display rigid behaviors.

Section VII b: Adding a line at the end of the paragraph to specify that the IQ test will be completed only if the subject is capable of doing so.
Following change was made in the ICF: the signature section on pg 9- i) Caregiver/ Legal Guardian is being changed to Parent/ Legal Guardian and, ii) Printed Name of Participant is being changed to Printed Name of Parent/ Legal Guardian.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 01/29/2020 with reference #060025.
Recruitment flyer and text for Montefiore e-screens, and Einstein update email text was approved to help facilitate recruitment efforts.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 03/14/2020 with reference #060950, combined with # 059792.
Addition of IB edition 8, issue date December 2019. Changes to the ICF based on request received from the study sponsor (02/24/2020) in reference to changes in the IB edition 8.
Summary of changes based on new information in the IB edition 8, which don't affect risk versus benefit relationship to the study protocol, and which included the following addition to the ICF (pp. 6-7):

"Risks of Taking CBDV
To date, 282 people (adult patients and healthy adult volunteers) have taken GWP42006 as a research participant in a GW-sponsored clinical trial. The side effects reported by people who have taken GWP42006 were generally mild to moderate in severity.
In a GW-sponsored trial which looked at convulsions in a type of epilepsy, the following common side effects were reported:

Very common side effects (affected more than 1 person in every 10)
- Diarrhea.
- Feeling sleepy.

Common side effects (affected 3 or more people in every 100)
- Feeling nauseous.
- Headache.
- Feeling dizzy.
- Stomach-ache/pain.
- Changes in blood tests that look at how the liver works.
- Changes in anticonvulsant drug levels in the blood.
- Low sodium levels in blood.
- Anemia (low iron levels in blood).
- Back pain.
- Feeling itchy
- Rash.
- Convulsions (only in people who have had convulsions before).

There may be other risks of CBDV that are currently unknown. If you are concerned, please contact your child's trial physician. As GWP42006 may affect the results of some blood tests, if your child needs a blood test please tell the tester that he/she is taking cannabidivarin as part of a clinical trial."

Following non-substantial changes were made and approved by Einstein IRB#1, East on 03/18/2020 with reference # 062721.
Letter to enrolled subjects to inform them of changes approved in the amendment #059792. Due to COVID-19 situation, letter was approved to be sent via email.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 04/11/2020 with reference #063312 and clarification # 063901 approved on 05/04/2020.
Addition of dietary diary and body composition analysis via bioimpedance to the protocol and ICF at baseline and all subsequent visits. Addition of information on specimen and information banking to the ICF.
Addition of these measures (dietary diary and body composition analysis via bioimpedance) doesn't change risk versus benefit relationship for the study protocol.
Clarification #063901 specifies that amendment #063312 introduced changes are only applicable for Montefiore-Einstein site and not NYU site.

Following acknowledgement approved by Einstein IRB#1, East on 06/11/2020 with reference #065366.
Correspondence from GW Pharmaceuticals and their correspondence with DEA on the de-scheduling of CBDV.

Following non-substantial changes to ensure participant safety and reduce participant burden during the COVID-19 pandemic were made and approved by Einstein IRB#1, East on 07/06/2020 with reference # 066128.

1) Reducing the number of on-site visits. Screening, week 2, week 6, week 10 and week 14 visits will be done remotely.
2) Informed consent will be done remotely at the screening visit and in person at the baseline visit.
3) Physical and Neurological exam will be done at all on-site visits only.
4) IQ test will be done at the baseline visit.
5)ECG will be done at the baseline visit and at week 12.
6) ADOS-2 will be done at the baseline visit not at screening.
7) C-SSRS will be done at all visits except for screening.
8) Dietary Diary will be done at all visits except for screening.
9) Vital signs, weight and BMI will be done at all on-site visits only.
10) Pregnancy test at all visits except for week 10 and week 14.
11) AE monitoring at all visits except for screening and baseline
12) CBC with differential and basic metabolic panel at screening and all on site visits
13) Liver function enzymes and AED drug levels at all visits except for week 10 and week 14.
14) Menstrual Diary at all visits except for screening and week 14.
15) Medication dispensed only at baseline, week 4 and week 8.
16) ABC scale to be done at screening, baseline, week 4, week 8, week 12, and week 14.
17) RBS-R to be done at baseline, week 4, week 8, week 12, and week 14.
18) MERS scale to be done at baseline, week 4, week 8, week 12, and week 14.
19) PedsQL to be done at baseline, week 4, week 8, week 12, and week 14.
20) CGI-I to be done at week 4, week 8, week 12, and week 14.
21) CGI-S to be done at screening, baseline, week 4, week 8, week 12, and week 14.

All labs during remote visits will be done at a local Quest Diagnostics facility convenient to the participant's location.

Following non-substantial changes to ensure participant safety and reduce participant burden during the COVID-19 pandemic were made and approved by Einstein IRB#1, East on 08/10/2020 with reference # 067116 and following formatting change was approved with reference #067785 on 08/17/2020.

a) Including Quest Diagnostics in the IRB application as an external site Not Engaged in Research. All lab work for the study during remote visits will be done at a Quest Diagnostics location convenient to the participant. Also editing the informed consent process section of the application-- 25.2 and 25.4.
b) Revising the protocol to incorporate the changes below:

1) Added ABC sub-scales to be secondary measures
2) Added ADI-R as a substitute for ADOS-2 if ADOS-2 can't be performed due to site restriction (face mask)
3) Allow ADOS-2/ADI-R from within 12 months to be used to meet eligibility
4) Reduced the number of AED drug testing to baseline, weeks 4, 8, 12 only
5) Updated schedule of events
6) Revised languages in section C5 safety assessment to reflect the changes in schedule of events
7) Clarified language in section C8.2 secondary measures to reflect frequencies per schedule of events
8) Updated language in section C9.1 on consent
9) Updated language in section C9.2 to clarify remote and in-person visit details
10) Added section C 9.3 for special circumstances (ie COVID-19)

c) Revising the consent to reflect the changes mentioned above. The new Einstein IRB ICF template for Greater than Minimal Risk studies has been used for this version.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 9/18/2020 with reference # 068273:
Submission of revised assent form with removal of witness signature field and time field to match the current assent formatting standards.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 10/30/2020 with reference # 070030:
1) Submission of the Montefiore social media request form which will be used to post ads for our study on Montefiore's social media pages
2) Revision of the phone screening script to be consistent with the recent version of the protocol

Following non-substantial changes were made and approved by Einstein IRB#1, East on 12/07/2020 with reference # 070974: addition of the second DSMC meeting minutes and blinded data tables.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 02/04/2021 with reference # 073117: Addition of recruitment materials in Montefiore/ Einstein social media advertising templates.

Following non-substantial changes were made and approved by Einstein IRB#1, East on 07/19/2021 with reference #078073: Adding most recent IDSMC committee meeting minutes and blinded data tables.

Finalize recruitment materials.

Drafting of recruitment materials, including flyers, advertisements, and referral solicitations was completed. Recruitment flyer and text for Montefiore e-screens, and Einstein update email was approved by Einstein IRB #1 on 01/29/2020 with reference # 060025 to help facilitate recruitment efforts.

Coordinate with sites for the US Army Medical Research and Material Command Office of Research Protections (ORP) and the Human Research Protection Office (HRPO) Submission.

HRPO received our initial submission on 10/31/17. The following items were included:
-HRPO Protocol Submission Form
-Principal Investigator CV, Independent Medical Monitor bio-sketch
-Documentation of Human Subjects Training for all the Investigators at the Einstein site
-Updated FDA Form 1572
-Informed Consent, Assent, HIPAA forms
-Letters of Support from Collaborating Institutions (NYU, GW Pharma)
-Updated Research Protocol
-Peer and Programmatic Review of Protocol
-Email Correspondence with Protocol Changes
-Study Instruments and Data Collection Forms. The following items were included:
-Aberrant Behavior Checklist (ABC) – Irritability, Social Withdrawal
-Repetitive Behavior Scale – Revised (RBS-R)
-Pediatric Quality of Life Inventory (PedsQL) Family Impact Module
-Vineland Adaptive Behavior Scale – II (VABS-II)
-Clinical Global Impression Scale – Improvement, Severity (CGI-I, CGI-S)
-IQ Test: Stanford-Binet Intelligence Scales, 5e
-Autism Diagnostic Observation Schedule – II (ADOS-II)
-Social Responsiveness Scale (SRS)

On 02/08/2018 upon request we submitted to Dr. Margaret Frederick additional items from AECOM/MMC site, and then our application was routed by her to the Approval Authority by COB for HRPO pre-review. Following items were included in this additional submission:

-Investigators Brochure Edition 6, GW Pharmaceuticals
-Einstein IRB conditional approval notice
-Conditionally IRB approved Research Protocol
-Conditionally IRB approved Informed Consent, Assent and informed consent feedback tool.
-IND acknowledgement letter
-CITI GCP certificate of Dr. A. Djukic (medical monitor)
- Human subject's protection training of Dr. A.Djukic (medical monitor)

On 02/13/18 we received following comments from HRPO:
A. Required Information/Documents.

1. Provide the final IRB submission package, approval memo, and stamped consent and assent forms when available.
2. Ensure that the IRB approval memo or other communication from the IRB states the risk category for the inclusion of children (either 45 CFR 46.404, 45 CFR 46.405, or 45 CFR 46.406).

B. Revisions to be made to the protocol.

1. The role and responsibilities of the Research Monitor, Dr. Djukic, must include the following.

a) May discuss the protocol with the investigators, interview subjects, and consult with others outside the study about the research.

b) Shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB can assess the Monitor's report.

c) Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official.

d) Is required to review all unanticipated problems involving risks to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor must comment on the outcomes of the event or problem and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator.

e) Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and report of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO.

2. BENEFITS to subjects - The protocol states in Section C7 that "It may or may not directly benefit the subjects." The consent form states on page 7 that "There may or may not be a direct medical benefit to you from being in this research study." As this protocol involves an experiment where the primary purpose is to determine the effect of the intervention, the requirements under 10 USC 980 apply. Since the protocol requires the informed consent from a legally authorized representative or parent, the protocol must describe how participation in the research is intended to be beneficial to each subject participating in the study. This cannot include monetary compensation or possible benefit from the study drug, as the benefit is not yet known and not all subjects will receive the study drug. The benefit(s) must apply to both the treatment and placebo groups. The benefit(s) can take many forms. The protocol could possibly include providing education to the families on strategies to handle irritability or other symptoms of ASD, or providing feedback to the families on the results from the behavioral testing that may help them handle symptoms of ASD. These are just suggestions. The PI must revised Section C7 to describe the benefit(s) to the subjects.

C. Revisions to be made to the consent form (Version dated 9 February 2017).

1.BENEFITS to subjects - Once the protocol has been updated to describe the benefit to the subjects, the description of this benefit must be added to the consent form page 7.

They were addressed as summarized above and approved by HRPO for the IRB submission.
On 03/23/18 revised protocol and ICF were submitted to IRB and were approved on 03/28/18. After obtaining FDA study May Proceed letter on 04/02/18, we then submitted it to HRPO as well on 04/03/2018. On 04/13/18 we confirmed with Dr. Gloria Lawrence, Dr. Margaret Frederick successor from ORP HRPO, that the only outstanding item for HRPO approval is unconditional IRB approval (pending BNE and DEA approval).

We submitted NYU IRB approval to HRPO ORP on 05/02/2018 as it was received.

On 05/16/2018 we submitted to ORP HRPO Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) Approval letter #035849. ORP HRPO approval for AECOM/MMC site HRPO Log Number A-20351.a was granted on 05/25/2018.
On 12/19/2018 Albert Einstein College of Medicine Continuing Review report (Log Number A-20351.a) was submitted to ORP HRPO. On 01/14/2019 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report and supporting documents.
ORP HRPO approval for NYU site HRPO Log Number A-20351.b was granted on 02/05/2019. NYU site Continuing review report was accepted by HRPO ORP on 04/01/2019 and on 04/16/2020.

On 05/02/2019 AECOM/MMC submitted following amendment to the study protocol to ORP HRPO (Log Number A-20351.a)
1) change in Independent Research Monitor name to Dr. Battaglia (his CV and GCP training are on file)
2) addition of exclusion criteria (allergy to sesame oil) on page 12 of the protocol.

On 11/13/2019 AECOM/MMC site submitted Continuing Review Report to ORP HRPO (Log Number A-20351.a).
On 01/27/2020 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report and supporting documents.

On 08/19/2020 AECOM/MMC site submitted following amendments to the study protocol to ORP HRPO (Log Number A-20351.a) and it was acknowledged on 08/21/2020. We further received a confirmation on 08/25/2020 that changes being made do not meet ORP HRPO criteria for substantive amendments and do not require HRPO approval prior to implementation.

On 10/29/2020 AECOM/MMC site submitted Continuing Review Report to ORP HRPO (Log Number A-20351.a).

On 11/4/2020 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report and supporting documents.

On 03/04/2021 NYU site submitted Continuing Review Report to ORP HRPO (Log Number A-20351.b).
On 03/24/2021 ORP HRPO accepted NYU Continuing Review Report (Log Number A-20351.b) and supporting documents.

Coordinate with sites for IRB protocol submission.
Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) received our initial IRB submission on 11/1/17. The following items were included: research protocol, parent consent/HIPAA forms, child assent form.

On 12/13/2017 Dr. Eric Hollander (AECOM/MMC) participated in Einstein IRB review meeting of the study protocol. Questions on frequency of Independent Data Safety Monitoring Committee (IDSMC) meetings, benefits to the child and psychoactive properties of CBDV have arisen, and were addressed during the meeting and in following the meeting correspondence.

On 01/08/2018 AECOM/MMC site received conditional IRB approval pending IND approval from the FDA, DEA license for schedule I substance, approval from Bureau of Narcotics, DEA approval to Pharmacy for additional storage space.

On 04/03/2018 AECOM/MMC site received conditional IRB approval pending approval from Bureau of Narcotics, DEA license for schedule I substance and DEA approval to Pharmacy for additional storage space.

On 05/11/2018 AECOM/MMC site received conditional IRB approval pending DEA license for schedule I substance and DEA approval to Pharmacy for additional storage space.

On 05/15/2018 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) Approval letter #035849.

DEA license for Schedule I substance and DEA approval to Pharmacy for additional storage space (letter from Clemencia Solorzano were submitted as amendment and acknowledged by Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East).

Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 11/2/2018. Study protocol was reapproved on 11/12/2018 with reference # 046713.

On 12/13/2018 Albert Einstein College of Medicine Institutional Review Board (Einstein IRB#1, East) approved protocol amendment with reference # 047991. Non-substantial changes were made as outlined on pp.10.

On 03/27/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #051122 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 04/16/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #051762 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 10/04/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval # 056458 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 10/17/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #057064 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 10/11/2019. Study protocol was reapproved on 10/28/2019 with reference # 057029.

On 11/13/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #057577 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 01/29/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #060025 for the protocol amendment. Non-substantial changes were made as outlined on pp.12.

On 03/14/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #060950 combined with #059792.Non-substantial changes were made as outlined on pp.12

On 03/18/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #062721. Non-substantial changes were made as outlined on pp.12.

On 04/11/202 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #063312 and subsequent clarification # 063901 was approved on 05/04/2020. Non-substantial changes were made as outlined on pp.12.

On 06/11/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board acknowledgement with reference #065366. Non-substantial changes were made as outlined on pp.12.

On 07/06/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #066128. Non-substantial changes were made as outlined on pp.13.

On 08/10/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference # 067116 and formatting change was approved with reference #067785 on 08/17/2020. Non-substantial changes were made as outlined on pp.13.

On 08/19/2020 AECOM/MMC site submitted amendments to the study protocol to ORP HRPO (Log Number A-20351.a) and it was acknowledged on 08/21/2020. We further received a confirmation on 08/25/2020 that changes being made do not meet ORP HRPO criteria for substantive amendments and do not require HRPO approval prior to implementation.

Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 09/16/2020 with reference # 068608. Study protocol was reapproved on 10/08/2020 with reference # 068608.

On 09/18/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #068273. Non-substantial changes were made as outlined on pp.13.

On 10/29/2020 AECOM/MMC site submitted Continuing Review Report to ORP HRPO (Log Number A-20351.a).
On 11/4/2020 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report and supporting documents.

On 10/30/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #070030. Non-substantial changes were made as outlined on pp.13.

On 12/07/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #070974. Non-substantial changes were made as outlined on pp.14.

On 02/04/2021 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #073177. Non-substantial changes were made as outlined on pp.14.

On 07/19/2021 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #078073. Non-substantial changes were made as outlined on pp.14.

Dr. Orrin Devinsky submitted to the New York University Langone Medical Center IRB #6 on 02/16/2018. His submission was delayed by the requirement to obtain IND # prior to the submission. AECOM/MMC site shared all IRB approved documents with NYU site to facilitate their submission, as we obtained IND#138371 as of 02/07/2018 in the mail. NYU site subsequently amended their IRB submission once HRPO comments were addressed by AECOM/MMC site and FDA study May Proceed letter was received on 04/02/2018. NYU site received IRB approval on 05/02/2018. This approval was submitted as amendment and acknowledged by Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East).
NYU site submitted Continuing review to their IRB on 03/15/2019 and received protocol reapproval on 03/20/2019.
NYU site submitted protocol amendments (same as Einstein site, outlined on p.7) on 04/19/19 and received approval on 05/14/2019. They subsequently submitted protocol amendments (same as Einstein site, outlined on p.7) and received approvals on 1/7/2020, 1/15/2020 and 2/4/2020.
NYU site Continuing Review #2 was submitted to their IRB on 3/5/2020 and received protocol reapproval on 3/17/2020.
Modification #12 for NYU site was on submitted 3/13/2020, and approved on 3/17/2020 (removal of personnel Kimberly Menzer, NP). Modification #13 for NYU site was submitted on 3/19/2020 and approved 3/27/2020 (addition of personnel Dana Price, MD). On 8/24/2020 NYU site submitted COVID-19 related amendment to their IRB which consisted in 1) updating protocol that some visits and procedures to be completed remotely by telephone or computer in order to

decrease exposure risk during the COVID-19 pandemic. For these remote visits, any required labs will be collected at the subject's local Quest location; 2) updating main consent, 7-11 y.o. and 12-14 y.o. assents, and key information form to reflect protocol changes. NYU IRB approval for the COVID-19 related amendment was obtained on 9/30/2020.
NYU site Continuing Review #3 was submitted to their IRB on 02/19/2021 and received protocol reapproval on 03/09/2021.

Coordinate with sites for CTSA-sponsored Clinical Research Center (CRC) approval.

Application for Einstein-Montefiore CTSA-sponsored ICTR Clinical Research Center (CRC) was received on 11/10/2017. The following items were included: research protocol, parent consent form, child assent form, PI bio-sketch, study budget, projected use of CRC resources. We obtained ICTR approval notice on 11/28/2017 pending unconditional IRB approval. Following Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) Approval letter #035849 receipt on 05/15/2018 we submitted it to ICTR and conducted CRC initiation visit. Dr. Orrin Devinsky completed submission for NYU CTSA-sponsored CRC. His submission was delayed by the requirement to obtain IND # prior to the submission. NYU site IRB approval was granted on 05/02/2018.

Submit amendments, adverse events, and protocol deviations as needed.
All amendments, adverse events, and protocol deviations will be reported and documented as they arise.
Coordinate with sites for annual IRB report for continuing review.
As the study progressed, investigators and coordinators at AECOM/MMC, NYU, and GW Pharma communicated regularly in regard to initial IRB submissions and remain accountable for timely review of all regulatory activities relevant to annual IRB reporting. Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 11/2/2018. Study protocol was reapproved on 11/12/2018 with reference # 046713. Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 10/11/2019. Study protocol was reapproved on 10/28/2019 with reference # 057029. Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 09/16/2020 with reference # 068608. Study protocol was reapproved on 10/08/2020 with reference # 068608.
NYU site submitted Continuing review#1 to their IRB on 03/15/2019 and received reapproval on 03/20/2019.
NYU site Continuing Review #2 was submitted to their IRB on 03/5/2020 and received reapproval on 03/17/2020.
NYU site Continuing Review #3 was submitted to their IRB on 02/19/2021 and received reapproval on 03/09/2021.

Subtask 2: Training of Study Staff
Rater training was conducted on 06/01/2018. We completed regulatory binders and created manual of operations and IDSMC charter. Additional psychologist from NYU team, Dr. Michelle Lee, was trained on MERS scale on 09/20/2019.

Subtask 3: Facilitate Communication between Sites and Staff
We coordinate with NYU site weekly to review study goals and status of regulatory documents submission

Subtask 4: Assessments and Surveys Preparation
We finalized assessment measurements. We ordered study assessments and distributed them across sites.
We prepared patient study binders including surveys and source documents at AECOM/MMC site. NYU team did the same for NYU site.

Subtask 5: Receipt of Study Drug

We finalized with pharmacy protocol for storage and dispensing of study drug/placebo and randomization.
On 01/31/2018 Dr. Eric Hollander (AECOM/MMC) and Dr. Vera Nezgovorova (AECOM/MMC) met with Manager of Investigational Pharmacy at AECOM/MMC Dr. Clemencia Solorzano and Dr. Mark Sinett, Director of Clinical Services at AECOM/MMC to review dispensing of study drug/placebo strategy and confirmed storage space in GW designated safe, which is currently BNE and DEA approved.
As we were moving forward with BNE inspection, we were advised that an independent safe designated only for Dr. Hollander study would be required as a condition for BNE approval. Reconditioned Amsec Plate Steel Safe Model 2230 (right swing) UL-TL-30 1 Group 1R radiological lock: SG560 UL group 1 lock, SGD550 dial w/ 10” spindle; SGR167 ring, SC, SPKL was successfully ordered and installed on 04/16/18. We completed randomization protocol preparation on 08/01/2018.
On 09/27/2018, 10/19/18, 12/7/18, 1/9/19 we conducted a series of call between Dr. Clemencia Solorzano, Manager of Investigational Pharmacy at AECOM/MMC, Dr. Freda Afrifa (Pharmacist at AECOM/MMC) and GW Pharma team involved in drug shipment and operations (Hetal Patel, PharmD and Jan Joscak, PharmD). Randomization table was finalized. We prepared end-use letter for drug shipment. Due to unforeseen issues with drug labeling occurred from the GW side drug shipment was delayed, and was shipped from UK to the US depot on 02/05/2019. Study drug was received at the US depot on 02/15/2019, and was received at AECOM/MMC pharmacy on 03/20/19. On 03/25/2019 AECOM/MMC

site staff members completed training on pharmacy operating procedures required for successful execution of this study protocol. On 09/27/2019 NYU site staff members completed training on pharmacy operating procedures required for successful execution of this study protocol. NYU site was activated as of 10/23/2019 following regulatory clearance by GW pharmaceuticals to initiate drug shipment. NYU site received drug on site on 11/19/2019.

Subtask 6: Data Management Preparation

Follow-up communication, which included calls and emails in regards to budget justification were conducted bi-weekly during October 2018 between AECOM/MMC team and GW Pharma team. GW agreed to provide funding support for the creation of a study-specific EDC (electronic data capture solution) using the Bioforum data masters. On 11/16/18 GW Pharma finalized approval of the additional funding support, and the CTA contract between AECOM/MMC and GW Pharma was signed by GW Pharma. AECOM/MMC signed a contract with Bioforum Data Masters on 01/30/2019. We finalized eCRFs development and completed UAT (user acceptance testing) phase. Electronic data capture system went live on 12/11/2019 for data entry and data management. Data entry at both sites is ongoing. EDC system was adjusted for a protocol change due to COVID-19 Pandemic.

Major Task: Participant Recruitment and Enrollment.

Subtask 1: Begin Subject recruitment.

Currently, patients have been contacted in site databases, and 150 patients were identified. First patient was screened on 04/12/2019 and randomized on 04/26/2019 at AECOM MMC site. First patient was screened on 01/14/2020 and is waiting to be randomized after the COVID-19 pandemic situation subsides at NYU site.

Subtask 2: Conduct study.

First patient was screened on 04/12/2019 and randomized on 04/26/2019 at Montefiore Medical Center site. At the NYU site, the first patient was screened on 01/14/2020 and is waiting to be randomized after the COVID-19 pandemic situation subsides.
Study is ongoing. Prior to COVID-19 pandemic onset, we have projected a total of 84 patients enrolled by end of Q4 year 3, at the two sites, including AECOM/MMC and NYU (consisting of 16 subjects to be enrolled at year 2, i.e. 8 subjects at each site and 34 subjects to be enrolled at year 3, i.e. 17 subjects at each site; and 34 subjects to be enrolled at year 4, i.e. 17 subjects at each site).
Total number of subjects screened at AECOM/MMC site is 31 to date. Therefore 31 subjects were screened, 17 subjects have completed the study, 1 is active, 3 are screen failures, 6 have dropped out from the study and 4 are waiting to be randomized. Total number of subjects screened at NYU site is 9 to date. Therefore 9 subjects were screened, 2 subjects have completed the study, 1 is active, 4 are screen failures and 1 is waiting to be randomized.

Detailed information on enrollment for both sites, subject characteristics and listings of AEs is summarized in the tables 1a, 1b, 2a, 2b, 3a and 3b below.

Table 1a): Screening and Enrollment Data for AECOM/MMC site

Total Screened	Total Waiting to Randomize	Total Randomized	Currently Active	Total Completed	Total Screen Failures	Total Dropped Out
31	4	20	1	17	3	6

Table 1b): Screening and Enrollment Data for NYU site

Total Screened	Total Waiting to Randomize	Total Randomized	Currently Active	Total Completed	Total Screen Failures	Total Dropped Out
9	1	3	1	2	4	0

No study results have been obtained thus far. No serious adverse events have occurred.
Below is a demographic table for the randomized subjects:

Table 2a. Subject Characteristics Randomized at AECOM/MMC

Category	Statistic	Total (n=20)		
		Male	Female	Total
Gender	n (%)	17 (85%)	3 (15%)	20 (100%)
Age (yrs)	n mean (std) median min : max	17 10.6 (3.2) 10 6 : 17	3 14 (1.7) 13 13 : 16	20 11.1 (3.2) 11 6 : 17
Age (yrs)				
Category 1: 5-6	n (%)	1 (5%)	0 (0%)	1 (5%)
Category 2: 7-12	n (%)	12 (60%)	0 (0%)	12 (60%)
Category 3: 13-17	n (%)	4 (20%)	3 (15%)	7 (35%)
Race				
American Indian/Alaskan Native	n (%)	0 (0%)	0 (0%)	0 (0%)
Asian	n (%)	0 (0%)	0 (0%)	0 (0%)
Black / African American	n (%)	5 (25%)	0 (0%)	5 (25%)
Native Hawaiian / Pacific Islander	n (%)	0 (0%)	0 (0%)	0 (0%)
White	n (%)	11 (55%)	3 (15%)	14 (70%)
Mixed Race	n (%)	1 (5%)	0 (0%)	1 (5%)
Unknown/Not reported	n (%)	0 (0%)	0 (0%)	0 (0%)
Ethnicity				
Hispanic	n (%)	5 (25%)	0 (0%)	5 (25%)
Not Hispanic	n (%)	12 (60%)	3 (15%)	15 (75%)
Starting Height (cm)	n mean (std) median min : max	17 141.1 (18.8) 141 113 : 178.3	3 152.2 (14.6) 144 143.5 : 169	20 142.8 (18.3) 142.3 113:178.3
Starting Weight (kg)	n mean (std) median min : max	17 37.9 (11.7) 36.7 19.8 : 55.4	3 53.9 (3.9) 54.8 49.6 : 57.2	20 40.3 (12.3) 41.2 19.8 : 57.2
Starting BMI	n mean (std) median min : max	17 18.7 (3.1) 18.8 15.1 : 25.2	3 23.9 (5.6) 26.4 17.4 : 27.8	20 19.4 (3.9) 18.8 15.1 : 27.8
Starting BMI percentile	n mean (std) median min : max	17 59.9 (31.5) 58 3 : 99	3 67 (50.2) 95 9 : 97	20 60.98 (33.3) 61.5 3 : 99

Table 2b. Subject Characteristics Randomized at NYU

Category	Statistic	Total (n=3)		
		Male	Female	Total
Gender	n (%)	3 (100%)	0 (0%)	3 (100%)
Age (yrs)	n mean (std) median min : max	3 10.7 (4.9) 13.5 5 : 14	0	3 10.7 (4.9) 13.5 5 : 14
Age (yrs)				
Category 1: 5-6	n (%)	1 (33.3%)	0 (0%)	1 (33.3%)

Category 2: 7-12	n (%)	0 (0%)	0 (0%)	0 (0%)
Category 3: 13-17	n (%)	2 (66.6%)	0 (0%)	2 (66.6%)
Race				
American Indian/Alaskan Native	n (%)	0 (0%)	0 (0%)	0 (0%)
Asian	n (%)	0 (0%)	0 (0%)	0 (0%)
Black / African American	n (%)	0 (0%)	0 (0%)	0 (0%)
Native Hawaiian / Pacific Islander	n (%)	0 (0%)	0 (0%)	0 (0%)
White	n (%)	1 (33.3%)	0 (0%)	1 (33.3%)
Mixed Race	n (%)	0 (0%)	0 (0%)	0 (0%)
Unknown/Not reported	n (%)	0 (0%)	0 (0%)	0 (0%)
Hispanic	n (%)	2 (66.6%)	0 (0%)	2 (66.6%)
Starting Height (cm)	n mean (std) median min : max	3 147.2 (32.7) 164 109.5 : 168	0	3 147.2 (32.7) 164 109.5 : 168
Starting Weight (kg)	n mean (std) median min : max	3 49.2 (25.0) 49.8 23.9 : 73.9	0	3 49.2 (25.0) 49.8 23.9 : 73.9
Starting BMI	n mean (std) median min : max	3 21.9 (4.2) 20.3 18.8 : 26.6	0	3 21.9 (4.2) 20.3 18.8 : 26.6
Starting BMI percentile	n mean (std) median min : max	3 81.4 (26.6) 95.2 50.8: 98.3	0	3 81.4 (26.6) 95.2 50.8: 98.3

No serious adverse events have occurred. No IND safety reports have been submitted thus far. No subjects have died or dropped out of the study due to adverse events, and no additional information has been learned about the drugs actions. Mild adverse events reported in Table 3 below will be submitted to Einstein IRB with annual continuing review per existing SOPs in place.

Table 3a: Listing of Adverse Events at AECOM/MMC

System Organ Class	Adverse Event	Severity	Serious
General disorders and Administration site conditions	Fatigue (1)	Mild	No
	Fever (1)	Mild	No
	Poor Appetite (1)	Mild	No
	Sleep Disturbance (1)	Mild	No
	Sore Throat (1)	Mild	No
	Rhinorrhea (1)	Mild	No
	Hyperactivity (2)	Mild	No
Infection and Infestations			
	Upper Respiratory Infection (1)	Mild	No
	COVID-19 (1)	Mild	No
Ear and Labyrinth Disorders	Otitis Media – L (1)	Mild	No
	Otitis Media – R (1)	Mild	No
Skin and Subcutaneous Tissue Disorders	Perioral Rash (1)	Mild	No
	Irritation Dermatitis-Facial (1)	Mild	No
	Superficial skin infection (facial) (1)	Mild	No
Gastrointestinal Disorders	Vomiting (1)	Mild	No
	Constipation (1)	Mild	No
Nervous System Disorders	n/a		

Table 3b: Listing of Adverse Events at NYU

System Organ Class	Adverse Event	Severity	Serious
General disorders and Administration site conditions	Fever (1) Epistaxis (1)	Mild Mild Mild Mild Mild Mild Mild	No No No No No No No
Infection and Infestations	Strep throat (1)	 Mild Mild	 No No
Ear and Labyrinth Disorders	n/a	Mild Mild	No No
Skin and Subcutaneous Tissue Disorders	n/a	Mild Mild Mild	No No No
Gastrointestinal Disorders	Loose stool (1)	Mild Mild	No No
Nervous System Disorders	n/a		

Listing of Severe Adverse events: None reported to date at AECOM/MMC or NYU sites.

Human Use Regulatory Protocols

TOTAL PROTOCOLS: One human subject research protocol will be required to complete the Statement of Work.

PROTOCOL (1 of 1 total):

HRPO Log Number A-20351.a and b

Title: Cannabidivarin (CBDV) vs. Placebo in Children with Autism Spectrum Disorder (ASD) Target required for clinical significance: TBD

Target approved for clinical significance: TBD

SUBMITTED TO AND APPROVED BY:

ORP HRPO, initial submission 10/31/17, documents submitted by Dr. Margaret Frederick (HRPO) to the Approval Authority by COB for pre-review on 02/08/2018. Comments received on 02/13/18, addressed and approved by HRPO and Einstein IRB (03/28/18). NYU IRB approval submitted to ORP HRPO on 05/02/2018. Einstein IRB#1 Approval letter #035849 submitted to ORP HRPO on 05/16/2018.

ORP HRPO approval for AECOM/MMC site HRPO Log Number A-20351.a granted on 05/25/2018. ORP HRPO approval for NYU site HRPO Log Number A-20351.b was granted on 02/05/2019.

On 12/19/2018 Albert Einstein College of Medicine Continuing Review report (Log Number A-20351.a) was submitted to ORP HRPO. On 01/14/2019 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report (Log Number A-20351.a) and supporting documents.

NYU site Continuing Review report (Log Number A-20351.b) was received by ORP HRPO on 04/01/2019 and accepted by ORP HRPO on 05/28/2019.

On 11/13/2019 Albert Einstein College of Medicine Continuing Review report (Log Number A-20351.a) was submitted to ORP HRPO. On 01/27/2020 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report (Log Number A-20351.a) and supporting documents.

NYU site Continuing Review report (Log Number A-20351.b) was submitted to ORP HRPO on 04/09/2020 and accepted by ORP HRPO on 04/16/2020.

On 10/29/2020 AECOM/MMC site submitted Continuing Review Report (Log Number A-20351.a) to ORP HRPO.

On 11/4/2020 ORP HRPO accepted Albert Einstein College of Medicine Continuing Review report and supporting documents.

NYU site Continuing Review report (Log Number A-20351.b) was submitted to ORP HRPO on 03/04/2021 and was accepted on 03/24/2021.

Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) received initial study application submission on 11/6/17.

On 01/08/2018 AECOM/MMC site received conditional IRB approval pending IND approval from the FDA, DEA license for schedule I substance, approval from Bureau of Narcotics, DEA approval to Pharmacy for additional storage space.

On 04/03/2018 AECOM/MMC site received conditional IRB approval pending approval from Bureau of Narcotics, DEA license for schedule I substance, DEA approval to Pharmacy for additional storage space.

On 05/11/2018 AECOM/MMC site received conditional IRB approval pending DEA license for schedule I substance and DEA approval to Pharmacy for additional storage space.

On 05/15/2018 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) Approval letter #035849. Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 11/2/2018. It was reapproved on 11/12/2018 with reference # 046713

On 12/13/2018 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #047991 for the protocol amendment. Non-substantial changes were made as outlined above on pp.10.

On 03/27/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #051123 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 04/16/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #051762 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 10/04/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval # 056458 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 10/17/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #057064 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 10/11/2019. Study protocol was reapproved on 10/28/2019 with reference # 057029.

On 11/13/2019 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #057577 for the protocol amendment. Non-substantial changes were made as outlined on pp.11.

On 01/29/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #060025 for the protocol amendment. Non-substantial changes were made as outlined on pp.12.

On 03/14/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #060950 combined with #059792.Non-substantial changes were made as outlined on pp. 12.

On 03/18/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval #062721. Non-substantial changes were made as outlined on pp.12.

On 04/11/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #063312 and subsequent clarification # 063901 was approved on 05/04/2020. Non-substantial changes were made as outlined on pp.12.

On 06/11/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board acknowledgement with reference #065366. Non-substantial changes were made as outlined on pp.12

On 07/06/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #066128. Non-substantial changes were made as outlined on pp.13.

On 08/10/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #067116 and formatting change was approved with reference #067785 on 08/17/2020. Non-substantial changes were made as outlined on pp. 13.

Progress report was submitted to Albert Einstein College of Medicine Institutional Review Board (Einstein IRB #1, East) on 09/16/2020 with reference # 068608. Study protocol was reapproved on 10/08/2020 with reference # 068608.

On 09/18/20 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference # 068273. Non-substantial changes were made as outlined on pp.13.

On 10/30/20 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #070030. Non-substantial changes were made as outlined on pp.13.

On 12/07/2020 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference # 070974. Non-substantial changes were made as outlined on pp.14.

On 02/04/2021 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #073117. Non-substantial changes were made as outlined on pp. 14.

On 07/19/2021 AECOM/MMC site received Albert Einstein College of Medicine Institutional Review Board approval with reference #078073. Non-substantial changes were made as outlined on pp. 14.

New York University Langone Medical Center IRB #6, initially submitted 02/16/18 and amended after HRPO and IND non-hold comments. Approval letter received on 05/02/2018. NYU site submitted Continuing review to their IRB on 03/15/2019 and received protocol reapproval on 03/20/2019. NYU site submitted protocol amendments (same as Einstein site, outlined on p.7) on 04/19/19 and received approval on 05/14/2019. They subsequently submitted protocol amendments (same as Einstein site, outlined on p.7) and received approvals on 1/7/2020, 1/15/2020 and 2/4/2020.
NYU site Continuing Review #2 was submitted on 3/5/2020 to the Office of Science and Research IRB, NYU Langone Health and the protocol was reapproved on 3/17/2020.
Modification #12 for NYU site was on submitted 3/13/2020, and approved on 3/17/2020 (removal of personnel Kimberly Menzer, NP). Modification #13 for NYU site was submitted on 3/19/2020 and approved 3/27/2020 (addition of personnel Dana Price, MD).
On 8/24/2020 NYU site submitted COVID-19 related amendment to their IRB, which was approved on 09/30/2020.
New York State Department of Health, Bureau of Narcotic Enforcement (BNE), initial submission received 11/7/17. BNE inspection (including safe and site inspection) completed on 04/18/18 by Inspector Johnson. Class 7 Individual Researcher and Class 9 Importer licenses approvals received on 05/11/2018. Renewal of BNE Class 7 license application to engage in a controlled substance activity was submitted by Dr. Hollander on 02/03/2020 and was approved on 03/13/2020. BNE Class 7 license is effective from April 25 2020 to April 25 2022.
Drug Enforcement Administration (DEA), Drug and Chemical Evaluation Section, initial submission for Schedule I license received on 02/13/18. DEA inspection completed on 04/11/2018 by Inspector Rivera. Additional inspection for the new safe is scheduled for 05/18/2018 with Inspector Rivera. DEA schedule I license was granted on June 26 2018.
Upon DEA annual renewal requirements, it was renewed 09/05/2018 and 09/18/2019 and was valid through 10/31/2020. As of March 20, 2020, DEA formally notified GW/Greenwich Biosciences Inc. that they considered GW's products, including CBDV and GWP42006 to be non-controlled substances. Recently, DEA also published this information in the Federal Register. We also received a memo from GW/Greenwich Biosciences Inc. on June 8 2020, which was acknowledged by Einstein IRB, #1 East on 06/11/2020 with reference #065366. Since the CBDV used in the study is no longer a controlled substance, there is no further need for Dr. Hollander to have the DEA Schedule I Researcher registration i.e. to extend his registration.
NYU site Continuing Review #3 was submitted on 2/19/2021 to the Office of Science and Research IRB, NYU Langone Health and the protocol was reapproved on 3/09/2021.

STATUS:
Study is ongoing. Prior to COVID-19 pandemic onset, we have projected a total of 84 patients enrolled by end of Q4 year 3, at the two sites, including AECOM/MMC and NYU (consisting of 16 subjects to be enrolled at year 2, i.e. 8 subjects at each site and 34 subjects to be enrolled at year 3, i.e. 17 subjects at each site; and 34 subjects to be enrolled at year 4,

i.e. 17 subjects at each site). Total number of subjects screened at AECOM/MMC site is 31 to date. Therefore 31 subjects were screened, 17 subjects have completed the study, 1 is active, 3 are screen failures, 6 have dropped out from the study and 4 are waiting to be randomized. Total number of subjects screened at NYU site is 9 to date. Therefore 9 subjects were screened, 2 subjects have completed the study, 1 is active, 4 are screen failures and 1 is waiting to be randomized.

What opportunities for training and professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

We will continue to screen and enroll subjects at AECOM/MMC and NYU site COVID-19 pandemic allowing.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report.

Changes in approach and reasons for change.
Actual or anticipated problems or delays and actions or plans to resolve them.
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.
COVID-19 Pandemic situation has affected our enrollment at Montefiore and NYU sites. In 2020 during year 3 of the study, we have prepared and submitted to the IRBs and ORP HRPO an amendment of the study protocol adjusting our study to the COVID-19 Pandemic situation which included reducing in person visits of study subjects from 9 to 4, i.e. making 5 study visits remote. We are currently seeing patients in person at AECOM/MMC site.

Changes that had a significant impact on expenditures.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Significant changes in use or care of human subjects.

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

Nezgovorova V., Ferretti C.J., Taylor B.P., Shanahan E.,Uzunova G., Hong K., Devinsky O., Hollander E. Potential of cannabinoids as treatments for autism spectrum disorders. J Psychiatr Res. 2021 May;137:194-201.

Books or other non-periodical, one-time publications.

Nezgovorova V., Ferretti C., Taylor B., Hollander E. Cannabinoids in Autism Spectrum Disorders. Chapter in APPI Textbook of Autism Spectrum Disorders, 2d Edition, APA Publishing. Submitted. 2020.

Other publications, conference papers and presentations.

- In previous reporting period study outline was presented at Department of Psychiatry Grand Rounds on 11/15/2018 at AECOM/MMC; on 11/30/2018 at Annual Isabelle Rapin Conference on Communication Disorders at Rose F.

Kennedy Intellectual and Developmental Disabilities Research Center (IDDRC) at AECOM/MMC; in November 2018 at the Autism Speaks conference in New York; in December 2018 at American College of Neuropsychopharmacology conference panel entitled “No Longer Tarred With the Same Brush? Evidence for the Therapeutic Potential of Cannabidiol: Implications for Regulatory Policy”. Interview with Dr. Hollander about the study outline was aired on NPR on 12/4/2018.

- Study outline was presented by Dr. Eric Hollander on April 25 2019 at the Department of Psychiatry and Behavioral Sciences Grand Rounds at Stanford University.
- Study outline was presented by Dr. Eric Hollander on May 18 at American Psychiatric Association meeting in San-Francisco at the “Essentials of cutting-edge evidence based treatments for Autism Spectrum Disorders” symposia, chaired by Dr. Eric Hollander (PI).
- As we enrolled first patient in the study, publication about the study appeared at Einstein magazine (http://magazine.einstein.yu.edu/summer-fall-2019/search-for-autism-therapies). Following CNN article about the study (https://fox6now.com/2019/09/29/new-study-aims-to-find-out-if-marijuana-help-treat-autism-symptoms),165 local television reports were generated and reached nearly 2 million people in various markets across the country.
- Examples include:

  https://app.criticalmention.com/app/#/clip/public/ed76b72c-1692-4dff-b054-c1f7f290f028;
  https://app.criticalmention.com/app/#/clip/public/759e937a-ab09-4768-b42f-bc3b2d3861e7?show_sentiment=false
- CNN has followed two patients through the DOD CBDV ASD study for WEED documentary of Dr. Gupta. The media team, coordinated by Keri Enriquez from CNN, attended at least one study visit for each of the two currently enrolled patients (Patient 01-001: August 7, 2019& Patient 01-008: December 6, 2019). CNN interviewed Dr. Hollander on August 2 2021. Updated documentary will be broadcasted on CNN in October 2021.
- **Website(s) or other Internet site(s)**
- https://www.autismeye.com/us-army-cannabis/ Autism Eye publication
  https://www.childrenshospitals.org/newsroom/childrens-hospitals- today/articles/2018/03/military-funds-research-of-cannabis-based-autism-treatment-for-kids Children’s Hospital association newsroom
  http://www.montefiore.org/body.cfm?id=1738&action=detail&ref=1375
- Montefiore news release
- https://www.newsweek.com/2018/02/23/really-good-weed-why-cannabis-may-be-worlds-most- effective-remedy-core-806758.html
- Newsweek magazine publication
- https://nypost.com/2018/05/02/clinical-trials-will-test-if-cannabis-compound-can-treat-autism/ NY post publication

Montefiore news release
https://www.newsweek.com/2018/02/23/really-good-weed-why-cannabis-may-be-worlds-most- effective-remedy-core-806758.html
Newsweek magazine publication
https://nypost.com/2018/05/02/clinical-trials-will-test-if-cannabis-compound-can-treat-autism/ NY post publication

Other Products

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Not applicable at this point

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Funding support:

Name: Eric Hollander
Project Role: Principal Investigator (AECOM/MMC Site):
Nearest Person Month Worked: 1.8 months, 15%
Contribution to Project: Dr. Hollander has performed work in monitoring study progress and development, protocol finalization. He oversaw regulatory documents submissions and assured that award reporting requirements are met.
Funding support: no change

Name: Bonnie Taylor, PhD
Project Role: Study Psychologist/Rater:
Nearest Person Month Worked: 1.8 months, 15%
Contribution to Project: Dr. Taylor has performed work in the areas of regulatory and source documents preparation and protocol development.
Funding support: no change

Name: Casara Ferretti
Project Role: Study Coordinator/Rater
Nearest Person Month Worked: 0.6 months, 5%
Contribution to Project: Ms. Ferretti has performed work in the areas of grant writing, protocol drafting, and advertising materials preparation
Funding support: no change

Name: Vera Nezgovorova, MD
Project Role: Study/Regulatory Coordinator:
Nearest Person Month Worked: 3 months, 25%
Contribution to Project: Dr. Nezgovorova has performed work in the areas of regulatory documents preparation, protocol development, NYS BNE/DEA licensure, inter-sites communication and report writing.
Funding support: AECOM/MMC

Name: Yin Zhao, MD
Project Role: Co-Investigator
Nearest Person Month Worked: 0.6 months, 5%
Contribution to Project: Dr. Zhao has performed work in the areas of regulatory documents preparation and protocol development.
Funding support: AECOM/MMC

Name: Orrin Devinsky
Project Role: Principal Investigator (NYU Site)
Nearest Person Month Worked: 1.2 months, 10%
Contribution to Project: Dr. Devinsky has performed work in monitoring study progress and development, protocol finalization. He oversaw regulatory documents submissions and assured that award reporting requirements are met.
Funding support: no change

Name: Latoya King
Project Role: Study Coordinator (NYU Site):
Nearest Person Month Worked: 3 months, 25%
Contribution to Project: Ms. King has performed work in the areas of regulatory documents preparation and submission.
Funding support: no change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

NYU Langone Comprehensive Epilepsy Center Collaboration **(co-PI Dr. Orrin Devinsky).**
GW Pharmaceuticals

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES:

Journal of Psychiatric Research 137 (2021) 194–201

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Review article

Potential of cannabinoids as treatments for autism spectrum disorders

V. Nezgovorova [a], C.J. Ferretti [a], B.P. Taylor [a], E. Shanahan [a], G. Uzunova [a], K. Hong [a], O. Devinsky [b], E. Hollander [a,*]

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ARTICLE INFO

Keywords:
Autism spectrum disorders
Cannabinoids

ABSTRACT

Current treatments for autism spectrum disorders (ASD) are limited in efficacy and are often associated with substantial side effects. These medications typically ameliorate problem behaviors associated with ASD, but do not target core symptom domains. As a result, there is a significant amount of research underway for development of novel experimental therapeutics. Endocannabinoids are arachidonic acid-derived lipid neuromodulators, which, in combination with their receptors and associated metabolic enzymes, constitute the endocannabinoid (EC) system. Cannabinoid signaling may be involved in the social impairment and repetitive behaviors observed in those with ASD. In this review, we discuss a possible role of the EC system in excitatory-inhibitory (E-I) imbalance and immune dysregulation in ASD. Novel treatments for the core symptom domains of ASD are needed and phytocannabinoids could be useful experimental therapeutics for core symptoms and associated domains.

1. Introduction

The Center for Disease Control and Prevention (CDC) estimates that 1 in 59 children in the US have autism spectrum disorder (ASD) (Baio et al., 2018). The World Health Organization estimated 0.76% of the world's children had ASD in 2010 (Baxter et al., 2015). Recently, Lyall et al. (2017) based on the prevalence estimates of several studies across multiple countries, estimated the population prevalence of ASD to be around 1.5% in developed countries around the world. ASD is characterized by two core symptoms - deficits in social communication/interaction and the presence of restricted/repetitive behaviors (RRBs) - and is often accompanied by irritability and impulsivity (Lecavalier et al., 2006). In the US, nearly 11% of youth with ASD undergo psychiatric hospitalization and based on recent analyses of data from Medicaid and US commercial healthcare claims databases 65% of outpatient youth are treated with psychotropic medications, which only ameliorate associated symptoms of ASD, and frequently cause disabling side-effects (Wink et al., 2018).

The etiology of ASD involves complex interactions of genetic, immunological and environmental factors (Loke et al., 2015). This complex mechanistic network constrains the development of targeted treatments that extend beyond small subgroups. ASD can also be divided into both idiopathic and non-idiopathic (syndromal) forms. Syndromal forms of ASD are characterized by an identified genetic cause and include Prader-Willi Syndrome (PWS), Tuberous-Sclerosis Complex (TSC), Angelman Syndrome and Fragile X syndrome. By studying treatments in established subgroups of ASD, we can define the treatment response before applying it to a larger heterogeneous population.

Endocannabinoids are arachidonic acid-derived lipid neuromodulators, which, in combination with their receptors and associated metabolic enzymes, constitute the endocannabinoid (EC) system. Cannabinoid signaling may be involved in the social impairment observed in those with ASD (Wei et al., 2016). There is also a link between the endocannabinoid system and signaling of oxytocin, a hormone fundamental for social processes and involved in ASD. It was recently found that anandamide-mediated signaling at CB_1 receptors, driven by oxytocin, controls social reward (Wei et al., 2015). Deficits in this signaling mechanism might contribute to social impairment in ASD (Wei et al., 2015). Increasing anandamide activity at CB_1 receptors might improve ASD-related social impairment (Wei et al., 2016). There is also a potential in modulation of the EC and oxytocinergic systems as a potential treatment approach for social anxiety disorder (Dos Santos et al., 2019).

In this review, we discuss the use of cannabinoids for a treatment of

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https://doi.org/10.1016/j.jpsychires.2021.02.048
Received 27 January 2021; Accepted 22 February 2021
Available online 26 February 2021
0022-3956/

ASD, including a possible role of the EC system in the following current ASD hypotheses: 1) immune dysregulation and 2) excitatory-inhibitory imbalance hypotheses. Novel treatments for the core symptom domains of ASD are needed, and the EC system could be a target for those therapies through the administration of exogenous cannabinoids.

2. Overview of autism spectrum disorder (ASD)

The DSM-5 diagnostic criteria for ASD list two core symptom domains: social communication and RRBs (Mazurek et al., 2019; Doernberg and Hollander, 2016). Deficits in social communication and social interaction occur in diverse contexts: social-emotional reciprocity, nonverbal communication and initiating, maintaining and understanding of interpersonal relationships. Symptoms can manifest as an impaired verbal and non-verbal communication, delayed and reduced interactions with peers, lack of enjoyment and interest in experiences with peers, and lack of social judgment and insight. Anxiety, which often increases with age, can exacerbate social communication deficits (Duvekot et al., 2018). There are currently no FDA-approved treatments for social communication deficits associated with ASD. The second core symptom domain - restricted and repetitive patterns of behavior (RRBs), interests or activities - includes stereotyped or repetitive motor movements, insistence on sameness and rigidity, highly fixated interests, and hypo- or hyper-reactivity to sensory input (Mazurek et al., 2019; Doernberg and Hollander, 2016; Harrop, 2015; Harrop et al., 2015; Leekam et al., 2011). RRBs vary widely in frequency and intensity among children and adolescents with ASD (Scahill et al., 2015), and may or may not cause distress. RRBs include *"lower level"* repetitive sensory and motor behaviors (e.g., hand flapping, rocking, humming, motoric compulsions and some self-injurious behaviors), and *"higher-level"* behaviors (e.g., insistence on sameness, pursuit of narrow circumscribed interests, insistence that routines), similar to repetitive language. Individuals with ASD may use RRBs for self-stimulation or to decrease arousal, or both. RRBs also occur in other neurodevelopmental and psychiatric disorders (Joseph et al., 2013). Additionally, those with ASD frequently have serious behavioral disturbances including irritability, which may manifest as aggression, self-injurious behavior and tantrums. Irritability is often defined as a "feeling state characterized by reduced control over temper which usually results in irascible verbal or behavioral outbursts," and results from emotion dysregulation or stimulus hyper-responsivity (Fung et al., 2016). These behavioral problems can severely disrupt school and family environments, further impairing education and social interactions.

3. The endocannabinoid (EC) system and ASD

Pathophysiological mechanisms that are thought to underlie the neurobehavioral deficits present in ASD include aberrant synaptic plasticity (Nelson and Valakh, 2015; Pardo et al., 2005; Liu et al., 2017), immune dysfunction (Careaga et al., 2010), and metabolic disturbances (Frye and James, 2014). Many of these mechanisms can be modulated by the EC system (Chakrabarti et al., 2015; Pacher et al., 2006; Zamberletti et al., 2017). Here, we provide a review of the EC system functioning and its dysfunction in the ASD population, describe preclinical evidence of EC system involvement in neurodevelopmental processes related to ASD and summarize two hypotheses of ASD pathogenesis, namely the immunological dysfunction hypothesis and the excitatory-inhibitory hypothesis. We also describe a role of the endocannabinoids and the exogenous cannabinoids within these hypotheses as summarized in Fig. 1 and Fig. 2.

3.1. Review of the EC system functioning

The EC system exerts its effects through multiple receptors and channels as summarized in Fig. 1. These include the G-protein coupled (GPCR) CB_1 and CB_2 cannabinoid receptors(27), transient receptor potential (TRP) channels (Iannotti et al., 2014) which modulate calcium flux (Ryan et al., 2009), the α3 and α1 glycine receptors (Devinsky et al., 2014; Li et al., 2011), and nuclear peroxisome proliferator-activated receptors (PPARs) (Battista et al., 2012). To date, there is no published evidence on interaction of AEA and 2AG with 5-HT1A receptors (Mastinu et al., 2018).

CB_1 receptors are among the most widely expressed GPCRs in the brain, and are mostly present in the forebrain including the allocortex, neocortex, thalamus, and basal ganglia areas, in addition to the peripheral nerves and non-neuronal tissues (Prenderville et al., 2015). Generally, CB_1 receptors are expressed pre-synaptically on glutamatergic and γ-aminobutyric acid-ergic (GABA-ergic) interneurons. CB_1 receptor activation results in glutamate release and inhibition of synaptic transmission (Perucca, 2017). CB_2 receptors are predominantly expressed in cells of the immune system, but are also expressed in the adrenal gland, heart, lung, prostate, uterus, ovary, testes, bone and

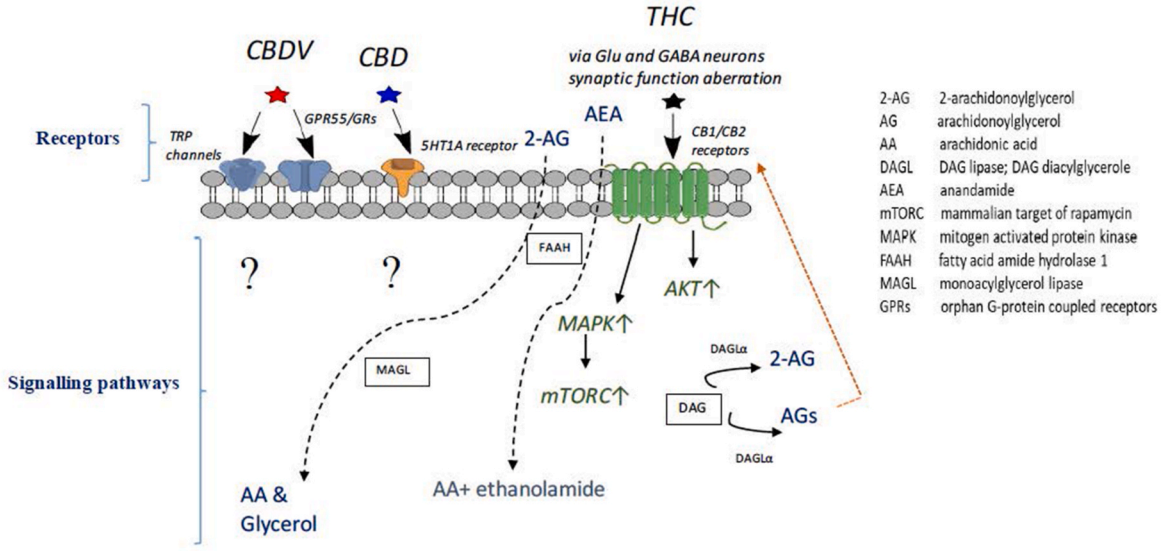

Fig. 1. Components of EC system and potential mechanisms of action of exogenous cannabinoids in ASD.

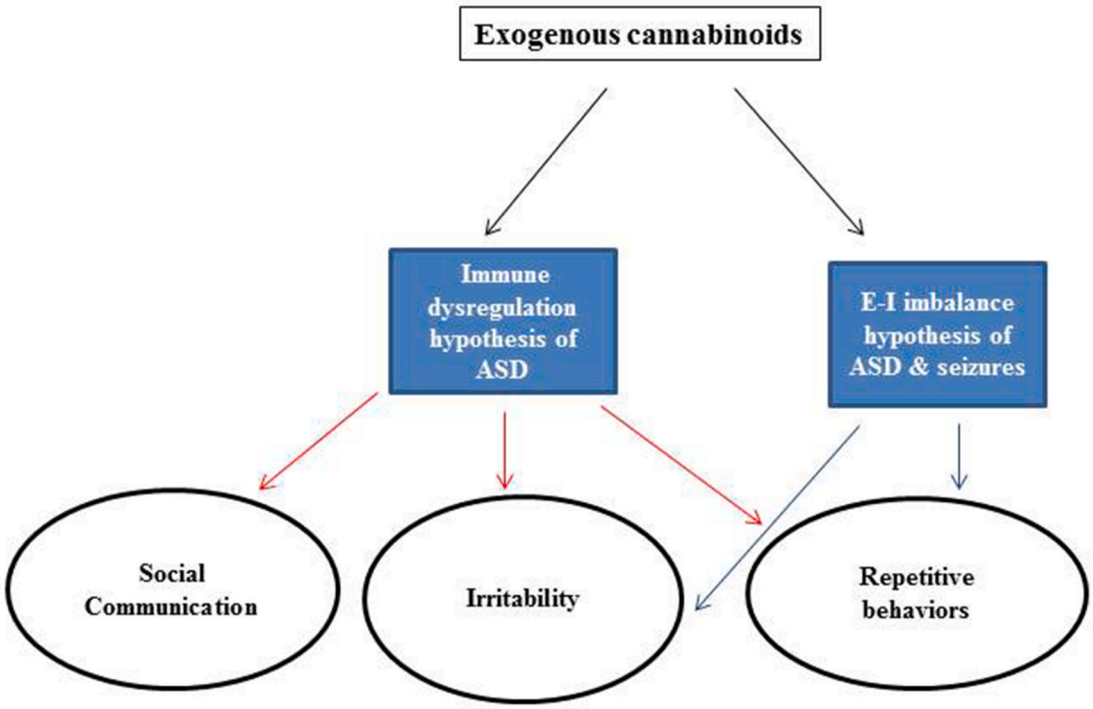

Fig. 2. Possible mechanisms of actions of exogenously administered cannabinoids in ASD.

pancreas in a number of mammalian species (Siniscalco et al., 2013; Turner et al., 2017). They are also expressed in the microglia(37) and astrocytes(38), which might be critical to the mechanism of ASD-related neuroinflammation(39) and indicative of a trend towards neural and systemic pro-inflammatory status of the immune system in ASD(40). Thus, recent studies have shown prominent microglia activation and increased levels of inflammatory cytokine and chemokine production in the brain tissue and cerebral spinal fluid as a trend towards neural and systemic pro-inflammatory status of the immune system in ASD(40).

Endocannabinoids are synthesized on demand and are modulated by ligand binding to the CB_1 and CB_2 receptors. This leads to protein kinase B (PKB, also known as Akt), mitogen activated protein kinase (MAPK) and mammalian target of rapamycin complex (mTORC) pathway activation, which is responsible for cell differentiation and proliferation (Prenderville et al., 2015). It can also lead to the inhibition of cellular EC uptake or the modulation of the intracellular metabolism of EC by specific enzymes. These enzymes might include the diacylglycerol lipase alpha (DAGLα), fatty acid amide hydrolase 1 (FAAH) and monoacylglycerol lipase (Battista et al., 2012), which in turn are responsible for the synthesis and degradation of endogenous cannabinoids such as arachidonoylglycerol (2-AG) and anandamide (arachidonoylethanolamide, AEA) (Turner et al., 2017) as summarized at Fig. 1. AEA (an endocannabinoid) and other bioactive long-chain N-acylethanolamines (NAEs) are formed by direct release from N-acyl-phosphatidylethanolamine (N-acyl-PE) by phospholipase D (PLD) (Sun et al., 2004). It is structurally related to N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA) (Aran et al., 2019a). OEA and PEA are widely distributed in the CNS, but their classification as endocannabinoids is debatable, as they lack affinity for CB_1 and CB_2 receptors (Aran et al., 2019a). Similar to AEA, they are, however, agonists for PPARα and GPR119(42). AEA regulates ion-channel activity and neurotransmitter release via CB_1 receptor activation (Silva et al., 2013). Interestingly, in a recent pilot study, plasma concentrations of AEA were found to be lower in children with ASD (n = 59) in comparison to healthy controls (n = 53) (Karhson et al., 2018). It was also recently shown that serum levels of AEA, PEA and OEA were lower in children with ASD (n = 93) (Aran et al., 2019a). Further studies are needed to determine whether circulating EC levels can be used as biomarkers in ASD research.

3.2. *Preclinical evidence of EC system involvement in neurodevelopmental processes related to ASD and role of exogenous cannabinoids in it*

EC signaling in the brain is complex. The EC system has an important role in neurodevelopment (Basavarajappa et al., 2009) and is transiently activated during stressful conditions (Steiner and Wotjak, 2008). Additionally, EC are key modulators of synaptic function, which is believed to be disrupted in ASD (Castillo et al., 2012). Numerous studies reported a link between the EC system and ASD (Chakrabarti et al., 2015). Disruption of this system may impair social communication, social play and reciprocity (Kerr et al., 2013). Here we summarize findings in selected ASD mice models.

BTBR T + Itpr3tf/J (BTBR) mouse strain is an useful tool to understand the neural circuit aberrations that underlie ASD due to its unique behavioral profile as such mice exhibit reduced play and social approach behavior in comparison to other strains (Guo and Commons, 2017). It is important to note that polymorphisms in the CB_1 receptor gene may adversely affect social reward processing in ASD (Chakrabarti and Baron-Cohen, 2011). It was shown in the BTBR mouse autism model that increasing AEA activity at CB_1 receptors improves ASD-related social impairment (Wei et al., 2016). Using the BTBR mouse autism model it was also shown that treatment with an EC reduces locomotor activity, suggesting an impact on irritability and repetitive behaviors that are commonly observed in those with ASD (Onaivi et al., 2011).

Prenatal exposure to valproic acid is an important environmental risk factor for ASD. Of note, humans prenatally exposed to valproic acid have higher rates of ASD (Christensen et al., 2013; Meador and Loring, 2013). Altered phosphorylation of CB_1 receptors in different brain areas, associated with changes in AEA metabolism from infancy to adulthood was found in prenatal exposure to valproic acid ASD models in rats (Wei et al., 2016; Servadio et al., 2016). *Rats prenatally exposed to VPA* exhibit ASD-like behavioral changes including reduced sociability and increased anxiety-related behavior, as well as alterations to cannabinoid targets. In particular, they have reduced expression of mRNA for PPARα

and GPR55 (a primary cannabidiol (CBD) target) at EC receptor targets in the hippocampus and frontal cortex (Kerr et al., 2013). Additional studies supporting a role for AEA signaling in the VPA model have been recently published and included studies of inhibition of fatty acid amide hydrolase, and its possible role in amelioration of the EC-mediated synaptic dysfunction (Melancia et al., 2018; Wu et al., 2020). In a study on male rats prenatally exposed to valproic acid (500 mg/kg i.p.; gestation day 12.5) Cannabidivarin (CBDV) treatment provided preclinical evidence in support of its ability to ameliorate behavioral abnormalities resembling core and associated symptoms of ASD and to restore hippocampal endocannabinoid signaling and neuroinflammation induced by prenatal valproic acid exposure(57).

Rare mutations in neuroligins might predispose to autism, including a neuroligin-3 amino-acid substitution (R451C) and a neuroligin-3 deletion. Intriguingly, EC-mediated signaling at inhibitory synapses is dysregulated in mouse models of autism-associated *Neuroligin 3 mutations* and notably in a mouse model of neurogilin 3 knockouts (Foldy et al., 2013). It was recently shown in the Neuroligin-3 $(NL3)^{R451C}$ mouse model of ASD, which has a heightened aggressive phenotype, that modulation of CB_1 receptors with a CB_1 receptor agonist (WIN55,212-2 (WIN)) could potentially reduce aggressive behavior (Hosie et al., 2018).

Roles for 2-AG signaling and CB_1/CB_2 receptors modulation have been demonstrated in the *Fmr1 KO model* of Fragile X syndrome (Maccarrone et al., 2010; Busquets-Garcia et al., 2013). Thus, CB_1 receptor blockade in the Fmr1 KO model could potentially normalized cognitive impairment, nociceptive desensitization and susceptibility to audiogenic seizures, whereas pharmacological blockade of the CB_2 receptor might normalize anxiolytic-like behavior(61). Modulation of EC signaling could be a novel target for the treatment of the Fragile X syndrome (Maccarrone et al., 2010).

As a result of this heightened interest and the noted benefits of CBD and its variants in the treatment of certain medical and neurodevelopmental conditions, there has also been an increase in pre-clinical research in this area. For example, after the administration of CBD in a mouse model in C57BL/6J mice submitted to the marble-burying test (*MBT*), an animal model proposed to reflect compulsive behaviour, there was an alleviation of marble-burying behavior, which is analogous to repetitive and compulsive behaviors observed in ASD(62). There is also preclinical evidence that supports the use of CBD for improvement of autism-like social behavior in mice with Dravet syndrome (Kaplan et al., 2017).

A therapeutic potential for CBDV is supported by recent preclinical studies that investigated its effects in Rett syndrome models (Bonini et al., 2018; Poleg et al., 2019; Gloss, 2015). Rett syndrome (RTT) is a rare neurodevelopmental disorder characterized by severe behavioral and physiological symptoms and is caused by mutations in the MECP2 gene in about 95% of cases and to date no cure is available (Vigli et al., 2018). In a recent study using a validated RTT mice model (MeCP2-308 male mice), systemic treatment with CBDV (2, 20, 100 mg/kg ip for 14 days) restored the compromised general health status, the sociability and the brain weight in mice(64). In the different study, CBDV administration exerted a rescue of memory deficits in *Mecp2 mutant mice* (Zamberletti et al., 2019b).

3.3. Immune dysfunction hypothesis of ASD and the EC system

EC have potent anti-inflammatory and immunosuppressive properties (Devinsky et al., 2014; Jean-Gilles et al., 2010; Klein and Cabral, 2006; Siniscalco et al., 2014). They have been identified in immune cells, such as monocytes, macrophages, basophils, lymphocytes and dendritic cells (Cabral et al., 2015), and reciprocal regulation has been described between EC and the cytokine-mediated immune system (Jean-Gilles et al., 2010). There is preclinical evidence of a possible contribution of the EC system in the immune dysfunctions underlying ASD (Habib et al., 2017; Brigida et al., 2017; Doenni et al., 2016). The immune system has an important role in brain development, memory and neurogenesis, and immune dysfunction is hypothesized to play a role in ASD onset(40). Alterations in EC signaling following postnatal inflammation could contribute to impairments in social behavior during adolescence (Doenni et al., 2016). EC system dysfunction in a monocyte and macrophagic cellular model of autism has been demonstrated by showing that the mRNA and protein for the CB_2 receptor and EC enzymes were significantly dysregulated(70). Several studies have reported increased autoimmune activity in patients with ASD, accompanied by marked immune dysfunction and heightened inflammatory responses, as evidenced by microglial activation (Goines and Ashwood, 2013; Hsiao, 2013; Masi et al., 2015; Onore et al., 2012; Rose and Ashwood, 2014; Vargas et al., 2005; Kalkman and Feuerbach, 2017). More specifically, in children with ASD elevated levels of pro-inflammatory interleukins IL-1β, IL-6, IL-8 and IL-12p40 (Ashwood et al., 2011; Tonhajzerova et al., 2015) and decreased levels of IL-10 and transforming growth factor-β (TGF-β) anti-inflammatory cytokines have been observed (Masi et al., 2015; Estes and McAllister, 2015). It was found that increases in pro-inflammatory cytokines IL-1β, IL-6, IL-8 and IL-12p40 are associated with more regressive forms of autism and more pronounced stereotypical behaviors (Akintunde et al., 2015). These changes in cytokine levels in ASD may be developmentally regulated as they differ when measured during the neonatal period as compared to later developmental periods (Estes and McAllister, 2015; Abdallah et al., 2012). Children with ASD also have elevated levels of the enzyme Nagalase, which is responsible for proper macrophage function (via Gc Protein-Derived Macrophage Activating Factor, GcMAF) (Bradstreet and Thyer, 2012). Treatment with GcMAF ameliorates symptoms of ASD in some children (Siniscalco et al., 2014) which may be due to its effects on gene expression of the EC system and CB_2 receptor protein (CB2R), and down-regulation of the over-activated blood monocyte-derived macrophages. These alterations and treatment responses indicate that the EC system might be involved in ASD pathogenesis.

3.4. Excitatory-inhibitory imbalance hypothesis of ASD and the EC system

Another widely held hypothesis of ASD etiology proposes there is an excitatory-inhibitory (E-I) imbalance in neural circuits created by local hyperconnectivity and long-range hypoconnectivity and disconnection (Uzunova et al., 2016). E-I imbalance might be associated with an increase in glutamatergic or decrease in GABA-ergic signaling, although initial deficits and homeostatic responses could be hard to discriminate (Nelson and Valakh, 2015; Uzunova et al., 2016). It could also lead to altered synaptic plasticity affecting learning and memory, increased seizure susceptibility and thus epilepsy pathogenesis, especially in syndromic forms of ASD (Uzunova et al., 2016). Autistic patients develop epilepsy at a rate up to 25 times that of the general population. Epilepsy prevalence in ASD varies between 8% and 30% (Jokiranta et al., 2014). The prevalence of epileptiform EEG without overt seizures is even higher. Risk factors that increase the presence of both ASD and epilepsy/seizures include intellectual disability, female gender, and age (Jokiranta et al., 2014; Blackmon et al., 2016). Intellectual disability increases the risk of epilepsy in ASD by ~3–5 fold (Strasser et al., 2018).

An increased E-I ratio in the prefrontal cortex is thought to be partly responsible for behavioral and social impairments observed in ASD. Additionally, E-I imbalance can result in seizures, behavioral changes and social dysfunction, including irritability, repetitive and disruptive behaviors, and social avoidance and withdrawal (Uzunova et al., 2016).

For example, ASD is a common comorbidity in Lennox-Gastaut syndrome (LGS), a severe epileptic encephalopathy, characterized by treatment-resistant tonic (and other) seizures, specific EEG abnormalities (i.e., slow spike-wave discharges, generalized paroxysmal fast activity) and cognitive impairment(89). Risk for both epilepsy and ASD symptoms are also increased in patients with Rett syndrome, Dravet syndrome, TSC and Fragile X syndrome, and many other genetic

epilepsies (Strasser et al., 2018). TSC is an autosomal-dominant disease, affecting 1 in 6000 patients, associated with cortical tubers and dysplasia, which could be related to ASD symptom severity, especially in the social communication domain(90). Dravet Syndrome, which is also known as severe myoclonic epilepsy in infancy (SMEI) (Berkvens et al., 2015), is an epileptic encephalopathy presenting in the first year of life with multiple seizures febrile and afebrile types (Devinsky et al., 2017). Thus, timely interventions addressing seizures and/or social-cognitive deficits could significantly improve outcomes, especially for young children with ASD and epilepsy(93, 94).

Exogenous activation of CB_1 receptors or enhancement of EC signaling to some extent might partially rescue imbalance between excitatory and inhibitory neurotransmission (Martella et al., 2018; Speed et al., 2015). More translational studies in this domain are warranted.

4. Mechanisms of action of phytocannabinoids

Cannabinoids could be therapeutic in ASD even if ECs might not be directly involved in its pathogenesis.

Phytocannabinoids are synthesized naturally. *Cannabis* is produced from the flowering plant that includes three species: Sativa, Indica, and Ruderalis (Bonini et al., 2018; Poleg et al., 2019). *Cannabis sativa* can grow to 5–18 feet or more, and often has a few branches. *Cannabis indica* typically grows 2–4 feet tall and is compactly branched. *Cannabis ruderalis* contains very low levels of Δ-9-tetrahydocannabinol (THC) and flowers as a result of age (autoflowering) (Gloss, 2015). There are >700 strains of cannabis. The major cannabis psychoactive molecule is THC, which binds with high affinity to both the CB_1 and CB_2 receptor. Cannabis is often divided into several categories based on cannabinoid content: Type I, THC-predominant, Type II Cannabis that contains both THC and CBD, and CBD-predominant Type III Cannabis, which display CBD- and terpenoid-rich profiles (Lewis et al., 2018).

Olivetolic acid and divarinic acid are the two phytocannabinoid precursors that generate cannabigerolic acid (CBGA). CBGA is the central precursor for phytocannabinoids biosynthesis in *C. sativa*, from which tetrahydrocannabinolic acid (THCA), cannabichromenic acid (CBCA) and cannabidiolic acid (CBDA) originate. CBDA forms CBD, the most abundant non-psychotropic phytocannabinoid of *C. sativa* (Premoli et al., 2019). CBD has a very low affinity (in the micromolar range) for CB_1 and CB_2 receptors, and nevertheless CBD is able to bind to these receptors. CBD also interacts with numerous G proteins (GPCR) (Premoli et al., 2019). CBD shows a high affinity towards TRP channels, in particular towards TRPV1 and TRPV2 receptors(101). Other studies have shown that AEA - one of the main endocannabinoids in the brain, mediated signaling at CB_1 receptors, driven by oxytocin, controls social reward. Modulation of AEA has been reported to alter social behaviors in genetic models of ASD(56). Deficits in this signaling mechanism may contribute to social impairment in ASD and might offer an avenue to treat these conditions(8). Prenatal VPA exposure can alter phosphorylation of CB1 receptors in a sex-specific and age-specific manner. Enhancing anandamide signalling through inhibition of its degradation could potentially reverse the behavioral deficits displayed by VPA-exposed animals of both sexes(55) Recently, it was shown that social deficits, repetitive behaviors and abnormal emotion-related behaviors in VPA-exposed offspring were improved after treatment with an inhibitor of AEA degrading enzyme, URB597(56). These effects were mediated by the mechanism of removal of postsynaptic α-amino-3--hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) subunits GluA1 and GluA2 and could shed light on underlying AEA signaling in the prefrontal cortex in a VPA-induced model of ASD(56). There is also accumulating evidence for the efficacy of CBD in conditions associated with ASD, including social phobia(102) and epilepsy(103). However, the pathways involved in the biological responses of CBD remain poorly understood(101).

CBD might affect immune functions, which consists in reducing leukocytes transmigration and down-regulating the expression of the vascular cell adhesion molecule-1 (VCAM-1). Furthermore, a reduced activation of microglia and reduced expression of chemokine ligand 2 (CCL2), chemokine ligand 5 (CCL5) and Interleukin 1 beta (IL1-β) has been observed after CBD treatment in murine models (Premoli et al., 2019).

Finally, CBD is able to modulate different enzymes belonging to the cytochrome P450 (CYP450)]. CBD completely inhibits CYP29C and CYP2D6 and has a strongly inhibitory action on the CYP1 family, in particular on CYP1A1, CYP1A2 and CYP1B1. Finally, CBD inhibits members of the CYP3 family such as CYP3A5, CYP3A4 and CYP3A7. The ability of CBD to interact with hepatic cytochromes has still to be well defined. (Premoli et al., 2019).

Like CBD, CBDV is also promising among the cannabinoids family. It is a multi-target drug, interacting both with non-ECs s and within the EC. It is important to highlight that exact downstream signaling pathways of CBD and CBDV actions are not fully understood to this date as reflected in Fig. 1. Multiple studies have demonstrated the anticonvulsant effects of CBDV in a broad range of seizure models (Hill et al., 2013). CBDV may exert its effects through voltage dependent anion selective channel protein 1 (VDAC1), or through the activation and desensitization of transient receptor potential vanilloid (TRPV1) channels (Iannotti et al., 2014) as summarized on Fig. 1. It may also act similarly to its propyl analog, CBD, by reducing neuronal excitability and neuronal transmission, and engaging inflammatory pathways through the inhibition of adenosine reuptake or by modulating the release of pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) (Martin-Moreno et al., 2011).

This review also discusses the potential exploitation of some phytocannabinoids for treating ASD symptoms.

The therapeutic mechanisms of CBDV in humans with ASD can be deduced from their effects, and their homolog CBD's effects, on other neuropsychiatric conditions such as epilepsy, addiction, anxiety, depression and schizophrenia, as well as repetitive behaviors that are analogous to those observed in children with ASD. (Campos et al., 2012). CBDV has the potential to be a valuable therapeutic agent for individuals with ASD(57).

We summarize possible mechanisms of action of exogenously administered cannabinoids on ASD in Fig. 2.

In sum, the multiple links between the EC system and ASD suggest both potential mechanisms and treatment targets.

5. Currently ongoing large clinical trials involving cannabinoids

The potential therapeutic mechanisms of both CBD and CBDV in ASD are currently being investigated.

Media and parent communities have recently demonstrated heightened interest in artisanal marijuana strains with high ratios of CBD:THC. Current large clinical trials using cannabinoids for treatment of ASD and other disorders are summarized in Table 1. As seizures highly correlate with ASD symptoms and cannabinoids were extensively studied in seizures disorders, we provide a brief outline of these studies there. There is a phase II double blind, randomized, placebo-controlled trial with crossover that aims to assess safety, tolerability and efficacy of a cannabinoids mix for behavioral problems in children and adolescents with ASD, which was completed in Israel (Dr. A. Aran, NCT02956226). Our research center at Montefiore Medical Center, Albert Einstein College of Medicine is also conducting a Phase 2 double-blind, randomized, placebo-controlled trial of CBDV, discussed further below, in children and adolescents with ASD (NCT03202303).

There is also an increased awareness of the ability of cannabinoids to control seizures in children with treatment-resistant epilepsy, which is often comorbid with syndromal forms of autism. The Charlotte's Web preparation of CBD oil was named after a patient, Charlotte Figi, with refractory SCN1A-confirmed Dravet syndrome (Brodie and Ben-Menachem, 2018), and is a commonly used brand in this

Table 1
Key large clinical trials using cannabinoids for treatment of ASD.

Study principle investigator	Compound and dosage	*ClinicalTrials.gov Identifier NCT#*	Study design	Current status of the trial	Publication
Hollander E.	Weight-based dosing of 10 mg/kg/day oral solution of CBDV or matching placebo (Cannabidivarin) for 12 weeks	NCT03202303	100 children and adolescents (aged 5–18 years) with diagnosis of ASD confirmed by the ADOS-2 and DSM-5 criteria will be enrolled in a study. This study aims to examine the efficacy and safety of cannabidivarin (CBDV) with a primary aim of studying its effect on irritability in children with ASD. *Primary outcome measure* of this study includes change in Aberrant Behavior Checklist-Irritability Subscale (ABC-I) from baseline to endpoint.		NA
Castellanos X.	98% pure CBD (Greenwich Biosciences, Inc.) as 100 mg/mL oral solution for 6 weeks.	NCT03900923	30 participants (aged 7–17 y.o.) with diagnosis of ASD confirmed by the ADOS-2 and DSM-5 criteria will be enrolled in a single group assignment study. This study will identify primary and secondary outcomes for future controlled studies and will evaluate change in symptoms commonly associated with ASD. *Primary outcome measure* will include change in Clinical Global Impression Scale -Improvement (CGI-I) from baseline to end-point.	Recruiting	NA
Aran A.	cannabidiol (CBD), Δ9 tetrahydrocannabinol (THC) in a 20:1 ratio or placebo for 3 months	NCT02956226	150 children and adolescents (aged 5–21 years) with ASD and moderate or greater behavioral problems as measured by a rating of moderate or higher (≥4) on the Clinical Global Impression-Severity (CGI-S). *Primary Outcome measures* would include: change from baseline home situations questionnaire-Autism Spectrum Disorder (HSQ-ASD) score at three months and change in Clinical Global Impression scores (CGI, improvement and efficacy index items) at three months from baseline.	Completed	Aran et al., 2019. (Aran et al., 2019b)
Barnes G., GW Pharmaceuticals	GWP42006 (CBDV); 52-week, open-label trial sponsored by GW Pharmaceuticals to evaluate the safety and tolerability of GWP42006 (CBDV). . Patients who satisfy all eligibility criteria will titrate to a target GWP42006 dose of 10 mg/kg/day or 800 mg/day, whichever is smaller, during the first 4 weeks of treatment. If there is intolerance during titration, the patient may be maintained on a dose below 10 mg/kg/day. The maximum dose patients aged 6 years or older can receive will be 20 mg/kg/day or 1600 mg/day, whichever is smaller.	NCT03849456	This study investigates safety and tolerability of GWP42006 (CBDV) in children and young adults with ASD (aged 4–18 y.o.) and to examine the effect of GWP42006 on communication, social interactions, sleep, behavior, and cognition profiles. *Primary outcome measure* of the study includes number of patients who experienced severe treatment-emergent Adverse Events (TEAEs) from baseline to endpoint.	recruiting	NA
McAlohan G.	single acute dose of 600 mg of CBD or 600 mg of CBDV	NCT03537950	This study investigates brain response to single acute dose of cannabidiol, cannabidivarin, and placebo in healthy men (n = 38, aged 18–50 years) with and without autism spectrum disorder. Outcome measures would include: brain biochemistry response to pharmacological stimulation (primary outcome measure); measurement of low frequency brain activity using resting state fMRI; measurement of brain functional connectivity using resting state fMRI	Active, not recruiting	NA
Athena Zuppa, MD, Children's Hospital of Philadelphia, Zelda Therapeutics.	Part 1 of the study consists of building a patient registry. Part 2 of the study consists of pharmacokinetic (PK) evaluation of select subjects. Part 3 of study related activities will include analysis and summary of the data on approximately a six month basis.	NCT03699527	The overall goals of this observational research (n = 200, up to 21 y.o) is to describe the 1) natural history of current use and disposition of medical cannabis products including CBD products, being administered to children as standard of care for the treatment of ASD, 2) understand the pharmacokinetic and pharmacodynamics of medical cannabis products and 3) provide educational feedback on what is learned to families and care providers to provide evidenced based dosing guidance for these products to the pediatric community.	Completed	

population. In a phase II randomized double-blind placebo-controlled study of 120 children and young adults with Dravet syndrome, the use of CBD led to a greater reduction in convulsive-seizure frequency than placebo (Devinsky et al., 2017), demonstrating the success of this treatment in those with seizure disorders. CBD may also be efficacious and well-tolerated for the treatment of drop seizures in Lennox-Gastault syndrome, and is currently being studied (Thiele et al., 2018).

6. Conclusion

This review discusses the use of novel therapeutic agents, CBD and CBDV as well as cannabis, for the treatment of ASD, including how the proposed potential mechanisms relate to current ASD hypotheses. Cannabinoids could be therapeutic in ASD even if endocannabinoids might not be directly involved in its pathogenesis.

Current treatments for ASD are limited in efficacy and are associated with debilitating side effects. Additionally, ASD is a common comorbidity in treatment-resistant epilepsy (TRE), a population in which CBD and CBDV have been shown to be effective. CBD is effective in several treatment-resistant childhood onset epilepsies (i.e., Lennox-Gastaut and Dravet Syndromes), but has not been studied in a large randomized double-blind clinical trial in ASD. Like CBD, CBDV could also be a promising treatment for ASD, acting upon both epileptiform and immune dysfunction hypotheses of ASD. Thus, the effect of CBD and CBDV on multiple mechanisms dysfunctional in ASD and their apparent low toxicity and lack of THC psychoactive properties make them attractive therapeutic agents, worthy of additional research.

Funding

No sources of funding were used to assist with the preparation of this review.

Declaration of competing interest

Dr. Hollander received support from Department of Defense (award# W81XWH-17-1-0253) and GW Pharmaceuticals for a study of Cannabidivarin (CBDV) vs. Placebo in Children with Autism Spectrum Disorder (ASD).

References

Abdallah, M.W., Larsen, N., Mortensen, E.L., Atladottir, H.O., Norgaard-Pedersen, B., Bonefeld-Jorgensen, E.C., et al., 2012. Neonatal levels of cytokines and risk of autism spectrum disorders: an exploratory register-based historic birth cohort study utilizing the Danish Newborn Screening Biobank. J. Neuroimmunol. 252 (1–2), 75–82.

Akintunde, M.E., Rose, M., Krakowiak, P., Heuer, L., Ashwood, P., Hansen, R., et al., 2015. Increased production of IL-17 in children with autism spectrum disorders and co-morbid asthma. J. Neuroimmunol. 286, 33–41.

Aran, A., Eylon, M., Harel, M., Polianski, L., Nemirovski, A., Tepper, S., et al., 2019a. Lower circulating endocannabinoid levels in children with autism spectrum disorder. Mol. Autism. 10, 2.

Aran, A., Cassuto, H., Lubotzky, A., Wattad, N., Hazan, E., 2019b. Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems-A retrospective feasibility study. J. Autism Dev. Disord. 49 (3), 1284–1288.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., Van de Water, J., 2011. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav. Immun. 25 (1), 40–45.

Baio, J., Wiggins, L., Christensen, D.L., Maenner, M.J., Daniels, J., Warren, Z., et al., 2018. Prevalence of autism spectrum disorder among children aged 8 Years - autism and developmental disabilities monitoring network, 11 sites, United States, 2014. MMWR Surveill. Summ. 67 (6), 1–23.

Basavarajappa, B.S., Nixon, R.A., Arancio, O., 2009. Endocannabinoid system: emerging role from neurodevelopment to neurodegeneration. Mini Rev. Med. Chem. 9 (4), 448–462.

Battista, N., Di Tommaso, M., Bari, M., Maccarrone, M., 2012. The endocannabinoid system: an overview. Front. Behav. Neurosci. 6, 9.

Baxter, A.J., Brugha, T.S., Erskine, H.E., Scheurer, R.W., Vos, T., Scott, J.G., 2015. The epidemiology and global burden of autism spectrum disorders. Psychol. Med. 45 (3), 601–613.

Berkvens, J.J., Veugen, I., Veendrick-Meekes, M.J., Snoeijen-Schouwenaars, F.M., Schelhaas, H.J., Willemsen, M.H., et al., 2015. Autism and behavior in adult patients with Dravet syndrome (DS). Epilepsy Behav. 47, 11–16.

Blackmon, K., Bluvstein, J., MacAllister, W.S., Avallone, J., Misajon, J., Hedlund, J., et al., 2016. Treatment resistant epilepsy in autism spectrum disorder: increased risk for females. Autism Res. 9 (2), 311–320.

Bonini, S.A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, M., et al., 2018. Cannabis sativa: a comprehensive ethnopharmacological review of a medicinal plant with a long history. J. Ethnopharmacol. 227, 300–315.

Bradstreet, J.J.V.E., Thyer, L., 2012. Initial observations of elevated alpha-N-acetylgalactosaminidase activity associated with autism and observed reductions from GcProtein-Macrophage Activating Factor injections. Autism Insights (4), 31–38.

Brigida, A.L., Schultz, S., Cascone, M., Antonucci, N., Siniscalco, D., 2017. Endocannabinod signal dysregulation in autism spectrum disorders: a correlation link between inflammatory state and neuro-immune alterations. Int. J. Mol. Sci. 18 (7).

Brodie, M.J., Ben-Menachem, E., 2018. Cannabinoids for epilepsy: what do we know and where do we go? Epilepsia 59 (2), 291–296.

Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., Agustin-Pavon, C., Pastor, A., Mato, S., et al., 2013. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat. Med. 19 (5), 603–607.

Cabral, G.A., Rogers, T.J., Lichtman, A.H., 2015. Turning over a new leaf: cannabinoid and endocannabinoid modulation of immune function. J. Neuroimmune Pharmacol. 10 (2), 193–203.

Campos, A.C., Moreira, F.A., Gomes, F.V., Del Bel, E.A., Guimaraes, F.S., 2012. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (1607), 3364–3378.

Careaga, M., Van de Water, J., Ashwood, P., 2010. Immune dysfunction in autism: a pathway to treatment. Neurotherapeutics 7 (3), 283–292.

Castillo, P.E., Younts, T.J., Chavez, A.E., Hashimotodani, Y., 2012. Endocannabinoid signaling and synaptic function. Neuron 76 (1), 70–81.

Chakrabarti, B., Baron-Cohen, S., 2011. Variation in the human cannabinoid receptor CNR1 gene modulates gaze duration for happy faces. Mol. Autism. 2 (1), 10.

Chakrabarti, B., Persico, A., Battista, N., Maccarrone, M., 2015. Endocannabinoid signaling in autism. Neurotherapeutics 12 (4), 837–847.

Christensen, J., Gronborg, T.K., Sorensen, M.J., Schendel, D., Parner, E.T., Pedersen, L. H., et al., 2013. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. J. Am. Med. Assoc. 309 (16), 1696–1703.

Devinsky, O., Cilio, M.R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., et al., 2014. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia 55 (6), 791–802.

Devinsky, O., Cross, J.H., Laux, L., Marsh, E., Miller, I., Nabbout, R., et al., 2017. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N. Engl. J. Med. 376 (21), 2011–2020.

Doenni, V.M., Gray, J.M., Song, C.M., Patel, S., Hill, M.N., Pittman, Q.J., 2016. Deficient adolescent social behavior following early-life inflammation is ameliorated by augmentation of anandamide signaling. Brain Behav. Immun. 58, 237–247.

Doernberg, E., Hollander, E., 2016. Neurodevelopmental disorders (ASD and ADHD): DSM-5, ICD-10, and ICD-11. CNS Spectr. 21 (4), 295–299.

Dos Santos, R.G., de Lima Osorio, F., Martin-Santos, R., Zuardi, A.W., Hallak, J.E.C., Crippa, J.A.S., 2019. Modulation of the endocannabinoid and oxytocinergic systems as a potential treatment approach for social anxiety disorder. CNS Drugs 33 (10), 1031–1038.

Duvekot, J., van der Ende, J., Verhulst, F.C., Greaves-Lord, K., 2018. Examining bidirectional effects between the autism spectrum disorder (ASD) core symptom domains and anxiety in children with ASD. JCPP (J. Child Psychol. Psychiatry) 59 (3), 277–284.

Estes, M.L., McAllister, A.K., 2015. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. Nat. Rev. Neurosci. 16 (8), 469–486.

Foldy, C., Malenka, R.C., Sudhof, T.C., 2013. Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. Neuron 78 (3), 498–509.

Frye, R.E., James, S.J., 2014. Metabolic pathology of autism in relation to redox metabolism. Biomarkers Med. 8 (3), 321–330.

Fung, L.K., Mahajan, R., Nozzolillo, A., Bernal, P., Krasner, A., Jo, B., et al., 2016. Pharmacologic treatment of severe irritability and problem behaviors in autism: a systematic review and meta-analysis. Pediatrics 137 (Suppl. 2), S124–S135.

Gloss, D., 2015. An overview of products and bias in research. Neurotherapeutics 12 (4), 731–734.

Goines, P.E., Ashwood, P., 2013. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol. Teratol. 36, 67–81.

Guo, Y.P., Commons, K.G., 2017. Serotonin neuron abnormalities in the BTBR mouse model of autism. Autism Res. 10 (1), 66–77.

Habib, S.S., Al-Regaiey, K., Bashir, S., Iqbal, M., 2017. Role of endocannabinoids on neuroinflammation in autism spectrum disorder prevention. J. Clin. Diagn. Res. 11 (6), CE01–CE3.

Harrop, C., 2015. Evidence-based, parent-mediated interventions for young children with autism spectrum disorder: the case of restricted and repetitive behaviors. Autism 19 (6), 662–672.

Harrop, C., Gulsrud, A., Kasari, C., 2015. Does gender moderate core deficits in ASD? An investigation into restricted and repetitive behaviors in girls and boys with ASD. J. Autism Dev. Disord. 45 (11), 3644–3655.

Hill, T.D., Cascio, M.G., Romano, B., Duncan, M., Pertwee, R.G., Williams, C.M., et al., 2013. Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB1 receptor-independent mechanism. Br. J. Pharmacol. 170 (3), 679–692.

Hosie, S., Malone, D.T., Liu, S., Glass, M., Adlard, P.A., Hannan, A.J., et al., 2018. Altered amygdala excitation and CB1 receptor modulation of aggressive behavior in the neuroligin-3(r451C) mouse model of autism. Front. Cell. Neurosci. 12, 234.
Hsiao, E.Y., 2013. Immune dysregulation in autism spectrum disorder. Int. Rev. Neurobiol. 113, 269–302.
Iannotti, F.A., Hill, C.L., Leo, A., Alhusaini, A., Soubrane, C., Mazzarella, E., et al., 2014. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. ACS Chem. Neurosci. 5 (11), 1131–1141.
Jean-Gilles, L., Gran, B., Constantinescu, C.S., 2010. Interaction between cytokines, cannabinoids and the nervous system. Immunobiology 215 (8), 606–610.
Jokiranta, E., Sourander, A., Suominen, A., Timonen-Soivio, L., Brown, A.S., Sillanpaa, M., 2014. Epilepsy among children and adolescents with autism spectrum disorders: a population-based study. J. Autism Dev. Disord. 44 (10), 2547–2557.
Joseph, L., Thurm, A., Farmer, C., Shumway, S., 2013. Repetitive behavior and restricted interests in young children with autism: comparisons with controls and stability over 2 years. Autism Res. 6 (6), 584–595.
Kalkman, H.O., Feuerbach, D., 2017. Microglia M2A polarization as potential link between food allergy and autism spectrum disorders. Pharmaceuticals 10 (4).
Kaplan, J.S., Stella, N., Catterall, W.A., Westenbroek, R.E., 2017. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc. Natl. Acad. Sci. U. S. A. 114 (42), 11229–11234.
Karhson, D.S., Krasinska, K.M., Dallaire, J.A., Libove, R.A., Phillips, J.M., Chien, A.S., et al., 2018. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol. Autism. 9, 18.
Kerr, D.M., Downey, L., Conboy, M., Finn, D.P., Roche, M., 2013. Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav. Brain Res. 249, 124–132.
Klein, T.W., Cabral, G.A., 2006. Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. J. Neuroimmune Pharmacol. 1 (1), 50–64.
Lecavalier, L., Leone, S., Wiltz, J., 2006. The impact of behaviour problems on caregiver stress in young people with autism spectrum disorders. J. Intellect. Disabil. Res. 50 (Pt 3), 172–183.
Leekam, S.R., Prior, M.R., Uljarevic, M., 2011. Restricted and repetitive behaviors in autism spectrum disorders: a review of research in the last decade. Psychol. Bull. 137 (4), 562–593.
Lewis, M.A., Russo, E.B., Smith, K.M., 2018. Pharmacological foundations of cannabis chemovars. Planta Med. 84 (4), 225–233.
Li, C., Jones, P.M., Persaud, S.J., 2011. Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. Pharmacol. Ther. 129 (3), 307–320.
Liu, X., Li, Z., Fan, C., Zhang, D., Chen, J., 2017. Genetics implicate common mechanisms in autism and schizophrenia: synaptic activity and immunity. J. Med. Genet. 54 (8), 511–520.
Loke, Y.J., Hannan, A.J., Craig, J.M., 2015. The role of epigenetic change in autism spectrum disorders. Front. Neurol. 6, 107.
Lyall, K., Croen, L., Daniels, J., Fallin, M.D., Ladd-Acosta, C., Lee, B.K., et al., 2017. The changing epidemiology of autism spectrum disorders. Annu. Rev. Publ. Health 38, 81–102.
Maccarrone, M., Rossi, S., Bari, M., De Chiara, V., Rapino, C., Musella, A., et al., 2010. Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology 35 (7), 1500–1509.
Martella, G., Meringolo, M., Trobiani, L., De Jaco, A., Pisani, A., Bonsi, P., 2018. The neurobiological bases of autism spectrum disorders: the R451C-neuroligin 3 mutation hampers the expression of long-term synaptic depression in the dorsal striatum. Eur. J. Neurosci. 47 (6), 701–708.
Martin-Moreno, A.M., Reigada, D., Ramirez, B.G., Mechoulam, R., Innamorato, N., Cuadrado, A., et al., 2011. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. Mol. Pharmacol. 79 (6), 964–973.
Masi, A., Quintana, D.S., Glozier, N., Lloyd, A.R., Hickie, I.B., Guastella, A.J., 2015. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. Mol. Psychiatr. 20 (4), 440–446.
Mastinu, A., Premoli, M., Ferrari-Toninelli, G., Tambaro, S., Maccarinelli, G., Memo, M., et al., 2018. Cannabinoids in health and disease: pharmacological potential in metabolic syndrome and neuroinflammation. Horm. Mol. Biol. Clin. Invest. 36 (2).
Mazurek, M.O., Lu, F., Macklin, E.A., Handen, B.L., 2019 Feb. Factors associated with DSM-5 severity level ratings for autism spectrum disorder. Autism 23 (2), 468–476. https://pubmed.ncbi.nlm.nih.gov/29390871/.
Meador, K.J., Loring, D.W., 2013. Prenatal valproate exposure is associated with autism spectrum disorder and childhood autism. J. Pediatr. 163 (3), 924.
Melancia, F., Schiavi, S., Servadio, M., Cartocci, V., Campolongo, P., Palmery, M., et al., 2018. Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. Br. J. Pharmacol. 175 (18), 3699–3712.
Nelson, S.B., Valakh, V., 2015. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 87 (4), 684–698.
Onaivi, E.S., Benno, R., Halpern, T., Mehanovic, M., Schanz, N., Sanders, C., et al., 2011. Consequences of cannabinoid and monoaminergic system disruption in a mouse model of autism spectrum disorders. Curr. Neuropharmacol. 9 (1), 209–214.
Onore, C., Careaga, M., Ashwood, P., 2012. The role of immune dysfunction in the pathophysiology of autism. Brain Behav. Immun. 26 (3), 383–392.
Pacher, P., Batkai, S., Kunos, G., 2006. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol. Rev. 58 (3), 389–462.
Pardo, C.A., Vargas, D.L., Zimmerman, A.W., 2005. Immunity, neuroglia and neuroinflammation in autism. Int. Rev. Psychiatr. 17 (6), 485–495.
Perucca, E., 2017. Cannabinoids in the treatment of epilepsy: hard evidence at last? J. Epilepsy Res. 7 (2), 61–76.
Poleg, S., Golubchik, P., Offen, D., Weizman, A., 2019. Cannabidiol as a suggested candidate for treatment of autism spectrum disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 89, 90–96.
Premoli, M., Aria, F., Bonini, S.A., Maccarinelli, G., Gianoncelli, A., Pina, S.D., et al., 2019. Cannabidiol: recent advances and new insights for neuropsychiatric disorders treatment. Life Sci. 224, 120–127.
Prenderville, J.A., Kelly, A.M., Downer, E.J., 2015. The role of cannabinoids in adult neurogenesis. Br. J. Pharmacol. 172 (16), 3950–3963.
Rose, D., Ashwood, P., 2014. Potential cytokine biomarkers in autism spectrum disorders. Biomarkers Med. 8 (9), 1171–1181.
Ryan, D., Drysdale, A.J., Lafourcade, C., Pertwee, R.G., Platt, B., 2009. Cannabidiol targets mitochondria to regulate intracellular Ca2+ levels. J. Neurosci. 29 (7), 2053–2063.
Scahill, L., Aman, M.G., Lecavalier, L., Halladay, A.K., Bishop, S.L., Bodfish, J.W., et al., 2015. Measuring repetitive behaviors as a treatment endpoint in youth with autism spectrum disorder. Autism 19 (1), 38–52.
Servadio, M., Melancia, F., Manduca, A., di Masi, A., Schiavi, S., Cartocci, V., et al., 2016. Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. Transl. Psychiatry 6 (9), e902.
Silva, G.B., Atchison, D.K., Juncos, L.I., Garcia, N.H., 2013. Anandamide inhibits transport-related oxygen consumption in the loop of Henle by activating CB1 receptors. Am. J. Physiol. Ren. Physiol. 304 (4), F376–F381.
Siniscalco, D., Sapone, A., Giordano, C., Cirillo, A., de Magistris, L., Rossi, F., et al., 2013. Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. J. Autism Dev. Disord. 43 (11), 2686–2695.
Siniscalco, D., Bradstreet, J.J., Cirillo, A., Antonucci, N., 2014. The in vitro GcMAF effects on endocannabinoid system transcriptionomics, receptor formation, and cell activity of autism-derived macrophages. J. Neuroinflammation 11, 78.
Speed, H.E., Masiulis, I., Gibson, J.R., Powell, C.M., 2015. Increased cortical inhibition in autism-linked neuroligin-3r451C mice is due in part to loss of endocannabinoid signaling. PLoS One 10 (10), e0140638.
Steiner, M.A., Wotjak, C.T., 2008. Role of the endocannabinoid system in regulation of the hypothalamic-pituitary-adrenocortical axis. Prog. Brain Res. 170, 397–432.
Strasser, L., Downes, M., Kung, J., Cross, J.H., De Haan, M., 2018. Prevalence and risk factors for autism spectrum disorder in epilepsy: a systematic review and meta-analysis. Dev. Med. Child Neurol. 60 (1), 19–29.
Sun, Y.X., Tsuboi, K., Okamoto, Y., Tonai, T., Murakami, M., Kudo, I., et al., 2004. Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. Biochem. J. 380 (Pt 3), 749–756.
Thiele, E.A., Marsh, E.D., French, J.A., Mazurkiewicz-Beldzinska, M., Benbadis, S.R., Joshi, C., et al., 2018. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 391 (10125), 1085–1096.
Tonhajzerova, I., Ondrejka, I., Mestanik, M., Mikolka, P., Hrtanek, I., Mestanikova, A., et al., 2015. Inflammatory activity in autism spectrum disorder. Adv. Exp. Med. Biol. 861, 93–98.
Turner, S.E., Williams, C.M., Iversen, L., Whalley, B.J., 2017. Molecular pharmacology of phytocannabinoids. Prog. Chem. Org. Nat. Prod. 103, 61–101.
Uzunova, G., Pallanti, S., Hollander, E., 2016. Excitatory/inhibitory imbalance in autism spectrum disorders: implications for interventions and therapeutics. World J. Biol. Psychiatr. 17 (3), 174–186.
Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57 (1), 67–81.
Vigli, D., Cosentino, L., Raggi, C., Laviola, G., Woolley-Roberts, M., De Filippis, B., 2018. Chronic treatment with the phytocannabinoid Cannabidivarin (CBDV) rescues behavioural alterations and brain atrophy in a mouse model of Rett syndrome. Neuropharmacology 140, 121–129.
Wei, D., Lee, D., Cox, C.D., Karsten, C.A., Penagarikano, O., Geschwind, D.H., et al., 2015. Endocannabinoid signaling mediates oxytocin-driven social reward. Proc. Natl. Acad. Sci. U. S. A. 112 (45), 14084–14089.
Wei, D., Dinh, D., Lee, D., Li, D., Anguren, A., Moreno-Sanz, G., et al., 2016. Enhancement of anandamide-mediated endocannabinoid signaling corrects autism-related social impairment. Cannabis Cannabinoid Res. 1 (1), 81–89.
Wink, L.K., Pedapati, E.V., Adams, R., Erickson, C.A., Pedersen, K.A., Morrow, E.M., et al., 2018 Nov. Characterization of medication use in a multicenter sample of pediatric inpatients with autism spectrum disorder. J. Autism Dev. Disord. 48 (11), 3711–3719. https://pubmed.ncbi.nlm.nih.gov/28516426/.
Wu, H.F., Lu, T.Y., Chu, M.C., Chen, P.S., Lee, C.W., Lin, H.C., 2020. Targeting the inhibition of fatty acid amide hydrolase ameliorate the endocannabinoid-mediated synaptic dysfunction in a valproic acid-induced rat model of Autism. Neuropharmacology 162, 107736.
Zamberletti, E., Gabaglio, M., Parolaro, D., 2017. The endocannabinoid system and autism spectrum disorders: insights from animal models. Int. J. Mol. Sci. 18 (9).
Zamberletti, E., Gabaglio, M., Piscitelli, F., Brodie, J.S., Woolley-Roberts, M., Barbiero, I., et al., 2019. Cannabidivarin completely rescues cognitive deficits and delays neurological and motor defects in male Mecp2 mutant mice. J. Psychopharmacol. 33 (7), 894–907.