Aus dem Institut für experimentelle Anästhesiologie der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

„The efficacy of Cannabidivarin for HIV-associated neuropathic pain – a randomized, blinded, controlled clinical trial“

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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von

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1 Abstract

In the current opioid crisis, the need for alternative analgesics is high and cannabinoids are an intensely discussed treatment option. Cannabinoids have shown promising results in both clinical and animal studies but are limited due to side effects such as euphoria or feeling ´high´. We conducted a randomized, double-blind, placebo-controlled cross-over study to evaluate the effects of cannabidivarin on HIV-associated neuropathic pain. Patients received the phytocannabinoid cannabidivarin (400 mg/d) or placebo in two successive phases (4 weeks each) in a randomized order. In between the two phases, a wash-out-phase of 3 weeks was interposed to eliminate potential carry-over-effects. After the second treatment phase, patients were followed up for 3 additional weeks. The primary endpoint was pain intensity on an 11-point numeric rating scale, recorded in a diary. Secondary endpoints were supplemental pain medication, pain characteristics and quality of life. 32 patients were included. The mean decrease in pain intensity under placebo was 0.32 points higher compared to cannabidivarin (p=0.38; 95% CI -0.42 to 1.05). Cannabidivarin did not influence any secondary endpoints (amount of additional pain medication, pain characteristics or quality of life). Cannabidivarin and placebo showed similar incidences of adverse events. No significant adverse reactions occurred during either treatment. Cannabidivarin was safe but failed to reduce neuropathic pain in HIV-patients. Based on current knowledge that cannabinoid-induced pain relief is dependent on cannabinoid receptors, we assume that this is due to a lack of cannabinoid receptor activation. A larger patient number might have improved the validity of the collected data. However, the recorded differences are far from statistical significance. Therefore, we assume that the same conclusion would be drawn from a bigger sample size. Based on our findings, we do not recommend cannabidivarin as a treatment option for HIV-associated neuropathic pain.

2 Zusammenfassung

In der derzeitigen Opioidkrise wächst der Bedarf an alternativen Analgetika. Dabei werden auch Cannabinoide häufig als eine Behandlungsoption diskutiert. Sowohl in klinischen als auch in tierexperimentellen Studien zu neuropathischen Schmerzen zeigten Cannabinoide vielversprechende Resultate. Allerdings ist ihr Einsatz auf Grund von Nebenwirkungen, wie zum Beispiel Euphorie oder das Gefühl „high“ zu sein, limitiert. In dieser randomisierten, doppelblinden, Placebo-kontrollierten Cross-over Studie untersuchten wir Cannabidivarin, ein Phytocannabinoid ohne nennenswerte Affinität an Cannabisrezeptoren, in Patienten mit HIV-assoziierten neuropathischen Schmerzen. Die Patienten erhielten Cannabidivarin (400 mg/d) oder ein Placebo in zwei aufeinanderfolgenden jeweils 4-wöchigen Behandlungsphasen. Dazwischen lag eine 3-wöchige Auswaschphase, um einen Carry-Over-Effekt auszuschließen. Anschließend wurden die Patienten für 3 weitere Wochen nachverfolgt. Der primäre Endpunkt war Schmerzintensität auf einer numerischen 11 Punkte Skala, aufgezeichnet in einem Patiententagebuch. Sekundäre Endpunkte waren zusätzlich eingenommene Schmerzmedikation, Schmerzcharakteristika und Lebensqualität. Es wurden 32 Patienten eingeschlossen. Die durchschnittliche Reduktion der Schmerzintensität war unter Placebo um 0.32 Punkte höher als unter Cannabidivarin (p=0.38; 95% KI -0.42 bis 1.05). Cannabidivarin hatte keinen Einfluss auf die sekundären Endpunkte (zusätzlich eingenommene Schmerzmedikation, Schmerzcharakteristika, Lebensqualität). Cannabidivarin und Placebo zeigten ähnlich häufige Nebenwirkungen. Weder unter Cannabidivarin noch unter Placebo traten Verdachtsfälle schwerwiegender Nebenwirkungen auf. Zusammenfassend zeigte Cannabidivarin keine schädlichen Nebenwirkungen, hatte aber keinen Einfluss auf HIV-assoziierte neuropathische Schmerzen. Dies könnte durch die fehlende Aktivierung von Cannabinoidrezeptoren erklärt werden. Eine größere Patientenkohorte hätte die Validität der Daten möglicherweise gesteigert. Da die Differenzen zwischen den beiden Gruppen jedoch weit von statistischer Signifikanz entfernt sind, versprechen wir uns auch bei größeren Patientenanzahlen keine wesentlich abweichenden Resultate. Daher empfehlen wir Cannabidivarin derzeit nicht als Behandlungsoption für HIV-assoziierte neuropathische Schmerzen.

3 Introduction

As of 2019, 38 million people worldwide were living with the Human immunodeficiency virus (HIV) [1]. A cure is not yet available, and the number of people living with HIV is still rising [1]. Among many complications of an HIV-infection, neuropathic pain belongs to most common [2] and affects more than 30% of people living with HIV [3]. Patients often suffer from typical neuropathic symptoms, such as allodynia or loss of pinprick sensation [4]. The pathophysiological mechanisms of HIV-associated neuropathic pain are not fully understood. Inflammatory effects mediated by HIV-infected macrophages as well as neurodegenerative effects of antiretroviral drugs are discussed [4,5]. Since causal treatment is not available, current treatment concentrates on slowing down the progress and minimizing the symptoms. Therefore, combined antiretroviral therapy should start early to prevent late stages of HIV infections, and neurotoxic antiretroviral drugs should be avoided [6,7] . Symptomatic treatment often focuses on antidepressants, anticonvulsants and opioids [8]. Due to detrimental side effects of such analgesics and rising concerns regarding the opioid crisis, the need for new, effective treatment options is high. In this context cannabinoids are often discussed [9].

Cannabinoids have been used for medical purposes for thousands of years [10], but their efficacy is still not established. The endocannabinoid system consisting of the two endocannabinoids 2-arachidonylglycerol and anandamide, as well as the two cannabinoid (CB) receptors 1 and 2 modulate the transmission of pain signals [11]. Cannabis-based analgesia may either be achieved by exogenous cannabinoids or by influencing the endocannabinoid system [11]. Even though some exogenous cannabinoids were effective in humans, they often show disturbing side effects like nausea or drowsiness [12–14]. Drugs inhibiting endocannabinoid-degrading enzymes showed detrimental side effects in humans [15]. However, new cannabinoids not primarily activating CB receptors have shown analgesic effects in animal studies [16] and are considered a treatment option in neuropathic pain [11]. In this study we investigated cannabidivarin (CBDV) a natural component of the cannabis plant with very low affinity to CB receptors [17,18] in patients with HIV-associated neuropathic pain. We

conducted a double-blind-placebo controlled cross-over trial and assessed CBDV´s influence on pain intensity, pain characteristics, side effects and quality of life.

4 Methods

4.1 Study design

This was a randomized, placebo-controlled, double-blind cross-over phase II trial conducted from 1st January 2017 to 8th January 2019 in a single-center outpatient setting. All patients enrolled were treated with two agents (CBDV and placebo) in two successive phases. The allocation to the order of treatments (CBDV-placebo [C-P] or placebo-CBDV [P-C]) was determined by randomization. After screening, suitable patients were included and baseline values for pain intensity, supplemental pain medication, pain characteristics and quality of life were recorded during a one-week baseline phase. Patients received placebo or CBDV, respectively, during two 4-week treatment phases (A and B). Treatment phase A was followed by a 3-week washout phase plus another 1-week phase to collect baseline values before treatment phase B (see **Figure 1**). The wash-out phase was inserted to prevent potential carry-over effects of the treatment received in phase A. Because previous studies had shown that cannabinoids accumulate in fatty tissue with a half-life of about 5 days after long-term oral administration [19], a 3 week wash-out phase seemed reasonable. Afterwards, patients were followed up for another 3 weeks. Thus, each patient was monitored for a total of 16 weeks. Subjects were seen by a clinician before each baseline phase, and before, during and after each treatment phase. All examinations were carried out in the study center at Charité Campus Benjamin Franklin, except for one telephone interview before baseline B. Throughout the study, the patients self-documented pain values, additional pain medication and side effects in pre-printed tables and additional drug effects on the Drug Effects-Questionnaire [20] in paper-based diaries.

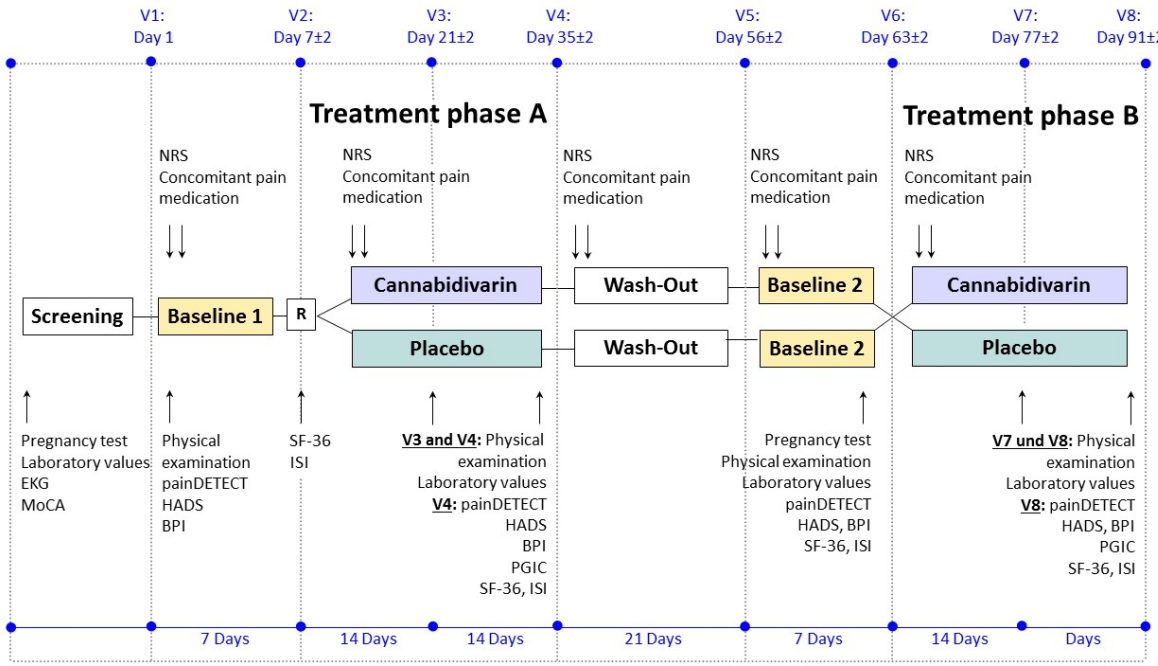

Figure 1 **Study design** (adapted from Eibach et al. 2020 [35])

4.2 Study participants

We recruited patients through personal contacts to Berlin-based HIV-specialists, patient-advocacy groups and by advertisement in the Berlin public transportation system. Patients were contacted via phone and informed about the study. Suitable subjects were invited to the study center and were screened for in- and exclusion criteria.

Inclusion criteria:

- Male and female patients with chronic, painful HIV-associated neuropathy (numeric rating scale score ≥ 4); women who were post-menopausal for more than one year; other female patients were allowed to participate only if they were permanently sterilized (e.g. tubal occlusion, hysterectomy) or if they provided a negative pregnancy test and were willing to use a highly effective method of contraception (e.g. hormonal contraceptives) during the course of the study and for three months thereafter.
- Age: 18-65 years
- Body mass index (BMI): 18-30 kg/m^2

- Fluency in German language
- Signed written informed consent

Exclusion criteria:

- Individuals related to or dependent on the sponsor, the trial site or the investigator
- Individuals housed in institutions due to official or judicial orders
- Severe diseases of the central nervous system (e.g. dementia)
- Major psychiatric conditions
- Acute neurological disorders with functional limitations, and/or limitations to neurological assessment
- Limited mental capacity or knowledge of the German language
- Chronic or previous abuse of recreational drugs and/or alcohol
- Pregnancy and lactation, or planning pregnancy during the course of the study and for three months thereafter
- Men and women of childbearing potential not using adequate contraception during the trial and three months thereafter
- Intolerance to the study medication or to components of the study medication
- Hepatic diseases where
    - the level of alanine aminotransferase (ALT) or the level of aspartate aminotransferase (AST) exceed three times the upper limit of normal range, bilirubin exceeded two times the upper limit of normal range, or the international normalized ratio (INR) exceeded 1.5 times the upper limit of normal range
    - the levels of ALT or AST exceed three times the upper limit of normal range, in combination with symptoms (fatigue, nausea, vomiting, pain or tenderness in the right upper quadrant, fever, rash and/or eosinophilia)
    - the levels of ALT or AST alone exceeded eight times the upper limit of normal range

    - the levels of ALT or AST exceed five times the upper limit of normal range for longer than two weeks
- Chronic renal insufficiency (with significant deviation of the creatinine-level from normal range)
- Electrocardiogram-Parameters outside following reference ranges:
    - PR-interval: 120 ms (lower limit), 220 ms (upper limit)
    - QRS-duration: 0 ms (lower limit), 120 ms (upper limit)
    - QT-interval: 0 ms (lower limit), 500 ms (upper limit)
    - QTcF-Interval (males): 0 ms (lower limit), 430 ms (upper limit)
    - QTcF (females): 0 ms (lower limit), 450 ms (upper limit)
- Clinically significant cardiovascular or metabolic diseases:
    - uncontrolled hypertension (lower limit: 90/40 mmHg, upper limit: 140/90 mmHg (18-45 years), 160/90 mmHg (>45 years))
    - severe heart failure (NYHA > III)
    - abnormal heart rate (lower limit: 40 min^{-1} (18-45 years), 50 min^{-1} (>45 years), upper limit: 90 min^{-1})
    - heart attack within the past 12 months
- Active participation in other clinical trials within three months before or during this study

A clinician confirmed the diagnosis of HIV-associated painful sensory neuropathy based on patient history, the Douleur Neuropathique 4 interview (DN4i) and the Clinical HIV-Associated Neuropathy Tool (CHANT) assessing the presence of neuropathic pain by different specific neuropathic pain characteristics (DN4i and CHANT) and signs (only CHANT) [21,22]. Laboratory values (full blood count, liver function tests, electrolytes, glucose, urea, cholesterol, creatinine, creatinine kinase, protein and INR) were recorded on the day of screening, before, during and after each treatment phase. Electrocardiograms were recorded and analyzed by a cardiologist before inclusion. The Montreal Cognitive Assessment (MoCA) was performed to exclude dementia before inclusion [23].

Patients were allowed to use concomitant analgesics (including antidepressants and anticonvulsants) as needed and documented every dosage in the patient diary.

4.3 Outcome measurements

The primary outcome was pain intensity under CBDV as compared to placebo. Patients were instructed to document the pain intensity thrice a day (8:30 AM, 1:00 PM and 7:00 PM) on an 11-point NRS (0 = no pain, 10 = worst pain imaginable) in their diary. For analysis, the mean value of the NRS scores was calculated for the last 7 days of baseline and treatment, respectively. A treatment response was defined as a pain reduction of at least 20% between the last 7 days of baseline and the last 7 days of treatment, as postulated in previous studies on neuropathic pain [24,25]. The number of treatment responders was calculated for each treatment phase.

Secondary endpoints (pain characteristics, quality of life and sleep, and subjective impression of change) were measured by questionnaires. For an overview of questionnaires, see **Table 1**. We compared the results of all questionnaires before (visit 1/2 and 6) and after each treatment phase (visit 4 and 8), except PGIC, which was only used at the end of each treatment phase (see **Figure 1**).

On the first day, patients received a patient diary in which NRS values, all adverse or unusual events, use of concomitant pain medication and subjective treatment effects were recorded. We used the Medication Quantification Scale (MQS) in its 3rd version to analyze the use of concomitant pain medication [26]. For analysis of concomitant pain medication, mean MQS values were calculated for the last 7 days of baseline and treatment, respectively.

The entries in the diary were discussed with a study physician at each visit and adverse events were then documented in paper-based tables. All adverse events were classified using the Common Terminology Criteria for Adverse Events, Version 4.03. Any deviations from standard laboratory values were recorded as adverse events as well.

Table 1 **Overview of questionnaires**

Questionnaire	**Characteristics**
painDETECT [27]	• Presence of neuropathic pain • Neuropathic pain characteristics
DN4i [22]	• Presence of neuropathic pain • Neuropathic pain characteristics
Brief Pain Inventory [28]	• Pain severity • Quality of life
Hospital Anxiety and Depression Scale [29]	• Severity of anxiety and depression
36-Item Short Form Survey [30]	• Quality of life
Insomnia Severity Index [31]	• Quality of sleep
Patient Global Impression of Change [32]	• Subjective impression of change after treatment as compared to before treatment

4.4 Randomization, allocation concealment and blinding

Patients were allocated to the treatment groups in a randomized manner in blocks of 4. The computer-generated random lists were stored in a locked cabinet in the study center. Every patient´s allocation to the sequence group was kept in sealed envelopes which were always accessible in case of emergency. All patients and staff involved in patient contacts and assessment of outcomes were blinded until the end of the study.

4.5 Monitoring

The study was monitored by two independent colleagues of the Charité Comprehensive Cancer Center who secured patient safety and adherence to good clinical practice (GCP) principles throughout the trial.

4.6 Investigational Medicinal Products (IMP)

Patients received the active agent and placebo as two identically appearing and tasting solutions depending on the treatment phase. GW Pharmaceuticals provided the IMP in amber-glass bottles which were marked with the patient ID and treatment phase for each patient. One ml of CBDV solution consisted of 50 mg CBDV, 79 mg anhydrous ethanol, 0.5 mg sucralose, 0.2 mg strawberry flavor and quantum satis to 1.0 ml refined sesame oil. 1 ml of placebo solution consisted of 79 mg anhydrous

ethanol, 0.5 mg sucralose, 0.2 mg strawberry flavor and quantum satis to 1.0 ml refined sesame oil. Based on preclinical and clinical phase-I-studies [17] a daily dose of 400 mg CBDV (8ml of IMP in both phases) was chosen. Every bottle was weighed before and after each treatment phase to document the exact amount of IMP taken by each patient.

4.7 Statistics

Statistical analysis and sample size calculations were performed by the Charité Coordinating Center for Clinical studies (KKS). Prior to conduct of the study, a sample size calculation was carried out using nQuery Advisor® 7.0. The primary endpoint (pain score on NRS) and the cross-over study-design were taken into account. Previous literature showed that a cannabinoid-induced pain reduction of 20% compared to placebo and a common standard deviation (SD) for the period differences of 2.5 seemed realistic [24,25,33]. These calculations resulted in a planned sample size of 21 patients per sequence group to show this effect with a power of 85% and a two-sided type-I error of 0.05 using a paired t-test for 2x2 crossover designs. A dropout rate of 15% was estimated, resulting in a planned sample size of 50 patients in total.

Statistical analysis was based on the intention-to-treat principle. Every patient who was dosed with IMP and delivered at least one post-baseline measurement of the primary end point was included in the efficacy analysis. Every patient treated with IMP was included in the population analyzed for adverse side effects (safety population). Continuous variables are displayed as mean, SD and range. Categorical parameters are given as absolute and relative frequency.

For all continuous endpoints, first, the difference between sequence-specific baseline and then the value after treatment was calculated. In the next step the difference between the two treatment effects (CBDV - placebo) was calculated for every patient. For a direct comparison of the two treatment groups, we used a paired t-test taking period effects into account. In case of non-normality of data distribution, a non-parametric version was applied. For the treatment effects, 95% confidence intervals (CI) were calculated. The primary endpoint was also analyzed using a random subject intercept mixed

model. In this model the change of NRS values from baseline to post-treatment was a dependent variable, and treatment, phase, and NRS phase baseline value were independent variables. P values resulting from the analyses are to be considered as non-confirmatory. A p value ≤ 0.05 was considered significant. All analyses were done using R (version 3.5.0) [34].

For the primary endpoint, missing values were not replaced, and means were used. Missing values in questionnaires were treated according to the guidelines of the questionnaire.

4.8 Study approval

All patients signed written informed consent prior to inclusion in the study. The trial protocol, patient information and informed consent sheets were approved by the ethics committee of the state regulatory authority of Berlin (Landesamt für Gesundheit und Soziales; 15/0255 EK 13) and the German Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte; 61-3910-4040377). The CONSORT guidelines and checklist, GCP principles and the Declaration of Helsinki were strictly followed. The study was registered at EudraCT (https://www.clinicaltrialsregister.eu/) under number 2014-005344-17.

5 Results

5.1 Patient population

Patient screening was conducted from January 2015 to September 2018 and was terminated at the end of financial support by the EU grant FP7-HEALTH-2013-INNOVATION-1; No. 602891-2. During this time 194 patients were contacted by email or phone. 55 were invited to the study center and screened, of which 34 were assessed eligible and were assigned a patient identification number (ID). Two patients were excluded from efficacy analysis due to missing data (the patient did not bring his diary) and a screening failure (the patient did not meet inclusion criteria) but were not excluded from the safety population. 32 patients were included in the efficacy analysis. Of these, 4 patients dropped out during the study but were not excluded from efficacy analysis. Characteristics of all 32 patients included in the full analysis are shown in **Table 2.**

Table 2: **Demographic data at day of screening** (adapted from Eibach et al. 2020 *[35]*)

Characteristic	**Level**	**C-P (n=16)**	**P-C (n=16)**	**Total (n=32)**
Sex, n (%)	Male	16 (100)	15 (93.8)	31 (96.9)
	female	0 (0)	1 (6.2)	1 (3.1)
Age, y (SD)	mean (SD)	52.31 (8.06)	48.31 (9.62)	50.31 (8.96)
	range	36; 65	31; 65	31; 65
Body height, m (SD)	mean	1.82 (0.06)	1.77 (0.06)	1.8 (0.07)
	range	1.7; 2	1.6; 1.9	1.6; 2
NRS score at screening (SD)	mean	6.12 (1.15)	6.44 (1.59)	6.28 (1.37)
	Range	4; 8	4; 9	4; 9
DN4i score (0-7) (SD)	mean	5.19 (1.17)	5 (0.89)	5.09 (1.03)
	range	3; 7	4; 6	3; 7
Pain in the feet, n (%)	yes	16 (100)	16 (100)	32 (100)
	no	2 (12.5)	5 (31.2)	7 (21.9)
Numbness in at least one foot, n (%)	yes	14 (87.5)	11 (68.8)	25 /78.1)
Reduced vibration sensation in at least one foot, n (%)	no	1 (6.2)	2 (12.5)	3 (9.4)
	yes	15 (93.8)	14 (87.5)	29 (90.6)
Reduced ankle reflexes in at least one foot, n (%)	no	2 (12.5)	4 (25)	6 (18.8)
	yes	14 (87.5)	12 (75)	26 (81.2)
MOCA score (0-30) (SD)	mean	26.62 (2.03)	26.38 (2.09)	26.5 (2.03)
	Range	24; 30	22; 30	22; 30

5.2 Primary endpoint

The mean decrease in pain intensity on the last 7 days of CBDV was 0.32 points lower as compared to placebo; this difference was not statistically significant (p=0.38; 95% CI -0.42 to 1.05) (see **Figure 2** and **3**). An overview of pain scores throughout the study is given in **Table 3.** The mean NRS values during the last 7 days of follow-up (3 weeks after end of treatment phase B) were 2.71 (SD: 1.57) in the C-P group and 3.72 (SD: 2.43) in the P-C group. 13 patients were classified as CBDV responders, whereas 16 patients responded to placebo.

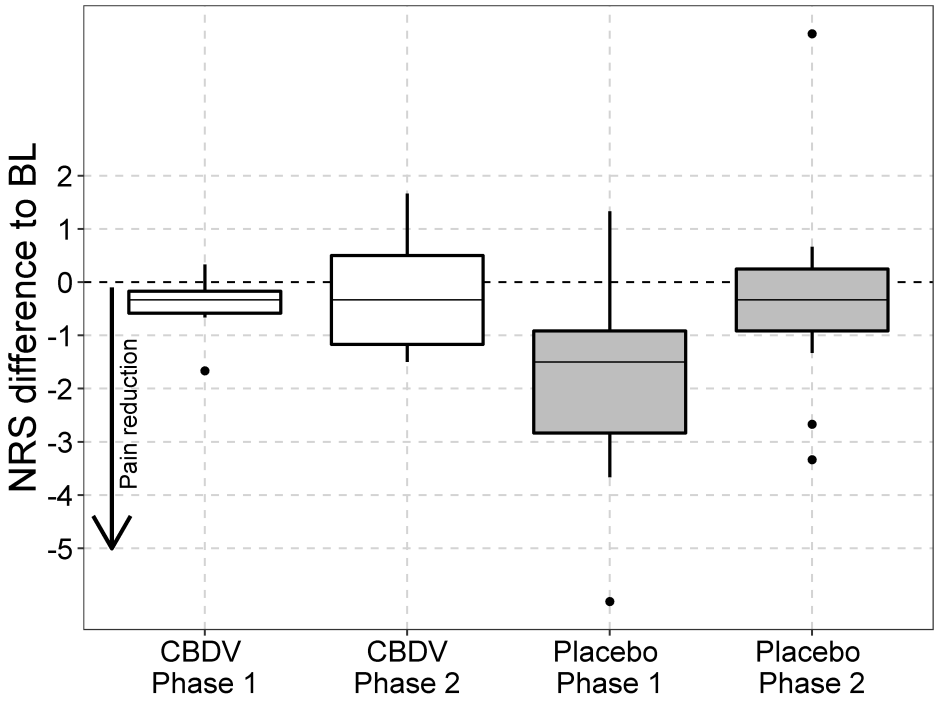

Figure 2 **Differences in pain intensity by treatment and phase.** The boxplots show differences between mean pain values under cannabidivarin (CBDV) (white) and placebo (grey) during the last 7 days of treatment and baseline (BL). Negative values indicate pain reduction compared to baseline; bars indicate minimum and maximum values; dots indicate values outside of 1.5* interquartile range (paired t-test; n=32). (adapted from Eibach et al. 2020 [35])

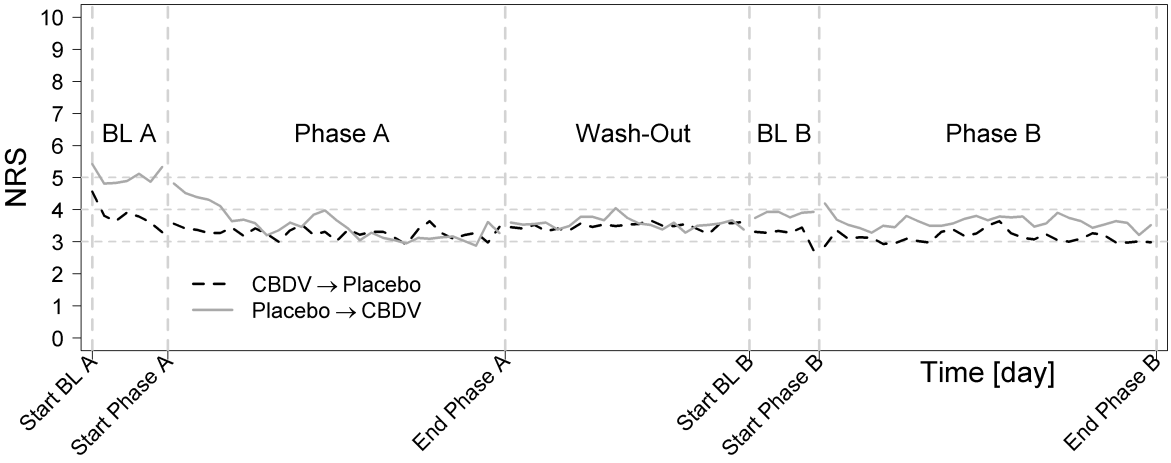

Figure 3 **Descriptive presentation of pain intensity over time.** Displayed are mean NRS values per day by treatment sequence. Cannabidivarin (CBDV)-placebo (black, broken line); placebo-CBDV (grey, continuous line). BL, baseline; NRS, Numeric Rating Scale. Figure taken from Eibach et al. [35]

Table 3: **NRS values of both treatment groups at different time points**

Characteristic	**Level**	**C-P**	**P-C**	**Total**
NRS last 7 days base line phase A	n	16	16	32
	Mean (SD)	3.84 (1.59)	5.07 (1.99)	4.45 (1.88)
	Median (IQR)	3.38 (2.6; 5.1)	5.09 (3.7; 6)	4.32 (2.8; 5.8)
	Range	1.7; 6.8	1.9; 9.7	1.7; 9.7
NRS last 7 days treatment phase A	Mean (SD)	3.09 (1.79)	3.35 (2.35)	3.22 (2.06)
	Median (IQR)	2.68 (1.5; 4.3)	2.9 (2.3; 3.8)	2.77 (1.8; 4.3)
	Range	1.1; 6.6	0.2; 9.9	0.2; 9.9
NRS difference treatment phase A	Mean (SD)	-0.75 (0.8)	-1.71 (1.52)	-1.23 (1.29)
	Median (IQR)	-0.63 (-1.3; -0.1)	-1.62 (-2; -0.9)	-1.07 (-1.7; -0.1)
	Range	-2.7; 0.3	-5.2; 0.4	-5.2; 0.4
Pain reduction ≥ 20% treatment phase A (%)		8 (50)	12 (75)	20 (62.5)
NRS last 7 days base line phase B	n	14	14	28
	Mean (SD)	3.29 (2.05)	3.88 (2.59)	3.58 (2.31)
	Median (IQR)	2.88 (1.7; 4.9)	3.12 (2.4; 5.2)	2.97 (2; 5.2)
	Range	0.2; 7	0.3; 9.7	0.2; 9.7
NRS last 7 days treatment phase B	Mean (SD)	3.07 (2.14)	3.5 (1.81)	3.29 (1.96)
	Median (IQR)	2.43 (1.4; 3.7)	3.31 (2.5; 4.8)	3.05 (1.6; 4.3)
	Range	1.1; 8.4	0.8; 6.8	0.8; 8.4
NRS difference treatment phase B	Mean (SD)	-0.22 (1.05)	-0.37 (1.18)	-0.3 (1.1)
	Median (IQR)	-0.01 (-0.7; 0.2)	-0.19 (-1; 0.1)	-0.05 (-0.9; 0.1)
	Range	-2.8; 1.4	-2.9; 2	-2.9; 2
Pain reduction ≥ 20% treatment phase B		4 (28.6)	5 (35.7)	9 (32.1)

5.3 Secondary endpoints

Secondary endpoints included pain characteristics, quality of life and sleep and subjective impression of change. No statistically significant differences between CBDV and placebo were detectable by any of these questionnaires. In particular, no significant differences in specific pain parameters assessed in the painDETECT questionnaire were detectable (see **Figure 4**). We did not observe any statistical differences in the intake of additional pain medication between CBDV and placebo (mean treatment effect of CBDV compared to placebo = -0.16, p=0.81, 95% CI -1.50 to 1.19 – see **Figure 5**), nor any influence of supplemental pain medication on the treatment effect of CBDV or placebo.

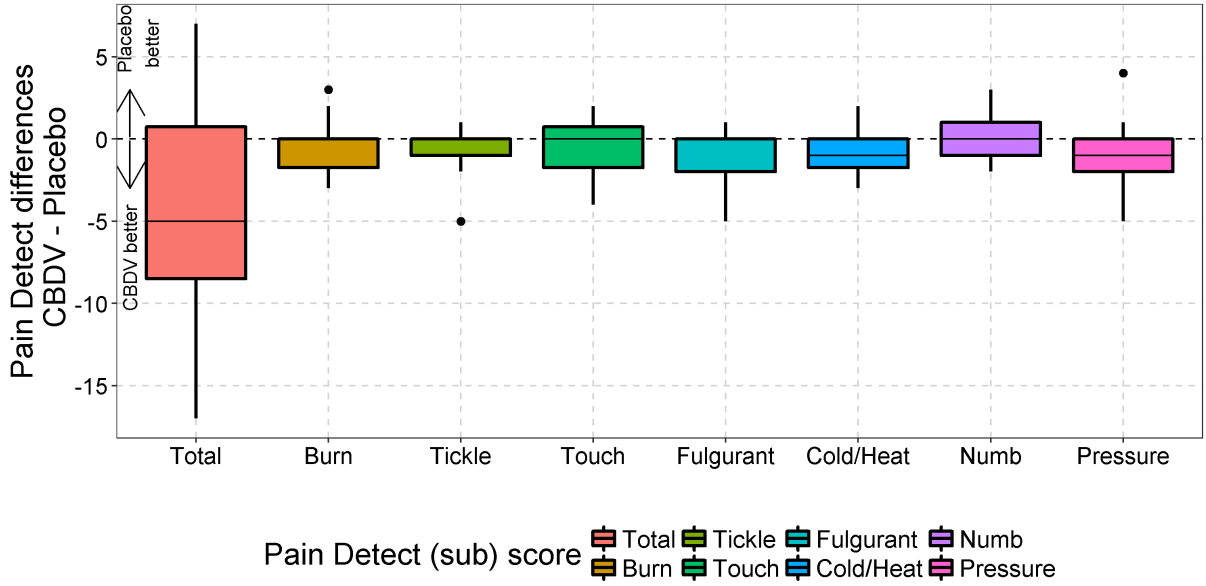

Figure 4 **Treatment effects on painDETECT score and sub-scores.** Differences (medians; mean for total effect) between cannabidivarin (CBDV) and placebo effects. Bars indicate minimum and maximum values; dots indicate values outside of 1.5* interquartile range (paired t-test; n = 32).

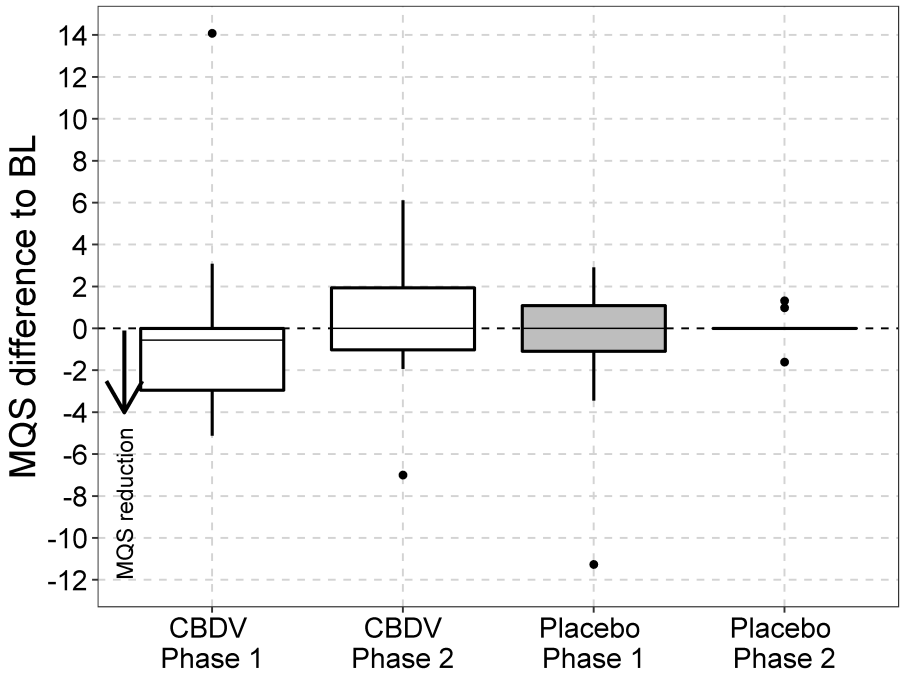

Figure 5 **Differences in use of concomitant pain medication by treatment and phase.** Compared are MQS values under cannabidivarin (CBDV) (white) and placebo (grey) on the last 7 days of treatment and baseline (BL). Negative values indicate lower use of concomitant pain medication compared to baseline; bars indicate minimum and maximum; dots indicate values outside of 1.5* interquartile range.

5.4 Adverse events

34 patients were included in the population analyzed for safety (adverse side effects), of which 31 (91.2%) experienced at least one adverse event during CBDV treatment; 27 patients (79.4%) had at least one adverse event during placebo. During each treatment (CBDV or placebo), 9 patients (26.5 %) experienced an adverse event that was considered to be related to study medication (see **Table 4**) Overall, the prevalence of adverse events during both treatment phases was similar and all adverse events were of low to moderate severity. The most frequently recorded adverse events (related to study medication) were dry mouth and diarrhea (3 cases during each treatment). Only one subject withdrew due to an adverse event (cough). This happened during CBDV treatment. We considered this adverse event related to study medication. We did not recognize any clinically significant changes of laboratory values related to study medication.

There were 2 serious adverse events (SAE) recorded. One patient experienced an acute myocardial infarction during CBDV treatment. This patient (male, 62 years) had the following cardiovascular risk

factors: History of arterial hypertension, transient ischemic attack, pulmonary embolism and factor-V-Leiden mutation. Therefore, this event was not considered related to study medication. Another patient experienced acute back pain which led to hospitalization. This SAE occurred during follow-up phase and was not considered related to study medication either.

Table 4 **Overview of Adverse Events considered related to study medication** (adapted from Eibach et al. 2020 [35])

	Number of patients (%)	
AE	**CBDV**	**Placebo**
Dry mouth	3 (8.8)	5 (14.7)
Diarrhea	3 (8.8)	1 (2.9)
Headache	1 (2.9)	3 (8.8)
Concentration disturbance	1 (2.9)	1 (2.9)
Dizziness	0 (0)	2 (5.9)
Hyperhidrosis	0 (0)	2 (5.9)
Pruritus	1 (2.9)	1 (2.9)
Constipation	0 (0)	1 (2.9)
Dysesthesia	0 (0)	1 (2.9)
Dyspepsia	0 (0)	1 (2.9)
Fatigue	0 (0)	1 (2.9)
Gastrointestinal irritation	0 (0)	1 (2.9)
Hypertrichosis	1 (2.9)	0 (0)
Insomnia	0 (0)	1 (2.9)
Mood disturbances	0 (0)	1 (2.9)
Nausea	0 (0)	2 (5.9)
Numbness in neck	1 (2.9)	0 (0)
Tachycardia	0 (0)	1 (2.9)
Vision disturbance	0 (0)	1 (2.9)

6 Discussion

In this study, CBDV did not elicit major adverse side effects, but did also not influence pain intensity or any other parameters related to chronic neuropathic pain as compared to placebo. In particular, we did not observe any influence on specific pain characteristics or the use of concomitant pain medication.

In experimental studies, cannabinoid analgesic effects were mainly mediated by activation of CB receptors [13,36]. However, other mechanisms were also proposed in animal studies [37]. For example, allosteric effects of cannabidiol and CBDV on the CB1 receptor were postulated [38]. We tested CBDV, a compound that does not exhibit significant orthosteric binding to CB receptors, according to preclinical data [17,18]. In our study, CBDV failed to reduce neuropathic pain. This supports the common theory that anti-nociceptive effects of cannabinoids are mainly CB receptor mediated [39]. On the other hand, a lack of efficacy may also be explained by the type of administration. Maldonado et al. claimed that cannabinoids provide less pain relief when administered orally compared to smoked or vaporized administration [11].

Furthermore, activation of TRPV1-receptors by cannabinoids was observed in previous studies [16]. TRPV1-receptors localized on peripheral sensory neurons, are mainly responsible for heat sensations and are known to be dysfunctional in neuropathic pain [40], which can lead to burning sensations. Some authors have postulated a cannabinoid-mediated desensitization of TRPV1-receptors comparable to responses after locally administered capsaicin, an effective treatment for neuropathic pain [8,41]. We could not observe any effects of CBDV on specific pain characteristics, especially on burning pain.

In our study, CBDV was administered at a dose of 400 mg/d. This dose was considered safe in preclinical and clinical phase I-studies [17]. Studies with CBDV for other indications, such as autism spectrum disorders, have titrated CBDV to doses of up to 1600 mg/d in children [42]. Thus, an underdosing of CBDV might also explain the lack of efficacy in our trial.

The most readily visible (but statistically nonsignificant) reduction of pain scores was observed during treatment phase 1 in the placebo group (**Figure 3**). As mentioned in the corresponding paper, we explained this by ´the enhanced attention to the patients in the setting of a clinical trial´ [35]. These findings are in line with current observations that neuropathic pain patients, and especially HIV-patients, show high placebo response rates [43,44] .

Medical use of cannabinoids is limited by CB receptor-mediated side effects such as euphoria or feeling ´high´ [45,46]. CBDV does not bind to CB receptors [17,18] and did not show typical cannabinoid side effects. We observed no differences in side effects between CBDV and placebo. The most common side effects were diarrhea and dry mouth. These did not differ between the two treatment groups. It is conceivable that these effects were due to the vehicle (sesame oil solution or strawberry flavor). Of two SAEs, only one (myocardial infarction) occurred during treatment with CBDV. There is data suggesting that cannabinoids increase the risk of cardiovascular events via a CB receptor-mediated mechanism [47]. As mentioned above, CBDV does not bind to CB receptors [17,18] and it did not show any increase in cardiovascular risk in preclinical or clinical studies [unpublished confidential data supplied by the manufacturer]. This makes a relation between CBDV and this event unlikely. In summary, CBDV did not elicit major side effects in this trial. Even though the term ´safety population´ is widely used, it is questionable whether “safety” analyses should be based on such small sample sizes as in early phase I/II trials. For a profound safety analysis, much bigger sample sizes with thousands of patients are recommended [48,49] . Nevertheless, the data collected in this study may be used for future safety analyses of CBDV.

Consistent with statistics showing that HIV in Germany affects mainly men [50], we could only enroll one female patient. Interestingly, this patient perceived marked pain reduction under CBDV (9.7 to 6.8) compared to placebo (9.7 to 9.9). Of course, this solitary observation cannot be used to formally evaluate sex differences in cannabinoid-mediated analgesia but it is in line with findings from Redmond et al. showing that cannabinoids produce analgesia in women but not in men [51].

Patients suffering from chronic pain often use many different pain medications [52]. We not only aimed to reduce neuropathic pain but also to reduce the amount of concomitant pain medication.

According to our MQS values, CBDV did not have any effects on the use of concomitant pain medication compared to placebo. This is consistent with the results on the primary endpoint suggesting that CBDV is not a suitable treatment option for neuropathic pain.

Causal treatment for HIV-associated neuropathic pain is still not available and symptomatic treatment predominates. However, many patients are not correctly diagnosed with neuropathic pain in the first place [53] and many of those who are, are not sufficiently pain controlled [52]. Therefore, special attention on early diagnosis and therapy is necessary. For these purposes, screening tools such as DN4i, painDETECT and CHANT are available [21,22,27]. To prevent the development of neuropathic pain in HIV patients, combined antiretroviral therapy should be started early with neurotoxic agents, such as stavudine [54], to be avoided [7]. Currently the use of cannabinoids is not recommended as a first line therapy [8] which is underlined by the results of our study. As mentioned in the corresponding paper, we observed (statistically nonsignificant) pain relief under placebo treatment which might be associated with psychological effects [35]. Pain, and especially chronic HIV-associated neuropathic pain, is known to be influenced significantly by psychological components and placebo response rates [43,44,55]. Thus, a multimodal treatment approach is highly recommended, especially since multiple non-pharmacological treatments have shown efficacy in HIV-associated pain [56,57].

The exact analgesic mechanisms of cannabinoids are still subject to research. An activation of CB receptors is considered the major mechanism. However, other mechanisms such as influencing the endocannabinoid system have been described [11,16,36,37,41,58]. An inhibition of endocannabinoid degrading enzymes might be a useful approach but phase I-studies of the FAAH-inhibitor BIA 10-2474 resulted in lethal outcomes [15]. Since the combination of opioids and cannabinoids has shown synergistic analgesic effects [11], this might be a further option to treat neuropathic pain. In this study however, patients receiving opioids did not show greater pain reduction under CBDV compared to placebo. It is conceivable that development of novel cannabinoid therapeutics could improve pain therapy. But knowledge about cannabinoids is limited and a more thorough understanding of the endocannabinoid system and its analgesic mechanisms is crucial.

The CONSORT-Guidelines and GCP-principles were strictly followed. Nevertheless, this study had the following limitations: Due to limited financial resources we could not enroll more patients, nor could we offer a high remuneration which resulted in unexpected recruitment difficulties. Thus, we did not reach the planned sample size of 21 per treatment group. However, since our observed differences between pain scores were far from statistical significance (p=0.38) we would not expect any other conclusions with a bigger sample size. Even interpreting the lower border of the 95% CI, we do not expect any clinical relevance.

The mean NRS values during baselines A and B (4.45 and 3.58, respectively) were much lower compared to the mean value obtained on the screening visits (6.28) (see **Table 2 and 3**). We used NRS=6 in our sample size calculation. However, the values on the day of screening may not be very reliable because the patients were asked about their mean pain intensity during the last several weeks. For example, one might assume that higher pain values were reported to be able to participate in our trial, or patients might have remembered their pain values worse than they really were.

During baseline phase A, pain values between the treatment groups diverged. However, patients were randomized and did not receive any treatment at this time point, so that this can only be explained by chance. Therefore, statistical testing is not recommended [59]. Other causes such as differences in sex or age are unlikely since both groups had similar demographic characteristics (see **Table 2**)

Patient adherence to prescribed medication is a recurring issue, especially in chronic pain patients [60]. Our patients received CBDV in an oily solution. Patients had to measure 8 ml every day and drink the solution. Even though oral medications result in better compliance [61], an oily solution is complicated for both patients and caregivers. The administration takes more time and effort and some patients reported difficulty swallowing the solution and bad taste. Furthermore, the most common adverse events (diarrhea and dry mouth) might have been due to the sesame oil solution or strawberry flavor in our study. In addition, clinical trials investigating cannabinoids often suffer from poor blinding due to the typical side effects. In our study, no patient reported feeling ´high´ so that we assume that blinding was sufficient.

7 Conclusion

In summary, this study examining the administration of CBDV showed no significant change of neuropathic pain intensity, supplemental pain medication or associated pain characteristics in HIV-patients. No major side effects of CBDV were reported. Therefore, we postulate that an activation of CB receptors is necessary for clinically relevant cannabinoid-mediated analgesia as indicated in the literature [39]. CBDV has not been investigated for neuropathic pain before. For a comprehensive evaluation, however, further trials with larger sample sizes and other types of pain are necessary. At this point in time, CBDV cannot be recommended as a treatment option for HIV-associated neuropathic pain.

8 References

1. GHO | By category | Number of people (all ages) living with HIV - Estimates by WHO region. WHO. [cited 2018 Oct 23]. Available from: http://apps.who.int/gho/data/view.main.22100WHO?lang=en

2. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol.* 2010 Aug;9(8):807–19.

3. Evans SR, Ellis RJ, Chen H, Yeh T, Lee AJ, Schifitto G, Wu K, Bosch RJ, McArthur JC, Simpson DM, Clifford DB. Peripheral neuropathy in HIV: prevalence and risk factors. *AIDS.* 2011 Apr 24;25(7):919–28.

4. Aziz-Donnelly A, Harrison TB. Update of HIV-Associated Sensory Neuropathies. *Curr Treat Options Neurol.* 2017 Oct 1;19(10):36.

5. Stavros K, Simpson DM. Understanding the Etiology and Management of HIV-Associated Peripheral Neuropathy. *Curr HIV/AIDS Rep.* 2014 Jun 27;11(3):195–201.

6. Martin C, Solders G, Sönnerborg A, Hansson P. Antiretroviral therapy may improve sensory function in HIV-infected patients: a pilot study. *Neurology.* 2000 Jun 13;54(11):2120–7.

7. McArthur JC, Brew BJ, Nath A. Neurological complications of HIV infection. *Lancet Neurol.* 2005 Sep 1;4(9):543–55.

8. Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, Gilron I, Haanpää M, Hansson P, Jensen TS, Kamerman PR, Lund K, Moore A, Raja SN, Rice ASC, Rowbotham M, Sena E, Siddall P, Smith BH, Wallace M. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol.* 2015 Feb 1;14(2):162–73.

9. Modesto-Lowe V, Bojka R, Alvarado C. Cannabis for peripheral neuropathy: The good, the bad, and the unknown. *Cleve Clin J Med.* 2018 Dec;85(12):943–9.

10. Russo EB. History of cannabis and its preparations in saga, science, and sobriquet. *Chem Biodivers.* 2007 Aug;4(8):1614–48.

11. Maldonado R, Baños JE, Cabañero D. The endocannabinoid system and neuropathic pain: *PAIN.* 2016 Feb;157:S23–32.

12. Stockings E, Campbell G, Hall WD, Nielsen S, Zagic D, Rahman R, Murnion B, Farrell M, Weier M, Degenhardt L. Cannabis and cannabinoids for the treatment of people with chronic noncancer pain conditions: a systematic review and meta-analysis of controlled and observational studies. *PAIN.* 2018 Oct;159(10):1932.

13. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *Br J Pharmacol.* 2008 Jan;153(2):199–215.

14. Starowicz K, Finn DP. Chapter Thirteen - Cannabinoids and Pain: Sites and Mechanisms of Action. In *Advances in Pharmacology.* Vol. 80 (eds Kendall D, Alexander SPH) 437-475 (Academic Press, Cambridge, 2017)

15. Kerbrat A, Ferré J-C, Fillatre P, Ronzière T, Vannier S, Carsin-Nicol B, Lavoué S, Vérin M, Gauvrit J-Y, Le Tulzo Y, Edan G. Acute Neurologic Disorder from an Inhibitor of Fatty Acid Amide Hydrolase. *N Engl J Med.* 375, 1717–1725 (2016).

16. Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazzarella E, Russo E, Whalley BJ, Di Marzo V, Stephens GJ. 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.* 2014 Nov 19;5(11):1131–41.

17. Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS. Progress report on new antiepileptic drugs: A summary of the Thirteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XIII). *Epilepsia.* 2017;58(2):181–221.

18. Hill A, Mercier M, Hill T, Glyn S, Jones N, Yamasaki Y, Futamura T, Duncan M, Stott C, Stephens G, Williams C, Whalley B. Cannabidivarin is anticonvulsant in mouse and rat. *Br J Pharmacol.* 2012 Dec;167(8):1629–42.

19. Millar SA, Stone NL, Yates AS, O'Sullivan SE. A Systematic Review on the Pharmacokinetics of Cannabidiol in Humans. *Front Pharmacol*. 9, 1365 (2018).

20. Morean ME, de Wit H, King AC, Sofuoglu M, Rueger SY, O'Malley SS. The Drug Effects Questionnaire: Psychometric Support across Three Drug Types. *Psychopharmacology*. 2013 May;227(1):177–92.

21. Woldeamanuel YW, Kamerman PR, Veliotes DGA, Phillips TJ, Asboe D, Boffito M, Rice ASC. Development, Validation, and Field-Testing of an Instrument for Clinical Assessment of HIV-Associated Neuropathy and Neuropathic Pain in Resource-Restricted and Large Population Study Settings. *PLoS One*. 11, e0164994 (2016).

22. Bouhassira D, Attal N, Alchaar H, Boureau F, Brochet B, Bruxelle J, Cunin G, Fermanian J, Ginies P, Grun-overdyking A, Jafari-schluep H, Lantéri-minet M, Laurent B, Mick G, Serrie A, Valade D, Vicaut E. comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (dn4). *PAIN*. 2005 Mar 1;114(1):29–36.

23. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, Chertkow H. The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. *J Am Geriatr Soc*. 2005 Apr 1;53(4):695–9.

24. Abrams DI, Jay CA, Shade SB, Vizoso H, Reda H, Press S, Kelly ME, Rowbotham MC, Petersen KL. Cannabis in painful HIV-associated sensory neuropathy A randomized placebo-controlled trial. *Neurology.* 2007 Feb 13;68(7):515–21.

25. Demant DT, Lund K, Vollert J, Maier C, Segerdahl M, Finnerup NB, Jensen TS, Sindrup SH. The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: a randomised, double-blind, placebo-controlled phenotype-stratified study. *PAIN*. 2014 Nov;155(11):2263–73.

26. Harden RN, Weinland SR, Remble TA, Houle TT, Colio S, Steedman S, Kee WG. Medication Quantification Scale Version III: Update in Medication Classes and Revised Detriment Weights by Survey of American Pain Society Physicians. *J Pain.* 2005 Jun;6(6):364–71.

27. Freynhagen R, Baron R, Gockel U, Tölle TR. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Curr Med Res Opin*. 2006 Oct 1;22(10):1911–20.

28. Tan G, Jensen MP, Thornby JI, Shanti BF. Validation of the brief pain inventory for chronic nonmalignant pain. *J Pain.* 2004 Mar 1;5(2):133–7.

29. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand.* 1983 Jun 1;67(6):361–70.

30. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care.* 1992 Jun;30(6):473–83.

31. Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med.* 2001 Jul 1;2(4):297–307.

32. Rampakakis E, Ste-Marie PA, Sampalis JS, Karellis A, Shir Y, Fitzcharles M-A. Real-life assessment of the validity of patient global impression of change in fibromyalgia. *RMD Open.* 1, e000146 (2015).

33. Ellis RJ, Toperoff W, Vaida F, van den Brande G, Gonzales J, Gouaux B, Bentley H, Atkinson JH. Smoked Medicinal Cannabis for Neuropathic Pain in HIV: A Randomized, Crossover Clinical Trial. Neuropsychopharmacology. 2009 Feb;34(3):672–80.

34. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienne, Austria: R Foundation for Statistical Computing; Available from: https://www.r-project.org/

35. Eibach L, Scheffel S, Cardebring M, Lettau M, Celik MÖ, Morguet A, Roehle R, Stein C. Cannabidivarin for HIV-Associated Neuropathic Pain: A Randomized, Blinded, Controlled Clinical Trial. *Clin Pharmacol Ther* 2020 Aug 8 doi: 10.1002/cpt.2016. Epub ahead of print. PMID: 32770831.

36. Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ, Rubino T, Michalski CW, Marsicano G, Monory K, Mackie K, Marian C, Batkai S, Parolaro D, Fischer MJ, Reeh P, Kunos G, Kress M, Lutz B, Woolf CJ, Kuner R. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci.* 2007 Jul;10(7):870–9.

37. Costa B, Comelli F, Bettoni I, Colleoni M, Giagnoni G. The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB1, TRPV1 and PPARγ receptors and neurotrophic factors. *PAIN.* 2008 Oct;139(3):541.

38. Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright EM. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br J Pharmacol.* 2015 Oct;172(20):4790–805.

39. Lötsch J, Weyer-Menkhoff I, Tegeder I. Current evidence of cannabinoid-based analgesia obtained in preclinical and human experimental settings. *Eur J Pain.* 2018 Mar 1;22(3):471–84.

40. Muller C, Morales P, Reggio PH. Cannabinoid Ligands Targeting TRP Channels. *Front Mol Neurosci.* 11, (2019).

41. Petrocellis LD, Ligresti A, Moriello AS, Allarà M, Bisogno T, Petrosino S, Stott CG, Marzo VD. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol.* 2011 Aug;163(7):1479.

42. GW Research Ltd. Safety and Tolerability of GWP42006 in Children and Young Adults With Autism Spectrum Disorder. clinicaltrials.gov; 2020 Jan [cited 2020 Jul 22]. Report No.: NCT03849456. Available from: https://clinicaltrials.gov/ct2/show/NCT03849456

43. Vase L, Skyt I, Hall KT. Placebo, nocebo, and neuropathic pain. *PAIN.* 2016 Feb;157:S98.

44. Cepeda MS, Berlin JA, Gao CY, Wiegand F, Wada DR. Placebo Response Changes Depending on the Neuropathic Pain Syndrome: Results of a Systematic Review and Meta-Analysis. *Pain Med.* 2012 Apr 1;13(4):575–95.

45. Dekker N, Linszen DH, Haan LD. Reasons for Cannabis Use and Effects of Cannabis Use as Reported by Patients with Psychotic Disorders. *PSP.* 2009;42(6):350–60.

46. Moreira FA, Grieb M, Lutz B. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab* 2009 Feb 1;23(1):133–44.

47. Lee J, Sharma N, Kazi F, Youssef I, Myers A, Marmur JD, Salifu MO, McFarlane SI. Cannabis and Myocardial Infarction: Risk Factors and Pathogenetic Insights. *Scifed J Cardiol.* 2017;1(1).

48. Chuang-Stein C. Safety Analysis in Controlled Clinical Trials: *Drug Inf J.* 1998;(32(1_suppl)):1363S-1372S.

49. Avorn J, Kesselheim AS. Up Is Down — Pharmaceutical Industry Caution vs. Federal Acceleration of Covid-19 Vaccine Approval. *N Engl J Med*. 2020 Sep 15;0(0):null.

50. RKI - HIV/AIDS - HIV/AIDS: Eckdaten und Trends für Deutschland und für die Bundesländer [cited 2020 Aug 1]. Available from: https://www.rki.de/DE/Content/InfAZ/H/HIVAIDS/Eckdaten/Eckdaten.html

51. Redmond WJ, Goffaux P, Potvin S, Marchand S. Analgesic and antihyperalgesic effects of nabilone on experimental heat pain. *Curr Med Res Opin.* 2008 Apr 1;24(4):1017–24.

52. Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: results of a French nationwide survey. *PAIN.* 2011 Dec;152(12):2836–43.

53. Puplampu P, Ganu V, Kenu E, Kudzi W, Adjei P, Grize L, Käser M. Peripheral neuropathy in patients with human immunodeficiency viral infection at a tertiary hospital in Ghana. *J Neurovirol.* 2019;25(4):464–74.

54. Cherry CL, Wadley AL, Kamerman PR. Painful HIV-associated sensory neuropathy. *Pain Manag.* 2012 Nov 1;2(6):543–52.

55. Lucey BP, Clifford DB, Creighton J, Edwards RR, McArthur JC, Haythornthwaite J. Relationship of Depression and Catastrophizing to Pain, Disability, and Medication Adherence in Patients with HIV-Associated Sensory Neuropathy. *AIDS Care.* 2011 Aug;23(8):921–8.

56. Phillips KD, Skelton WD, Hand GA. Effect of Acupuncture Administered in a Group Setting on Pain and Subjective Peripheral Neuropathy in Persons with Human Immunodeficiency Virus Disease. *The Journal of Alternative and Complementary Medicine.* 2004 Jun 1;10(3):449–55.

57. Maharaj SS, Yakasai AM. Does a Rehabilitation Program of Aerobic and Progressive Resisted Exercises Influence HIV-Induced Distal Neuropathic Pain? *Am J Phys Med Rehabil.* 2018 May;97(5):364–369.

58. Fine PG, Rosenfeld MJ. Cannabinoids for Neuropathic Pain. *Curr Pain Headache Rep.* 2014 Oct 1;18(10):451.

59. Consort-Statement > CONSORT 2010 > Baseline Data. [cited 2020 Jul 15]. Available from: http://www.consort-statement.org/checklists/view/32--consort-2010/510-baseline-data

60. Broekmans S, Dobbels F, Milisen K, Morlion B, Vanderschueren S. Medication adherence in patients with chronic non-malignant pain: Is there a problem? *Eur J Pain.* 2009;13(2):115–23.

61. Jin J, Sklar GE, Min Sen Oh V, Chuen Li S. Factors affecting therapeutic compliance: A review from the patient's perspective. *Ther Clin Risk Manag.* 2008 Feb;4(1):269–86.

9 Eidesstattliche Versicherung

„Ich, Luca Nils Eibach, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „The efficacy of Cannabidivarin for HIV-associated neuropathic pain – a randomized, blinded, controlled clinical trial“ „Die Wirksamkeit von Cannabidivarin bei HIV-assoziierten neuropathischen Schmerzen – eine randomisierte, verblindete kontrollierte klinische Studie“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

10 Ausführliche Anteilserklärung an der erfolgten Publikation als Top-Journal im Rahmen der Promotionsverfahren zum Dr. med.

Publikation: Eibach L, Scheffel S, Cardebring M, Lettau M, Celik MÖ, Morguet A, Roehle R, Stein C. Cannabidivarin for HIV-associated neuropathic pain – a randomized, blinded, controlled clinical trial. Clinical Pharmacology & Therapeutics [published online]. [cited 2020 Aug 17];n/a(n/a). Available from: https://ascpt.onlinelibrary.wiley.com/doi/abs/10.1002/cpt.2016

Beitrag im Einzelnen: My participation in this project started during the planning stage of this clinical trial. Dr. Simone Scheffel and Prof. Dr. Christoph Stein had already started to design the study and have written the trial protocol. The initial power analysis for the sample size calculation was carried out by Dr. Alexander Krannich. Therefore, I was involved in the final planning procedures and initiation of this trial. Together with Dr. Simone Scheffel and Madeleine Cardebring I recruited the patients.

The data collection phase of the study was conducted by Madeleine Cardebring, Dr. Marie Lettau, Prof. Dr. Christoph Stein and myself. Since I was not licensed to practice medicine at this time point, all of my activities were supervised by licensed physicians (Prof. Dr. Christoph Stein, Madeleine Cardebring or Dr. Marie Lettau).

Dr. Özgür Celik was responsible for the processing of blood samples for genetic analyses described in the publication. PD Dr. Andreas Morguet assessed the ECGs which were recorded during initial patient screening visits.

The statistical analysis was planned by Dr. Marie Lettau, Robert Röhle, Prof. Dr. Christoph Stein and myself. The statistical analysis plan was written by Robert Röhle and edited by Prof. Dr. Christoph Stein and myself and was conducted by Robert Röhle. Figure 1 in the publication as well as figure 1 in this text were prepared by myself, all other figures were prepared by Robert Röhle. All tables in the publication and in this text were prepared by myself. The whole publication was written by myself, except for the method section ´Inactivation of HIV in blood samples, DNA isolation, and genetic analysis´, which was written by Dr. Özgür Celik. It was then edited by Prof. Dr. Christoph Stein, Robert Röhle and myself.

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Unterschrift, Datum und Stempel des erstbetreuenden Hochschullehrers

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Unterschrift des Doktoranden

11 Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI Selected Categories: **"PHARMACOLOGY and PHARMACY"** Selected Category Scheme: WoS

Gesamtanzahl: 267 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE REVIEWS DRUG DISCOVERY	32,266	57.618	0.054890
2	PHARMACOLOGICAL REVIEWS	12,653	18.886	0.011950
3	ADVANCED DRUG DELIVERY REVIEWS	36,350	15.519	0.037430
4	Annual Review of Pharmacology and Toxicology	7,820	12.103	0.009900
5	DRUG RESISTANCE UPDATES	2,856	11.708	0.003590
6	TRENDS IN PHARMACOLOGICAL SCIENCES	12,317	11.523	0.018180
7	MEDICINAL RESEARCH REVIEWS	4,560	9.791	0.004920
8	PHARMACOLOGY & THERAPEUTICS	15,434	9.396	0.022540
9	JOURNAL OF CONTROLLED RELEASE	47,630	7.901	0.052240
10	ALIMENTARY PHARMACOLOGY & THERAPEUTICS	20,998	7.731	0.033430
11	NEUROPSYCHOPHARMACOLOGY	25,672	7.160	0.039090
12	DRUG DISCOVERY TODAY	14,244	6.880	0.021560
13	European Heart Journal-Cardiovascular Pharmacotherapy	442	6.723	0.001430
14	BRITISH JOURNAL OF PHARMACOLOGY	34,006	6.583	0.033440
15	CLINICAL PHARMACOLOGY & THERAPEUTICS	16,170	6.336	0.016950
16	Reviews of Physiology Biochemistry and Pharmacology	738	6.214	0.000540
17	Acta Pharmaceutica Sinica B	2,418	5.808	0.004930

12 Chosen publication

Cannabidivarin for HIV-Associated Neuropathic Pain: A Randomized, Blinded, Controlled Clinical Trial

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HIV remains a major burden to the health care system and neuropathic pain is the most common neurological complication of HIV infection. Because current treatment strategies often lack satisfying pain relief, cannabinoids (CBs) are discussed as a new option. We investigated cannabidivarin (CBDV) as treatment for HIV-associated neuropathic pain. We conducted a randomized, double-blind, placebo-controlled crossover study. Patients underwent two successive treatment phases (4 weeks each) and were treated with CBDV (400 mg/day) or placebo in a randomized order. A 3-week washout phase was designed to eliminate potential carry-over effects. Patients were followed up for 3 weeks after the end of the second treatment phase. The primary end point was pain intensity on an 11-point numeric rating scale, recorded in a diary. Secondary end points were additional pain medication, pain characteristics, and quality of life. We included 32 patients. The mean pain intensity under CBDV was 0.62 points higher compared with placebo (P = 0.16, 95% confidence interval −0.27 to 1.51). CBDV did not influence the amount of additional pain medication, pain characteristics, or quality of life. The incidence of adverse events was similar during both treatments. No suspected unexpected adverse reactions occurred during either treatment. CBDV was safe but failed to reduce neuropathic pain in patients with HIV. This may be explained by a lack of CB receptor activation, as indicated by preclinical experiments. Although a larger patient number might be desirable, we would not expect a change in the conclusions because the present differences are far from statistical significance. Therefore, we would currently not consider CBDV as a clinically meaningful treatment option for neuropathic pain.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Pain relief in patients with HIV-associated neuropathic pain is often unsatisfying but cannabinoids (CBs) have shown promising results in preclinical studies.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Can pain relief be achieved by the novel phytocannabinoid cannabidivarin (CBDV) in patients with HIV-associated neuropathic pain?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ CBDV was safe but failed to improve neuropathic pain or quality of life in patients with HIV.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Despite encouraging preclinical data, CBDV is not a promising substance for treatment of patients with HIV-associated neuropathic pain. We presume that clinical pain relief is unlikely to be achieved without activation of CB receptors.

Approximately 7–8% of the general population have neuropathic pain, defined as "pain that arises as a direct consequence of lesions or diseases affecting the somatosensory system."[1,2] Chronic neuropathic pain impairs quality of life and negatively affects the patients' social relationships.[3] Among various diseases that can underlie neuropathic pain, HIV infection belongs to the most prevalent.[4] Despite the development of highly effective antiretroviral therapy, HIV remains a major burden to the health system.[5]

HIV-associated neuropathic pain usually occurs together with distal sensory neuropathy with symptoms of burning or dysesthesia in combination with numbness in stocking-like or glove-like distribution,[6] and may be caused by the inflammatory effects of HIV-infected macrophages and other neurodegenerative mechanisms.[6,7]

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Furthermore, antiretroviral drugs, mainly dideoxynucleoside reverse transcriptase inhibitors, can cause mitochondrial and nerve damage[7] so that they are no longer recommended.[8] Despite novel, more effective, and less neurotoxic antiretroviral drugs, the prevalence of neuropathic pain in HIV-infected patients is still high and causal treatment is not available.[6] Although treatment of chronic neuropathic pain should be based on both pharmacological and interdisciplinary nonpharmacological approaches (e.g., behavioral, physical, and/or occupational therapy),[4] pharmacological therapy often predominates. Antidepressants, anticonvulsants, and opioid analgesics are medications of choice.[9] However, they often lack efficacy[4] and are limited by side effects, such as respiratory depression, addiction, and sedative effects,[10] resulting in extensive additional costs and reduced quality of life.[3,11,12]

Endocannabinoids (e.g., 2-arachidonylglycerol and anandamide) influence the transmission of pain signals by acting on cannabinoid (CB)-receptors 1 and 2.[13] Some exogenous cannabinoids (CBs) have shown promising results in the treatment of neuropathic pain but they were limited by complicated dosing of smoked cannabis and side effects like nausea or drowsiness.[14–16] Therefore, improved CB and opioid analgesics are being developed.[9,13,17,18]

In this study, we investigated cannabidivarin (CBDV) a novel phytocannabinoid derived from the *Cannabis sativa* L. plant, in patients with HIV-associated neuropathic pain. Using a double-blind crossover trial design, we assessed pain, side effects, and quality of life, and sought to correlate treatment responses to the patients' genotype.

METHODS

Study design

Data were collected from January 1, 2017, to January 8, 2019. We conducted a randomized, placebo-controlled, double-blind crossover phase II trial in a single-center outpatient setting. All patients received both treatments (CBDV and placebo) in two successive phases. The order of treatments (CBDV-placebo (C-P) or placebo-CBDV (P-C)) was allocated by chance (randomized). Each patient was monitored for 13 weeks. After the screening phase, baseline values on pain scales, questionnaires, and medications were recorded during a 1-week phase (**Figure 1**). This was followed by 4-week treatment phase A with either placebo or CBDV. A subsequent 3-week washout phase was included to eliminate potential carry-over effects. The duration of the washout phase was based on data showing an accumulation of cannabinoids (CBs) in fatty tissue resulting in a half-life of about 5 days after long-term oral administration.[19] Thereafter, another 1-week baseline phase ensued, followed by treatment phase B. Patients were then followed up for another 3 weeks. Throughout the study, the patients documented data in diaries (see also study protocol in **Supplementary Materials**).

Study participants

Participants were recruited through personal contacts to physicians and patient-advocacy groups in the greater Berlin area, as well as by advertisement in the Berlin public transportation system. Before inclusion, subjects were screened for age (18–65 years), vital signs, and pain intensity (≥ 4 on an 11-point numeric rating scale (NRS)). The diagnosis of HIV-associated sensory neuropathy was confirmed by a clinician (C.S., M.C., or M.L.) based on patient history, the Douleur Neuropathique 4 interview (DN4i), and the Clinical HIV-associated Neuropathy Tool.[20,21] Exclusion criteria were pregnancy and lactation, major psychiatric conditions, severe diseases of the central nervous system, hepatic, renal, or cardiovascular diseases, or use of conventional cannabinoids (CBs), examined by blood test. Electrocardiograms were recorded on the day of screening and analyzed for abnormalities by an experienced cardiologist (A.M.). Infection with hepatitis virus B or C and AIDS-defining diseases were debarred by consulting HIV specialists. The use of concomitant analgesics (including antidepressants and anticonvulsants) as needed was permitted throughout the study. Standard laboratory values (full blood count, liver function tests, electrolytes, glucose, urea, cholesterol, creatinine, creatinine kinase, protein, and international normalized ratio) were recorded on the day of screening and during the trial.

Outcome measurements

The primary outcome was pain intensity measured thrice a day (8:30 AM, 1:00 PM, and 7:00 PM) by an 11-point NRS (0 = no pain to 10 = worst pain imaginable), as documented in the patient diary. For each day, the arithmetic mean of the three NRS scores was determined. According to several previous studies on neuropathic pain,[22,23] a decrease of mean NRS values by at least 20% between the last day of baseline measurement and the last day of treatment was defined as a clinically relevant effect (responder). The number of responders and nonresponders to each treatment was determined. Secondary end points were pain characteristics, quality of life, and sleep, measured by questionnaires. We used painDETECT,[24] the Brief Pain Inventory,[25] and the DN4i[21] for evaluation of pain intensity and pain characteristics, the Hospital Anxiety and Depression Scale[26] to evaluate anxiety and depression, and the 36-Item Short Form Survey,[27] the Patient Global Impression of Change,[28] and the Insomnia Severity Index for quality of life and sleep,[29] respectively. All questionnaires were applied on the last day of each baseline phase and on the last day of each treatment phase, except Patient Global Impression of Change, which was only used at the end of each treatment phase. Concomitant medication and side effects were recorded in the patient diary. For the analysis of concomitant pain medication, we used the Medication

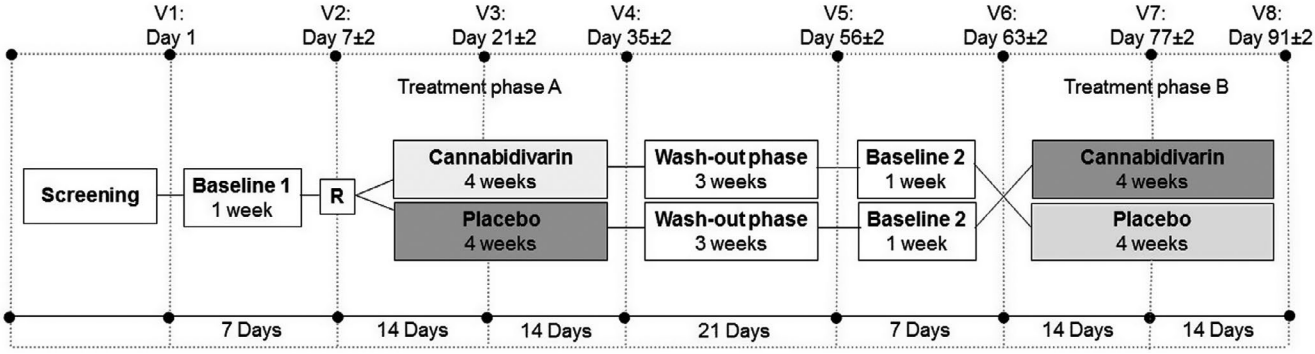

Figure 1 Study design. R, randomization; V, visit.

Quantification Scale (MQS) in its third version, which assigns a score (on an unlimited scale) based on the detrimental effects and dose of each pain medication.[30] For analysis of side effects, patients were asked to document any adverse or unusual events. These were discussed with a study physician at each visit. For standardized documentation, we used paper-based tables and classified the events with the Common Terminology Criteria for Adverse Events, version 4.03.

Randomization, allocation concealment, and blinding

Randomization to the sequence of treatments occurred in blocks of four by use of paper-based, computer-generated random lists, which were stored in a locked cabinet. Included patients were pseudonymized by generating a serial number (ID). Allocation to the treatment sequence was documented and kept in sealed envelopes. All patients and staff involved in patient contacts and assessment of outcomes were blinded until the end of the study.

Monitoring

Two independent monitors conducted unblinded monitoring of patient safety and adherence to good clinical practice principles throughout the trial.

Investigational Medicinal Products

The active agent and placebo, both dissolved in sesame oil, were identically appearing and tasting solutions. The Investigational Medical Products was packaged in amber-glass bottles by GW Pharmaceuticals. All bottles were subject-specific and marked with the patient ID. The bottles with active agent contained 50 mg CBDV/mL. Patients were instructed to use 8 mL of the solution orally every morning at 9 AM, corresponding to 400 mg CBDV in the verum treatment phase (for detailed information see **Table S1**). The dose was chosen based on preclinical and clinical phase I studies, showing that daily doses between 200 and 800 mg were well-tolerated.[31]

Inactivation of HIV in blood samples, DNA isolation, and genetic analysis

Blood samples were obtained during the last visit from 28 patients who gave consent for genetic analysis. Five mL of peripheral venous blood was mixed with 15 mL of red cell lysis solution (Epicentre R) and incubated at room temperature for 10 minutes. After centrifugation, supernatant was discarded, and the pellet was dissolved in 7.5 mL tissue and cell lysis solution (Epicentre R). The solution was kept at 65°C for 1 hour for inactivation of HIV and cell lysis. Samples were then stored and transported at −20°C until genotyping by deCODE Genetics (Reykjavik, Iceland). Whole genome sequencing was performed by the Infinium Global Screening array (GSA24, Illumina).

Statistics

Sample size was calculated by nQuery Advisor 7.0 based on the primary end point (NRS scale) and the crossover study design. According to previous literature, a pain reduction by 20% upon verum compared with placebo and a common SD for the period differences of 2.5 seemed to be achievable and would have been clinically meaningful.[22,23,32] We calculated that 21 patients per sequence group were sufficient to show this effect (e.g., a reduction of 20% from 6 points to 4.8 points) with a power of 85% and a two-sided type I error of 0.05 using a paired *t*-test for 2 × 2 crossover designs. To account for an estimated 15% dropouts, we aimed at a total of 50 patients. Because some guidelines define higher pain reductions as clinically relevant,[33] we also provide 30% and 50% pain reduction analyses to allow our data to be used in data syntheses.

Statistical analysis was based on the intention-to-treat principle (i.e., every patient who started treatment and had at least one post-baseline measurement of the primary end point was included in the full set for the efficacy analysis). Continuous variables are shown as mean, SD, and range, whereas categorical parameters are given as absolute and relative frequency. For the continuous end points, first, the difference between sequence-specific baseline and the value after treatment was calculated. Then, for each individual the difference between the two treatment effects (C-P) was determined. A paired *t*-test taking period effects into account was used for comparing the two treatments. In case of non-normality of data distribution, a nonparametric version was applied instead. The 95% confidence intervals (CIs) were calculated for the treatment effects. Further, for the primary end point, a random subject intercept mixed model was calculated. This model used the change of NRS values from phase baseline to post-treatment as dependent variable, and treatment, phase, and NRS phase baseline value as independent variables. All *P* values resulting from the analyses have to be considered as nonconfirmatory using a cutoff of 0.05. All analyses were done using R (version 3.5.0)[34] (see also statistical analysis plan in **Supplementary Materials**).

Study approval

Written informed consent was obtained from all participants prior to inclusion in the study. The trial protocol, patient information, and informed consent sheets were approved by the ethics committee of the state regulatory authority Berlin (Landesamt für Gesundheit und Soziales; 15/0255 EK 13) and the German Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte; 61-3910-4040377). The CONSORT guidelines and checklist, good clinical practice principles, and the Declaration of Helsinki were strictly followed. The study was registered at EudraCT (https://www.clinicaltrialsregister.eu/) under number 2014-005344-17.

RESULTS

Patient population

From January 2015 to September 2018, a total of 194 patients were contacted by email or phone, of which 55 were screened in the study center. Screening was terminated as planned at the end of financial support. Thirty-four patients gave informed consent and were assigned a patient ID. The data of two patients could not be used for final efficacy analysis due to missing data or screening failure but were included in the safety population (for more information see **Figure S1**). Characteristics of the remaining 32 patients included in the efficacy analysis are shown in **Table 1**. All patients met the inclusion criterion of a positive DN4i (≥ 3) and Clinical HIV-associated Neuropathy Tool. Of the remaining 32 patients, 4 dropped out during the study but were not excluded from analysis. Patients were randomized to receive CBDV in treatment phase A followed by placebo in treatment phase B (C-P), or placebo in phase A followed by CBDV in phase B (P-C).

Primary end point

Overall, mean pain intensity (NRS) at the end of CBDV treatment was 0.62 points higher compared with placebo; this difference was not significant (P = 0.16, 95% CI −0.27 to 1.51) (**Figure 2**, **Figure 3**, **Table S2**). The mixed model provided very similar results (difference 0.63, 95% CI −0.05 to 1.32). The differences between mean NRS at the end of the treatment and baseline were not statistically significant for any substance or treatment phase (**Figure 3**). The mean NRS value at the end

Table 1 Data on day of initial screening

		Treatment sequence CBDV-Placebo	Treatment sequence Placebo-CBDV	Total
Male, *n*		16	15	31
Female, *n*		0	1	1
Age, years	Mean (SD)	52.31 (8.06)	48.31 (9.62)	50.31 (8.96)
	range	36–65	31–65	31–65
NRS score (0–10)	Mean (SD)	6.12 (1.15)	6.44 (1.59)	6.28 (1.37)
	range	4–8	4–9	4–9
DN4i (0–7)	Mean (SD)	5.19 (1.17)	5 (0.89)	5.09 (1.03)
	range	3–7	4–6	3–7
Duration of pain, years	Mean (SD)	16.47 (7.91)	9.94 (8.77)	13.1 (8.87)
	range	2–30	1–27	1–30
Duration of HIV infection, years	Mean (SD)	24.88 (9.17)	17.81 (10.81)	21.4 (10.2)
	range	3–33	2–32	2–33
On cART, *n*		16	15	31

cART, combined antiretroviral therapy; CBDV, cannabidivarin; DN4i, Douleur Neuropathique 4 interview; NRS, Numeric Rating Scale.

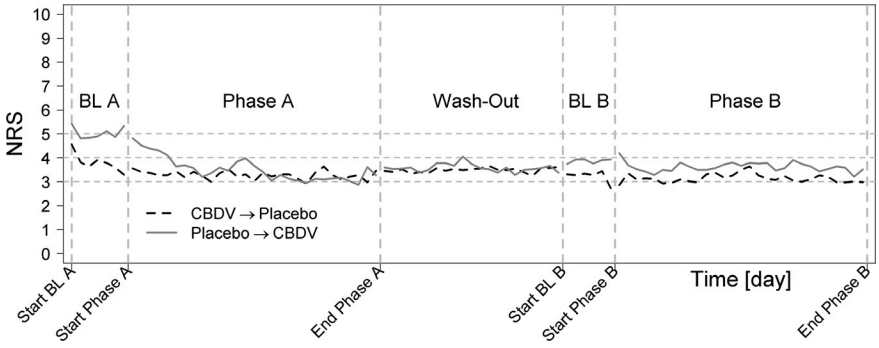

Figure 2 Pain intensity over time. Descriptive presentation of pain intensities per day (means) by treatment sequence. Cannabidivarin (CBDV)-placebo (black, broken line); placebo-CBDV (grey, continuous line). BL, baseline; NRS, Numeric Rating Scale.

of follow-up (3 weeks after end of treatment phase B) was 2.74 (SD = 1.47) in the C-P group and 3.67 (SD: 2.62) in the P-C group. During CBDV treatment, 9 patients experienced a mean pain reduction of at least 20% and were therefore classified as CBDV responders. By the same criteria, 19 patients were classified as placebo responders. Based on a 30% pain reduction, 6 patients were CBDV-responders and 13 patients responded to placebo. A 50% pain reduction was experienced by 1 patient under CBDV and by 9 patients under placebo.

Secondary end points

No statistical differences between CBDV and placebo were detectable by any of the questionnaires analyzing pain characteristics, sleep quality, subjective impression of change, or quality of life (**Table 2**, **Figure 4**). No significant changes in specific parameters in the painDETECT questionnaire were detectable. Overall, the intake of additional pain medication, measured by the MQS, was not significantly different between CBDV and placebo (median treatment effect of CBDV compared with placebo = 0, $P = 0.52$, 95% CI −0.05 to 2.85; nonparametric rank sum test; **Figure 5**).

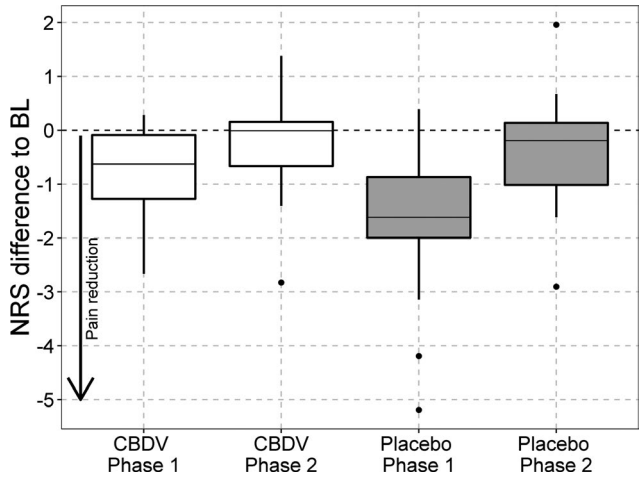

Figure 3 Pain intensity difference by treatment and phase. Differences (medians) between numeric rating scale (NRS) values on the last day of cannabidivarin (CBDV; white) and placebo (grey) phases and baseline (BL) values, respectively. Negative values indicate pain reduction; bars indicate minimum and maximum values; dots indicate values outside of 1.5* interquartile range (paired *t*-test; $n = 32$).

Table 2 Effects of CBDV vs. placebo assessed by questionnaires

Questionnaire (score range)	Effect CBDV vs. placebo
painDETECT (0–38)	−0.84 (*P* = 0.53, 95% CI −3.59 to 1.91)
DN4i (0–7)	−0.50 (*P* = 0.18, 95% CI −1 to 0.50)
BPI (pain intensity) (0–10)	+0.23 (*P* = 0.76, 95% CI −0.63 to 1.25)
BPI (influence on daily living) (0–10)	−0.35 (*P* = 0.22, 95% CI −1.36 to 0.43)
HADS (anxiety) (0–21)	−0.60 (*P* = 0.51, 95% CI −2.44 to 1.24)
HADS (depression) (0–21)	0 (*P* = 0.91, 95% CI −1.50 to 1.50)
ISI (0–28)	−1.50 (*P* = 0.24, 95% CI −5.50 to 1)
PGIC (0–7)	−0.50 (*P* = 0.26, 95% CI −1.50 to 0.50)

painDETECT and DN4i: higher values indicate presence of neuropathic pain; PGIC: higher values indicate a subjective improvement; all others: lower values indicate lower impairment. Paired *t*-tests, see Methods.
BPI, Brief Pain Inventory; CBDV, cannabidivarin; CI, confidence interval; DN4i, Douleur Neuropathique 4 interview; HADS, Hospital Anxiety and Depression Scale; ISI, Insomnia Severity Index; PGIC, Patient Global Impression of Change.

After CBDV treatment, the differences in MQS values between baseline and end of treatment were + 1.13 (SD = 7.13) in the C-P group and −0.16 (SD = 0.61) in the P-C group. After placebo treatment, these differences were + 0.11 (SD = 3.79) and −1.87 (SD = 5.26) in the C-P group and P-C group, respectively.

Adverse events

Thirty-one patients (91.2%) experienced at least one adverse event (AE) during CBDV treatment; and 27 patients (79.4%) had at least one AE during placebo. During each treatment (CBDV or placebo), nine patients (26.5%) experienced an AE that was considered to be related to study medication (**Table S3**). One serious AE (acute myocardial infarction) was recorded during CBDV treatment but was judged as not related to study medication. This patient (male, 62 years) had the following cardiovascular risk factors: history of arterial hypertension, transient ischemic attack, pulmonary embolism, and factor-V-Leiden mutation. The most common AEs were diarrhea and dry mouth (3 cases during each treatment; **Table S3**). The incidence of AEs was similar in both treatment phases. All AEs were of low or moderate severity; one patient withdrew study participation due to an AE (cough) during CBDV treatment. This was considered related to treatment. No clinically relevant or medication-related changes of laboratory values were noted.

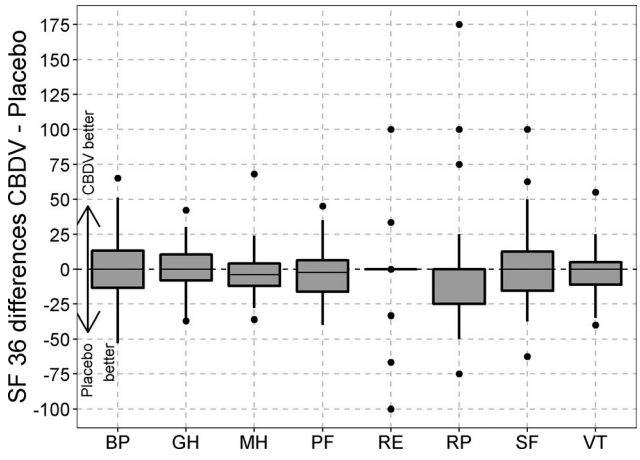

Figure 4 Treatment effects on quality of life. Differences (medians) between cannabidivarin (CBDV) and placebo effects as measured by SF-36. Bars indicate minimum and maximum values; dots indicate values outside of 1.5* interquartile range (paired *t*-test; *n* = 32). BP, bodily pain; GH, general health; MH, mental health; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, 36-Item Short Form Survey; VT, vitality.

Genetic analysis

Samples from 28 patients who gave consent to genetic analysis were genotyped using the Infinium Global Screening array (GSA24; Illumina), and whole genome sequencing was performed on this subset of patients by deCODE Genetics (Reykjavik, Iceland). The small sample size did not allow a meaningful genomewide association analysis of response. However, these data may have utility in future meta-analysis efforts, and can be queried for the role of individual markers identified in other studies.

DISCUSSION

CBDV failed to reduce neuropathic pain intensity in patients with HIV. Additionally, we could not observe any statistically or clinically significant effects on use of supplementary pain medication, specific pain characteristics, or quality of life. CBDV and placebo produced similar rates of AEs, which were of mild to moderate severity.

According to data on CB receptor knock-out mice and pharmacological studies, the mechanisms underlying analgesic effects of CBs are thought to be based on the activation of CB1 and/or CB2 receptors, leading to an inhibition of pain signal transmission and/or anti-inflammatory effects.[13,35,36] This may either be achieved by exogenous CBs or by inhibiting enzymes degrading endocannabinoids (fatty acid amide hydrolase and/or monoacylglycerol lipase). Costa *et al.* also showed that antinociception can be produced by a CB re-uptake inhibitor in rats.[37] In addition, effects of phytocannabinoids not primarily activating CB receptors have been described.[36]

CBDV is mainly known for its anticonvulsant effects.[38] Limited preclinical data indicated the occurrence of antinociceptive effects without binding to CB receptors.[31] Antinociceptive effects of CBs not activating CB receptors were observed in animal studies[39] but not in humans so far. Different mechanisms of action were hypothesized, such as inhibition of diacylglycerol lipase-α,[40] another enzyme influencing endocannabinoid. Some groups observed an activation of transient receptor potentials[40] and postulated that this activation could lead to desensitization of sensory neurons.[41]

To evaluate clinical effects, we assessed both pain intensity and the amount of supplemental pain medication. A dose reduction

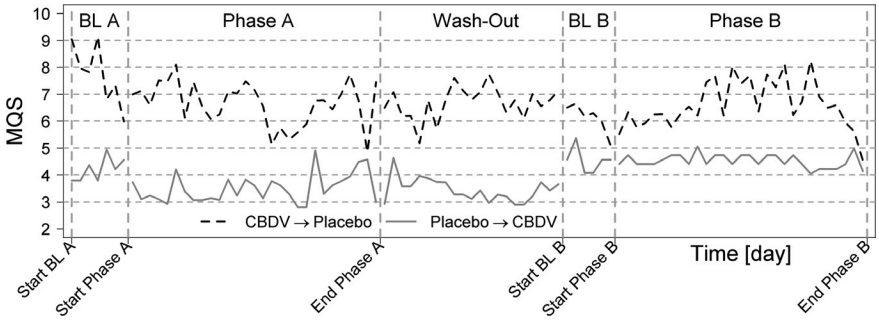

Figure 5 Medication quantification scale (MQS) values over time. Descriptive presentation of MQS values per day (means) by treatment sequence. Cannabidivarin (CBDV)-placebo (black, broken line); placebo-CBDV (grey, continuous line); baseline (BL); *n* = 32.

of additional pain medication can minimize detrimental side effects and can therefore be useful. CBDV, however, did not significantly change pain intensity or the use of additional pain medication as compared with placebo. Potentially promising effects may be assumed in **Figure 5** but should be considered visually misleading because real differences on the unlimited MQS were small and not statistically significant. We also examined whether CBDV can influence pain characteristics, such as burning sensation, numbness, or heat hyperalgesia. Due to the possible involvement of TRPV1,[40] a receptor that is responsible for heat sensation,[42] one might assume that CBDV can alleviate burning sensations in patients with neuropathic pain. In the painDETECT questionnaire, however, CBDV did not influence any specific pain characteristics. To our knowledge, this is the first study investigating the influence of CBDV on such parameters.

Overall, CBDV was ineffective in our trial. This is in line with recent extensive meta-analyses that did not detect clinically relevant analgesic effects of CBs in humans with chronic noncancer pain.[14,43] The analysis by Stockings *et al*. included all CBs, all study designs, considered all outcomes recommended by the IMMPACT group, and it assessed the clinical relevance of these findings.[14] In our study, a notable (but statistically nonsignificant) pain reduction was observed in patients receiving placebo during the first phase (P-C) and a difference between the groups was visible at baseline A (**Figure 2**). However, on the day of screening, the NRS scores were quite similar (**Table 1**). Because patients were randomized and did not receive any test substances before baseline A, this NRS difference was due to chance. Another NRS difference is visible in group P-C between baselines A and B (**Table S2**). To account for baseline variations in the statistical analysis, we included sequence-specific baseline values into a linear mixed model. It is conceivable that patients who were not treated sufficiently for pain before entering our study benefitted psychologically due to the enhanced attention in the setting of a clinical trial. Similar findings were reported in several previous studies and meta-analyses on neuropathic pain in patients with HIV.[44–46] This underlines the importance of a multidisciplinary approach, including psychotherapy, to treat chronic pain.

Chronic pain negatively influences many other facets of the patient's life according to the biopsychosocial model of pain.[3,12,47] CBs are known to influence emotional processes. For example, the CB receptor agonist Δ9-tetrahydrocannabinol may reduce the unpleasantness but not the intensity of pain.[48] We did not ask our patients about previous use of CBs. However, CBDV failed to improve any of these features in the current study. Again, this is in agreement with previous meta-analyses that did not find significant impacts of CBs on physical or emotional functioning in patients with chronic noncancer pain.[14]

CBDV does not bind to CB-receptors[31,38] and therefore should not show typical CB receptor-mediated psychotropic side effects, such as euphoria, reduced anxiety, or feeling "high,"[49] consistent with our findings. Because the most common side effects (diarrhea and dry mouth) did not differ between CBDV and placebo, we do not consider these AEs related to CBDV treatment. However, they could be associated with the sesame oil solution. We only observed side effects of low to moderate severity and only one patient withdrew due to such effects. For a more detailed analysis, a larger number of patients may be advantageous.

One serious AE (myocardial infarction) occurred during treatment with CBDV but was not considered related to CBDV. There are data supporting increased cardiovascular risk due to cannabinoids, but these data suggest a CB-receptor mediated mechanism.[50] CBDV and its major metabolites lack appreciable affinity and functional activity at the CB1-receptor[38] and neither clinical nor preclinical data point out any increase in cardiovascular risk. Therefore, the available information suggests that an association between CBDV and myocardial ischemia is unlikely.

We were able to obtain blood samples from most patients, but this sample size was not sufficient for a meaningful genomewide association study regarding treatment responses. However, these data are available upon request and may have utility in future meta-analysis efforts.

The time frame for patient inclusion was limited by the end of financial support. Due to additional, unexpected recruitment difficulties (many patients lost interest because we could not offer a satisfying remuneration), we could only enroll 16 patients per treatment sequence group instead of a planned sample size of 21. Although a larger patient number might have been desirable, we would not expect a marked change in the conclusions because the present results are far from statistical significance. Even the lower border of the 95% CI of the mean differences in NRS score does not promise any clinical relevance.

To conclude, this study showed that CBDV did not elicit more adverse side effects than placebo but failed to alleviate neuropathic

pain or associated parameters in patients with HIV. We presume that activation of CB receptors is necessary for significant analgesia. This was the first study investigating CBDV for neuropathic pain and further research with larger numbers of patients and possibly other types of neuropathic pain is desirable. However, because our results did not reveal any significant differences, we would not consider CBDV a clinically meaningful treatment option for HIV-associated neuropathic pain.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

L.E., R.R., and C.S. wrote the manuscript. S.S., L.E., and C.S. designed the research. L.E., S.S., M.C., M.L., Ö.C., A.M., and C.S. performed the research. R.R., L.E., and C.S. analyzed the data.

1. Bouhassira, D., Lantéri-Minet, M., Attal, N., Laurent, B. & Touboul, C. Prevalence of chronic pain with neuropathic characteristics in the general population. *Pain* **136**, 380–387 (2008).
2. Treede, R.-D. *et al*. Neuropathic pain redefinition and a grading system for clinical and research purposes. *Neurology* **70**, 1630–1635 (2008).
3. Dueñas, M., Ojeda, B., Salazar, A., Mico, J.A. & Failde, I. A review of chronic pain impact on patients, their social environment and the health care system. *J. Pain Res.* **9**, 457–467 (2016).
4. Baron, R., Binder, A. & Wasner, G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol.* **9**, 807–819 (2010).
5. World Health Organization (WHO). WHO | Data and statistics. WHO <http://www.who.int/hiv/data/en/> (2020). Accessed July 1, 2020.
6. Aziz-Donnelly, A. & Harrison, T.B. Update of HIV-associated sensory neuropathies. *Curr. Treat. Options Neurol.* **19**, 36 (2017).
7. Stavros, K. & Simpson, D.M. Understanding the etiology and management of HIV-associated peripheral neuropathy. *Curr. HIV/AIDS Rep.* **11**, 195–201 (2014).
8. World Health Organization (WHO). *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach.* (World Health Organization, Geneva, Switzerland, 2016).
9. Finnerup, N.B. *et al*. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol.* **14**, 162–173 (2015).
10. Carter, G.T. *et al*. Side effects of commonly prescribed analgesic medications. *Phys. Med. Rehabil. Clin. N. Am.* **25**, 457–470 (2014).
11. Mayer, S. *et al*. The societal costs of chronic pain and its determinants: the case of Austria. *PLoS One* **14**, e0213889 (2019).
12. Simons, L.E., Kaczynski, K.J., Conroy, C. & Logan, D.E. Fear of pain in the context of intensive pain rehabilitation among children and adolescents with neuropathic pain: associations with treatment response. *J. Pain* **13**, 1151–1161 (2012).
13. Maldonado, R., Baños, J.E. & Cabañero, D. The endocannabinoid system and neuropathic pain. *Pain* **157**, 23–32 (2016).
14. Stockings, E. *et al*. Cannabis and cannabinoids for the treatment of people with chronic noncancer pain conditions: a systematic review and meta-analysis of controlled and observational studies. *Pain* **159**, 1932–1954 (2018).
15. Pertwee, R.G. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *Br. J. Pharmacol.* **153**, 199–215 (2008).
16. Starowicz, K. & Finn, D.P. Chapter thirteen - cannabinoids and pain: sites and mechanisms of action. In *Advances in Pharmacology* Vol. **80** (eds, Kendall, D., & Alexander, S.P.H.) 437–475. (Academic Press, Cambridge, 2017).
17. Woodhams, S.G., Chapman, V., Finn, D.P., Hohmann, A.G. & Neugebauer, V. The cannabinoid system and pain. *Neuropharmacology* **124**, 105–120 (2017).
18. Spahn, V. *et al*. A nontoxic pain killer designed by modeling of pathological receptor conformations. *Science* **355**, 966–969 (2017).
19. Millar, S.A., Stone, N.L., Yates, A.S. & O'Sullivan, S.E. A systematic review on the pharmacokinetics of cannabidiol in humans. *Front. Pharmacol.* **9**, 1365 (2018).
20. Woldeamanuel, Y.W. *et al*. Development, validation, and field-testing of an instrument for clinical assessment of HIV-associated neuropathy and neuropathic pain in resource-restricted and large population study settings. *PLoS One* **11**, e0164994 (2016).
21. Bouhassira, D. *et al*. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* **114**, 29–36 (2005).
22. Abrams, D.I. *et al*. Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo-controlled trial. *Neurology* **68**, 515–521 (2007).
23. Demant, D.T. *et al*. The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: a randomised, double-blind, placebo-controlled phenotype-stratified study. *Pain* **155**, 2263–2273 (2014).
24. Freynhagen, R., Baron, R., Gockel, U. & Tölle, T.R. painDETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Curr. Med. Res. Opin.* **22**, 1911–1920 (2006).
25. Tan, G., Jensen, M.P., Thornby, J.I. & Shanti, B.F. Validation of the brief pain inventory for chronic nonmalignant pain. *J. Pain* **5**, 133–137 (2004).
26. Zigmond, A.S. & Snaith, R.P. The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* **67**, 361–370 (1983).
27. Ware, J.E. & Sherbourne, C.D. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med. Care* **30**, 473–483 (1992).
28. Rampakakis, E. *et al*. Real-life assessment of the validity of patient global impression of change in fibromyalgia. *RMD Open* **1**, e000146 (2015).

29. Bastien, C.H., Vallières, A. & Morin, C.M. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med.* **2**, 297–307 (2001).
30. Harden, R.N. *et al*. Medication quantification scale version III: update in medication classes and revised detriment weights by survey of American Pain Society Physicians. *J. Pain* **6**, 364–371 (2005).
31. Bialer, M. *et al*. Progress report on new antiepileptic drugs: a summary of the Thirteenth Eilat conference on new antiepileptic drugs and devices (EILAT XIII). *Epilepsia* **58**, 181–221 (2017).
32. Ellis, R.J. *et al*. Smoked medicinal cannabis for neuropathic pain in HIV: a randomized, crossover clinical trial. *Neuropsychopharmacology* **34**, 672–680 (2009).
33. Dworkin, R.H. *et al*. Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: IMMPACT recommendations. *J. Pain* **9**, 105–121 (2008).
34. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2020) <https://www.r-project.org/>. Accessed July 1, 2020.
35. Agarwal, N. *et al*. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat. Neurosci.* **10**, 870–879 (2007).
36. Fine, P.G. & Rosenfeld, M.J. Cannabinoids for neuropathic pain. *Curr. Pain Headache Rep.* **18**, 451 (2014).
37. Costa, B. *et al*. AM404, an inhibitor of anandamide uptake, prevents pain behaviour and modulates cytokine and apoptotic pathways in a rat model of neuropathic pain. *Br. J. Pharmacol.* **148**, 1022–1032 (2006).
38. Hill, A. *et al*. Cannabidivarin is anticonvulsant in mouse and rat. *Br. J. Pharmacol.* **167**, 1629–1642 (2012).
39. Costa, B., Comelli, F., Bettoni, I., Colleoni, M. & Giagnoni, G. The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB1, TRPV1 and PPARγ receptors and neurotrophic factors. *Pain* **139**, 541–550 (2008).
40. Petrocellis, L.D. *et al*. Effects of cannabinoids and cannabinoid-enriched cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br. J. Pharmacol.* **163**, 1479–1494 (2011).
41. Iannotti, F.A. *et al*. 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**, 1131–1141 (2014).
42. Rosenbaum, T. & Simon, S.A.TRPV1 receptors and signal transduction. In *TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascade*s (eds, Liedtke, W.B.&Heller, S.). (CRC Press/Taylor & Francis, Boca Raton (Florida), 2007).
43. Mücke, M., Phillips, T., Radbruch, L., Petzke, F. & Häuser, W. Cannabis-based medicines for chronic neuropathic pain in adults. *Cochrane Database Syst. Rev.* **3**, CD012182 (2018).
44. Cepeda, M.S., Berlin, J.A., Gao, C.Y., Wiegand, F. & Wada, D.R. Placebo response changes depending on the neuropathic pain syndrome: results of a systematic review and meta-analysis. *Pain Med.* **13**, 575–595 (2012).
45. Hahn, K. *et al*. A placebo-controlled trial of gabapentin for painful HIV-associated sensory neuropathies. *J. Neurol.* **251**, 1260–1266 (2004).
46. Kieburtz, K. *et al*. A randomized trial of amitriptyline and mexiletine for painful neuropathy in HIV infection. AIDS Clinical Trial Group 242 Protocol Team. *Neurology* **51**, 1682–1688 (1998).
47. Engel, G.L. The need for a new medical model: a challenge for biomedicine. *Science* **196**, 129–136 (1977).
48. Lötsch, J., Weyer-Menkhoff, I. & Tegeder, I. Current evidence of cannabinoid-based analgesia obtained in preclinical and human experimental settings. *Eur. J. Pain* **22**, 471–484 (2018).
49. Dekker, N., Linszen, D.H. & Haan, L.D. Reasons for cannabis use and effects of cannabis use as reported by patients with psychotic disorders. *Psychopathology* **42**, 350–360 (2009).
50. Lee, J. *et al*. Cannabis and myocardial infarction: risk factors and pathogenetic insights. *Scifed. J. Cardiol.* **1**, 1000004 (2017).

13 Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

14 List of publications

Eibach L, Scheffel S, Cardebring M, Lettau M, Celik MÖ, Morguet A, Roehle R, Stein C. Cannabidivarin for HIV-Associated Neuropathic Pain: A Randomized, Blinded, Controlled Clinical Trial. **Clinical Pharmacology & Therapeutics.** 2020 Aug 8 doi: 10.1002/cpt.2016. Epub ahead of print. PMID: 32770831.
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