UNIVERSITÀ DEGLI STUDI DI GENOVA

SCUOLA DI SCIENZE MEDICHE E FARMACEUTICHE

CORSO DI LAUREA IN MEDICINA E CHIRURGIA

Combined treatment with cannabidiol and fenfluramine in Dravet Syndrome: results from an international clinical trial

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Anno accademico 2021-2022

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ABSTRACT

Background: New therapeutic options have been approved as second-line treatments for drug-resistant epilepsies such as Dravet Syndrome (DS): cannabidiol (CBD) and fenfluramine (FFA), promising repurposed drugs. Evidence for their possible combination in the clinical practice is lacking. We evaluated CBD+FFA efficacy and tolerability in patients with DS.

Methods: Children and adults with DS were recruited through international collaboration and received add-on treatment with CBD and FFA up to 25 and 0.7 mg/Kg/day, respectively. Seizure endpoints were the percentage of patients with ≥50% (responders) or >25% <50% (partial responders) reduction in seizures, and the mean number of seizure-free days as compared to baseline. Clinical data, concomitant anti-seizure medications (ASMs), and adverse events (AEs) were recorded throughout the study.

Results: 12 patients (5 females) with a mean age of 12 years (range 1.1-31) were enrolled. The mean follow-up (FU) duration was 14.3 months (range 3-25). Eight out of twelve (67%) patients were responders, while 2/12 (17%) were partial responders. At baseline, the mean longest seizure-free interval was 15 days compared to 54.25 days at FU. Under combined treatment, 58% of the patients could reduce and 50% could stop one or more concomitant ASMs. Reported AEs were weight loss (33.3%), tremor (25%), irritability and facial tics (8.3%). All AEs were mild and did not lead to treatment withdrawal.

Conclusions: A significant reduction in seizures and longer seizure-free intervals were observed in DS patients under CBD+FFA treatment. Moreover, the combined treatment was well tolerated with only mild AEs reported.

INTRODUCTION

1.1 DRUG-RESISTANT EPILEPSY

Epilepsy is a disorder of the brain characterized by a persisting predisposition to generate epileptic seizures, and it leads to neurobiological, cognitive, psychological, and social consequences. [1] Epilepsy affects more than 65 million people in the world and represents the third leading contributor to the global burden of disease for the neurological field. [2,3] The definition of epilepsy requires the occurrence of at least one epileptic seizure [1] but a revised practical definition affirms that, in individuals who have other factors that are associated with a high probability of a persistently lowered seizure threshold and therefore a high recurrence risk, epilepsy could also be diagnosed after one unprovoked seizure. The mentioned risk should be equivalent to the recurrence risk of a third seizure in those people with two unprovoked seizures, approximately at least 60%. [4]

When an individual is seizure-free and older than the applicable age for an age-dependent epilepsy syndrome or, in alternative, when it is more than 10 years since the last seizure with no anti-seizure medication (ASM) for the past 5 years, epilepsy is considered resolved. [1]

The first-line treatment for epilepsy is represented by ASMs: more than 20 drugs have been approved by the US FDA and the European Medicines Agency (EMA). Despite this development of new drugs, almost 30% of people with epilepsy still have seizures, even if ASMs with different mechanisms of action are combined. [5–7]

The International League Against Epilepsy (ILAE) has defined drug-resistant epilepsy (DRE) as a failure of adequate trials of two well tolerated, appropriately chosen, and used ASMs schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom. [8]

DRE is more common in patients who have more than five seizures and a longer duration of epilepsy before the start of the treatment, an abnormal MRI, epileptiform discharges at the EEG, focal seizures, neurocognitive deficits or symptomatology, [9] and it is associated with an increased risk of injury and a higher standardized mortality ratio, greater medication burden and adverse events (AEs), increased psychiatric and neurocognitive comorbidities, socioeconomic disadvantage, and reduced quality of life (QoL). [10]

Notably, a 30-year longitudinal cohort study demonstrated that, despite the increased use of many new ASMs with different mechanisms of action over the past 2 decades, long-term outcomes in adolescent and adult patients who have been newly diagnosed with epilepsies have not improved. The probability of achieving seizure freedom decreases after every unsuccessful ASMs regimen. [11]

To be able to predict poor response to ASMs treatment and hopefully offer new treatment approaches, is important to study the basis of DRE. [12–14] The mechanisms of drug- resistance appear to be variable and multifactorial, depending to the underlying cause of DRE and to the drug's site of action. [5,15] Age also seems to affect treatment outcome: prognosis resulted better in seniors and adolescents than in the rest of the population. [13]

A recent review [16] reassumed the existing hypotheses aiming to explain pharmacoresistance: "*the significance of altered targets for anti-epileptic drugs, overexpression of brain, and/or peripheral efflux transporters, initial considerable seizure frequency, existence of aberrant brain networks and*

genetic polymorphisms. In the light of the above hypotheses, ASMs may not fully exert their effects, and their brain concentrations may not reach the therapeutic level. Also, a suppression of endogenous defense system against seizure activity, altered ASMs pharmacokinetics by genetic polymorphisms, increased inflammatory or mTOR signaling and production of free radicals, blood–brain barrier dysfunction, and disturbed renin–angiotensin system may contribute to pharmacoresistance as well".

1.2 DRAVET SYNDROME (DS)

A typical example of DRE is represented by Dravet syndrome (DS), previously known as severe myoclonic epilepsy of infancy (SMEI). With an incidence of 1:15500 [17,18], DS considered a rare disease. It was first described in France in 1978 [19] and then included as an epileptic syndrome in the ILAE classification in 1989. [20] DS is an epileptic encephalopathy, according to the definition of "*a condition in which the epileptiform abnormalities themselves are believed to contribute to the progressive disturbance in cerebral function*". [21]

Age at onset is usually between 5 and 8 months, but in 2006 an onset after 1 year has been reported.[22] The first seizures are typically clonic, generalized, or unilateral. Seizures can be triggered by fever but they can occur also due to vaccinations and hot baths: they are longer than a simple febrile seizure and could evolve into status epilepticus. [23–29] The incidence of heat-induced epilepsy decreases when the patient grows up, turning into the refractory epilepsy.

Patients with DS have multiple seizure types over time: generalized tonic-clonic seizures, generalized clonic seizures or alternating unilateral clonic seizures, myoclonic seizures, atypical absences and obtundation status, focal seizures, with or without secondary generalization, rarely, tonic seizures. [26–31] In some patients with DS, photosensitive epilepsy can also be observed. [32]

In addition to the epileptic seizures, the QoL of patients with DS is seriously affected by other comorbidities that can be combined: ataxia, premature death, language and motor development delay, cognitive impairment, sleep disorders, and sudden unexpected death in epilepsy (SUDEP). [33–38] Lack of attention, hyperactivity and recalcitrant behaviour appear to be the major factors responsible for the learning disabilities. Affected children are not interested in playing with educational toys and participating in the usual activities of their age group, they are restless and do not listen to adults, even if they are able to complete puzzles and to watch cartoons repetitively. [26,29,30,39]

An extensive survey of caregivers of patients with DS [40] demonstrated that the prevalence of autism was 42%, of ADHD was 24%, and of behavioral difficulties not related to autism or ADHD was 51%; 13% of all patients older than 5 years of age were reported as not speaking at all.

There is a variability in the long-term outcomes: some patients develop severe motor and cognitive disability, whereas others have mild cognitive impairment and can participate in social activities in a sheltered environment. [41]

Dravet distinguished three stages of DS: "*The febrile or diagnostic stage in the first year; the worsening stage between 1 and 5 years, with frequent seizures and statuses, behavioral deterioration, and neurologic signs; and the stabilization stage after 5 years when convulsive seizures decrease and occur mainly in sleep, myoclonic and absence seizures can disappear, focal seizures persist or decrease; mental development and behaviour tend to improve but cognitive impairment persists, although of variable degree*." [28]

In 2001, a study demonstrated that DS could have a genetic etiology and most of the cases are caused by de novo mutations in the *SCN1A* gene [42] , resulting in substantially decreased levels of the corresponding functional protein, the α-1 subunit of the neuronal, voltage-gated sodium channel $Na_V1.1$. [28] In the central nervous system, $Na_V1.1$ is a fundamental sodium channel highly expressed

in many GABAergic inhibitory neurons: the altered production of this protein leads to hyperexcitability of the neuronal network. [43]

To date, more than 1,800 mutations have been identified in *SCN1A*. [44,45] Familial mutations occur in 5–10% of cases and are usually missense in nature: in these cases, other family members with the *SCN1A* mutation have mild phenotypes. The rate of *SCN1A* mutations in that cohort of patients suggests that other factors may be involved in DS pathogenesis. [46–48] [46–48]

Mutations of *SCN9A* might be modifiers for the DS phenotype as suggested in a report of a single large family in which the mutation in a highly conserved amino acid residue of the *SCN9A* sodium channel alpha subunit is associated with a wide clinical spectrum of seizure phenotypes including simple focal seizures, self-limited afebrile seizures, and temporal lobe epilepsy. [49]

1.3 THERAPEUTIC ALGORITHM FOR DS

The aim of DS treatment is to reduce seizure frequency with limited toxicity since the high seizure frequency is associated with a lower QoL and could be correlated with cognitive dysfunction. Another therapeutic goal is to minimize comorbidities, including intellectual disability, behavioral and psychiatric problems, seizure-related injury and SUDEP. [26,39,50–53]

Since DS constitutes a major burden for patients and their caregivers/families, a multidisciplinary, individualized approach to care is required, which addresses each patient's medical, educational, psychological, and social needs throughout the course of their life, together with patient/caregiver values and preferences which are especially relevant when a disease cannot be definitively cured. [54,55]

Home rescue medication is required for all patients with DS, and they include rectal diazepam for very young children, and nasal or buccal midazolam for older children, adolescents, and adults.

A detailed seizure rescue plan should be also provided and followed if the home rescue medication is not successful in stopping the seizure. [56,57]

It is well recognized that certain ASMs, in particular the sodium channel-blocking agents, could trigger seizures in patients with DS: this pharmacologic intolerance is probably due to the fact that most patients have a nonsense or missense mutation in the *SCN1A* gene, a component of the Nav1.1 sodium channel.[58] Carbamazepine (CBZ)'s role in exacerbating seizures in DS has been particularly well described [59]; for this reason, CBZ and its analogs (oxcarbazepine, and eslicarbazepine) as well as phenytoin are contraindicated. Phenytoin has also been reported to result in dose-dependent and reversible choreoathetosis. [60] Furthermore, a recent study [61] demonstrated that a longer duration of contraindicated medication use was significantly associated with a worse cognitive outcome at time of inclusion, and with lower interpolated intelligence quotient/developmental quotient scores after the first 5 years of disease in DS patients.

Studies noted that lamotrigine (LTG) [62] and rufinamide (RUF) [63] exacerbate seizures in patients with DS and so they do not seem to be suitable options for this condition.

Vigabatrin (VGB) was reported to exacerbate seizures in patients with DS and to worsen cognitive outcomes and thus is not recommended. [61,64] It has been suggested that phenobarbital (PB) should also be avoided in DS as it can result in the exacerbation of seizures.[64] Use of PB in status epilepticus has also been reported to predispose to anoxic-like lesions and poor psychomotor outcome. [65]

The two most recommended initial therapies include valproic acid (VPA) and clobazam (CLB), according to a North American Consensus Panel comprising epileptologists with nationally recognized expertise in DS and parents of children with DS. [66]

Most of the patients with DS progress to second-line agents since these current first-line therapies have only modest efficacy on reducing seizure frequency and preventing status epilepticus. Second-line agents include stiripentol (STP), topiramate (TPM) and, from two years onwards, cannabidiol and fenfluramine.[6757]

Third-line treatment includes clonazepam (CLZ), levetiracetam (LEV), zonisamide (ZNS), ethosuximide (ESM), and PB. [67]

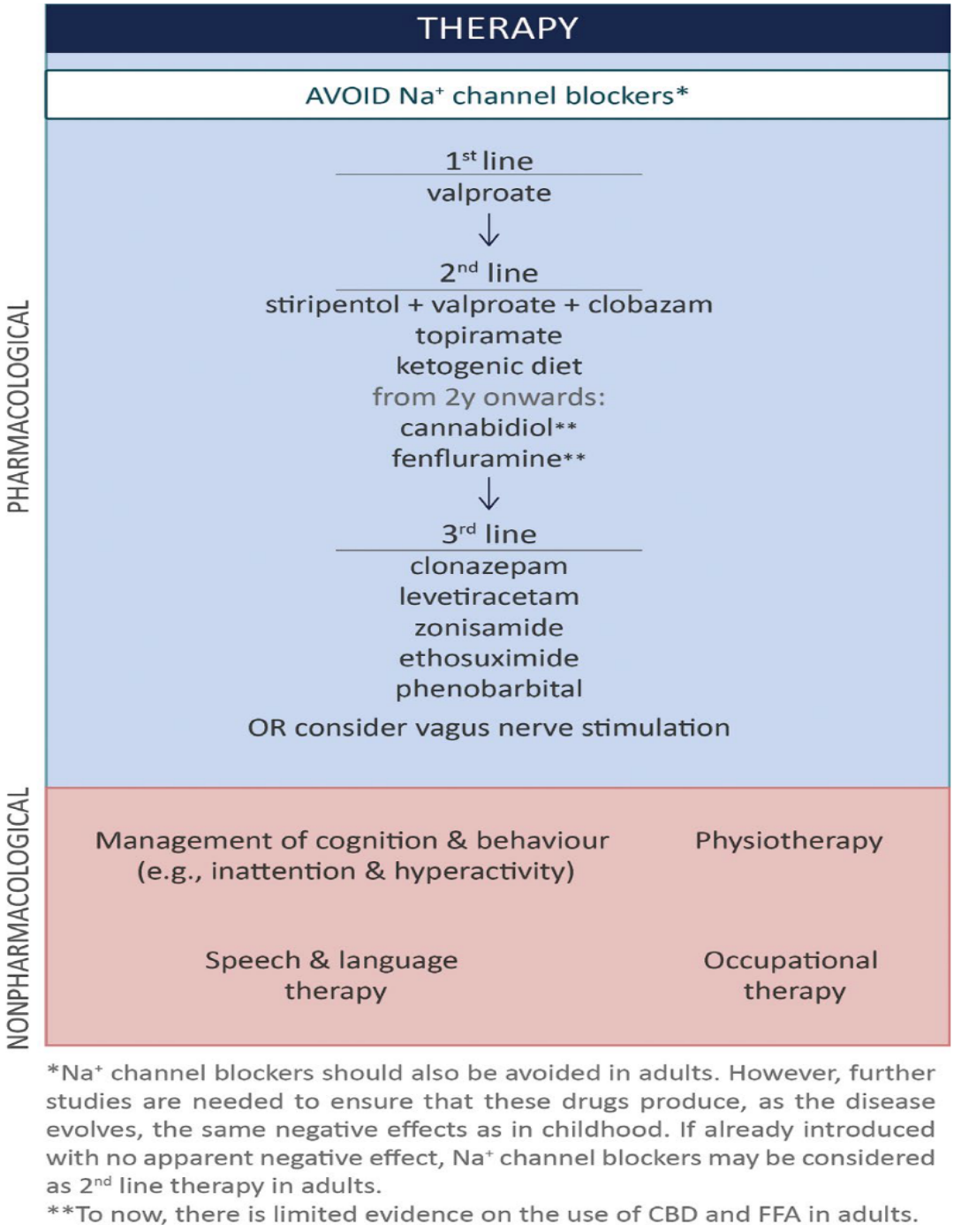

Figure 1. Guidance on Dravet syndrome from infant to adult care: Road map for treatment planning in Europe. (Cardenal-Muñoz, E. et al. Epilepsia Open. 2022)

VPA represents the first-line treatment for patients with DS. It is a broad-spectrum ASM effective across a range of focal and generalized seizure types including tonic-clonic, myoclonic, tonic and absence seizures. [68] This wide-ranging activity is likely a reflection of its complex mechanism of action modulating various targets implicated in the development of seizures, including the inhibitory neurotransmitter GABA, voltage-gated sodium channels and histone deacetylase.[69]

VPA has proved effective in generalized and unclassifiable epilepsies, and it is generally well tolerated. [70,71]. According to FDA "Depakene (VPA): prescribing information" and "Epilim (sodium valproate): summary of product characteristics" [72], the most common AEs include gastrointestinal disturbances (nausea, vomiting, abdominal pain, and diarrhea), headache, somnolence, tremor, and asthenia, although these are generally mild to moderate in severity and reduce over time. However, VPA has been rarely associated with serious and fatal hepatotoxicity in children < 2 years of age (especially those taking multiple ASMs) and people with mitochondrial disorders caused by mutations of the mitochondrial *DNA Polymerase γ* (*POLG*) gene, so VPA is contraindicated in patients with hepatic diseases. Before initiating VPA, liver function tests are required and patients should be monitored; a physical examination and a medical history should also be conducted as liver function tests are not always abnormal. [72] VPA is also associated with coagulation disorders (including thrombocytopenia, decreases in von Willebrand's factor, and platelet dysfunction) and hyperammonaemia. Therefore, laboratory tests should include blood count and ammonia. [68]

VPA is available in a variety of formulations including tablets and granules (modified release), oral solution, as well as a solution for injection or infusion. The starting dose of VPA is typically between 10 and 15 mg/Kg/day, divided into two or three doses, followed by gradual increases to target doses in the range of 25 to a maximum of 60 mg/Kg/day according to clinical response and tolerability.

In cases where acceptable clinical response has not been attained, plasma concentrations of VPA should be checked to see if they are in the generally accepted therapeutic range of 50–100 mcg/mL. Recently, a study demonstrated that chronic intraventricular administration of VPA is safe and effective in subjects with medically refractory epilepsy over many months. The procedure for implanting the infusion system is safe and well-tolerated, drug side effects were minimal. [73]

VPA alone is generally insufficient to control seizures in DS and persisting seizures leads to further add-on treatments. [68] Concomitant administration with TPM has been associated with encephalopathy and/or hyperammonaemia; the signs and symptoms should be carefully monitored especially in patients with pre-existing encephalopathy. [72]

CLB is widely used for the treatment of developmental and epileptic encephalopathies (DEEs) including DS, owing to its favorable tolerability, safety, and broad-spectrum anti-seizure activity. In the EU, CLB has a broad indication as an adjunctive treatment for epilepsy [74], while in the USA it is generally used off-label. [75] CLB is a 1,5-benzodiazepine with a greater selectivity for α2-GABA-A than α1-GABA-A subunits, which results in fewer sedative effects and a lower propensity for efficacy tolerance (i.e., losing efficacy over time). [76]

CLB has a good safety profile, the most common AEs of CLB include sedation or irritability, excessive salivation, dizziness, ataxia, and hypotonia. [57] Rare cases of Steven–Johnson syndrome and toxic epidermal necrolysis have also been reported. [77,78]

CLB is administered orally, the initial dose is 0.2 mg/Kg/day divided into two daily administrations, followed by gradual increases to reach target doses of 0.3–1 mg/Kg/day, up to a maximum of 2 mg/Kg/day.

Addition of CLB to VPA may increase the plasma concentrations of VPA, and clinical monitoring is recommended with a view to a possible dose adjustment of CLB. [75,76]

Non-pharmacological therapies include ketogenic diet (KD), vagal nerve stimulation (VNS), and other chirurgical approaches.

The efficacy of KD in DS has been evaluated in several studies [79–86] which were for the majority retrospective and used a classical diet: approximately two-thirds of children were responders, with a greater than 50% reduction in seizures. A significant minority of cases achieved a more significant reduction with rare cases becoming seizure-free. There is a consensus [66] that the KD should be considered as a second-line option for DS, utilizing the classical diet in patients aged younger than 2 years and a modified Atkins diet for teenagers and adults. [57]

Many studies have assessed the efficacy of VNS in DS [86–93] and a report from the North American Consensus Panel [66] indicated that it could be considered in DS but only after first- and second-line treatments (ideally including the KD) had been tried. The same approach has been suggested for callosotomy, which could be considered in a patient with DS with intractable drop seizures, but its benefits are unclear, and the potential risk/benefit ratio must be carefully discussed with the family. They also affirmed that there is no role for resective surgery, including temporal lobectomy. [57]

2.CANNABIDIOL (CBD)

2.1 CBD FORMULATIONS, INDICATIONS, AND DOSAGE

Cannabis is a generic term to refer to the products of the plant *Cannabis sativa L* which contains over 100 compounds, collectively called phytocannabinoids. Among these products, the two best characterized cannabinoids are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).[94] Compared with THC, CBD shows a more consistent anti-seizure activity profile in animal models without THC-like adverse psychoactive effects. [95,96]

The plant-derived pharmaceutical formulation of purified CBD oral solution (Epidiolex®) was approved in June 2018 by the US Food and Drug Administration as treatment and in July 2019 by the EMA as adjunctive therapy in conjunction with CLB for seizures associated with DS or Lennox-Gastaut syndrome (LGS) for patients aged 2 years and older. [97]

A "start slow" and "increase on a case-by-case basis" strategy is recommended. A starting dose of 5 mg/Kg /day, divided in two doses seems to be adequate. This dose should be increased to 10 mg/Kg/day after two weeks of treatment. Thereafter, the individual's response requires an observation time which strictly depends on baseline seizure frequency before the administration of CBD. If the drug is well tolerated but not sufficiently effective, the dose should be slowly increased in increments of 5 mg/Kg/day, if it is tolerated, up to a maximum of 20-25 mg/Kg/day. [98]

Oral administration is recommended. When necessary, CBD can be enterally administered via feeding tubes, such as nasogastric or gastrostomy tubes. Food may affect Epidiolex® levels. Consistent dosing

of Epidiolex® with respect to meals is recommended to reduce variability in CBD plasma exposure.[97]

2.2 EFFICACY OF CBD TREATMENT IN DRUG RESISTANT EPILEPSY

The anti-seizure effects of cannabis have been described, since the last century, in small studies of patients with refractory epilepsies.[99] A famous report detailed the treatment course of a 5-year-old girl with DS who experienced up to 50 generalized tonic-clonic seizures per month: CBD-rich cannabis treatment led to a > 90% reduction in seizures. This raised the interest in treating patients with epilepsy with CBD.[100,101] The first prospective data were collected in an observational interventional trial that included 214 children and adolescents with DRE of different etiologies: they all experienced frequent seizures (median about 60 seizures monthly). CBD was tapered up until intolerance or a maximum of 25 mg/Kg/day was achieved. The mean CBD dose was 22.9 mg/Kg (safety group) and 22.7 mg/Kg (efficacy group), respectively. The median monthly seizure change was − 34.6% for all seizures, − 55% for focal seizures, and − 54.3% for atonic seizures. In contrast, the efficacy for tonic-clonic seizures was worse (− 16%). Responder rates were 37% in all seizures, 56% in atonic seizures, 40% in tonic seizures, and 34% in tonic-clonic seizures, supporting the idea of CBD having different efficacies in different seizure types. The median reduction and responder rates for motor seizures were higher in the DS patient subgroup (49.8%). In the safety group, 79% of the patients reported AEs, and 12% reported drug-related serious AEs. AEs led to discontinuation in 3% of the patients. The most common AEs were somnolence (25%), decreased appetite (19%), diarrhea (19%), and fatigue (13%). Six patients experienced thrombocytopenia and 11 had elevated liver enzymes; all of those were

taking concomitant VPA. This study suggested a reduction in seizure frequency with CBD and an adequate safety profile.[102]

The first randomized, double-blind, placebo-controlled trial of CBD in DS (GWCARE1b) was carried out in 120 children and adolescents using 20 mg/Kg/day of CBD. Efficacy was assessed by comparing a 14-week treatment period with a baseline period. The median reduction in convulsive seizures was 38.9%. Responder rates were 42.6% for convulsive seizures. AEs occurred in the CBD group (93%) as well as in the placebo group (75%); most of the AEs were moderate or mild. Serious AEs were more common in the CBD group (16%). Common AEs were somnolence (36%) diarrhea (31%), decreased appetite (28%), and fatigue (20%) [103].

A second double-blind, placebo-controlled, randomized trial investigated 10 and 20 mg/Kg/day of CBD (GWPCARE2) in 199 children and adolescents using a similar protocol. Median convulsive seizure reduction was 48.7% in the CBD 10-mg/Kg/day group; 45.7% in the 20-mg/Kg/day group; and 26.9% in the placebo group. Responder rates were 43.9%, 49.3%, and 26.2%, respectively. AEs were common in all groups (87.5%, 89.9%, and 89.2%), 92% of the AEs were judged to be mild or moderate. The most common AEs were decreased appetite, diarrhea, somnolence, pyrexia, and fatigue. A higher incidence of AEs, such as somnolence, rash, and pneumonia, was found when co-medicating with CLB. Liver enzyme elevation (more than three times) was found in 12% of the CBD-treated patients, all of them in co-medication with VPA.[104]

Up until June 2019, 681 patients were included in long-term, follow-up studies (GWPCARE5). For the DS group, 264 patients with a mean dose of 21 mg/Kg/day and a median treatment duration of 274 days, a sustained convulsive seizure reduction (between 37.5 and 44.3%) was achieved. Again, AEs were common (93.8%) but mild (36.7%) or moderate (39%) and led to the withdrawal of 6.4%

of the patients. Diarrhea (34.5%), pyrexia (27.3%), decreased appetite (25.4%), and somnolence (24.6%) were the most common AEs.[105]

Longer follow-up data (up to 96 weeks) supported the long-term treatment option with a median monthly major seizure reduction of 50% and a reduction of 44% for all seizures for a group of 152 patients with DS/LGS. For 455 patients with different types of DRE, similar results were obtained.[106]

In 2021, a retrospective review study investigated the efficacy of CBD for a treatment period up to 60 months: 54 subjects with DRE were enrolled by the Massachusetts General Hospital's in the open-label EAP for CBD. The results indicated that CBD maintains its efficacy for controlling seizures from year 1 to the most recent study visit. The percentage of seizure responders remained similar at these time points (41.7%–42.6%), and the seizure response rate was also maintained. Although CBD use did not lead to an overall decrease in concomitant ASMs, most subjects reduced the dose of at least one concomitant ASM compared to baseline. CBD was generally well tolerated, with drowsiness and diarrhea as the primary adverse reactions. [107]

2.3 CBD PHARMACODINAMICS

CBD appears to have multiple mechanisms of action including altering neuronal excitability, antioxidant properties, and anti-inflammatory effects.[108] CBD anti-seizure activity has been documented in a wide range of experimental models.[109,110] The molecular mechanisms underlying these effects could be explained by the complex interactions of cannabinoids with a large number of receptors and biological systems, many of which have implications for effects on neuronal excitability.[111] A recent review suggested that three mechanisms are likely to be particularly relevant to the concentrations of CBD at which anti-seizure effects were demonstrated: antagonism of

G protein-coupled receptor 55 (GPR55), desensitization of transient receptor potential vanilloid type 1 (TRPV1) channels, and potentiation of adenosine-mediated signaling through inhibition of the equilibrative nucleoside transporter 1 (ENT-1).[112]

GPR55 activation triggers a sequence of events resulting in intracellular Ca^{2+} release from intracellular stores, and consequent modulation of neurotransmitter release and neuronal excitability.[113,114] CBD acts as a GPR55 antagonist and it has been found to reduce the frequency of spontaneous seizures and the severity and duration of thermally induce seizures, in a validated genetic mouse model of DS. CBD also attenuated autistic-like social interaction deficits in this model. These effects were associated with restoration of inhibitory neurotransmission in the hippocampal dentate gyrus and were mimicked and occluded by an antagonist of GPR55.[115]

CBD may modulate intracellular Ca^{2+}concentration also through an effect on TRPV1 channels which are widely expressed in the central nervous system (CNS)[116] , where they regulate neuronal activity through activation of intracellular Ca^{2+} influx.[117,118] There is clear evidence that TRPV1 antagonism can protect against seizures in experimental models.[119–122] Although CBD acts as a TRPV1 agonist, it also causes rapid desensitization of the channel [123] which, in turn, is considered to contribute to its anti-seizure activity.[124,125] Seizure protection afforded by CBD was found to be significantly attenuated, but not abolished, in TRPV1 knock-out mice compared with wild-type mice, suggesting that TRPV1 inhibition contributes to CBD's anticonvulsant effects.[126]

CBD is also a relatively potent inhibitor of the ENT1, which mediates the reuptake of adenosine.[127] Several pharmacological actions of CBD are thought to be mediated by inhibition of adenosine reuptake, and consequent augmentation of adenosine effects in various organs and systems.[128]

Elevation of extracellular adenosine concentration by CBD occurs also in the brain [129] where adenosine is considered to act as an endogenous anticonvulsant and seizure terminator, through stimulation of A1 and possibly other centrally-expressed adenosine receptors. [130,131] These findings support the hypothesis that adenosine-mediated effects can also contribute to CBD's anticonvulsant activity.[132]

CBD has been shown to act as a positive allosteric modulator at $GABA_A$ receptors. [133–135] This effect was recently confirmed in a study of brain tissue samples obtained post-mortem from patients with DS. [136] A GABA-mediated mechanism could be particularly relevant in these patients because the sodium channel mutation responsible for DS leads to impaired firing of GABAergic interneurons. [137] Interestingly, studies in animal models of DS indicate that CBD can potentiate pharmacodynamically the anti-seizure effect of CLB [133] this pharmacodynamic interaction is additive to the pharmacokinetic interaction whereby CBD increases several-fold the plasma levels of *N*-desmethylclobazam (nor-clobazam), the active primary metabolite of CLB.[138]

2.4 CBD PHARMACOKINETICS, DRUG INTERACTIONS

CBD has a time to maximum plasma concentration (Tmax) of 2.5 to 5 hours at steady state (Css). Coadministration of CBD with a high-fat/high-calorie meal increased Cmax by 5-fold, area under the curve (AUC) by 4-fold, and reduced the total variability, compared with the fasted state in healthy volunteers. Protein binding of the CBD and its metabolites was >94% in vitro.

The half-life of CBD in plasma is 56 to 61 hours after twice-daily dosing for 7 days. CBD is metabolized in the liver (primarily) and the gut by CYP2C19 and CYP3A4 enzymes, and UGT1A7, UGT1A9, and UGT2B7 isoforms.

After repeat dosing, the active metabolite of CBD, 7-OH-CBD, has a 38% lower AUC than the parent drug. The 7-OH-CBD metabolite is converted to 7-COOH-CBD, which has an approximately 40-fold higher AUC than the parent drug. Based on preclinical models of seizure, the 7-OH-CBD metabolite is active; however, the 7-COOH-CBD metabolite is not active. CBD is excreted in feces, with minor renal clearance. [97]

A bidirectional pharmacokinetic interaction exists between CBD and CLB: CBD can inhibit the catalytic activity of CYP2C19 and elevate by 2- to 6-fold the plasma levels of N-desmethylclobazam (N-CLB), a biologically active metabolite of CLB.[139] In parallel, CLB leads to a 47% increase in the exposure to 7-OH-CBD, a CBD active metabolite, likely via inhibition of glucuronidation. Nonetheless, CBD is a safe and effective treatment for refractory epilepsy in patients receiving CLB treatment. [140] Increased systemic levels of N-CLB and 7-OH-CBD may lead to enhanced pharmacological effects and increased AEs, mainly somnolence or sedation. [141,142] CBD-CLB interaction may be less marked or absent in presence of STP and should be considered if the latter is modified in the treatment schedule. [143]

STP and CBD interact bidirectionally with a clinical relevance which is still unknown. STP slightly decreased the concentration of 7-OH-CBD (−29%) and 7-COOH-CBD (−13%), whereas CBD led to a small increase in STP exposure due to a 17% increase in C_{max} and 30% increase in AUC. [139,144]

CBD does not seem to affect systemic exposure to VPA and *vice versa* [139]; however, a 17% reduction in VPA's AUC has been reported while it was not linked to clinical relevance.[144] The concomitant administration of these drugs is associated with the risk of reversible elevations in transaminase levels and, more recently, to thrombocytopenia.[145] In addition to the hepatotoxic profile of CBD and VPA alone, there could be an additive toxic effect when the ASMs are combined, due to the fact that the chemical structure of the 7-COOH-CBD derivatives reveals similarities with the VPA metabolite associated with hepatotoxic properties.[143,146]

Linear increases in serum levels of TPM and RUF with increasing CBD dose were seen in pediatric and adult patients. A significant increase in serum levels of ZNS with increasing CBD dose was seen in adults and not in pediatric patients, and eslicarbazepine increase has been studied only in adults.[147] Adjunctive treatment with CBD resulted in increased brivaracetam (BRV) levels by 95% to 280% in a case series of five patients: one possible mechanism contributing at least partially to increasing BRV level is the inhibition of CYP2C19 by CBD. Only two patients reported mild AEs, leading to a reduction of BRV in one patient.[148]

No significant changes in drug levels of LEV, CLZ, VGB and pregabalin have been observed with CBD dose titration.[147]

2.5 ADVERSE EVENTS

The most common AEs observed in both randomized, placebo-controlled, and open-label studies include somnolence, decreased appetite, and gastrointestinal symptoms. An increase in serum

transaminases resulted also associated with CBD administration. Less commonly reported AEs include upper respiratory tract infection, vomiting, fatigue, and pyrexia. [149,150]

Somnolence is the most frequent AE observed during CBD treatment and it is dose related. In pivotal trials, it was reported by around 35%, 25%, and 10% of the patients treated with CBD at 20 mg/Kg/day, 10 mg/Kg/day and placebo, respectively. [149] The incidence of somnolence is higher in patients concomitantly taking CLB: when CBD is added to therapeutic regimen encompassing CLB, slower titration may be also advised and dose adjustment of CLB may be required. Therapeutic drug monitoring (TDM) might be recommended before CBD administration and after any dose increase since it is clinically difficult to distinguish benzodiazepine toxicity from side effects due to CBD. [151]

Decreased appetite, diarrhea, and weight loss are common AEs associated with CBD treatment, and they are generally mild to moderate in severity. Reduced appetite and diarrhea usually become evident within the first 3 months of treatment. The effects of CBD on gut microbiome and the oil-based drug vehicle may contribute to the diarrhea, due to the seemingly opportunistic increase in *Akkermansia muciniphila* at the expense of other bacterial species. [152] The lack of appetite and subsequent weight loss occur independently of the diarrhea and may be directly related to CBD.[153]

In randomized controlled trials (RCTs), half of the drug withdrawals were due to increased serum transaminases, which represented the most frequent severe AE.[149] All cases resolved either spontaneously, or after the dose of a concomitant ASM was reduced, or after CBD was tapered or discontinued. No events suggested lasting liver damage and no patients met the criteria for severe drug-induced liver injury. [154]

Transaminases elevations generally appear during the first 30 days and rarely after 100 days from starting CBD[154] and the main risk factors associated with elevation of liver enzymes are the concomitant administration of VPA, which is known to have hepatotoxic effects, CBD dose, and baseline transaminases levels.[97] More than two-thirds of aminotransferases elevations occurred in patients taking concomitant VPA, and some cases resolved while on CBD after the decrease in VPA dose. Concomitant administration of CLB also raises the risk of transaminases elevations, although to a lesser extent. [151]

Because of the risk of hepatocellular injury, serum transaminases and total bilirubin levels should be assessed at baseline in all patients, monitored at one month, three months, and six months after CBD initiation, and checked periodically thereafter or as clinically indicated. More frequent monitoring is warranted in patients who take VPA or have elevated liver enzymes at baseline. Liver functions should be also measured within one month following changes in CBD dose and addition of or changes in medications with known effect on the liver, like VPA and CLB. [97]

In the case of transaminases elevations, weekly laboratory monitoring over 15 days can be useful to verify whether spontaneous normalization occurs. If liver abnormalities persist, a reduction in the dosage of CBD or concomitant drugs (VPA and/or CLB), and control of transaminases every two weeks until normalization is warranted. [151]

3.FENFLURAMINE (FFA)

3.1 FFA FORMULATIONS, INDICATIONS AND DOSAGE

Fenfluramine (FFA) is indicated for the treatment of seizures associated with DS as an add-on therapy to other ASMs for patients 2 years of age and older. The starting dose (first week) is 0.1 mg/Kg taken twice daily (0.2 mg/Kg/day); at day 7, the dosage can be increased from 0.2 mg/Kg twice daily (0.4 mg/Kg/day) to a maximum of 26 mg (13 mg twice daily i.e., 6.0 mL twice daily). In patients taking STP, maximum dosage is 17 mg (8.6 mg twice daily i.e., 4.0 mL twice daily). FFA may be taken with or without food and it is compatible with commercially available gastric and nasogastric feeding tubes and a KD. [155]

3.2 EFFICACY OF FFA TREATMENT IN DRUG RESISTANT EPILEPSY

In 1985, Aicardi and Gastaut observed a reduction in seizure frequency of self-induced photosensitive epilepsy patients treated with FFA: FFA seemed to reduce or suppress the compulsive need for self-induction of seizures through its antipsychotic properties and a direct anti-epileptic mechanism could have been considered, since the photo convulsive response disappeared in one subject. [156] In 1987, the use of FFA was studied in 33 patients with severe childhood epilepsy and the primary diagnosis of DRE: this follow-up pilot study showed more than 50% reduction in seizure frequency in 46% of the patients when adding 0.5–1.5 mg/Kg/day of FFA in their current ASMs regimen. [157]

A case report in 1988 about a 5- month-old child with LGS and self-induced seizures highlighted that the addition of FFA (10 mg twice a day) to VPA, CBZ and bromocriptine mesylate resulted in child's

inability to induce seizure and long-lasting seizure control: patient's interest turned from seizures to his environment, and he became educable at a special school. [158]

A study published in 1996 evaluated add-on therapy of FFA in intractable self-induced epilepsy: FFA was given in a dosage varying between 0.5 mg/Kg/day and 1 mg/Kg/day. A single daily dosage was given in 6 patients, but in 4 a better result was obtained by giving 2 dosages, and in one patient the best result came with 3 dosages. After the introduction of FFA, a better seizure control was obtained in two or three weeks. Seven patients became seizure-free and in 4 patients a greater than 75% decrease in seizure frequency was obtained. There was no influence on the blood levels of other ASMs and no influence on body weight. Enhanced social participation in all patients and a decrease of overactivity in three patients were additional benefits of FFA. Self-inducing acts completely disappeared in the seven seizure-free patients and decreased in frequency in the other four patients. [159]

Later, *SCN1A* mutations were found in five of those patients: these mutations were a newly defined cause for DS and so FFA could be considered an interesting ASM for this DRE. [160]

In 2012, FFA was studied in a retrospective case series in patients with DS: at a mean dosage of 0.34 mg/Kg/day, 7 of the 12 patients became seizure-free (in six of them, seizure freedom was achieved within 3 days after the start of FFA treatment), 1 patient had 75% reduction in seizure frequency, 2 patients no significant response, 1 patient withdrew due to lack of efficacy, and 1 patient withdrew because controlled with another ASM. Interestingly, three seizure-free patients had a recurrence of seizures after discontinuing FFA and became seizure-free again after restarting the medication. [161]

In 2017, a prospective study assessed the safety and effectiveness of low-dose FFA in a new cohort of patients with DS. Nine patients were enrolled in the study and were treated with FFA for a median duration of 1.5 years. Median frequency of major motor seizures was 15.0/month in the baseline period and all patients demonstrated a reduction in seizure frequency during the treatment period with a median reduction of 75%. Seven patients (78%) experienced a ≥50% reduction in major motor seizure frequency. No evidence of cardiac valvulopathy or pulmonary hypertension was observed. In addition, a tendency towards a positive effect on the sleep quality and QoL of both patients and parents was seen in combination with an improved global impression. [162]

Two important studies were conducted in 2019. The first one was a double-blind, parallel-group, placebo-controlled, phase 3 RCT involving 87 pediatric patients with DS who did not have any underlying cardiac or valvular insufficiency. The patients were randomized to receive either FFA 0.4 mg/Kg/day in addition to their treatment or regimen (based on STP plus CLB or VPA) or a placebo medication. The patients who received FFA were estimated to have a 54.0% greater reduction in monthly convulsive seizure frequency (MCSF) compared to the patient's receiving the placebo medication. The treatment group also had significantly more patients experiencing a clinically meaningful (determined to be >= 50%) reduction in mean MCSF that the placebo group. Twenty-two out of 43 patients in the treatment group compared to 2 of 44 patients in the placebo group experienced a clinically meaningful reduction in mean MCSF. The treatment group also experienced significantly longer seizure-free intervals. Therefore, FFA may be an effective treatment option for patients with DS whose seizures are not adequately controlled. [163]

The second study was a similar randomized, double-blind, placebo-controlled clinical trial that also demonstrated encouraging results for the use of FFA in patients with DS. Patients were treated at

baseline with a mean of 2.4 ASMs (which most included VPA, TPM, CLB, TPM, and LEV) without concomitant STP use. The study enrolled 119 patients ages 2–18 with DS who were randomly assigned to receive either FFA 0.2 mg/Kg, FFA 0.7 mg/Kg per day or placebo. The study found a median reduction in seizure frequency of 74.9% in the FFA 0.7 mg/Kg/day group. This represents a decrease in seizures from 20.7 seizures per 28 days to 4.7 seizures per 28 days. Both doses of FFA showed statistically significant reduction in mean MCSF as compared to the placebo group. Also in this work, FFA provided significantly greater reduction in convulsive seizure frequency as compared with the placebo, it was well tolerated, and no valvular heart disease or pulmonary arterial hypertension were observed. [164]

Patients who completed these two studies were enrolled in an open-label extension (OLE) study conducted by Zogenix, the productors of Fintepla (Fenfluramine). The aim was to assess long-term safety and efficacy of FFA. They found a median 70.6% reduction in convulsive seizure frequency after optimization of dosing for a median 256 days of treatment. In addition, FFA continued to be well tolerated, no valvular heart disease or pulmonary arterial hypertension was observed during longitudinal echocardiographic assessments performed during the OLE study. [165]

A recent report suggested the potential use of FFA for the acute treatment of non-convulsive status epilepticus in patients with DS: these patients followed a faster titration to 30 mg over 4 days, they recovered, and no AEs were reported. [166]

A 2020 real-world study demonstrated that FFA provided a clinically meaningful reduction in convulsive seizure frequency in most patients with DS and was well tolerated. A reduction in the number of associated ASMs was also observed, resulting in discontinuation of STP in 13.4% of patients, and of a different drug in 26.9%. None of the patients withdrew FFA due to AEs and the

treatment was accompanied by improved behaviour, autonomy, communication, and motor skills (assessment obtained by the Clinical Global Impressions scale). [167]

In 2021, a multicenter, retrospective, observational study in Germany described the efficacy, tolerability, and retention of FFA within the compassionate use program (CUP). 78 patients with DS were treated for a median duration of 255.5 days with add-on therapy of oral FFA gradually titrated to a target dose between 0.13 and 0.7 mg/Kg/day. Responder rates (≥50% reduction; n = 78) and seizure-freedom rates at 3 months were 68% and 14% for total seizures, respectively, and 67% and 23% for generalized tonic–clonic seizures. Responder rates were consistent at 6 and 12 months (n = 66 and n = 43, respectively). Median seizure days per month decreased from 10.0 to 3.0 in the 3-month period before and after FFA treatment. During FFA treatment, 35 (45%) patients could discontinue a concomitant ASM. At the last FU date, 66 (85%) patients remained on treatment with FFA. Forty-eight (62%) patients were reported as having a meaningful global clinical improvement. FFA demonstrated efficacy in a clinically significant reduction in convulsive seizures and was well tolerated. [168]

Another study [169] explored the relationships between reduction in convulsive seizure frequency and everyday executive functions (EFs) in a population of children and young adults with DS treated with adjunctive FFA for 1 year and suggested that substantial reduction in convulsive seizure frequency over extended periods of time could be important for improving everyday EF, in particular emotion regulation and management of cognitive function. This was the first study to highlight a positive association between ASM use and everyday EF in a DS population characterized by high seizure burden at baseline.

3.3 FFA PHARMACODINAMICS

FFA is a synthetic monoamine deriving from amphetamine and it consists of a racemic mixture: dexfenfluramine (d-fenfluramine), which promotes serotonin (5-HT)-mediated neurotransmission; and levofenfluramine (l-fenfluramine) which can suppress dopaminergic transmission. [170,171] D-fenfluramine and its enantiomers have been used in the past to help obese patients to adhere a diet and to maintain the weight loss achieved.[172] This is due to the fact that d-fenfluramine might resect the balance between 5-HT release and the stimulation of 5-HT_{2c} receptors, which have a role in controlling food intake and satiety. [170]

D-Fenfluramine acts through a carrier-dependent mechanism to release 5-HT by disrupting the vesicles storage of 5-HT and by inhibiting its re-uptake from the synapse, leading to an increase of 5-HT concentrations in the synaptic cleft and causing functional changes secondary to enhanced serotoninergic neurotransmission. [173]

FFA is metabolized into norfenfluramine (nor-FFA), which shows greater affinity and agonist activity at serotonin 5-HT2 receptors than FFA. [174]

Pre-clinical [175] and clinical studies demonstrated a seizure improvement with selective 5-HT reuptake inhibitors [176] , suggesting that elevated 5-HT levels have beneficial effects on focal and generalized seizures: noradrenergic and serotonergic deficiencies contribute to seizure predisposition; indeed, the antidepressants have the potential to overcome seizure predisposition in epilepsy. [177] These considerations explain why racemic FFA was an ASM that primarily affected the serotoninergic system.

FFA explicates anti-epileptic activity as an agonist towards 5-HT_{1D} and 5-HT_{2C} receptors, while the 5-HT_{2B} receptor is not involved in anti-epileptic activity, and the role of the 5-HT_{2A} receptor is not clear [178].

A recent study indicated that seizure-induced sudden death could be prevented by 5-HT_4 receptor agonists either alone or in combination with FFA: the action of FFA to prevent SUDEP in DBA/1 mice is mediated primarily by activation of 5-HT_4 receptors. [179]

Other mechanisms involving σ1-receptors (σ1-R) contribute and potentiate the effect of direct 5-HT receptor agonists: σ1-R are a group of chaperones that exert a neuro-modulatory effect on other neurotransmitters involved in the excitatory or inhibitory processes linked to the initiation or maintenance of a seizure (e.g., glutamate and GABA). [180–182] A σ1-R antagonism by FFA was suggested by a *SCN1A* mutant Zebrafish model [178] and this was later confirmed in a mouse model with induced seizures: FFA and nor-FFA disrupted the regulatory association of the σ1R with NR1 subunits of glutamate *N*-methyl-D-aspartate receptors (NMDAR). The antagonists removed σ1R bound to NMDAR NR1 subunits enabling calcium-regulated calmodulin (CaM) to bind to those subunits: CaM may inhibit calcium permeation through NMDARs. The convulsive syndrome promoted by NMDAR overactivation could be prevented by serotoninergic activity of FFA at 5-HT_{2AR} and at 5-HT_{2CR}, in addition with its activity at σ1Rs. FFA enhanced the inhibitory coupling of G protein-coupled receptors such as 5-HT_{1AR} and cannabinoid type 1 receptor with NMDARs, thus allowing the more effective restrain of NMDAR activity, avoiding the negative side effects of direct NMDAR antagonists and improving the QoL of patients. [182]

Interestingly, a recent study illustrated that FFA activity at 5-HT receptors is complemented synergistically by functional activation of σ1R in vitro and *ex vivo*, as well as *in vivo*. These effects

are concentration-dependent and may be responsible for the profound and long-lasting efficacy but also for executive function improvement in the treatment of seizures in children and young adults with DS. [183]

Additionally, FFA might be involved in the inhibition or stimulation of the NPY mRNA expression. [184,185] NPY is known to have anti-seizure effects in animal models [186,187] , so FFA could protect against seizures also trough the NPY action. [188]

3.4 FFA PHARMACOKINETICS, DRUG INTERACTIONS

The absolute bioavailability of FFA administered as an oral solution is approximately 75-83% [155] and it is not affected by concomitant intake of food. [189]

FFA pharmacokinetics appear to be dose proportional over the 0.35 to 0.7 mg/Kg/day single dose range; nor-FFA plasma concentration increases less than proportionally with increasing dose [155].

FFA is partially converted *in vivo* to active metabolites d(+)- (6)- and l(−)-(1)-nor-FFA, by cytochrome P450 (CYP) 1A2, CYP2B6, and CYP2D6, with additional metabolism by CYP2C9, CYP2C19, and CYP3A4. Metabolic interactions with other drugs might be minimized thanks to this extensive metabolism involving different CYPs. [190,191] Both FFA and nor-FFA are about 50% bound to plasma proteins. [192]

No significant differences were found between plasma levels the two enantiomers: they are detectable 1-2 hours after ingestion of the parent drug and reach maximum plasma concentrations in 4 hours and their AUC values are comparable. The half–life of FFA in plasma estimated after a single dose by

using a non-enantioselective assay is about 20 h, whereas the half-life of nor-FFA in plasma is longer, with mean reported values of 24 to 48 h. [189,192] Steady state is usually reached after 3–4 days of treatment. [191]

The fraction of the dose excreted in urine as unchanged FFA and nor-FFA is about 6 to 24%, it decreases with alkalinization of urine and increases when urine is acidified. [193]

FFA has no significant effect on the PK of VPA, STP, CLB: no dose adjustments are needed for these ASMs which are commonly prescribed in subjects with DS. However, a moderate interaction is present when FFA is used in combination with the STP regimen (STP, CLB, and VPA administered together), and thus, an adjustment of the FFA dosage is recommended in an STP regimen. [190]

FFA is partially metabolized by CYP1A2, CYP2B6, and CYP2D6, with additional metabolism by CYP2C9, CYP2C19, and CYP3A4. STP is reported to be a strong inhibitor of CYP2C19 and CYP3A4 as well as CYP1A2, CYP2C9, and CYP2D6. [194,195] Because FFA is a substrate for some of these CYP450 enzymes, the inhibition of these enzymes by the STP regimen could result in increased levels of FFA due to reduced metabolism of FFA to nor-FFA, which is not a strong substrate for any CYP450 enzyme.

The administration of other drugs that modulate the activity of CYP450 isozymes could not affect the clearance pathway of FFA because it is characterized by renal elimination. Interference with a single pathway is unlikely to cause a large change in FFA clearance, since FFA has multiple pathways of elimination. [196,197]

Zogenix data indicate that no clinically relevant drug-drug interactions or dose alterations are likely with combination FFA and CBD based on in vitro drug-drug interaction and preliminary Phase 1 results. [198]

3.5 ADVERSE EVENTS

The main AEs related to FFA are anorexia, diarrhea, fatigue, sedation, lethargy, vomiting, nasopharyngitis, and pyrexia, but they are generally mild and transient and there is a degree of dose dependency. [199] [162]

When FFA was used as appetite suppressant, its dosages were higher (60–120 mg/day) and there had been reports of an increased incidence rate of pulmonary hypertension and heart valve disease.

Recent studies demonstrated that at the treatment dosages used in the management of individuals with DS (0.2–0.7 mg/Kg) no cardiovascular AEs occur and only a minority of individuals experimented a moderate weight loss. [200] [201]

A long-term follow-up of FFA in DS highlighted that efficacy and safety of FFA appears to be maintained also in adulthood and no clinical symptoms or clinically meaningful echocardiographic findings of cardiac valvulopathy or pulmonary hypertension were seen in the patients. [199]

Despite the risk of developing valvulopathy related to FFA seems correlated with its dosage, mandatory annual cardiac screening while on FFA therapy has been suggested by the FDA.

A recent study demonstrated that the treatment with FFA in patients with DS has no long-term effects on weight and growth. [202]

4.THERAPEUTIC DRUG MONITORING AND VAMS MICROSAMPLING

4.1 THERAPEUTIC DRUG MONITORING

Therapeutic drug monitoring (TDM) represents an important tool in the clinical practice, guiding the dosage regimen to maintain drug levels in therapeutic ranges. TDM implies the measuring of patient's blood or plasma drug levels at the specified time, it enables the assessment of certain drugs' efficacy and safety trough combining pharmaceutical, pharmacokinetics, and pharmacodynamic notions. [203] [204] In polypharmacy, TDM can prevent drug interactions by guiding dose adjustments and minimizing toxicity. [205]

TDM has been applied mainly to the first-generation ASMs (CBZ, PB, phenytoin, primidone, and VPA) and in the last years also the newer ASMs plasma levels have been monitored through TDM. [206 , 207]

In patient with DRE, it has been demonstrated that TDM improves seizure control and reduces ASMs AEs. [208]

The correlation between clinical effect and serum/plasma concentration could be negatively influenced by factors including tolerance of the drug, irreversibility of drug action and active metabolites. For drugs with active metabolites, such as CBD, TDM can include measurement of the concentrations of both parent drug and its metabolites.

4.2 VAMS MICROSAMPLIG

Miniaturized sampling approaches allow blood collection in an easier and less invasive way than conventional sampling, which are performed through intravenous blood collection. Volumetric absorbitive microsampling (VAMS) are porous hydrophilic tips used to collect 10-30μL of blood sample directly from a finger prick. This technique is well tolerated by patients, it guarantees the result avoiding the effect of hematocrit on the analytical performances and the shipment process is very simple. [209] [210] [211]

VAMS devices successfully applied to several quantitative TDM methods, including ASMs. [212] A recent study evaluated VAMS in combination with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the quantification of CBD blood levels to be used in clinical practice to personalize the cannabis-based treatment of DRE: it demonstrated that VAMS can be used as a valuable support for patients with refractory epilepsy allowing control of CBD concentrations and dosage regulation, minimizing interindividual pharmacokinetic and pharmacodynamic problems, obtaining an effective personalized treatment and better control of therapeutic adherence. [206]

An adjunctive actual advantage of VAMS technique is that the patient can do the procedure at home, allowing TDM during the COVID-19 pandemic, which required quarantine. [213]

5. AIM OF THE STUDY

We aimed to evaluate the efficacy and tolerability of the combined treatment of purified GW Cannabidiol (Epidiolex®) and Fenfluramine (FFA) (Fintepla®) in children and adults with highly refractory DS. The need for concomitant ASMs dose-adjustments and the effect on other "areas" (e.g., overall QoL) during treatment was evaluated as well.

6. PATIENTS AND METHODS

6.1 PATIENTS' SELECTION

Children and adults with drug-resistant DS were recruited within the NETRE (Network for Therapy in Rare Epilepsies) from European tertiary pediatric Centres. Patients received add-on treatment with GW purified Cannabidol (CBD) up to 25 m/Kg/day and FFA up to 0.7 mg/Kg/day or 0.4 mg/Kg/day if in concomitant treatment with STP, as per EMA guidelines. Written informed consent was provided by patients or their parents/caregivers. The study was conduct following the Good Clinical Practice guidelines and local standard operating procedures.

Clinical and treatment data (ASMs tried and efficacy, age at the beginning of the combined therapy, duration of the therapy) were recorded during the study through a structured clinical sheet addressed to the referring clinicians. Concomitant ASMs were recorded at baseline and during all the treatment period. CBD, FFA, and concomitant ASMs doses modification, as well as adding/removing co-ASMs, were allowed as clinically indicated.

6.2 ASSESSMENT OF EFFECTIVENESS AND TOLERABILITY

Seizures were defined as generalized (tonic, clonic, tonic-clonic, atonic, myoclonic, absences, or myoclonic-absences) or focal-onset seizures with or without impaired awareness basing on the criteria provided by the ILAE. [214]

A seizure diary was provided to patients' parents/caregivers and seizure frequency was provided per week since the baseline visit and efficacy outcome were assessed at follow up. Weekly seizure frequency was converted to frequency per 28 days (weekly frequency × 4). Percentage change in seizure frequency for each patient was calculated as ([seizure frequency per 28 days] − [seizure frequency at baseline]) / [seizure frequency at baseline] × 100.

Outcome analysis was based on the clinical information collected during their latest visit. Seizure endpoints were the percentage of patients with ≥50% (responders) or >25% <50% (partial responders) reduction in seizures, and the mean number of seizure-free days as compared to baseline.

Improvements in concentration, sleep, behaviour, speech, walking and other relevant aspects of the QoL were analyzed as self-reported secondary outcomes.

AEs were classified using the Medical Dictionary for Regulatory Activities (MedDRA, version 22.0).

7.RESULTS

7.1 CLINICAL FEATURES OF THE COHORT

A total of 12 patients (5 females) were enrolled. The mean age at epilepsy onset was 5.6 months (range, 3-11 months). More accurate demographic and clinical features are shown in **Table 1**.

Overall, the mean age at the beginning of the combination treatment was 12 years (range, 1.1-31 years). Mean CBD dose was 12.14 mg/Kg/day (range, 2-24 mg/Kg/day) and mean FFA dose was 0.44 mg/Kg/day (range, 0.2-0.75 mg/Kg/day). Ten out of twelve (83%) patients started CBD before FFA, while 2/12 (17%) started FFA before CBD.

The mean time under treatment combination was 14.3 months (range, 3-25 months). At baseline, the mean number of concomitant ASMs was 2.4 (range, 1-4). Concomitant medications were: CLB (83%), VPA (75%), potassium bromide (KBR) (25%), STP (25%), perampanel (PER) (8%), BRV (8%).

All the subjects (100%) presented a confirmed pathogenic variant in the *SCN1A*.

Mean age (years), range	12 (1.1-31)
Sex M (%), F (%)	7 (58%), 5 (42%)
Mean age (months) at seizure onset, range	5.6 (3-11)
Mean treatment duration (months), range	14.3 (3-25)
Mean CBD dosage mg/Kg/day, range	13 (2-24.5)
Mean FFA dosage mg/Kg/day, range	0.44 (0.2-0.75)
Mean number of concomitant ASMs, range	2.4 (1-4)
Mean monthly frequency of seizures, range	35.58 (4-72)

Table 1. Patients baseline demographic and clinical features

7.2 PRIMARY OUTCOMES

7.2.1 EFFECTIVENESS

At baseline, the mean monthly frequency of total seizures was 35.58 (range, 4-72), while at FU, it was 9.7 (range, 0-57), demonstrating a seizure reduction of 71% **(Figure 2)**.

Two out of twelve (17%) patients became seizure-free, whereas 6/12 (50%) patients achieved a seizure reduction of more than 50%. Two out of twelve (17%) patients achieved a seizure reduction of more than 25% but less than 50%, 2/12 (17%) patients achieved a seizure reduction of less than 25%. Patients considered responders resulted 8/12 (67%) while patients considered partial responders were 2/12 (17%). **(Figure 3).**

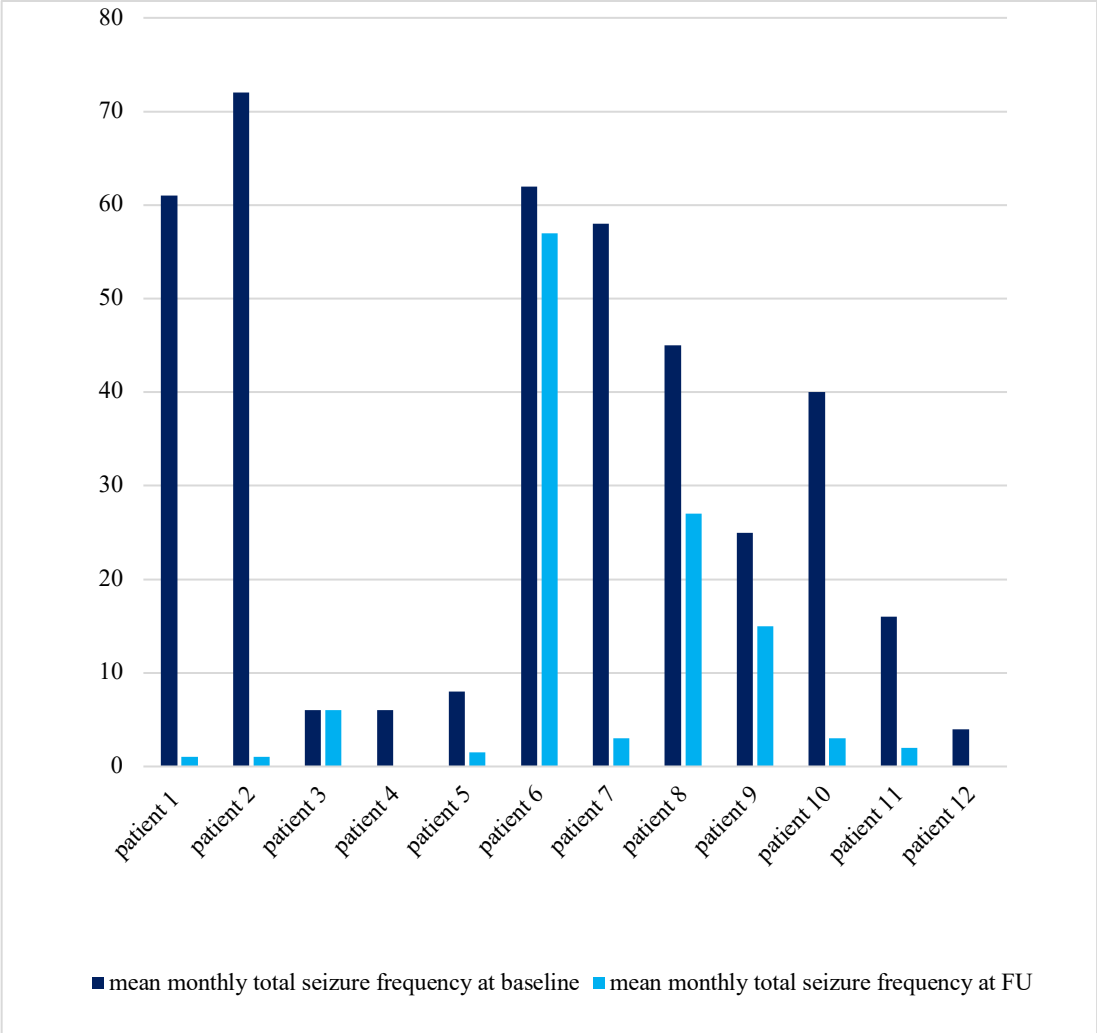

Figure 2. Mean monthly total seizure frequency at baseline compared to FU

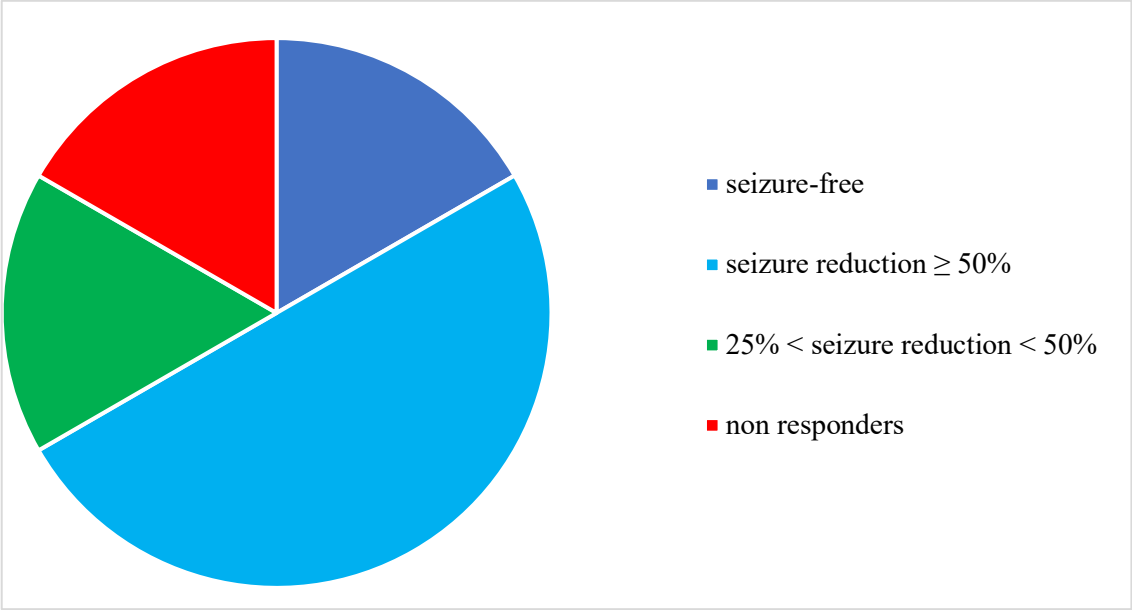

Figure 3. Responders rate

Seizure types included: generalized tonic-clonic seizures (GTC) (92%), myoclonies (50%), focal (42%), absences (25%), atonic (8%), hemiclonic (8%), eyelid myoclonia (8%), dialeptic (8%), drops (8%), GTC status (8%), non-convulsive status (8%), myoclonic status (8%), and hemiclonic status (8%).

Mean monthly seizure frequency for different type of seizure before combination therapy was: 6.11 for GTC, 18.4 for myclonies, 8.26 for focal, 10.67 for absences, 20 for atonic, 4 for hemiclonic, 28 for eyelid myoclonia, 28 for dialeptic, 3 for drops, 15 for GTC status, 4 for non-convulsive status, 20 for myoclonic status, 2 for hemiclonic status.

Mean monthly seizure frequency for every type of seizure at FU was: 2,27 for GTC, 8.16 for myoclonies, 2 for focal, 0,67 for absences, 0 for atonic, 1.5 for hemiclonic, 0 for eyelid myoclonia, 28 for dialeptic, 1 for drops, 0 for GTC status, 0 for non-convulsive status, 0 for myoclonic status, 1 for hemiclonic status. **(Figure 4).**

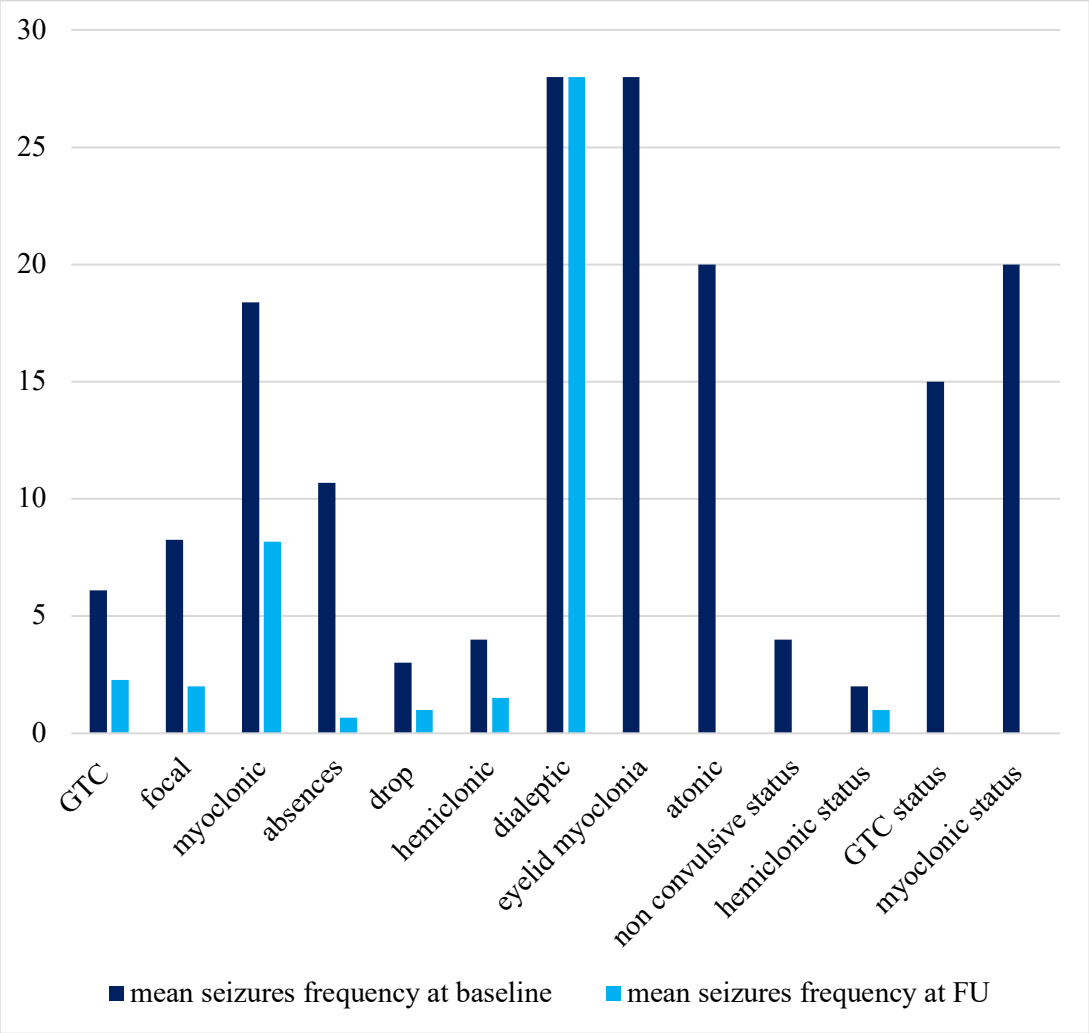

Figure 4. Mean seizure frequency for every type of seizure

No significant difference in achieving the responder status was found between patients co-treated with CLB and the one not taking CLB.

At baseline, mean longest seizure-free interval was 15 days (range, 0-112 days). At FU, mean longest seizure-free interval was 54.25 days (range, 2-168). **(Figure 5)**

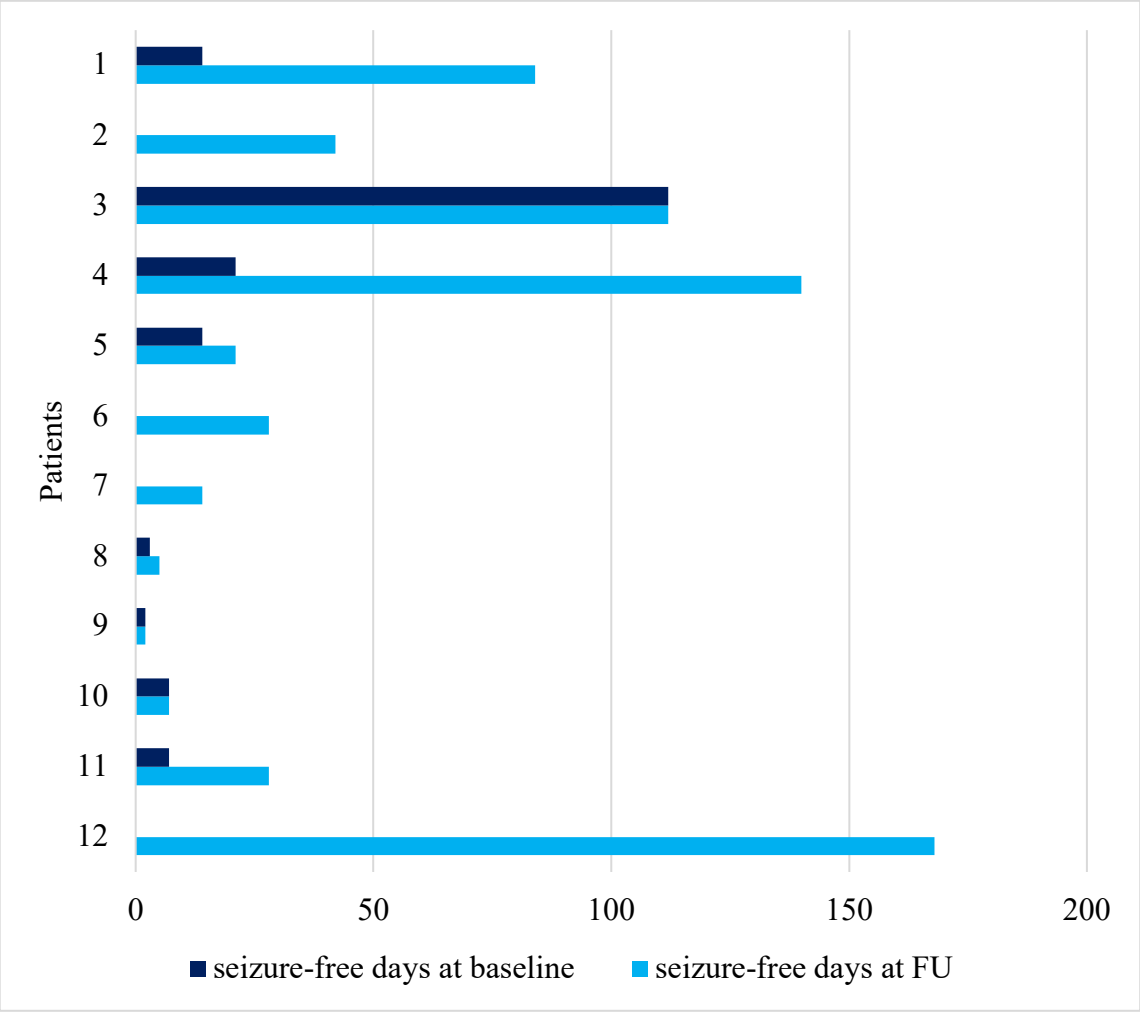

Figure 5. Seizure-free intervals at baseline compared to FU

7.2.2 TOLERABILITY

Overall, the most common AEs were represented by weight loss in 4/12 (33.3%) patients (then resolved in one case), tremor in 3/12 (25%), and irritability and facial tics in 1/12 (8.3%). None of the AEs have been classified as serious and none of the patients had to stop the treatment due to AEs. No thrombocytopenia (i.e., platelets count <140.000/microliter) or cardiac involvement were reported. **(Table 2).**

Overall AEs rate, n (%)	8 (67%)
Overall serious AEs rate, n (%)	0 (0%)
AEs leading to CBD+FFA discontinuation, n (%)	0 (0%)
Weight loss, n (%)	4 (33.3%)
Tremor, n (%)	3 (25%)
Irritability and facial tics, n (%)	1 (8.3%)

Table 2. Summary of all reported AEs

7.3 SECONDARY OUTCOMES

In ten out of twelve (83%) patients better concentration was observed, while in 6/12 (50%) a better behaviour was reported. Two out of twelve patients (17%) demonstrated better speech, 2/12 (17%) patients achieved better posturing and 2/12 (17%) patients improved their ability to walk. **(Figure 6).**

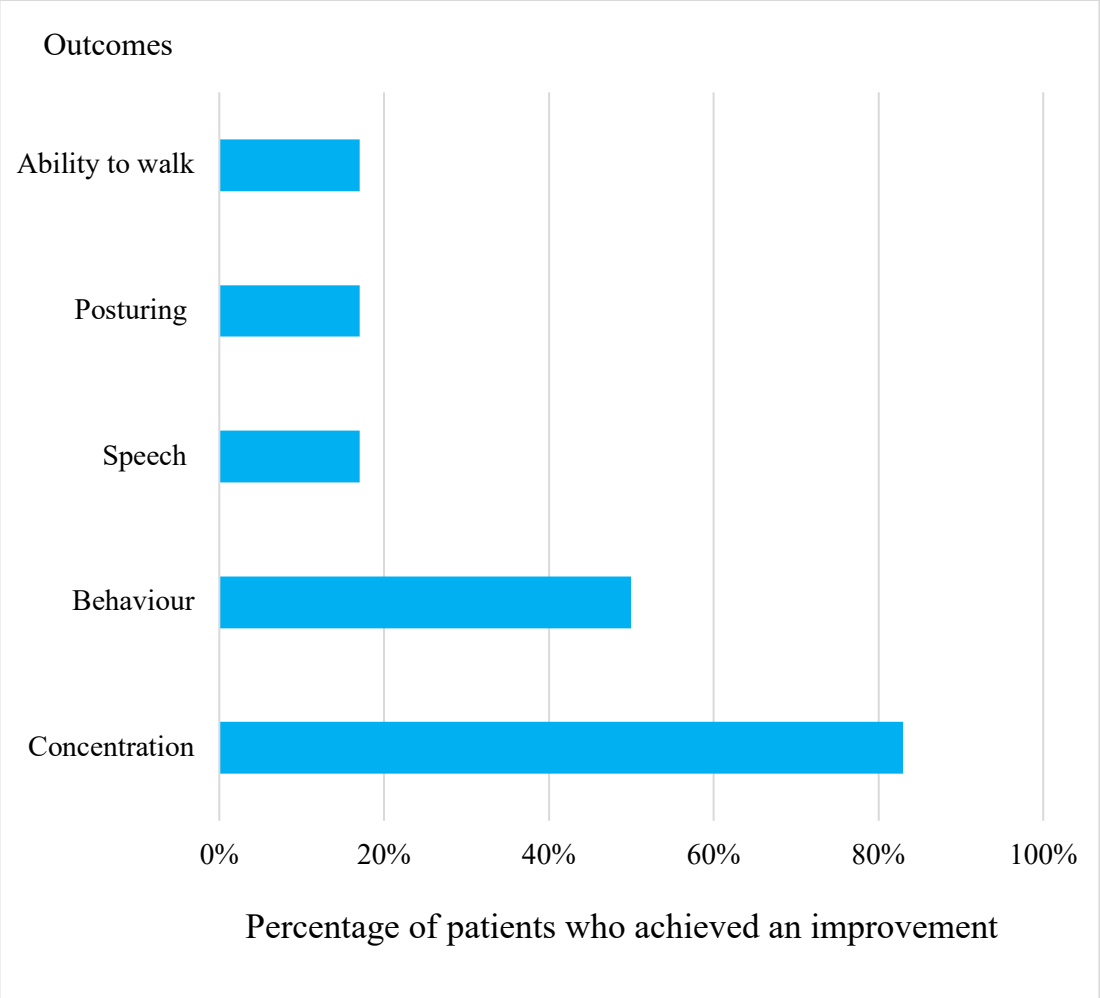

Figure 6. Percentage of patients who achieved an improvement in each outcome

7.4 CONCOMITANT ASMs DOSE-ADJUSTMENTS DURING THE TREATMENT

Seven out of twelve (58%) patients could reduce the dosage of other concomitant drugs after the combination of CBD+FFA, and 6/12 (50%) patients could stop one or more concomitant ASMs. **(Figure 7).**

One patient could reduce CLB dose by 50%. In one patient a withdrawal of KBR, a reduction of CLB by 30% and VPA by 50% were reported. In one patient VPA was reduced by 50%. In one patient CLB was decreased from 6 mg/day to 4 mg/day. In one patient, after FFA was added to the treatment, CBD could be decreased from 21 mg/Kg/day to 8.3mg/Kg/day and seizures relapsed when they tried to discontinue it while TPM was discontinued without a seizure relapse and CLB slightly increased. One patient stopped PER and reduced KBR. One patient stopped KBR. One patient stopped STP. One patient stopped TPM and reduced STP. One patient reduced STP. In two patients rescue medications were no longer needed.

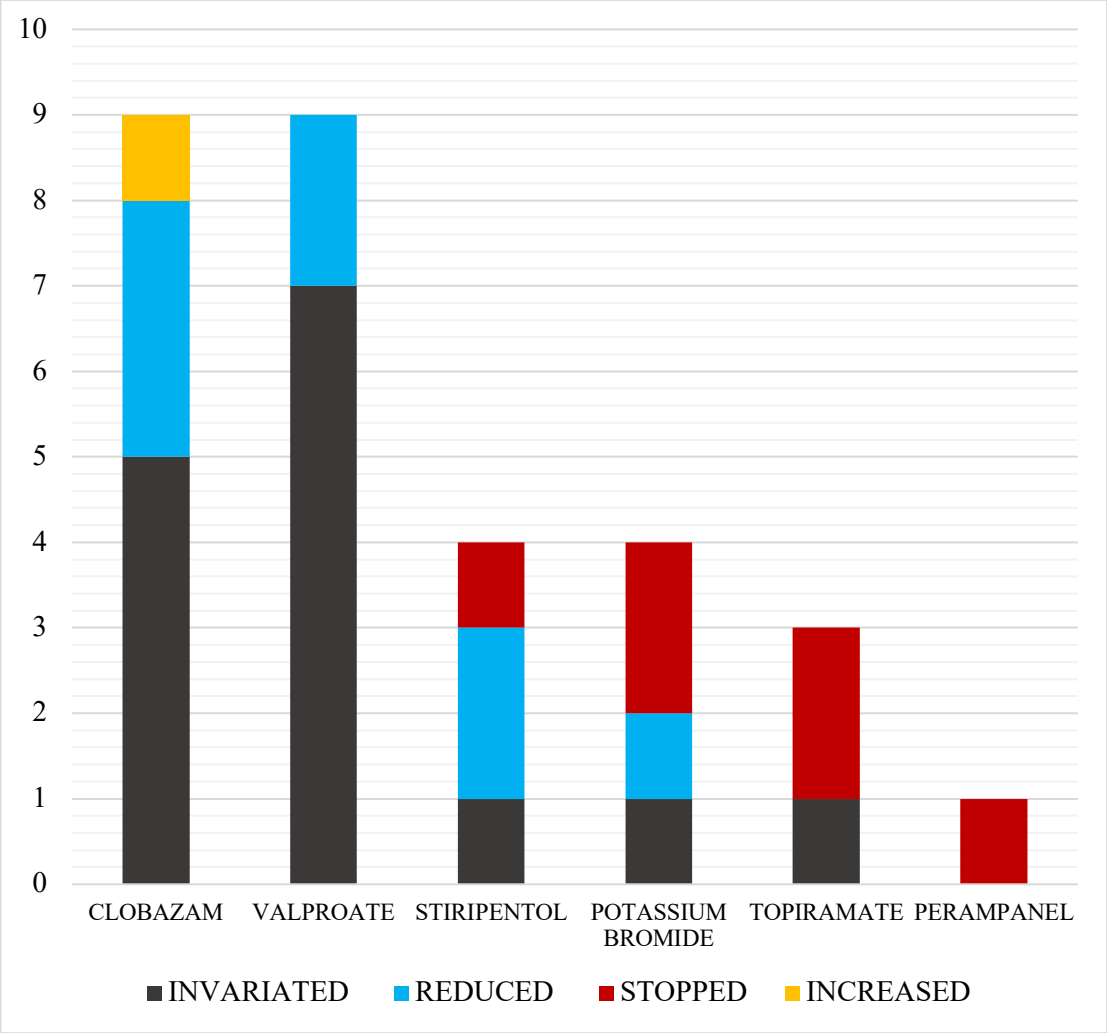

Figure 7. Concomitant ASMs dose-adjustments during the combination therapy

8.DISCUSSION

Therapeutic paradigm for DS recommends avoiding sodium channel blockers and considers VPA as first-line agent. VPA can decrease the number of seizures and the severity (duration) of the subsequent seizures, but the patients rarely become seizure-free. CLB represents and alternative first-line agent, in particular according to American guidelines. Second-line choices include STP in combination with VPA and CLB, TPM and KD. The most recent 2021 guidelines reviewed by Cardenal-Munoz also include CBD and FFA as possible second-line treatment. Third-line strategy includes CLZ, LEV, ZNS, ESM, PB or VNS. [67]

Seizures but also comorbidities (ataxia, premature death, language and motor development delay, cognitive impairment, sleep disorders, and SUDEP) represent an important burden for patients with DS. It is evident that optimizing therapeutic strategy is fundamental, also because an increased amount of ASMs could lead to an increased risk of AEs.

For these reasons, we aimed to investigate a new possible therapeutic option for DS, consisting in the combination of two recent ASMs for DS: CBD and FFA.

Our study shows that mean monthly total seizures frequency was 35.58 at baseline and 9.7 at FU, demonstrating that the combination therapy led to a reduction of 71% of total seizures per month. A high efficacy of the combination treatment on seizures was also proved by the fact that ten out of twelve (83%) patients responded to the treatment.

Secondary outcomes included better concentration (83%), better behaviour (50%), better speech (17%) and posturing (17%), improved ability to walk (17%): these results appear to be interesting concerning the improvement in the QoL that patients could achieve with the combination treatment.

Retention rate is a combined measure of effectiveness and tolerability aiming at evaluating how many patients stay on treatment in a given time-period. During the FU period, 100% of the patients of our cohort, with at least one month of treatment, remained on CBD+FFA.

Regarding tolerability, is important to highlight that the combination treatment did not induce serious AEs, none of the patients had to stop the treatment.

Interestingly, 7/12 (58%) patients reduced the dosage of other concomitant drugs after the combination of CBD+FFA and 6/12 (50%) patients stopped one or more concomitant ASMs. In particular, among the four patients who were taking STP, this drug was reduced in 2/4 (50%) and stopped in 1/4 (25%) patients: this might be an interesting outcome since the combination of FFA and STP could lead to weight loss.

The combination therapy of CBD+FFA could be an effective and well tolerated therapeutic option for patients with DS, and further FU might confirm this.

9.CONCLUSIONS

In conclusion, our study demonstrates the effectiveness and tolerability of CBD+FFA in highly refractory DS patients, also without the concomitant use of CLB. We report a significant reduction in total monthly seizures, longer seizure-free intervals as well as an improvement in concentration, behaviour, speech, posturing, walking of patients taking CBD+FFA. Interestingly, during the combination treatment, some patients were allowed to interrupt or decrease the dose of some other concomitant medications, indicating the efficacy of these new ASMs.

10. PERSPECTIVES

In view of a personalized treatment for DS, which is rare between diseases but frequent between DEEs, gene therapy could hopefully prevent DS evolution by directly relieving the specific genetic defect and possibly change the treatment paradigm for this condition.

Some major limitation could prevent the clinical application of gene therapy that is still set at the *in-animal* level for DS: the injection strategy is generally intrathecal, which represent a quite invasive approach requiring collaboration with experienced neurosurgeons and repeated interventions. Another great issue is the possibility of off-target editing, which may cause eventual disruption of gene function and permanent DNA alterations. [215,216]

The results and the course of the already applied gene therapies on other genetic conditions (e.g., spinal muscular atrophy) will provide results to really assess the effectiveness and the long-term safety and tolerability of this approach.

It is reliable that new strategies to deliver the genetic material into cells (with highly efficient target selection) will be prompted in the next few years, together with more practical administration routes for patients.

BIBLIOGRAPHY

1. Fisher, R. S. *et al.* Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* **46**, 470–472 (2005).

2. Vos, T. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **388**, 1545–1602 (2016).

3. Ngugi, A. K., Bottomley, C., Kleinschmidt, I., Sander, J. W. & Newton, C. R. Estimation of the burden of active and life-time epilepsy: a meta-analytic approach. *Epilepsia* **51**, 883–890 (2010).

4. Fisher, R. S. *et al.* ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* **55**, 475–482 (2014).

5. Kwan, P., Schachter, S. C. & Brodie, M. J. Drug-resistant epilepsy. *N Engl J Med* **365**, 919–26 (2011).

6. Golyala, A. & Kwan, P. Drug development for refractory epilepsy: The past 25 years and beyond. *Seizure* **44**, 147–156 (2017).

7. Kwan, P. & Brodie, M. J. Early identification of refractory epilepsy. *N Engl J Med* **342**, 314–319 (2000).

8. Kwan, P. *et al.* Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* **51**, 1069–1077 (2010).

9. Sillanp, M. & Schmidt, D. Early seizure frequency and aetiology predict long-term medical outcome in childhood-onset epilepsy. *Brain* **132**, 989–998 (2009).

10. Beghi, E. Drug-resistant epilepsy. *Neurologia Psichiatria Scienze Umane* **10**, 289–297 (1990).

11. Chen, Z., Brodie, M. J., Liew, D. & Kwan, P. Treatment Outcomes in Patients With Newly Diagnosed Epilepsy Treated With Established and New Antiepileptic Drugs: A 30-Year Longitudinal Cohort Study. *JAMA Neurol* **75**, 279–286 (2018).

12. Margineanu, D. G. & Klitgaard, H. Mechanisms of drug resistance in epilepsy: relevance for antiepileptic drug discovery. *Expert Opin Drug Discov* **4**, 23–32 (2009).

13. Mohanraj, R. & Brodie, M. J. Diagnosing refractory epilepsy: response to sequential treatment schedules. *Eur J Neurol* **13**, 277–282 (2006).

14. Dalic, L. & Cook, M. J. Managing drug-resistant epilepsy: challenges and solutions. *Neuropsychiatr Dis Treat* **12**, 2605–2616 (2016).

15. Semah, F. *et al.* Is the underlying cause of epilepsy a major prognostic factor for recurrence? *Neurology* **51**, 1256–1262 (1998).

16. Łukawski, K. & Czuczwar, S. J. Understanding mechanisms of drug resistance in epilepsy and strategies for overcoming it. *Expert Opin Drug Metab Toxicol* **17**, 1075–1090 (2021).

17. Symonds, J. D. *et al.* Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. *Brain* **142**, 2303–2318 (2019).

18. Durá Travé, T., Yoldi Petri, M. E. & Gallinas Victoriano, F. [Incidence of epilepsy in 0-15 year-olds]. *Anales de pediatria (Barcelona, Spain : 2003)* **67**, 37–43 (2007).

19. Dravet Charlotte. Les epilepsies graves de l'enfant. *Vie méd* (1978).

20. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. *Epilepsia* **30**, 389–399 (1989).

21. Engel, J. A Proposed Diagnostic Scheme for People with Epileptic Seizures and with Epilepsy: Report of the ILAE Task Force on Classification and Terminology. *Epilepsia* **42**, 796–803 (2001).

22. Kearney, J. A. *et al.* Recurrent de novo mutations of SCN1A in severe myoclonic epilepsy of infancy. *Pediatr Neurol* **34**, 116–120 (2006).

23. Auvin, S. *et al.* Altered vaccine-induced immunity in children with Dravet syndrome. *Epilepsia* **59**, e45–e50 (2018).

24. Scheffer, I. E. Diagnosis and long-term course of Dravet syndrome. *Eur J Paediatr Neurol* **16 Suppl 1**, S5 (2012).

25. Xu, X. *et al.* Early clinical features and diagnosis of Dravet syndrome in 138 Chinese patients with SCN1A mutations. *Brain Dev* **36**, 676–681 (2014).

26. Ragona, F. *et al.* Dravet syndrome: early clinical manifestations and cognitive outcome in 37 Italian patients. *Brain Dev* **32**, 71–77 (2010).

27. Ohki, T. *et al.* Severe myoclonic epilepsy in infancy: evolution of seizures. *Seizure* **6**, 219–224 (1997).

28. Dravet, C. The core Dravet syndrome phenotype. *Epilepsia* **52 Suppl 2**, 3–9 (2011).

29. Dravet, C., Bureau, M., Oguni, H., Fukuyama, Y. & Cokar, O. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol* **95**, 71–102 (2005).

30. Oguni, H., Hayashi, K., Awaya, Y., Fukuyama, Y. & Osawa, M. Severe myoclonic epilepsy in infants--a review based on the Tokyo Women's Medical University series of 84 cases. *Brain Dev* **23**, 736–748 (2001).

31. Nabbout, R. *et al.* An unexpected EEG course in Dravet syndrome. *Epilepsy Res* **81**, 90–95 (2008).

32. Verbeek, N. *et al.* Photosensitivity in Dravet syndrome is under-recognized and related to prognosis. *Clin Neurophysiol* **128**, 323–330 (2017).

33. Brown, A. *et al.* Cognitive, behavioral, and social functioning in children and adults with Dravet syndrome. *Epilepsy Behav* **112**, (2020).

34. Licheni, S. H., Mcmahon, J. M., Schneider, A. L., Davey, M. J. & Scheffer, I. E. Sleep problems in Dravet syndrome: a modifiable comorbidity. *Dev Med Child Neurol* **60**, 192–198 (2018).

35. Selvarajah, A., Zulfiqar-Ali, Q., Marques, P., Rong, M. & Andrade, D. M. A systematic review of adults with Dravet syndrome. *Seizure* **87**, 39–45 (2021).

36. Jansson, J. S., Hallböök, T. & Reilly, C. Intellectual functioning and behavior in Dravet syndrome: A systematic review. *Epilepsy Behav* **108**, (2020).

37. Ouss, L. *et al.* Autism spectrum disorder and cognitive profile in children with Dravet syndrome: Delineation of a specific phenotype. *Epilepsia Open* **4**, 40–53 (2018).

38. Cooper, M. S. *et al.* Mortality in Dravet syndrome. *Epilepsy Res* **128**, 43–47 (2016).

39. Wolff, M., Cassé-Perrot, C. & Dravet, C. Severe myoclonic epilepsy of infants (Dravet syndrome): natural history and neuropsychological findings. *Epilepsia* **47 Suppl 2**, 45–48 (2006).

40. Lagae, L., Brambilla, I., Mingorance, A., Gibson, E. & Battersby, A. Quality of life and comorbidities associated with Dravet syndrome severity: a multinational cohort survey. *Dev Med Child Neurol* **60**, 63–72 (2018).

41. Dravet, C. Dravet syndrome history. *Dev Med Child Neurol* **53 Suppl 2**, 1–6 (2011).

42. Claes, L. *et al.* De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* **68**, 1327–1332 (2001).

43. Catterall, W. A., Kalume, F. & Oakley, J. C. NaV1.1 channels and epilepsy. *J Physiol* **588**, 1849–1859 (2010).

44. Mantegazza, M. & Broccoli, V. SCN1A/Na V 1.1 channelopathies: Mechanisms in expression systems, animal models, and human iPSC models. *Epilepsia* **60 Suppl 3**, S25–S38 (2019).

45. Mei, D., Cetica, V., Marini, C. & Guerrini, R. Dravet syndrome as part of the clinical and genetic spectrum of sodium channel epilepsies and encephalopathies. *Epilepsia* **60 Suppl 3**, S2–S7 (2019).

46. Nabbout, R. *et al.* Spectrum of SCN1A mutations in severe myoclonic epilepsy of infancy. *Neurology* **60**, 1961–1967 (2003).

47. Wallace, R. H. *et al.* Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology* **61**, 765–769 (2003).

48. Fujiwara, T. *et al.* Mutations of sodium channel alpha subunit type 1 (SCN1A) in intractable childhood epilepsies with frequent generalized tonic-clonic seizures. *Brain* **126**, 531–546 (2003).

49. Singh, N. A. *et al.* A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet* **5**, (2009).

50. Wirrell, E. C. Treatment of Dravet Syndrome. *Can J Neurol Sci* **43 Suppl 3**, S13–S18 (2016).

51. Nabbout, R. *et al.* Encephalopathy in children with Dravet syndrome is not a pure consequence of epilepsy. *Orphanet J Rare Dis* **8**, (2013).

52. Brunklaus, A., Ellis, R., Reavey, E., Forbes, G. H. & Zuberi, S. M. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. *Brain* **135**, 2329–2336 (2012).

53. Lagae, L., Brambilla, I., Mingorance, A., Gibson, E. & Battersby, A. Quality of life and comorbidities associated with Dravet syndrome severity: a multinational cohort survey. *Dev Med Child Neurol* **60**, 63–72 (2018).

54. Brigo, F., Striano, P., Balagura, G. & Belcastro, V. Emerging drugs for the treatment of Dravet syndrome. *Expert Opin Emerg Drugs* **23**, 261–269 (2018).

55. Fallah, A. Moving beyond evidence-based medicine: Incorporating patient values and preferences. *Epilepsy Behav* **53**, 209–210 (2015).

56. Wirrell, E. C. *et al.* Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel. *Pediatr Neurol* **68**, 18-34.e3 (2017).

57. Wirrell, E. C. & Nabbout, R. Recent Advances in the Drug Treatment of Dravet Syndrome. *CNS Drugs* **33**, 867–881 (2019).

58. Wallace, A., Wirrell, E. & Kenney-Jung, D. L. Pharmacotherapy for Dravet Syndrome. *Paediatr Drugs* **18**, 197–208 (2016).

59. Dravet, C. & Oguni, H. Dravet syndrome (severe myoclonic epilepsy in infancy). *Handb Clin Neurol* **111**, 627–633 (2013).

60. Saito, Y., Oguni, H., Awaya, Y., Hayashi, K. & Osawa, M. Phenytoin-induced choreoathetosis in patients with severe myoclonic epilepsy in infancy. *Neuropediatrics* **32**, 231–235 (2001).

61. de Lange, I. M. *et al.* Influence of contraindicated medication use on cognitive outcome in Dravet syndrome and age at first afebrile seizure as a clinical predictor in SCN1A-related seizure phenotypes. *Epilepsia* **59**, 1154–1165 (2018).

62. Guerrini, R. *et al.* Lamotrigine and seizure aggravation in severe myoclonic epilepsy. *Epilepsia* **39**, 508–512 (1998).

63. Mueller, A. *et al.* Low long-term efficacy and tolerability of add-on rufinamide in patients with Dravet syndrome. *Epilepsy Behav* **21**, 282–284 (2011).

64. Nguyen Thanh, T. *et al.* [Long-term efficacy and tolerance of stiripentaol in severe myoclonic epilepsy of infancy (Dravet's syndrome)]. *Archives de pediatrie : organe officiel de la Societe francaise de pediatrie* **9**, 1120–1127 (2002).

65. Chipaux, M. *et al.* Unusual consequences of status epilepticus in Dravet syndrome. *Seizure* **19**, 190–194 (2010).

66. Wirrell, E. C. *et al.* Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel. *Pediatric Neurology* **68**, 18-34.e3 (2017).

67. Cardenal-Muñoz, E. *et al.* Guidance on Dravet syndrome from infant to adult care: Road map for treatment planning in Europe. (2021) doi:10.1002/epi4.12569.

68. Strzelczyk, A. & Schubert-Bast, S. A Practical Guide to the Treatment of Dravet Syndrome with Anti-Seizure Medication. *CNS Drugs* **36**, 217–237 (2022).

69. Nalivaeva, N. N., Belyaev, N. D. & Turner, A. J. Sodium valproate: an old drug with new roles. *Trends Pharmacol Sci* **30**, 509–514 (2009).

70. Marson, A. G. *et al.* The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. *Lancet* **369**, 1016–1026 (2007).

71. Marson, A. *et al.* The SANAD II study of the effectiveness and cost-effectiveness of valproate versus levetiracetam for newly diagnosed generalised and unclassifiable epilepsy: an open-label, non-inferiority, multicentre, phase 4, randomised controlled trial. *Lancet* **397**, 1375–1386 (2021).

72. Epilim 500 mg Gastro-resistant tablets - Summary of Product Characteristics (SmPC) - (emc). https://www.medicines.org.uk/emc/product/1446/smpc#gref.

73. Cook, M. *et al.* Anti-seizure therapy with a long-term, implanted intra-cerebroventricular delivery system for drug-resistant epilepsy: A first-in-man study. *EClinicalMedicine* **22**, (2020).

74. Frisium 10mg Tablets - Summary of Product Characteristics (SmPC) - (emc). https://www.medicines.org.uk/emc/product/1574/smpc#gref.

75. Fda. HIGHLIGHTS OF PRESCRIBING INFORMATION.

76. Gauthier, A. C. & Mattson, R. H. Clobazam: A Safe, Efficacious, and Newly Rediscovered Therapeutic for Epilepsy. *CNS Neurosci Ther* **21**, 543–548 (2015).

77. Redondo, P. *et al.* Photo-induced toxic epidermal necrolysis caused by clobazam. *Br J Dermatol* **135**, 999–1002 (1996).

78. Yapici, A. K. *et al.* Stevens-Johnson Syndrome triggered by a combination of clobazam, lamotrigine and valproic acid in a 7-year-old child. *Annals of Burns and Fire Disasters* **27**, 121 (2014).

79. Liu, F. *et al.* Efficacy of the ketogenic diet in Chinese children with Dravet syndrome: A focus on neuropsychological development. *Epilepsy Behav* **92**, 98–102 (2019).

80. Caraballo, R. H. Nonpharmacologic treatments of Dravet syndrome: focus on the ketogenic diet. *Epilepsia* **52 Suppl 2**, 79–82 (2011).

81. Yan, N. *et al.* Prospective study of the efficacy of a ketogenic diet in 20 patients with Dravet syndrome. *Seizure* **60**, 144–148 (2018).

82. Laux, L. & Blackford, R. The ketogenic diet in Dravet syndrome. *J Child Neurol* **28**, 1041–1044 (2013).

83. Nabbout, R. *et al.* Ketogenic diet also benefits Dravet syndrome patients receiving stiripentol: a prospective pilot study. *Epilepsia* **52**, (2011).

84. Hoon, C. K., Yong, J. K., Dong, W. K. & Heung, D. K. Efficacy and safety of the ketogenic diet for intractable childhood epilepsy: Korean multicentric experience. *Epilepsia* **46**, 272–279 (2005).

85. Kim, J. M. Ketogenic diet: Old treatment, new beginning. *Clin Neurophysiol Pract* **2**, 161–162 (2017).

86. Dressler, A. *et al.* Efficacy and tolerability of the ketogenic diet in Dravet syndrome - Comparison with various standard antiepileptic drug regimen. *Epilepsy Res* **109**, 81–89 (2015).

87. Dibué-Adjei, M., Fischer, I., Steiger, H. J. & Kamp, M. A. Efficacy of adjunctive vagus nerve stimulation in patients with Dravet syndrome: A meta-analysis of 68 patients. *Seizure* **50**, 147–152 (2017).

88. Fulton, S. P., van Poppel, K., McGregor, A. L., Mudigoudar, B. & Wheless, J. W. Vagus Nerve Stimulation in Intractable Epilepsy Associated With SCN1A Gene Abnormalities. *J Child Neurol* **32**, 494–498 (2017).

89. Dlouhy, B. J. *et al.* Palliative epilepsy surgery in Dravet syndrome-case series and review of the literature. *Childs Nerv Syst* **32**, 1703–1708 (2016).

90. Ali, R. *et al.* Use of social media to assess the effectiveness of vagal nerve stimulation in Dravet syndrome: A caregiver's perspective. *J Neurol Sci* **375**, 146–149 (2017).

91. Orosz, I. *et al.* Vagus nerve stimulation for drug-resistant epilepsy: a European long-term study up to 24 months in 347 children. *Epilepsia* **55**, 1576–1584 (2014).

92. Bremer, A., Lossius, M. I. & Nakken, K. O. Dravet syndrome--considerable delay in making the diagnosis. *Acta Neurol Scand* **125**, 359–362 (2012).

93. Zamponi, N., Passamonti, C., Cappanera, S. & Petrelli, C. Clinical course of young patients with Dravet syndrome after vagal nerve stimulation. *Eur J Paediatr Neurol* **15**, 8–14 (2011).

94. ElSohly, M. A. & Gul, W. Constituents of Cannabis Sativa. *Handbook of Cannabis* 3–22 (2015) doi:10.1093/ACPROF:OSO/9780199662685.003.0001.

95. Mechoulam, R., Shani, A., Edery, H. & Grunfeld, Y. Chemical basis of hashish activity. *Science* **169**, 611–612 (1970).

96. Assareh, N. *et al.* Cannabidiol disrupts conditioned fear expression and cannabidiolic acid reduces trauma-induced anxiety-related behaviour in mice. *Behavioural pharmacology* **31**, 591–596 (2020).

97. HIGHLIGHTS OF PRESCRIBING INFORMATION.

98. Arzimanoglou, A. *et al.* Epilepsy and cannabidiol: a guide to treatment. *Epileptic Disorders* **22**, 1–14 (2020).

99. Szaflarski, J. P. & Martina Bebin, E. Cannabis, cannabidiol, and epilepsy--from receptors to clinical response. *Epilepsy Behav* **41**, 277–282 (2014).

100. Maa, E. & Figi, P. The case for medical marijuana in epilepsy. *Epilepsia* **55**, 783–786 (2014).

101. von Wrede, R., Helmstaedter, C. & Surges, R. Cannabidiol in the Treatment of Epilepsy. *Clin Drug Investig* **41**, 211–220 (2021).

102. Devinsky, O. *et al.* Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol* **15**, 270–278 (2016).

103. Devinsky, O. *et al.* Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N Engl J Med* **376**, 2011–2020 (2017).

104. Miller, I. *et al.* Dose-Ranging Effect of Adjunctive Oral Cannabidiol vs Placebo on Convulsive Seizure Frequency in Dravet Syndrome: A Randomized Clinical Trial. *JAMA Neurol* **77**, 613–621 (2020).

105. Devinsky, O. *et al.* Long-term cannabidiol treatment in patients with Dravet syndrome: An open-label extension trial. *Epilepsia* **60**, 294–302 (2019).

106. Laux, L. C. *et al.* Long-term safety and efficacy of cannabidiol in children and adults with treatment resistant Lennox-Gastaut syndrome or Dravet syndrome: Expanded access program results. *Epilepsy Res* **154**, 13–20 (2019).

107. Patel, S. *et al.* The long-term efficacy of cannabidiol in the treatment of refractory epilepsy. *Epilepsia* **62**, 1594–1603 (2021).

108. Friedman, D. & Devinsky, O. Cannabinoids in the Treatment of Epilepsy. *N Engl J Med* **373**, 1048–1058 (2015).

109. Klein, B. D. *et al.* Evaluation of Cannabidiol in Animal Seizure Models by the Epilepsy Therapy Screening Program (ETSP). *Neurochem Res* **42**, 1939–1948 (2017).

110. Lazarini-Lopes, W., do Val-da Silva, R. A., da Silva-Júnior, R. M. P., Leite, J. P. & Garcia-Cairasco, N. The anticonvulsant effects of cannabidiol in experimental models of epileptic seizures: From behavior and mechanisms to clinical insights. *Neurosci Biobehav Rev* **111**, 166–182 (2020).

111. Senn, L., Cannazza, G. & Biagini, G. Receptors and Channels Possibly Mediating the Effects of Phytocannabinoids on Seizures and Epilepsy. *Pharmaceuticals (Basel)* **13**, 1–22 (2020).

112. Gray, R. A. & Whalley, B. J. The proposed mechanisms of action of CBD in epilepsy. *Epileptic Disord* **22**, S10–S15 (2020).

113. Sylantyev, S., Jensen, T. P., Ross, R. A. & Rusakov, D. A. Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proc Natl Acad Sci U S A* **110**, 5193–5198 (2013).

114. Alavi, M. S., Shamsizadeh, A., Azhdari-Zarmehri, H. & Roohbakhsh, A. Orphan G protein-coupled receptors: The role in CNS disorders. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **98**, 222–232 (2018).

115. Kaplan, J. S., Stella, N., Catterall, W. A. & Westenbroek, R. E. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc Natl Acad Sci U S A* **114**, 11229–11234 (2017).

116. Menigoz, A. & Boudes, M. The expression pattern of TRPV1 in brain. *J Neurosci* **31**, 13025–13027 (2011).

117. Xing, J. & Li, J. TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. *J Neurophysiol* **97**, 503–511 (2007).

118. Nazıroglu, M. TRPV1 Channel: A Potential Drug Target for Treating Epilepsy. *Curr Neuropharmacol* **13**, 239–247 (2015).

119. Cho, S. J., Vaca, M. A., Miranda, C. J. & N'Gouemo, P. Inhibition of transient potential receptor vanilloid type 1 suppresses seizure susceptibility in the genetically epilepsy-prone rat. *CNS Neurosci Ther* **24**, 18–28 (2018).

120. Gonzalez-Reyes, L. E., Ladas, T. P., Chiang, C. C. & Durand, D. M. TRPV1 antagonist capsazepine suppresses 4-AP-induced epileptiform activity in vitro and electrographic seizures in vivo. *Exp Neurol* **250**, 321–332 (2013).

121. Nazıroglu, M. TRPV1 Channel: A Potential Drug Target for Treating Epilepsy. *Curr Neuropharmacol* **13**, 239–247 (2015).

122. Shirazi, M. *et al.* Involvement of central TRPV1 receptors in pentylenetetrazole and amygdala-induced kindling in male rats. *Neurol Sci* **35**, 1235–1241 (2014).

123. de Petrocellis, L. *et al.* Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* **163**, 1479–1494 (2011).

124. 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).

125. Vilela, L. R. *et al.* Anticonvulsant effect of cannabidiol in the pentylenetetrazole model: Pharmacological mechanisms, electroencephalographic profile, and brain cytokine levels. *Epilepsy Behav* **75**, 29–35 (2017).

126. Gray, R. A. *et al.* Anticonvulsive Properties of Cannabidiol in a Model of Generalized Seizure Are Transient Receptor Potential Vanilloid 1 Dependent. *Cannabis Cannabinoid Res* **5**, 145–149 (2020).

127. Carrier, E. J., Auchampach, J. A. & Hillard, C. J. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci U S A* **103**, 7895–7900 (2006).

128. Jacobson, K. A. & Reitman, M. L. Adenosine-Related Mechanisms in Non-Adenosine Receptor Drugs. *Cells* **9**, (2020).

129. Mijangos-Moreno, S., Poot-Aké, A., Arankowsky-Sandoval, G. & Murillo-Rodríguez, E. Intrahypothalamic injection of cannabidiol increases the extracellular levels of adenosine in nucleus accumbens in rats. *Neurosci Res* **84**, 60–63 (2014).

130. Gray, R. A. & Whalley, B. J. The proposed mechanisms of action of CBD in epilepsy. *Epileptic Disord* **22**, S10–S15 (2020).

131. Weltha, L., Reemmer, J. & Boison, D. The role of adenosine in epilepsy. *Brain Res Bull* **151**, 46–54 (2019).

132. Franco, V., Bialer, M. & Perucca, E. Cannabidiol in the treatment of epilepsy: Current evidence and perspectives for further research. *Neuropharmacology* **185**, (2021).

133. Anderson, L. L. *et al.* Coadministered cannabidiol and clobazam: Preclinical evidence for both pharmacodynamic and pharmacokinetic interactions. *Epilepsia* **60**, 2224–2234 (2019).

134. Bakas, T. *et al.* The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABA A receptors. *Pharmacol Res* **119**, 358–370 (2017).

135. Cifelli, P. *et al.* Phytocannabinoids in Neurological Diseases: Could They Restore a Physiological GABAergic Transmission? *Int J Mol Sci* **21**, (2020).

136. Ruffolo, G. *et al.* A novel GABAergic dysfunction in human Dravet syndrome. *Epilepsia* **59**, 2106–2117 (2018).

137. Catterall, W. A. Dravet Syndrome: A Sodium Channel Interneuronopathy. *Curr Opin Physiol* **2**, 42–50 (2018).

138. Geffrey, A. L., Pollack, S. F., Bruno, P. L. & Thiele, E. A. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia* **56**, 1246–1251 (2015).

139. Morrison, G., Crockett, J., Blakey, G. & Sommerville, K. A Phase 1, Open-Label, Pharmacokinetic Trial to Investigate Possible Drug-Drug Interactions Between Clobazam,

Stiripentol, or Valproate and Cannabidiol in Healthy Subjects. *Clin Pharmacol Drug Dev* **8**, 1009–1031 (2019).

140. Geffrey, A. L., Pollack, S. F., Bruno, P. L. & Thiele, E. A. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia* **56**, 1246–1251 (2015).

141. Lattanzi, S. *et al.* Cannabidiol efficacy and clobazam status: A systematic review and meta-analysis. *Epilepsia* **61**, 1090–1098 (2020).

142. Lattanzi, S., Brigo, F. & Trinka, E. Cannabidiol efficacy and clobazam coadministration: Where do we stand now? *Epilepsia* **61**, 1795–1796 (2020).

143. Lattanzi, S. *et al.* Practical use of pharmaceutically purified oral cannabidiol in Dravet syndrome and Lennox-Gastaut syndrome. *Expert Rev Neurother* **21**, 99–110 (2021).

144. Ben-Menachem, E. *et al.* A Phase II Randomized Trial to Explore the Potential for Pharmacokinetic Drug-Drug Interactions with Stiripentol or Valproate when Combined with Cannabidiol in Patients with Epilepsy. *CNS Drugs* **34**, 661–672 (2020).

145. McNamara, N. A. *et al.* Thrombocytopenia in pediatric patients on concurrent cannabidiol and valproic acid. *Epilepsia* **61**, e85–e89 (2020).

146. Ujváry, I. & Hanuš, L. Human Metabolites of Cannabidiol: A Review on Their Formation, Biological Activity, and Relevance in Therapy. *Cannabis Cannabinoid Res* **1**, 90–101 (2016).

147. Gaston, T. E., Bebin, E. M., Cutter, G. R., Liu, Y. & Szaflarski, J. P. Interactions between cannabidiol and commonly used antiepileptic drugs. *Epilepsia* **58**, 1586–1592 (2017).

148. Klotz, K. A., Hirsch, M., Heers, M., Schulze-Bonhage, A. & Jacobs, J. Effects of cannabidiol on brivaracetam plasma levels. *Epilepsia* **60**, e74–e77 (2019).

149. Lattanzi, S. *et al.* Efficacy and Safety of Cannabidiol in Epilepsy: A Systematic Review and Meta-Analysis. *Drugs* **78**, 1791–1804 (2018).

150. Lattanzi, S. *et al.* Cannabidiol as adjunctive treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome. *Drugs Today (Barc)* **55**, 177–196 (2019).

151. Lattanzi, S. *et al.* Practical use of pharmaceutically purified oral cannabidiol in Dravet syndrome and Lennox-Gastaut syndrome. *Expert Rev Neurother* **21**, 99–110 (2021).

152. Skinner, C. M. *et al.* Potential Probiotic or Trigger of Gut Inflammation - The Janus-Faced Nature of Cannabidiol-Rich Cannabis Extract. *J Diet Suppl* **17**, 543–560 (2020).

153. Sands, T. T. *et al.* Long-Term Safety, Tolerability, and Efficacy of Cannabidiol in Children with Refractory Epilepsy: Results from an Expanded Access Program in the US. *CNS Drugs* **33**, 47–60 (2019).

154. FDA Briefing Document Peripheral and Central Nervous System Drugs Advisory Committee Meeting. (2018).

155. CHMP. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS.

156. Gastaut, H. [Efficacy of fenfluramine for the treatment of compulsive behavior disorders in psychotic children]. *Presse medicale (Paris, France : 1983)* **13**, 2024–5 (1984).

157. Gastaut, H., Zifkin, B. & Rufo, M. Compulsive respiratory stereotypies in children with autistic features: polygraphic recording and treatment with fenfluramine. *J Autism Dev Disord* **17**, 391–406 (1987).

158. Clemens, B. Dopamine agonist treatment of self-induced pattern-sensitive epilepsy. A case report. *Epilepsy Res* **2**, 340–343 (1988).

159. Boel, M. & Casaer, P. Add-on therapy of fenfluramine in intractable self-induced epilepsy. *Neuropediatrics* **27**, 171–173 (1996).

160. Claes, L. *et al.* De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* **68**, 1327–1332 (2001).

161. Ceulemans, B. *et al.* Successful use of fenfluramine as an add-on treatment for Dravet syndrome. *Epilepsia* **53**, 1131–1139 (2012).

162. Schoonjans, A. *et al.* Low-dose fenfluramine significantly reduces seizure frequency in Dravet syndrome: a prospective study of a new cohort of patients. *Eur J Neurol* **24**, 309–314 (2017).

163. Nabbout, R. *et al.* Fenfluramine for Treatment-Resistant Seizures in Patients With Dravet Syndrome Receiving Stiripentol-Inclusive Regimens: A Randomized Clinical Trial. *JAMA Neurol* **77**, 300–308 (2020).

164. Lagae, L. *et al.* Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet* **394**, 2243–2254 (2019).

165. Sullivan, J. *et al.* Fenfluramine HCl (Fintepla ®) provides long-term clinically meaningful reduction in seizure frequency: Analysis of an ongoing open-label extension study. *Epilepsia* **61**, 2396–2404 (2020).

166. Specchio, N., Pietrafusa, N., Ferretti, A., Trivisano, M. & Vigevano, F. Successful use of fenfluramine in nonconvulsive status epilepticus of Dravet syndrome. *Epilepsia* **61**, 831–833 (2020).

167. Specchio, N. *et al.* Efficacy and safety of Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: A real-world study. *Epilepsia* **61**, 2405–2414 (2020).

168. Strzelczyk, A. *et al.* Efficacy, tolerability, and retention of fenfluramine for the treatment of seizures in patients with Dravet syndrome: Compassionate use program in Germany. *Epilepsia* **62**, 2518–2527 (2021).

169. Bishop, K. I. *et al.* Improved everyday executive functioning following profound reduction in seizure frequency with fenfluramine: Analysis from a phase 3 long-term extension study in children/young adults with Dravet syndrome. *Epilepsy Behav* **121**, (2021).

170. Spedding, M. *et al.* Neural control of dieting. *Nature* **380**, 488 (1996).

171. Wurtman, R. J. & Wurtman, J. Fenfluramine: Back From the Dead. *Clin Ther* **40**, 1420–1422 (2018).

172. Guy-Grand, B. *et al.* International trial of long-term dexfenfluramine in obesity. *Lancet* **2**, 1142–1145 (1989).

173. Fuller, R. W., Snoddy, H. D. & Robertson, D. W. Mechanisms of effects of d-fenfluramine on brain serotonin metabolism in rats: uptake inhibition versus release. *Pharmacol Biochem Behav* **30**, 715–721 (1988).

174. Caccia, S., Conforti, I., Duchier, J. & Garattini, S. Pharmacokinetics of fenfluramine and norfenfluramine in volunteers given D- and DL-fenfluramine for 15 days. *Eur J Clin Pharmacol* **29**, 221–224 (1985).

175. BONNYCASTLE, D. D., GIARMAN, N. J. & PAASONEN, M. K. Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. *Br J Pharmacol Chemother* **12**, 228–231 (1957).

176. Favale, E., Rubino, V., Mainardi, P., Lunardi, G. & Albano, C. Anticonvulsant effect of fluoxetine in humans. *Neurology* **45**, 1926–1927 (1995).

177. Jobe, P. C. & Browning, R. A. The serotonergic and noradrenergic effects of antidepressant drugs are anticonvulsant, not proconvulsant. *Epilepsy Behav* **7**, 602–619 (2005).

178. Sourbron, J., Smolders, I., de Witte, P. & Lagae, L. Pharmacological Analysis of the Anti-epileptic Mechanisms of Fenfluramine in scn1a Mutant Zebrafish. *Front Pharmacol* **8**, (2017).

179. Tupal, S. & Faingold, C. L. Serotonin 5-HT 4 receptors play a critical role in the action of fenfluramine to block seizure-induced sudden death in a mouse model of SUDEP. *Epilepsy Res* **177**, (2021).

180. McNamara, J. O. Cellular and molecular basis of epilepsy. *J Neurosci* **14**, 3413–3425 (1994).

181. Mayer, M. L. Glutamate receptor ion channels. *Current Opinion in Neurobiology* **15**, 282–288 (2005).

182. Rodríguez-Muñoz, M., Sánchez-Blázquez, P. & Garzón, J. Fenfluramine diminishes NMDA receptor-mediated seizures via its mixed activity at serotonin 5HT2A and type 1 sigma receptors. *Oncotarget* **9**, 23373–23389 (2018).

183. Martin, P. *et al.* Fenfluramine acts as a positive modulator of sigma-1 receptors. *Epilepsy Behav* **105**, (2020).

184. Choi, S. J., Blake, V., Cole, S. & Fernstrom, J. D. Effects of chronic fenfluramine administration on hypothalamic neuropeptide mRNA expression. *Brain Res* **1087**, 83–86 (2006).

185. Boisvert, J. P., Boschuetz, T. J., Resch, J. M., Mueller, C. R. & Choi, S. J. Serotonin mediated changes in corticotropin releasing factor mRNA expression and feeding behavior isolated to the hypothalamic paraventricular nuclei. *Neurosci Lett* **498**, 213–217 (2011).

186. Cattaneo, S., Verlengia, G., Marino, P., Simonato, M. & Bettegazzi, B. NPY and Gene Therapy for Epilepsy: How, When,... and Y. *Front Mol Neurosci* **13**, (2021).

187. Richichi, C. *et al.* Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. *J Neurosci* **24**, 3051–3059 (2004).

188. Odi, R., Invernizzi, R. W., Gallily, T., Bialer, M. & Perucca, E. Fenfluramine repurposing from weight loss to epilepsy: What we do and do not know. *Pharmacol Ther* **226**, (2021).

189. Gammaitoni, A., Smith, S. & Boyd, B. The Lack of Effect of Food on the Pharmacokinetics of ZX008 (Fenfluramine Oral Solution): Results of a Single-dose, Two-period Crossover Study. *Clin Ther* **40**, 1338–1346 (2018).

190. Boyd, B., Smith, S., Gammaitoni, A., Galer, B. S. & Farfel, G. M. A phase I, randomized, open-label, single-dose, 3-period crossover study to evaluate the drug-drug interaction between ZX008 (fenfluramine HCl oral solution) and a regimen of stiripentol, clobazam, and valproate in healthy subjects. *Int J Clin Pharmacol Ther* **57**, 11–19 (2019).

191. Caccia, S., Ballabio, M. & de Ponte, P. Pharmacokinetics of fenfluramine enantiomers in man. *Eur J Drug Metab Pharmacokinet* **4**, 129–132 (1979).

192. Fintepla | European Medicines Agency. https://www.ema.europa.eu/en/medicines/human/EPAR/fintepla.

193. BECKETT, A. H. & BROOKES, L. G. The metabolism and urinary excretion in man of phentermine, and the influence of N-methyl and p-chloro-substitution. *Journal of Pharmacy and Pharmacology* **23**, 288–294 (1971).

194. Giraud, C. *et al.* In vitro and in vivo inhibitory effect of stiripentol on clobazam metabolism. *Drug Metab Dispos* **34**, 608–611 (2006).

195. Tran, A. *et al.* Influence of stiripentol on cytochrome P450-mediated metabolic pathways in humans: in vitro and in vivo comparison and calculation of in vivo inhibition constants. *Clin Pharmacol Ther* **62**, 490–504 (1997).

196. Gross, A. S., Phillips, A. C., Rieutord, A. & Shenfield, G. M. The influence of the sparteine/debrisoquine genetic polymorphism on the disposition of dexfenfluramine. *Br J Clin Pharmacol* **41**, 311–317 (1996).

197. BECKETT, A. H. & BROOKES, L. G. The metabolism and urinary excretion in man of phentermine, and the influence of N-methyl and p-chloro-substitution. *J Pharm Pharmacol* **23**, 288–294 (1971).

198. Wheless, J. W., Fulton, S. P. & Mudigoudar, B. D. Dravet Syndrome: A Review of Current Management. *Pediatr Neurol* **107**, 28–40 (2020).

199. Ceulemans, B., Schoonjans, A. S., Marchau, F., Paelinck, B. P. & Lagae, L. Five-year extended follow-up status of 10 patients with Dravet syndrome treated with fenfluramine. *Epilepsia* **57**, e129–e134 (2016).

200. Nabbout, R. *et al.* Fenfluramine for Treatment-Resistant Seizures in Patients With Dravet Syndrome Receiving Stiripentol-Inclusive Regimens: A Randomized Clinical Trial. *JAMA Neurol* **77**, 300–308 (2020).

201. Lai, W. W. *et al.* Cardiovascular safety of fenfluramine in the treatment of Dravet syndrome: Analysis of an ongoing long-term open-label safety extension study. *Epilepsia* **61**, 2386–2395 (2020).

202. Gil-Nagel, A. *et al.* Treatment with fenfluramine in patients with Dravet syndrome has no long-term effects on weight and growth. *Epilepsy Behav* **122**, (2021).

203. Kang, J. S. & Lee, M. H. Overview of therapeutic drug monitoring. *Korean J Intern Med* **24**, 1–10 (2009).

204. Buclin, T. *et al.* The Steps to Therapeutic Drug Monitoring: A Structured Approach Illustrated With Imatinib. *Front Pharmacol* **11**, (2020).

205. Patsalos, P. N., Spencer, E. P. & Berry, D. J. Therapeutic Drug Monitoring of Antiepileptic Drugs in Epilepsy: A 2018 Update. *Ther Drug Monit* **40**, 526–548 (2018).

206. Dubois, S. *et al.* A Volumetric Absorptive Microsampling Technique to Monitor Cannabidiol Levels in Epilepsy Patients. *Front Pharmacol* **11**, (2020).

207. Striano, S., Striano, P., Capone, D. & Pisani, F. Limited place for plasma monitoring of new antiepileptic drugs in clinical practice. *Med Sci Monit* **14**, (2008).

208. McKee, P. J. W., Percy-Robb, I. & Brodie, M. J. Therapeutic drug monitoring improves seizure control and reduces anticonvulsant side-effects in patients with refractory epilepsy. *Seizure* **1**, 275–279 (1992).

209. Guerra Valero, Y. C. *et al.* Clinical application of microsampling versus conventional sampling techniques in the quantitative bioanalysis of antibiotics: a systematic review. *Bioanalysis* **10**, 407–423 (2018).

210. Lei, B. U. W. & Prow, T. W. A review of microsampling techniques and their social impact. *Biomed Microdevices* **21**, (2019).

211. Barco, S. *et al.* A UHPLC-MS/MS method for the quantification of Δ9-tetrahydrocannabinol and cannabidiol in decoctions and in plasma samples for therapeutic monitoring of medical cannabis. *Bioanalysis* **10**, 2003–2014 (2018).

212. Velghe, S. & Stove, C. P. Volumetric absorptive microsampling as an alternative tool for therapeutic drug monitoring of first-generation anti-epileptic drugs. *Anal Bioanal Chem* **410**, 2331–2341 (2018).

213. Harahap, Y., Diptasaadya, R. & Purwanto, D. J. Volumetric Absorptive Microsampling as a Sampling Alternative in Clinical Trials and Therapeutic Drug Monitoring During the COVID-19 Pandemic: A Review. *Drug Des Devel Ther* **14**, 5757–5771 (2020).

214. Fisher, R. S. *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* **58**, 522–530 (2017).

215. Colasante, G. *et al.* dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice. *Mol Ther* **28**, 235–253 (2020).

216. Ricci, R. & Colasante, G. CRISPR/dCas9 as a Therapeutic Approach for Neurodevelopmental Disorders: Innovations and Limitations Compared to Traditional Strategies. *Dev Neurosci* **43**, 253–261 (2021).

ACKNOWLEDGMENTS

Desidero innanzitutto ringraziare il mio Relatore, il Professor Pasquale Striano, figura di riferimento brillante: grazie per aver riposto fiducia in me sin dal primo giorno e per avermi fatta interfacciare a questo interessante ambito della pediatria.
Ringrazio la mia Correlatrice, la Dottoressa Antonella Riva, per la precisione e la competenza e con cui mi ha seguita in tutti questi mesi di elaborazione della tesi e negli altri stimolanti progetti.

Ringrazio tutto il reparto di Neurologia pediatrica e Malattie muscolari, dove ho potuto imparare molto sia dal punto di vista umano che didattico.

Grazie alla Professoressa Elena Gardella per avermi accolta con dolcezza ed integrata nell'efficiente e divertente team di Filadelfia. Rappresenta per me un bellissimo esempio, sia come persona che come medico e ricercatrice. Porterò sempre nel cuore i mesi di tirocinio, le esperienze e gli studi svolti in Danimarca.

Ringrazio la Dottoressa Raffaella Re, pediatra talentosa, nutrizionista attenta e donna forte: mi ha ispirata ad intraprendere questo percorso, trasmettendomi sempre supporto e positività.

Sono estremamente grata ai miei genitori, Angeli di nome e di fatto: mi avete vista studiare per infinite ore e rinunciare a tante cose, mi avete incoraggiata nei momenti di ansia ed abbracciata ad ogni traguardo raggiunto. Mi avete conferito radici solide e ali vigorose: so di poter salpare verso i miei obiettivi avendo sempre un porto sicuro alle spalle.

Vi devo tanto e spero di rendervi sempre orgogliosi.

Grazie a mia Zia Marina e a mia sorella Lora, donne che stimo molto e che mi dedicano sempre incoraggiamenti e affetto profondo. Un ringraziamento anche a mio fratello e a mia cugina Valentina per tutto quello che abbiamo vissuto insieme; a mio Zio Piero, alle mie nipoti Elisa e Chiara, ai miei prozii Gian Carlo e Lina per i dolci abbracci.
Siete una famiglia meravigliosa.
"Chissà se riuscirò a vederti dottoressa" dicevi baciandomi la fronte china sui libri: grazie per le cose buone che preparavi e le canzoni con cui mi cullavi, cara nonna.

Ringrazio Marco e tutta la sua famiglia: non dimenticherò mai ciò che abbiamo condiviso, la gioia contagiosa dei nipotini, i racconti di Paolo e i gesti premurosi di Sandra.

Grazie ad Elena, Andreea, Marika, Elena e Chiara, sono fiera delle persone che siete e dell'amicizia preziosa che coltiviamo da tanti anni: in qualsiasi parte del mondo siate, so di poter sempre contare su di voi. Il bene che vi voglio non vede confini, ma solo luminosi orizzonti da perseguire insieme. Grazie anche a Guido, Stefano ed Esu, anime buone e sempre pronte a strapparmi una risata.

Ringrazio gli amici conosciuti in università e con cui spero di continuare a crescere: Margherita, inesauribile fonte di entusiasmo in ogni giorno di questi sei anni, tra aule studio anche a distanza in quarantena e sorellanza; Ginevra, coinquilina e confidente d'eccezione, compagna di ripetizioni dall'ora della sveglia all'ora in cui andare a dormire, ma anche di allenamenti rigeneranti e molto altro; Giulia, per l'incredibile sintonia che ci unisce in tutto e si riflette nella precisa organizzazione

sia degli esami che dei viaggi insieme, compiuti e da compiere; Virginia, travolgente e delicata brezza fresca, è per me una dinamica fonte di brillante innovazione; Erica, che splende di saggezza e tenacia, ed è sempre un piacere analizzare noi stesse e la realtà che ci circonda camminando insieme per ore; Sara, amica allegra e profonda, che ha sempre in serbo una battuta e un dolce consiglio; Gaia, saggia ed energica spalla sempre presente, per i nostri caratteri complementari che ballano insieme; Matteo, per lo stile di vita sano che apprezziamo e la fiducia che nutro nei tuoi confronti; grazie ad Arianna e Nicolò per aver alleggerito questi anni con la vostra ironia, gli spunti di riflessione ed il costante supporto reciproco; grazie anche ad Andrea e Francesca per le chiacchiere e le risate. Siamo un gruppo stupendo e ci auguro di continuare ad esserlo.

Ringrazio Giacomo, spirito altruista e pacato, per l'allegria e la melodia che trasmette alle mie corde anche quando sono tese, e per condividere con me riflessioni e progetti volti a fare del bene al prossimo.

Grazie all'Università di Genova e a questa città che è diventata casa, grazie a tutti i compagni con cui ho scambiato due chiacchiere confortanti prima di un esame oppure un sorriso dentro o fuori le aule.

Infine, ringrazio me stessa per aver affrontato questo percorso con passione, dedizione, empatia e curiosità: caratteristiche fondamentali del Medico che mi auguro di essere. Sono stati anni densi di sacrifici, ansia e rinunce, ma anche di apprendimenti e soddisfazioni. Sono orgogliosa di questo traguardo e della professione che mi accingo ad esercitare.
A tutto quello che è stato, a tutto quello che sarà.