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Italian Expanded Access Program data of Cannabidiol (Epidiolex) in Dravet Syndrome at Gaslini Children's Hospital.

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ABSTRACT

Background: Cannabidiol (CBD) is one of the major components of the Cannabis sativa plant. In recent years, CBD has shown anti-seizure properties without having psychoactive effects. We evaluated the efficacy, safety, and tolerability of a purified GW CBD (Epidiolex) for the treatment of highly refractory patients with Dravet syndrome (DS) through an ongoing expanded access program (EAP).

Methods: Children and young adults with DS were recruited. Patients received add-on treatment with purified GW CBD up to a maximum of 25 mg/kg/day. Adverse events (AEs) and blood laboratory tests were assessed at weeks 2, month 1, 3, 6 and 12 of treatment. Seizure endpoints were the percentage of patients with ≥50% (responders), or <50% (partial responders) reduction in both convulsive and total seizures as compared to baseline. Concomitant anti-seizure medications (ASMs) were recorded at baseline and monitored during the study.

Results: 6 patients were enrolled (age range 4-24). At baseline, the median monthly frequency of convulsive and total seizures was 5.5 (range, 1-56) and 6.0 (range, 1-84). At month 12, as compared to the baseline, 2 (33.3%) patients showed ≥ 50% reduction in both total and convulsive seizures frequency, while 2 (33,3%) patients showed <50% reduction. One (16.6%) patient early discontinued due to AEs. Main AEs were somnolence (16.6 %), inappetence (16.6%), and elevated liver enzymes (16.6%). Some patients decreased the dose of concomitant ASMs: 33.3% of the patients decreased Clobazam and Topiramate; Stiripentol and Valproic Acid were adjusted to lower dose in 25% and 16.6% of patients, respectively.

Conclusions: A significant reduction in both convulsive and total seizures, as well as an improvement in the quality of life and behaviour, was observed in patients treated with CBD. Interestingly, some patients decreased the dose of concomitant ASMs (particularly clobazam), pointing towards CBD effectiveness independently of other concomitant treatments.

INTRODUCTION

1.1 DRUG-RESISTANT EPILEPSY, DRAVET SYNDROME AND LENNOX-GASTAUT SYNDROME

Epilepsy is one of the most common brain disorders in the world. Approximately 70% of epilepsy patients become seizure-free with monotherapy and the remaining 30% require more extensive treatment with two or more Anti-Seizure Medications (ASMs) to control seizures. 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 antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" (1).

Hence, DRE is observed in those patients who do not achieve complete control of (disabling) seizures (2). For these patients, refractoriness to treatment mainly impact on the quality of life (QoL).

The definition of DRE depends also on the accuracy of the diagnosis, the natural history of the underlying epilepsy syndrome, and the available treatment options (3).

Lennox–Gastaut syndrome (LGS) and Dravet syndrome (DS) are two main examples of developmental and epileptic encephalopathies (DEE) typically refractory to common ASMs (4).

DS (previously known as severe myoclonic epilepsy of infancy (SMEI)), is a rare drug-resistant form of epilepsy that occurs in the first year of life in otherwise

previously healthy children and is accompanied by impaired psychomotor and neurologic development (5). In this syndrome, epilepsy is not limited to infancy and childhood, but persists through adulthood. In 2001, mutations in the sodium channel alpha1 subunit (SCN1A) gene were discovered in individuals with DS (6). SCN1A mutations, usually de novo, are found in 70-80% of patients with DS (6). Seizure typically start in the first year of life, usually between 5 and 8 months, with prolonged, febrile and afebrile, hemiclonic or generalized clonic seizures. The first seizure is typically clonic, generalized, or unilateral, triggered by fever and lasting longer than a simple febrile seizure. Nevertheless, in some cases the first seizures can be focal (5). Seizures may present in association with hyperthermia (for example, after a warm bath), fever, or vaccinations (7). During the following months, affected subjects often experience recurrent febrile and afebrile seizures that frequently affect alternate sides of the body. Between one and four years of age, other seizure types such as myoclonic and atypical absences, focal seizures, and generalized tonic-clonic seizures may develop (7). Focal seizures, with or without impairment of awareness, may be associated with imponent autonomic features such as pallor, cyanosis and drooling, and may evolve into a focal motor or bilateral convulsive seizure. Reflex seizures are frequent, and the most common trigger is hyperthermia (7). The most common types of seizures that patients with DS could experience throughout life are: convulsive seizures, consisting of generalized clonic seizures (GCS), generalized tonic–clonic seizures (GTCS), or alternating unilateral clonic seizures; myoclonic seizures; atypical absences and obtundation status; focal seizures, with or without secondary generalization; or, rarely, tonic seizures. Convulsive seizures, apparently generalized or unilateral, are

present throughout the evolution in all patients. Unilateral seizures are the most characteristic (5). There are also frequent episodes of status epilepticus (8). Neurodevelopment and formal neurological examination are typically normal at the time of seizure onset and diagnosis. However, there is slowing of the rate of the developmental progress along with variable decline in the developmental quotient over time (9). Phenotypes of DS patients are extremely different, including both epileptic and neurological/neuropsychological signs. Neuropsychological phenotypes, in particular, range from exceptional normal competence or specific partial defects up to severe global involvement of all abilities (8).

Cognitive and behavioral impairment usually appears during the second year of life or later, as shown by reports of different neuropsychological studies on early ages (8), (10), (11). There are several studies that show a progressive decline with a steep falling curve until four years of life, evolving later into a generally milder decrease, presenting a progressive worsening from a normal cognitive competence up to severe mental retardation, including several non-testable patients (10), (11), (12), (13). The decline in the first years of life does not correspond to a real cognitive deterioration but rather seems "due to the rising discrepancy between the steady mental age and the increasing chronological age" (10). At 25 years, 71% of patients show an IQ lower than 50 (14). Cognitive and neurological functions (such as motor skills, especially cerebellar and postural) are strongly impaired, being associated in several cases with severe behavioral disorders up to an "autistic" pattern, with a deterioration of mental abilities (15).

LGS is a severe form of DEE, characterized by several seizure types and severe cognitive impairment. In 75% of the cases the etiology of LGS is thought to be symptomatic, implying an identifiable cause, such as a cerebral malformation or hypoxic–ischemic injury (16). Identifiable causes are usually the result of a static brain disorder; progressive metabolic disorders are extremely rare. The other 25% is the cryptogenic group, which has no apparent cause and no neurologic precedents (17).

Seizures usually begin to occur before the age of 8 years, with a peak age of onset of 3–5 years of age, and persist into adulthood in more than 90% of patients (18). Drop seizures, due to an increase in (tonic) or loss of (atonic) motor tone are characteristic of this disorder and often result in serious injury (19). LGS syndrome is usually characterized by a triad of signs: multiple seizure types, slow spike-wave complexes on electroencephalographic (EEG) recordings, and impairment of cognitive function (20). All patients experience tonic seizures during sleep, that may be subtle. Cognitive impairment and behavioral problems are seen in almost all patients and lead to a life of dependency (19). Despite treatment, disabling seizures continue to occur in most patients (21) and nearly all have drug-resistant, lifelong epilepsy (19).

Cognitive impairments are clinically manifest in approximately half of the patients (20-60%) at the time of diagnosis (20). The cognitive impairment usually becomes more apparent over time, and within 5 years of onset, serious intellectual problems have been noted in 75–95% of patients (17). Along with cognitive problems, many

patients may develop behavioral and psychiatric disorders (22). Attentional problems, aggression, and autistic features can be very prominent in LGS.

1.2 TRADITIONAL ASMs IN DRAVET SYNDROME

The aim of the treatment in patients with DS is to significantly reduce seizure frequency (particularly that of prolonged events) with limited ASMs toxicity (23) Certain antiepileptic agents should be totally avoided, as they have been clearly shown to exacerbate seizures in DS. In particular, sodium channel blockers are known to exacerbate DS (24). This pharmacologic intolerance is most likely due to the fact that most patients have a nonsense or missense mutation in the in SCN1A gene, a component of the Nav1.1 sodium channel. Carbamazepine's role in exacerbating seizures in DS has been particularly well described (25); for this reason, carbamazepine and its analogs (oxcarbazepine, eslicarbazepine), as well as Phenytoin are contraindicated. Lamotrigine may also exacerbate seizures and is typically avoided (26).

First-line management typically involves either Valproic Acid (VPA) and Clobazam, but the refractoriness of this epilepsy syndrome typically leads to trials of a number of other therapies. The most efficacious second-line treatments include Topiramate, Stiripentol, and the ketogenic diet, although Levetiracetam and bromides may also be considered (24).

Approved by the EMA in 1967 and the FDA in 1978, VPA is a broad-spectrum ASM effective in the treatment of all generalized epilepsy syndromes in the absence of any specific contraindication (27). This molecule has multiple mechanisms of action:

it enhances gamma-aminobutyric acid (GABA) transmission by increasing GABA synthesis, reducing GABA turnover, and inhibiting GABA degradation; decreases the release of the excitatory amino acid β-hydroxybutyric acid; inhibits *N*-methyl-d-aspartate (NMDA) receptor-mediated excitatory transmission; blocks voltage gated sodium channels (VGSCs) and calcium channels; potentiates calcium-activated potassium currents; and modulates serotonergic and dopaminergic neurotransmission (28). Administration of VPA typically begins at 10–15 mg/kg/day, and most patients reach a maintenance dose of 30–60 mg/kg/day (24), titrated according to efficacy and side effects. Dosing regimens may depend on the formulation.

VPA is widely bound to plasma proteins, following a non-linear pharmacokinetic profile in terms of protein binding saturation, resulting in an increase in free-drug concentration with dose escalation. It is metabolized in the liver through β-oxidation (30%), glucuronidation (40%), ω-oxidation, ω-1 oxidation and other pathways, and its metabolites are renally excreted (29). Among pediatric populations, VPA clearance is significantly influenced by total body weight, daily dose, and concomitant therapy with other ASMs; moreover, children require higher mg/kg doses to obtain serum VPA concentrations comparable with those seen in adults. VPA has the potential for drug interactions. Enzyme-inducing medications, including ASMs as Phenobarbital (PB), Phenytoin (PHT), Carbamazepine (CBZ), Ethosuximide (ETX), and Topiramate (TPM) lower levels of VPA. On the other hand, other ASMs (felbamate, clobazam, and STP) increase VPA concentration. VPA inhibits the metabolism of several drugs and may result in intoxication if the dose of the comedication is not reduced, as shown for LTG, PB, and lorazepam (30).

Therapy with VPA in children has been associated with different Adverse events (AEs) that may increase morbidity and impair treatment adherence. The most frequent AEs are: somnolence, weight gain, fatigue, and headache, as well as hair loss, dizziness, hyperammonemia, and hypocarnitinemia (31). Thrombocytopenia can occur in 5–40% of children receiving VPA, and coagulopathies can occur in up to 4% (32); many of these effects are concentration dependent. The most serious AEs include hepatotoxicity and pancreatitis, which may lead to fatalities. Major risk factors for valproate-related fatalities are polytherapy and early age. Fatal cases of hepatotoxicity can occur at any age but are more common in children aged ≤ 6 years, with a peak incidence between 1 and 2 years because of their abnormal metabolism (33).

Clobazam is a drug of the benzodiazepine class. It is a 1,5-benzodiazepine and acts through potentiation of GABA-A receptors (34). It is a long-acting benzodiazepine with a median half-life of >36 hours (35). Its anticonvulsant and anxiolytic therapeutic effect has repeatedly demonstrated great efficacy and a high safety profile in refractory epilepsy as well as in a few monotherapy trials in both children and adults (36).

Clobazam dosage is between 5 mg and 40 mg a day, depending on patient weight, efficacy, and tolerability (36). It is typically started at a dose of 0.2–0.3 mg/ kg/day divided twice daily and increased over 2–3 weeks to an initial target dose of 0.5–1.0 mg/kg/day. If problematic seizures persist, the dose may be further increased to a maximum of 1.5–2.0 mg/kg/day; however, higher doses are often associated

with increased side effects. Clobazam undergoes hepatic metabolism, through CYP3A4 and CYP2C19.

Clobazam is generally considered safe to use, with only mild side effects when compared to other ASMs. As with any benzodiazepine, there is a risk for dependence. In addition, data from 50 clinical studies collected from over 3000 epileptic adult and pediatric patients show that the most common side effects include sedation, dizziness, and ataxia (37). These adverse effects are dose-dependent.

2.CANNABIDIOL

2.1 CBD FORMULATIONS

The cannabis plant (Cannabis sativa) is a plant of the Cannabaceae family which includes C. indica, C. ruderalis, and C. sativa. Cannabis consists of around 100 compounds known as phytocannabinoids and has 3 physiologically active components: cannabinoids, terpenoids, and flavonoids. Only 16 of the one hundred compounds exist in significant concentrations; these include Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabichromene (CBC), and cannabigerol (CBG) (38).

In recent years, one of these compounds, Cannabidiol (CBD), is receiving particular attention since its pharmacological profile has shown anti-seizure properties without having psychoactive effects such the ones that Δ9-tetrahydrocannabinol (THC) may have (39).

Purified CBD produced by GW pharma (EPIDYOLEX®) is the first of a new class of ASMs (40). This 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 (LGS) for patients aged 2 years and older.

2.2. CBD MECHANISM OF ACTION

Studies on animal models investigating the use of a purified CBD formulation have shown positive effects against several types of seizures and epilepsy (41)

Cannabidiol does not bind directly and does not activate the cannabinoid CB1 and CB2 receptors at clinically relevant concentrations, but it shows affinity and functional agonism or antagonism at multiple 7-transmembrane receptors, neurotransmitter transporters, and ion channels (42).

2.3. CBD PHARMACOKINETICS

The pharmacokinetics of CBD is extremely variable, depending on the different route of administration (e.g., oral, sublingual, intravenous, oromucosal spray, inhalation, and transdermal), the type of product administered, the concomitant intake of food or not, possible drug-drug interactions, and other factors (43).

Absorption of Cannabidiol from the gastrointestinal system is fast, with peak plasma concentrations occurring between 0.5–6 h after oral intake (44). The bioavailability of oral products administration, such as Epidiolex® (>98% CBD, 100 mg/mL), however, is limited (around 6%) due to important first-pass metabolism in the liver (45).

The relationship between bioavailability of CBD and food has been shown, based on observations regarding the administration of GW CBD (1500 mg) together with a high-calories and high-fat meal, assuming that food may affect the oral bioavailability of CBD. In healthy subjects, in fact, an increase of about 5 times the concentration of CBD in plasma occurs when CBD is taken with food. This results have therefore led to the suggestion that CBD should be taken consistently with food (46).

The elimination of CBD follows a biphasic pattern (42). Due to its very high lipophilic properties, CBD distributes extensively into tissues, resulting in a late-phase

terminal half-life of more than 24 hours. Nevertheless, the initial half-life values actually found during the first step of the elimination phase are much shorter, as reflected by an effective half-life in the range of 6-10 hours (42). The effective half-life provides a better estimation of fluctuations in plasma concentrations during the dosing interval, as well as of the time needed to reach a steady-state (about four effective half-lives) (43).

CBD is highly bound to plasma proteins (> 99%) (47) and extensively metabolized by cytochrome P450 (CYP) enzymes (in particular CYP3A4 and CYP2C19) and glucuronyltransferases. (47),(42) The major metabolic pathway involves hydroxylation and oxidation at C-7, followed by further hydroxylation in the pentyl and propenyl groups. The major oxidized metabolite identified is cannabidiol-7-oic acid containing a hydroxyethyl side chain. GW purified CBD is excreted in feces, with minor renal clearance (43).

The clearance of cannabidiol has been reported to be increased after co-administration with the enzyme inducer rifampicin.

2.4. CLINICAL INDICATIONS AND DOSAGES

Cannabidiol should be administered orally twice a day, in two equally divided doses, preferably with meals due to the increase of plasma concentration when administered with food. A dose titration is necessary to achieve the optimal therapeutic effect. (38) Therefore, a slow start and a gradual increase strategy is recommended. The recommended initial dosage is 2.5 mg/kg twice daily (5 mg/kg/day). After one week, the dosage can be increased to a maintenance dosage of 5 mg/kg twice daily (10 mg/kg/day). Patients who tolerate CBD at this dosage of

10 mg/kg/day and need a further reduction of seizures, may benefit from a further increase in the dosage, up to the maximum recommended daily maintenance dosage of 20 mg/kg/day, with weekly increases of 2.5 mg/kg twice daily. The generally tolerated and effective doses for the treatment of patients with Dravet syndrome and Lennox-Gastaut syndrome vary between 10 mg/kg/day and 20 mg/kg/day. When 10 mg/kg/day efficacy and safety is achieved, should pass at least one week before any further dose increase could be assessed. A dose increase above 10 mg/kg/day should be based on clinical response and safety assessment. In particular, liver function tests should be performed. Dose administration of 20 mg/kg/day has led to slightly higher reductions in seizure rates than the dose of 10 mg/kg/day, but with an increase in adverse reactions, so the target dose should be 10 mg/kg/day. In patients with moderate to severe hepatic impairment, slower dose titration may be necessary and dose adjustment is recommended. AEs and liver function tests should be performed approximately 2 weeks after treatment, 2 weeks after the last cannabidiol dose increase, regularly thereafter and on the occurrence of clinically relevant events. The dosage of 20 mg/kg/day should not be exceeded.

2.5. EFFICACY OF CBD TREATMENT IN DRUG-RESISTANT EPILEPSY

The first trials of purified CBD (Epidiolex) were launched as an expanded access program (EAP) in 2014 for patients with significant medically refractory epilepsy, which is associated with severe morbidity and increased mortality.

In 2016, a significant open-label, non-controlled trial for compassionate use made by Devisnky et al. (48) aimed to establish whether the addition of cannabidiol to existing anti-epileptic regimens would be safe, tolerated, and efficacious in children

and young adults with DRE. In this open-label trial, 214 patients (aged 1–30 years) with severe, intractable, childhood-onset, treatment-resistant epilepsy, who were receiving stable doses of ASMs before study entry, were enrolled in an expanded-access program at 11 epilepsy centres across the USA. Patients were given oral cannabidiol at 2–5 mg/kg/day, up-titrated until intolerance or to a maximum dose of 25 mg/kg or 50 mg/kg per day. The primary objective was to establish the safety and tolerability of cannabidiol and the primary efficacy endpoint was median percentage change in the mean monthly frequency of motor seizures at 12 weeks. The median monthly frequency of motor seizures was 30.0 at baseline and 15.8 over the 12week treatment period. The median reduction in monthly motor seizures was 36.5%.

Other encouraging results were published in 2018 by Szaflarski et al. (49), who reported results on the safety and efficacy of CBD in expanded access program patients treated through December 2016, supporting previous data showing that add-on CBD may be an efficacious long-term treatment option for DRE by revealing a reduction of median monthly convulsive seizures by 51% (52% with ≥50% seizure reduction) and total seizures by 48% at 12 weeks, with similar results over the 96 weeks.

In the following years, other controlled trials for Epidiolex were established for DS by Devinsky et al., 2017 and for LGS by Thiele et al., 2018 (50), Devinsky et al., 2018b.

In 2017, Devinsky et al (51) made a double-blind, placebo-controlled trial, where they randomly assigned 120 children and young adults with DS and drug-resistant

seizures to receive either CBD oral solution at a dose of 20 mg/kg/day or placebo, in addition to their standard antiepileptic treatment. The primary end point was the change in convulsive-seizure frequency over a 14-week treatment period, as compared with a 4-week baseline period.

In the cannabidiol group, the primary end point of convulsive-seizure frequency decreased from a median of 12.4 seizures per month at baseline to 5.9 over the entire treatment period, representing a median change of −38.9% (interquartile range, −69.5 to −4.8) from baseline. In the placebo group, the median monthly convulsive seizure frequency decreased from 14.9 to 14.1, representing a median change of −13.3% (interquartile range, −52.5 to 20.2). The adjusted median difference in convulsive seizures between the cannabidiol group and the placebo group was −22.8 percentage points (95% confidence interval [CI], −41.1 to −5.4; P=0.01). The percentage of patients who had at least a 50% reduction in convulsive seizure frequency was 43% with cannabidiol and 27% with placebo (odds ratio, 2.00; 95% CI, 0.93 to 4.30; P=0.08). The patient's overall condition improved by at least one category on the seven-category Caregiver Global Impression of Change (CaGI) scale in 62% of the CBD group as compared with 34% of the placebo group (P=0.02). The frequency of total seizures of all types was significantly reduced with cannabidiol (P=0.03), but there was no significant reduction in nonconvulsive seizures. The percentage of patients who became seizure-free was 5% with cannabidiol and 0% with placebo (P=0.08).

In 2019, this study continued as an open-label extension program (52) to evaluate long-term CBD treatment in patients with DS. This study confirms and extends

previous findings, demonstrating that add-on CBD treatment in patients with DS had an acceptable safety profile and reduced the frequency of total and convulsive seizures up to 48 weeks of treatment. Overall, the safety profile of CBD was similar to that observed in the previous 14-week, randomized controlled trial. The median percentage reduction in total seizures continued between 39% and 51% over 48 weeks.

2.6. DRUG INTERACTIONS

CBD modulates several cytochrome P450 (CYP) enzymes, which are of potential interest in investigating interactions with other medications (53). It is a potent inhibitor of CYP2C19, CYP2D6, CYP2C9, and a potential inhibitor of the CYP3 family.(54) Based on what is known about CBD's metabolism and the metabolism of other ASMs, we can assume that there could be many interactions due to the important involvement of the CYP enzymes in the metabolism of both CBD and other ASMs. Several studies have identified a pharmacokinetic interaction with clobazam. (55) In 2015, Geffrey et. al (56) studied 13 children who were taking concomitant clobazam with purified CBD. The mean clobazam and N-desmethylclobazam plasma levels were increased after treatment with CBD compared to baseline. These increased levels led to reduction of clobazam dose due to reports of sedation. This interaction was felt to be caused by CBD’s potent inhibition of CYP2C19, that is, the enzyme responsible for metabolizing N-desmethylclobazam. In fact, CBD increases the plasma concentrations of drugs metabolized by CYP2C19 such as diazepam or clobazam (57). This pharmacokinetic interaction may be at least partially responsible for the higher proportion of somnolence among patients receiving CLB as concomitant medication in the randomized placebo-controlled trials of add-on

CBD (58). Interestingly, in these studies the increase in serum concentrations of N-CLB did not occur in patients who were treated with stiripentol; this drug, similarly to CBD, is a potent CYP 2C19 inhibitor, and the lack of further elevation in N-CLB levels after exposure to CBD could be explained by prior saturation of the cytochrome isoenzyme.

In 2017, Gaston et al. measured the blood levels of all ASMs taken by 39 adult and 42 pediatric patients in the study prior to start treatment with CBD and at every study follow-up visit (53). With increasing CBD dose, there were statistically significant increases in levels of clobazam, N-desmethylclobazam, rufinamide, and topiramate in all patients. However, the mean changes in levels exceeded normal therapeutic range only for clobazam and N-desmethylclobazam. Patients taking concomitant valproate had statistically significant changes in mean aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) levels compared to others, though valproate levels did not change significantly from baseline.

In 2018, Devinsky et al (59). also focused on pharmacokinetics, confirming that approximately 30% of the patients receiving both CBD and valproate developed elevated transaminases liver enzymes. However, none of these elevations met criteria for drug- induced liver injury and all patients were reported to recover.

2.7. ADVERSE EVENTS

In humans receiving CBD for neuropsychiatric disorders, the most common AEs include somnolence, diarrhea, nausea, decreased appetite, and hepatotoxicity with transaminases elevation. Overall, the incidence of these events is low and, in

comparison with other drugs employed for the treatment of these diseases, CBD has a better side effect profile (57). Two of the most common AEs after CBD administration are somnolence and sedation (48). These effects are dose-related and potentiated by co-administration of the antiepileptic drugs, including clobazam. The vast majority of AEs were mild and moderate, dose-related and consistent with the tolerability profile that emerged during the open-label administration of CBD in severe refractory epilepsies (38). Serious AEs were far less common.

2.7.1. *Somnolence*

Somnolence was the most common AE encountered with CBD and it was more likely to occur when CBD was co-administered with CLB (55). The pharmacokinetic interaction between CLB and CBD may explain the more frequent occurrence of drowsiness compared to other patients. Therefore, in case of the introduction of cannabidiol, a reduction of clobazam dose may be considered in advance, suggesting the need to strictly monitor patients in treatment with CLB and adjust doses as necessary.

2.7.2. *Elevated Transaminases*

Transaminases elevation represent the other most frequent AE and account for half of the drug discontinuations across the phase III trials (55). Most transaminase elevations occurred within the first 30 days of use, although there were also cases starting after 6 months. Notably, the risk window was longer for patients taking concomitant VPA. An increase in ALT or AST concentrations by ≥ 3-fold the upper limit of the normal range was reported in approximately 15% of the patients randomized to CBD and represented the main reason for treatment withdrawal (58).

As CBD has no meaningful effects on VPA concentrations, the nature of this interaction is thought to be mostly pharmacodynamic rather than pharmacokinetic. All cases resolved either spontaneously during the treatment period or open-label extension trial, or after the dose of a concomitant ASM was reduced, or after CBD was tapered or discontinued. Slow titration and close monitoring of serum transaminases and signs suggestive of hepatic toxicity, above all during the initial phases of treatment and in patients concomitantly taking VPA, are recommended.

3. THERAPEUTIC DRUG MONITORING AND VAMS MICROSAMPLING

3.1 THERAPEUTIC DRUG MONITORING

ASMs have been one of the most common medications for which therapeutic drug monitoring (TDM) is performed. Traditionally, TDM has been applied mainly to the first-generation ASMs (carbamazepine, phenobarbital, phenytoin, primidone, and valproic acid) but in the last years also the newer antiepileptic drugs plasma levels have been monitored through TDM (60). In refractory epilepsy, the relationship between the administered dose and CBD blood levels has been demonstrated in some studies (56), (61) and has provided a starting point for the use of TDM also for CBD-based therapies. TDM is useful in clinical practice as it allows to obtain the ideal dose of cannabis-based therapy, based on the identification of the individual concentration associated with an optimal response. (60) Moreover, in polypharmacy TDM can prevent drug interactions by guiding dose adjustments and minimizing toxicity (62),(63). Factors that can negatively affect the correlation between clinical effect and serum/plasma concentration include 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.

3.2 VAMS MICROSAMPLING

Microsampling techniques based on dried blood spots allow a reliable and non-invasive collection of small blood volumes (60), (64). Recently, VAMS (Volumetric Absorptive Microsampling) have been introduced in the market, and successfully applied to several quantitative TDM methods. VAMS are porous hydrophilic tips that

allows the collection of a fixed volume of blood (10 or 30 µl) avoiding the effect of hematocrit (HCT) on the analytical performances (65). In a recent study (66) the use of VAMS was reported for the monitoring of various first-generation ASMs, i.e. valproate and carbamazepine. In 2020, (60) VAMS was evaluated 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 refractory epilepsy. In particular, CBD concentrations in capillary and venous blood obtained by micro-sampling was compared with CBD concentration in plasma, which is the matrix most frequently used for TDM in epilepsy patients.

4. AIM OF THE STUDY

We present an open-label, prospective, add-on study on the clinical use of a purified GW cannabidiol (CBD) Epidiolex® in children and young adults with highly refractory DS.

The aim of the study is to evaluate purified GW cannabidiol (CBD) Epidiolex® efficacy, safety, and tolerability, as well as the need for concomitant ASMs dose-adjustments in DS patients through a 12 months follow-up.

5.METHODS

5.1 PATIENTS

Children and young adults with DS were recruited at the Pediatric Neurology and Muscular Diseases Unit of the IRCCS Istituto Giannina Gaslini, Genoa, Italy between February 2019 and June 2021. They received add-on treatment with GW purified CBD at dosages up to a maximum of 25 mg/kg/day. Written informed consent was provided by patients or their parents/caregivers. The study was conducted following the Good Clinical Practice guidelines and local standard operating procedures.

5.2 PROCEDURES

Eligible patients underwent a "screening visit" and, after a 4-week baseline period, in which diaries of all countable seizures were provided, they received an oral solution of purified CBD (100mg/mL; Epidiolex GW Research Ltd) at starting dose ranging between 2-5 mg/Kg/die up to 18-25 mg/Kg/die.

Clinical and treatment data, as well as laboratory tests and reported AEs, were followed-up at regularly scheduled visits at week 2 and months 1, 3, 6 and 12 and periodically thereafter. AEs were classified using the Medical Dictionary for Regulatory Activities (MedDRA, version 22.0). Particular attention was paid to seizures, which were defined as generalized (tonic, clonic, tonic-clonic, atonic, myoclonic, absences, or myoclonic-absences) or focal-onset seizures with or without impaired consciousness basing on the criteria provided by the ILAE (67).

Concomitant ASMs were recorded at baseline and during all the treatment period. CBD and ASMs doses modification, as well as adding/removing co-ASMs, were allowed as clinically indicated.

All AEs were reported and detailed as severe or leading to discontinuation as appropriate. Finally, the incidence of AEs has been reported according to concomitant ASMs.

CBD blood concentration was monitored by both venipuncture and VAMS microsampling. After one month of treatment, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used for the analysis of cannabidiol dose in whole blood samples collected from patients by VAMS, a less painful fingerpicking (64), (60).

The laboratory tests roughly included FBC, serum sodium, potassium, chloride, creatinine, ALT, AST, total bilirubin, INR, glucose and were performed at baseline (within 2 weeks after initiation of cannabidiol treatment) and repeated during the follow-up visits.

Unscheduled visits were performed when clinically relevant for the evaluation of AEs.

5.3 ASSESSMENT OF EFFECTIVENESS

A seizure diary was provided to patients' parents/caregivers in order to strictly monitor the changes in the number of seizures throughout the study.

Seizure frequency was provided per week since the previous visit and efficacy outcome were assessed at 3, 6 and 12 months. According to other published studies (49), (68) 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. Median percentage changes in seizure frequency were calculated due to interpatient variability.

Seizure endpoints were the percentage of patients experiencing a reduction in monthly convulsive and total seizures as compared to 4-week baseline (responder rate). In particular, total responders (seizure reduction >50% or 100%) and partial responder patients (seizure reduction <50%). Additional variables evaluated were episodes of status epilepticus, use of rescue medications, and hospital admissions.

Questionnaires on quality of life (*i.e.,* QOLIE-31), sleep disturbance (*i.e.,* Sleep Disturbance Scale for Children, Epworth sleepiness scale), behavior (Neurological disorders depression inventory for epilepsy, Child Behavior Check List, Beck Depression Inventory for Primary Care) and the clinical global impression were also provided and collected during the study.

6.RESULTS

6.1 CLINICAL FEATURES

A total of 6 patients (1 female and 5 males) were enrolled. In the safety dataset, one patient dropped-out after 3 months of treatment due to lack of efficacy and reported AEs, particularly increase of absence seizures and somnolence. Overall, the mean (SD) treatment duration was 12 months, effectiveness data through 12 months was available for all patients.

The mean age of patients at baseline was 13.5 years (range 4-24 years), 100% had Dravet Syndrome, 83.3% were males and 16.7% females. More accurate demographic and clinical features at baseline are shown in **Table 1**. At baseline, the median and the mean number of concomitant ASMs was 3 (range 1-5).

Concomitant ASMs are detailed in **Table 3**. The most common concomitant medications were Valproic acid (100%), Stiripentol (66.7 %), Clobazam (50 %), and Topiramate (50%). The mean doses at baseline treatment were 673 mg/die for Valproate, 1063 mg/die for Stiripentol, 11.67 mg/die for Clobazam, and 133.33 mg/die for Topiramate. Levetiracetam and Phenobarbital was assumed in one patient each.

Table 1. Patients baseline demographic and clinical features

Age [years], mean	13.51
Sex M/F, n (%)	5 (83.33) / 1(16.66)
Body Weight [Kg], mean.	47
Paediatrics/adults, n (%)	5 (83.33) / 1(16.66)
Diagnosis	
Dravet, n (%)	6 (100%)
Lennox-Gastaut, n (%)	0 (0%)
Concomitant ASMs at baseline, median (Q1-Q3)	3 (3-3)
Convulsive seizures/28d, median (Q1-Q3) *	5.5 (8-3)
Total seizures/28d, median (Q1-Q3) *	6 (9-3)

*Legend: ASMs, antiseizure medications. *during 4-weeks baseline period*

6.2. SEIZURE OUTCOMES

At baseline, the median (Q1, Q3) monthly frequency of convulsive and total seizures was 5.5 (range from 1 to 56) and 6 (from 1 to 84) (**Table 1**). At 3 months of follow-up, compared to the 28-day baseline period, the percentage of patients with at least a 50% reduction in both total and convulsive seizure frequency was 33.3% (0 % seizure-free), whereas 16.6 % had a reduction < 50%; 16.6 % for total seizure and 33.3% for convulsive seizures had no change , 33.3 % for total seizure and 16.6% for convulsive experienced seizures worsening (Table 2). At 6 months follow-up, the percentage of patients with at least a 50% reduction in seizure frequency was

16.6% for both total and convulsive seizures (0 % seizure-free), whereas 50 % had a reduction < 50%, 0 % had no change, and 16.6 % showed seizures worsening. Lastly, at months 12, the percentage of patients with at least a 50% reduction in both total and convulsive seizure frequency was 33.3 % (0 % seizure-free), whereas 16.6 % had a reduction < 50%, 16.6 % had no change and 16.6 % seizures worsening (**Table 2**). No significant difference in achieving the responder status at months 12 was found between patients co-treated with Clobazam and those not taking Clobazam.

The mean and median dose of CBD between months 3 and 12 were 14.5 mg/Kg/die and 16 mg/kg/die, respectively. No patients required CBD dose reduction at any time during the follow-up, but one patient dropped-out at month 3. Approximately 50 % of the patients taking concomitant Clobazam and/or Valproate modified their dose from baseline during the study (33.3% modified VPA and 16.6% CLB) (**Table 4**).

Table 2. Responder rates for convulsive seizures (A) and total seizures (B).

Table 2 (A): Convulsive seizures

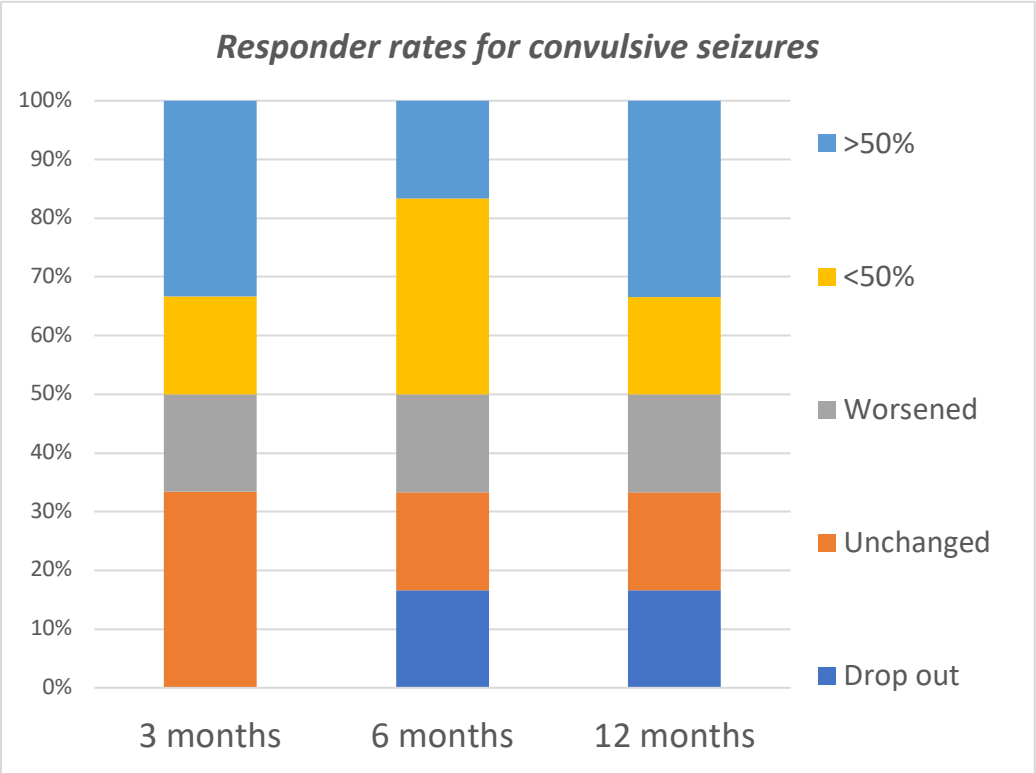
	Full cohort	***Unchanged***	***Worsened***	***<50%***	***>50%***	***Seizure free***
3 months, n (%)	6 (100%)	2 (33,3%)	1 (16,6%)	1 (16,6%)	2 (33,3%)	0 (0%)
6 months, n (%)	5 (83,3%)	1 (16,6%)	1 (16,6%)	2 (33,3%)	1 (16,6%)	0 (0%)
12 months, n (%)	5 (83,3%)	1 (16,6%)	1 (16,6%)	1 (16,6%)	2 (33,3%)	0 (0%)

Table 2 (B): Total seizures

	Full cohort	***Unchanged***	***Worsened***	***<50%***	***>50%***	***Seizure free***
3 months, n (%)	6 (100%)	1 (16,6%)	2 (33,3%)	1 (16,6%)	2 (33,3%)	0 (0%)
6 months, n (%)	5 (83,3%)	0 (0%)	1 (16,6%)	3 (50%)	1 (16,6%)	0 (0%)
12 months, n (%)	5 (83,3%)	1 (16,6%)	1 (16,6%)	1 (16,6%)	2 (33,3%)	0 (0%)

Total seizures included convulsive seizures (i.e., clonic, tonic, tonic-clonic, atonic, focal-onset with secondary generalization) and nonconvulsive seizures (i.e., myoclonic, absence, myoclonic-absence, focal with and without impaired consciousness).

Figure 1. Responder rates for convulsive and total seizures.



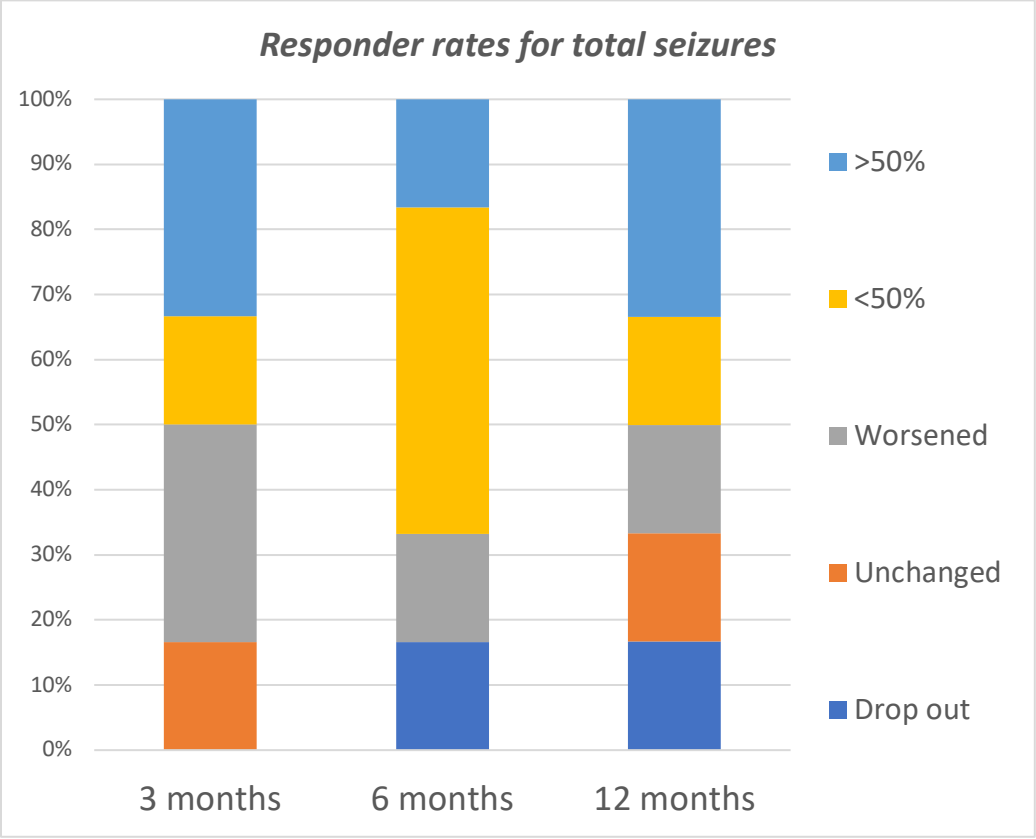
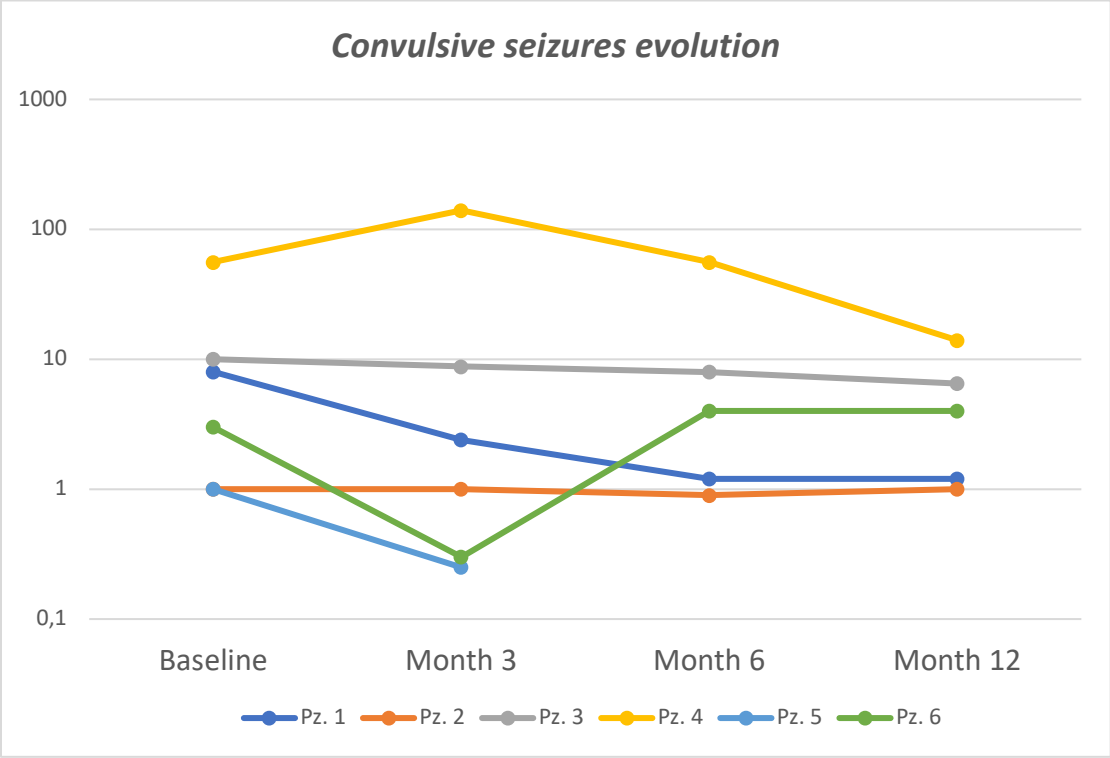
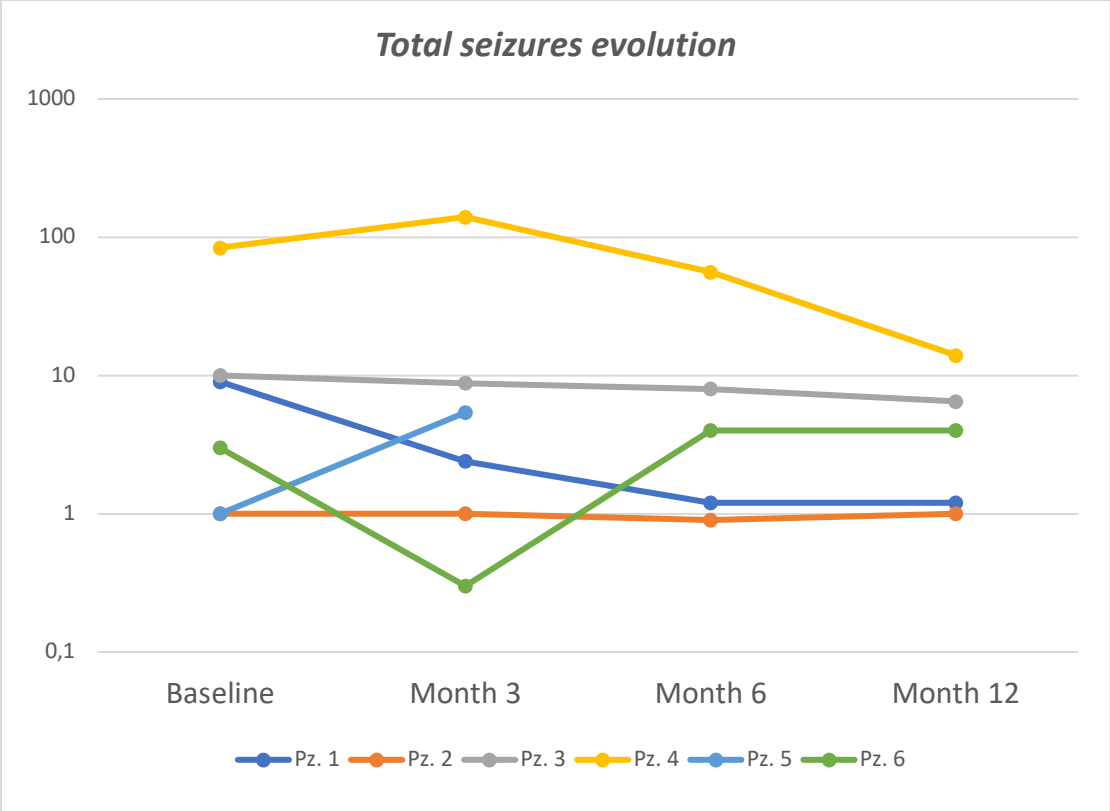


Figure 2. Mean monthly convulsive and total seizures evolution through the study.





6.3. TOLERABILITY

During the 12 months of follow-up, 50 % of the patients experienced at least one AE. Overall, the most common AEs reported were somnolence (16.6%), inappetence (16.6%), and elevated liver enzymes (16.6%) (aspartate aminotransferase >3 upper than the normal limit) (**Table 3**). None of the AEs have been classified as serious. Only one AE (1/6; 16.6 %) led to CBD discontinuation due to both increase of absence seizures and somnolence. One patient taking concomitant Valproic Acid showed clinically significant elevated liver enzymes. No thrombocytopenia (*i.e.,* platelets count <140.000/microliter) was reported. All AE are detailed in table 3.

Table 3. Summary of all reported AEs during the study.

	CBD dose (mg/kg/die)			
	0-1	**11-15**	**16-25**	**All**
Overall AE rate, n (%)	1 (16,67%)	1 (16,67%)	1 (16,67%)	3 (50%)
Overall serious AE rate, n (%)	0%	0%	0%	0%
AEs leading to CBD discontinuation, n (%)	1 (16,67%)	0%	0%	1 (16,67%)
AEs reported in ≥2% in any group:				
Loss of appetite, n (%)	0%	1 (16,67%)	0%	1 (16,67%)
Somnolence, n (%)	1 (16,67%)	0%	0%	1 (16,67%)
Transaminases elevated, n (%)	0%	0%	1 (16,67%)	1 (16,67%)

6.4. EPIDIOLEX AND OTHER ASMS DOSE ADJUSTMENTS DURING TREATMENT

All patients in the study were co-treated with other ASMs during Epidiolex treatment. In particular, the most common concomitant medications were Valproic acid (100%), Stiripentol (66,7 %), Clobazam (50 %), and Topiramate (50%). Levetiracetam and Phenobarbital were assumed in one patient each. The median number of concomitant ASMs was 3 at baseline and during the follow up period. Almost all the concomitant ASMs doses remained stable through the 12 months follow up, but: 33.3% of the patients decreased Clobazam and Topiramate; Stiripentol and Valproic Acid were adjusted to lower dose in 25% and 16.6% of patients, respectively.

At month 6 one patient reduced the Stiripentol dose (from 500 mg/die to 400 mg/die) and another patient reduced the Topiramate dose (from 100 mg/die to 87.5 mg/die). One patient, instead, increased Valproic Acid (from 1000 mg/die to 1250 mg/die). At month 12 one patient reduced Valproic Acid (from 387.5 mg/die to 350 mg/die), one reduced Stiripentol (from 400 mg/die to 375 mg/die) and another patient reduced both Clobazam (from 15 mg/die to 10 mg/die) and Levetiracetam (from 1600 mg/die to 1400 mg/die). All doses adjustments are detailed in **Table 4** and Figures 3, 4, 5, 6.

Table 4. Dose adjustments of co-administered ASMs.

ASMs dose adjustment at all visits, n (%)	Valproic Acid (n=6)	Clobazam (n=3)	Stiripentol (n=4)	Topiramate (n=3)
Baseline dose stable	4 (66,7%)	2 (66,6%)	2 (50%)	2 (66,7%)
Baseline dose increased	1 (16,6%)	0	0	0
Baseline dose decreased	1 (16,6%)	1 (33,3%)	2 (50%)	1 (33,3%)
Baseline dose increased and decreased	0	0	0	0

Figure 3. Epidiolex dose adjustments during the study.

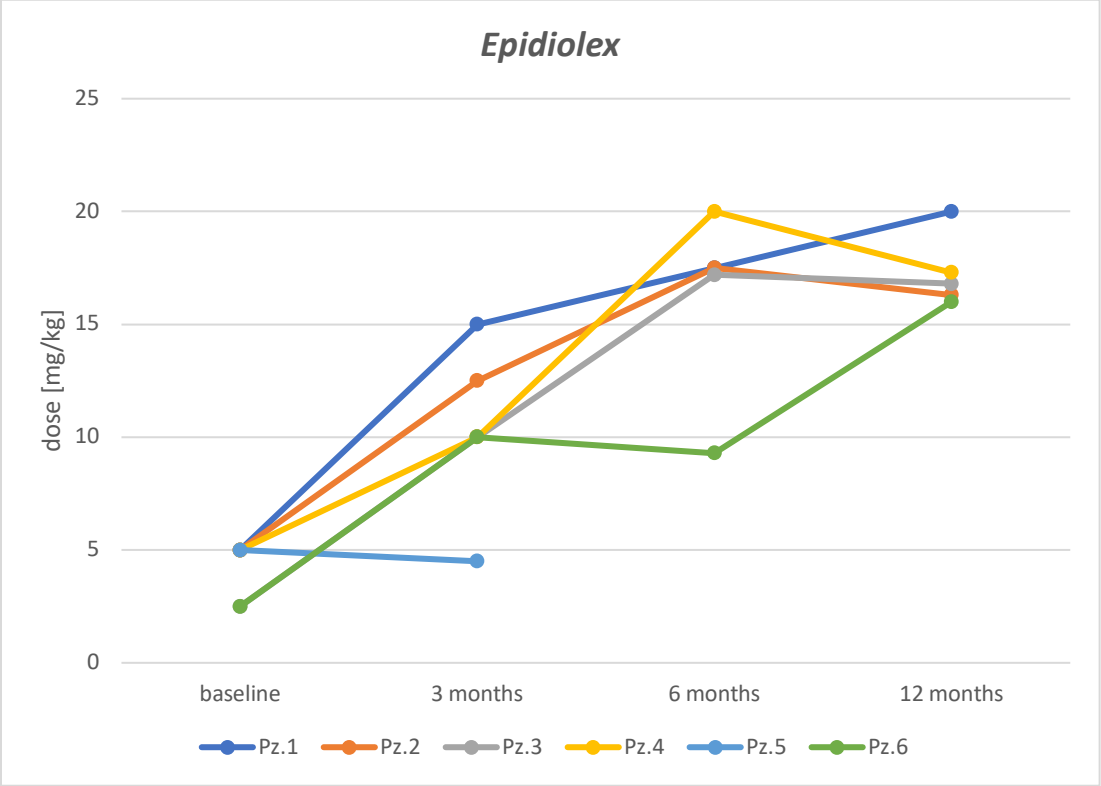
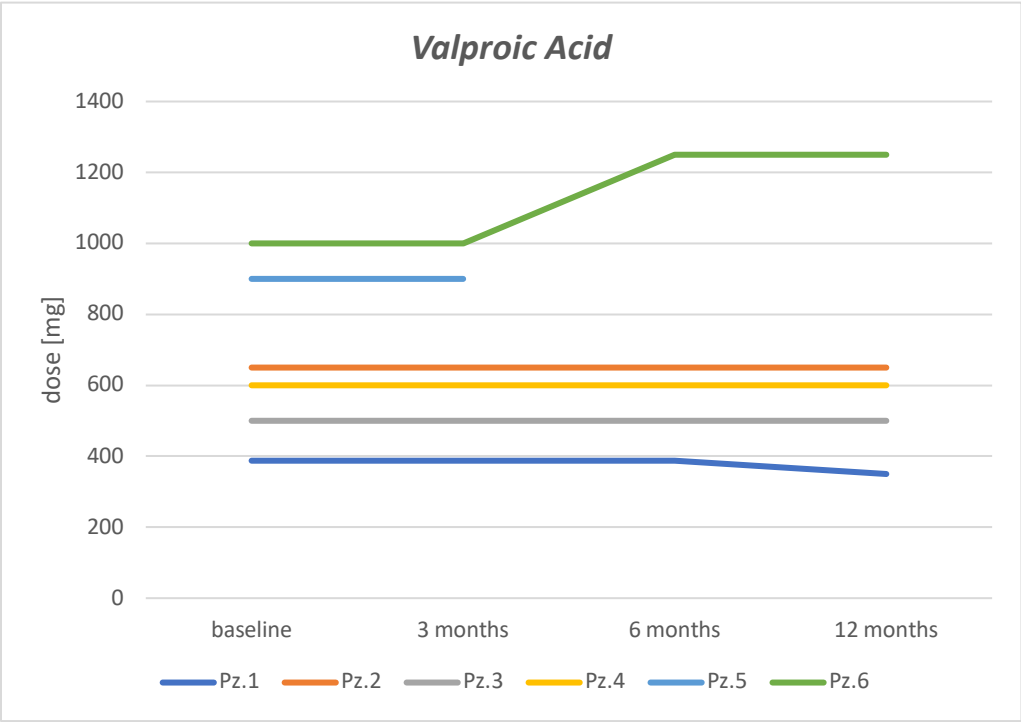
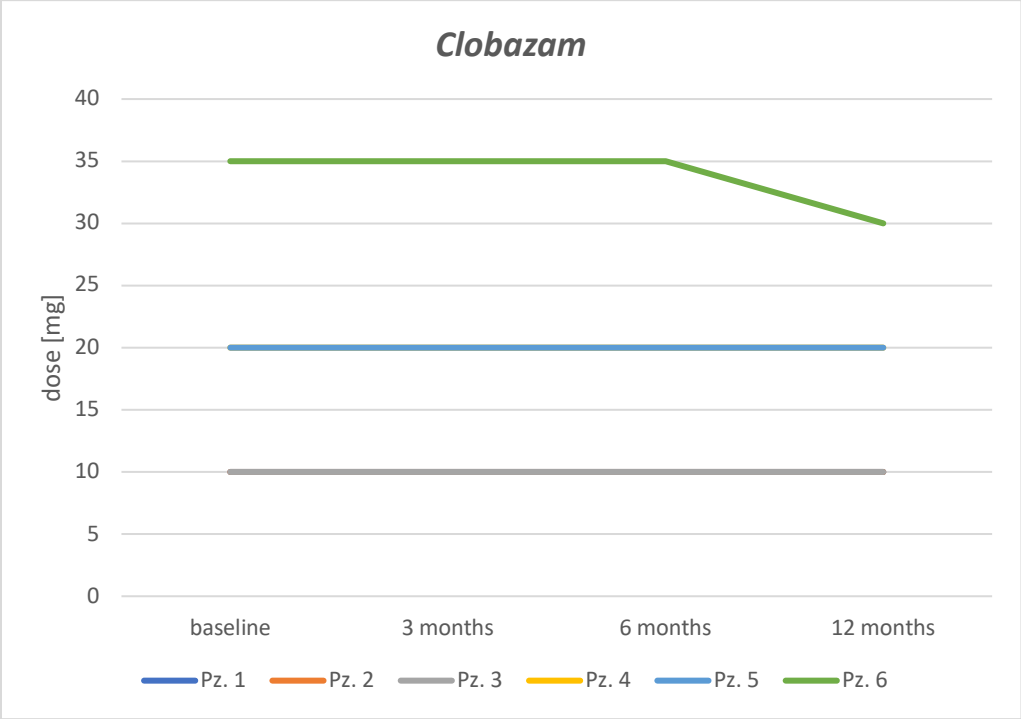
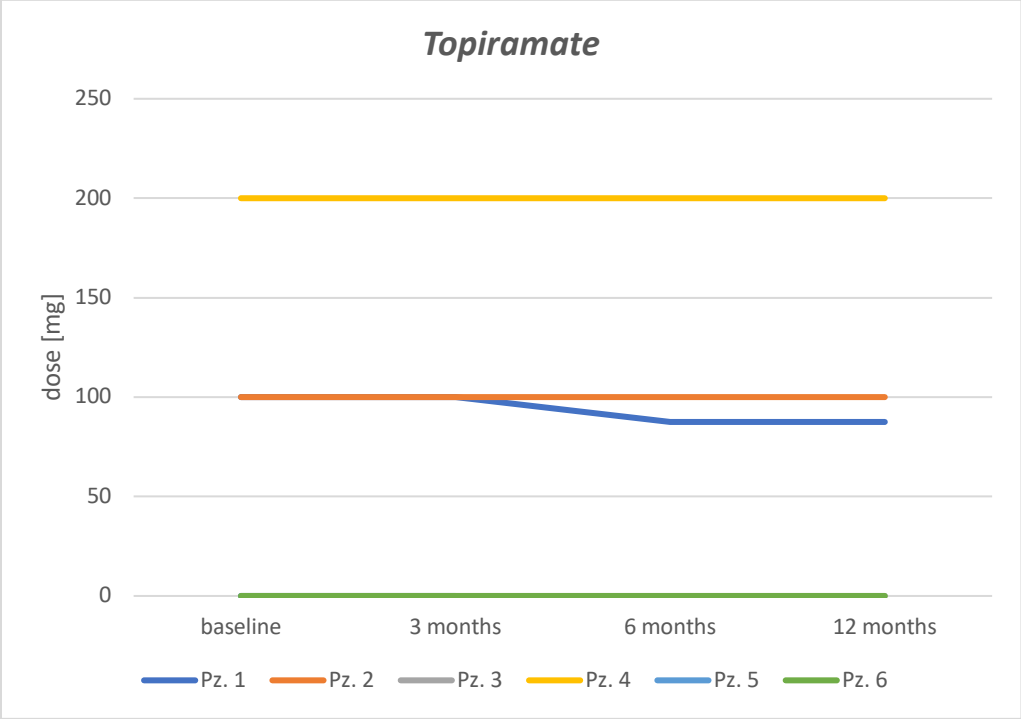
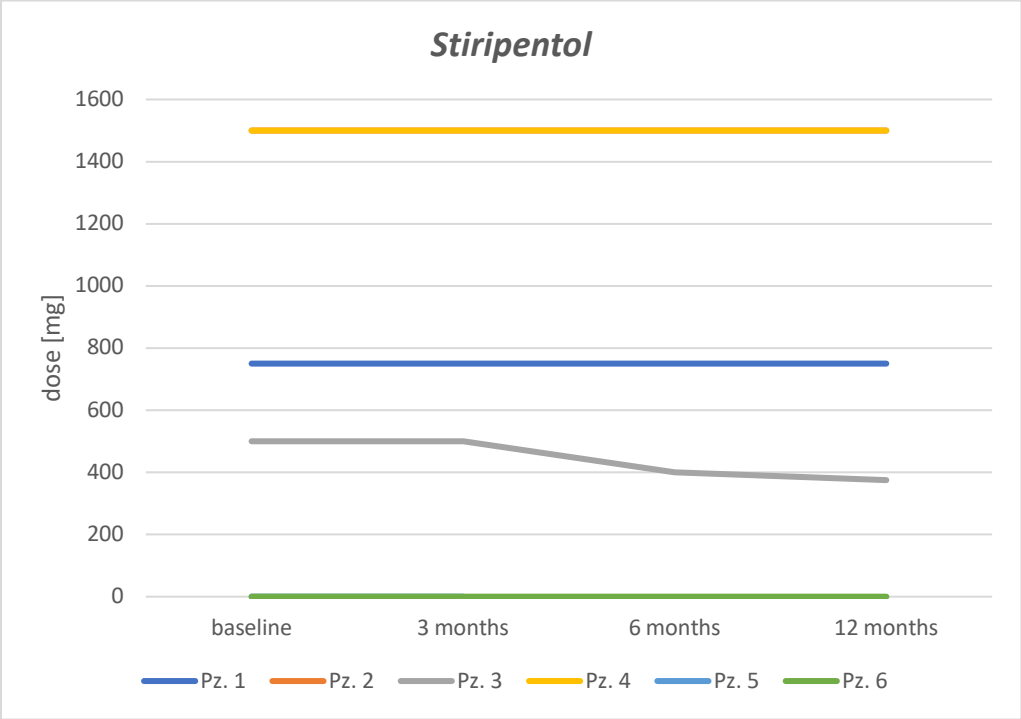


Figure 4. Concomitant ASMs adjustments during treatment









In Figure 3 and 4 Epidiolex and other concomitant ASMs daily doses modification through the 12 months follow up are shown.

Figure 5. Epidiolex dose adjustments during the study.

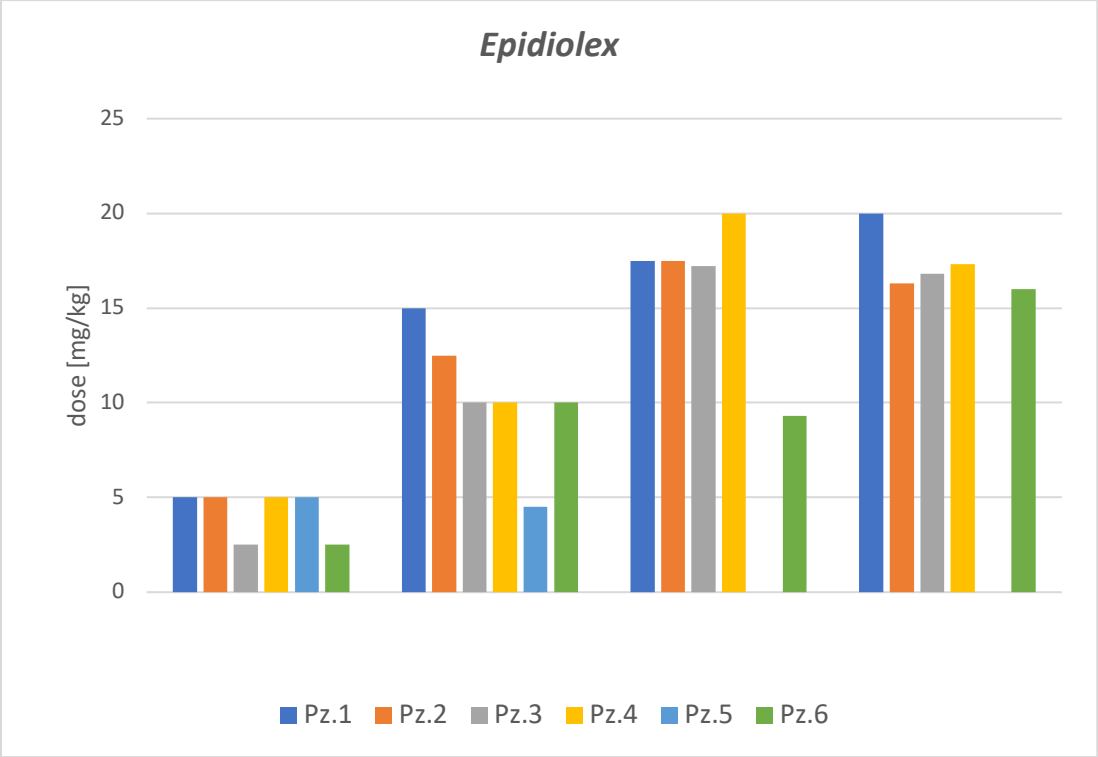
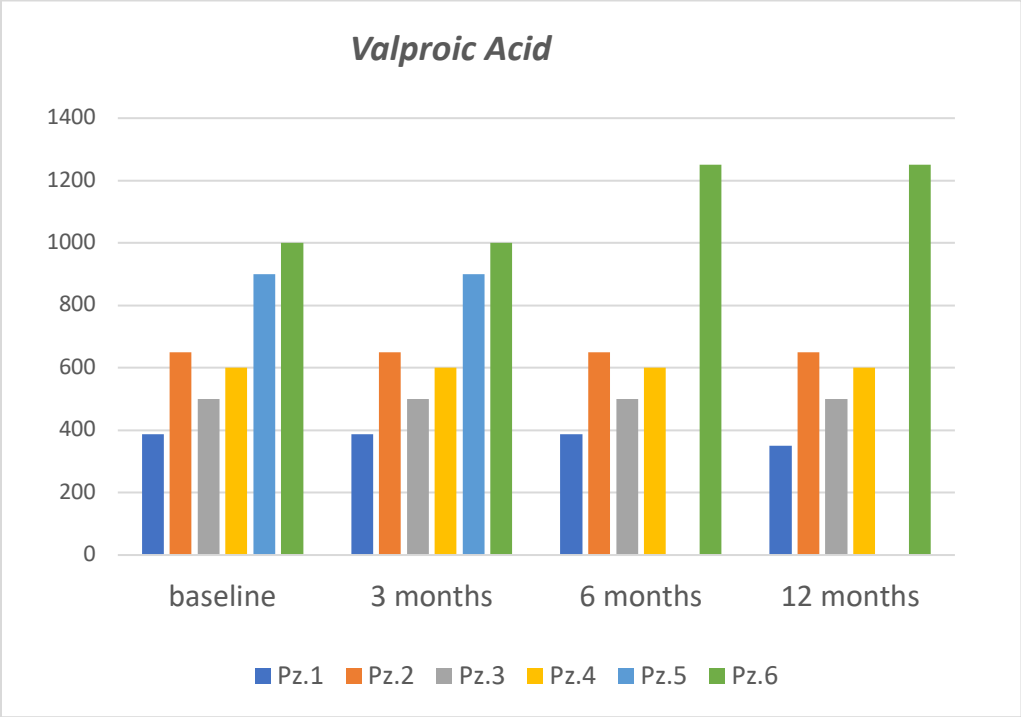
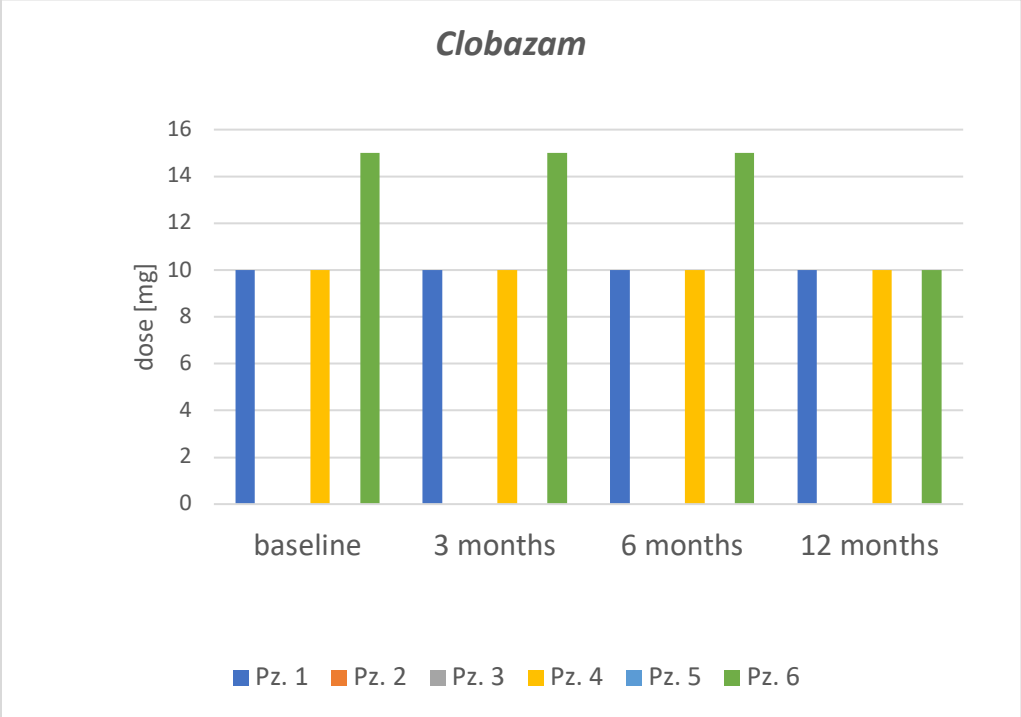
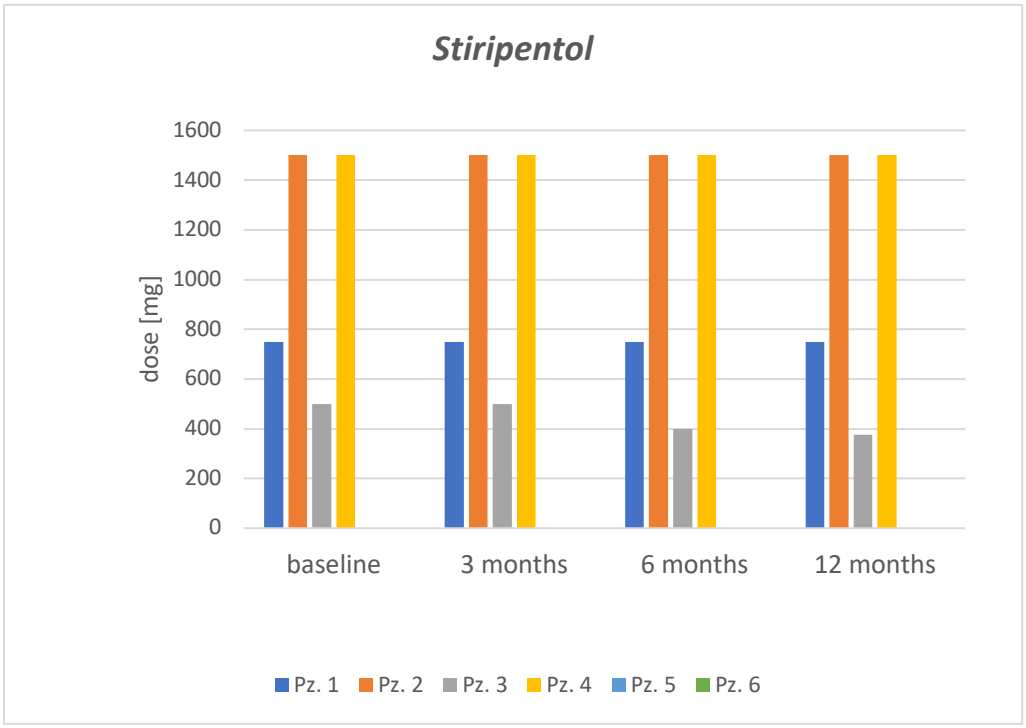
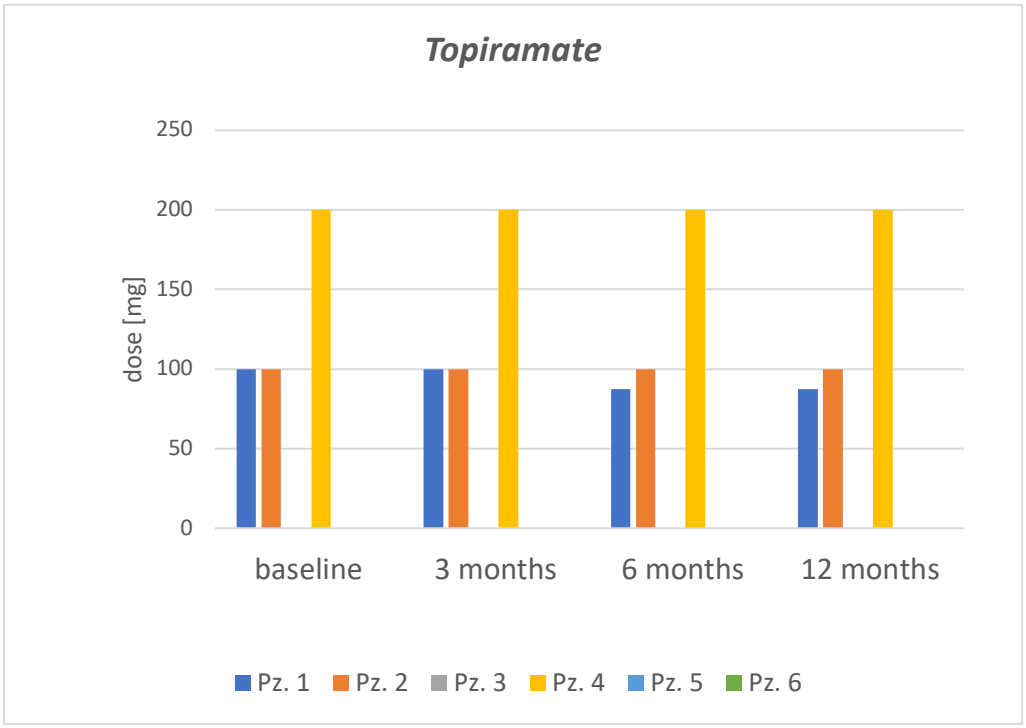


Figure 6. Concomitant ASMs adjustments during treatment






Stiripentol
1600
1400
1200
1000
800
600
400
200
0
dose [mg]
baseline
3 months
6 months
12 months
Pz. 1
Pz. 2
Pz. 3
Pz. 4
Pz. 5
Pz. 6


Topiramate
250
200
150
100
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3 months
6 months
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Pz. 2
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Pz. 4
Pz. 5
Pz. 6

7.DISCUSSION

In our cohort of highly refractory DS patients, the add-on treatment with purified GW cannabidiol (CBD) Epidiolex® for 12 months was associated with a reduction in seizure frequency and was generally well tolerated without severe AEs. CBD also reduced behavioural impairment, as reported by patients and caregivers during the follow-up visits.

Overall, the percentage of patients achieving a seizure reduction ≥50% was 33.3% for both total and convulsive seizures. A partial seizure reduction (<50%) was achieved in a range from 16.6% to 50% patients. No significant difference in median seizures frequency reductions has been found between patients on concomitant CLB and those without. These findings confirm that CBD has antiseizure activity independent to concomitant CLB.

The AEs rates were lower (50%) than expected, the most frequent being loss of appetite (16.6%), somnolence (16.6%), and elevation of liver enzymes (16.6%). None of the patients experienced serious AEs such as status epilepticus or vomiting, but, on the other hand, one patient (16.6%) discontinued CBD due to adverse events (increased absence seizures and somnolence). Notably, the overall incidence of AEs was the same between the different Epidiolex dosages group, as shown in Table 3. This is in contrast with the suggested dose effect (mainly for somnolence) reported in previous studies (57),(38). Recently, one study has reported thrombocytopenia in one-third of patients treated with CBD and concomitant

Valproic Acid (71). However, in our study, no cases occurred, even though 100% of patients were co-treated with Valproic Acid.

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 follow-up period, until 12 months for some patients, 83.3% of the patients with at least one month of treatment, remained on CBD.

The median CBD dosage at baseline (5mg/kg/die) increased at month 3 to 10 mg/kg/die. Then, the median CBD dose remained stable or increased (median dose at month 6 was 17.5 mg/kg/die) although one patient slightly reduced the dose, as allowed by the protocol.

Cannabidiol has well known bidirectional drug-drug interactions with Clobazam (increasing nordesmehyl-clobazam and 7-hydroxy-CBD), and Valproic Acid (probably pharmacodynamic rather than pharmacokinetic interactions) and probably one AE has been reported due to drugs interactions (elevated liver enzymes in patient co-treated with Valproic Acid). As a matter of fact, in our cohort, the only patient reporting transaminase elevation was taking concomitant Valproic Acid. This further confirms the potential interaction of CBD and Valproic Acid in the development of such AEs, as reported in literature (59).

8.CONCLUSIONS

In conclusion, we confirm CBD effectiveness and tolerability in highly refractory DS patients, also without the concomitant use of Clobazam. Of note, dose-dependency for both efficacy and tolerability are not evident. We report a significant reduction in both convulsive and total seizures, as well as an improvement in the QoL and behaviour of patients taking CBD. Interestingly, during CBD treatment, some patients decreased the dose of other concomitant medications (33.3% of the patients decreased Clobazam and Topiramate; Stiripentol and Valproic Acid were adjusted to lower dose in 25% and 16.6% of patients, respectively) indicating the efficacy of this new ASM.

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